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CANCER RESEARCH INSTITUTE REPORT

2003 - 2005

KANAZAWA UNIVERSITY

Cancer Research

Institute Report

2003－2005

SCIENTIFIC REPORTS

Division
of
Molecular Biology

General Summary of Division of Molecular Biology

The division has been dedicated to basic researches on Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), the two predominant causes of chronic hepatitis and eventual hepatocellular carcinoma (HCC) among the world. We focused two molecular targets of the viruses; HBV X protein (HBx) that has been long-suspected but not approved to be oncogenic, and HCV NS5B that is RNA-dependent RNA polymerase (RdRP) responsible for HCV replication. Also we have opened the researches on telomere and telomerase in yeast and human, that are tightly regulated in normal cells but deregulated in cancer cells. The outcomes of these projects in the last three years have been much contributed to understanding new aspects of the regulatory processes and the putative molecular partners of these target molecules. Unfortunately, the division will be closed by the end of March 2006 since the chief has to retire.

A) HBx and related targets.

To elucidate the molecular role of HBx in transcriptional modulation, we further analyzed the nuclear target of HBx, RNA polymerase II subunit 5 (RPB5) by a two or three-step alanine scanning strategy. Among the identified residues critical for the bindings to HBx and RAP30, T111 and S113 are close to DNA and critical for both TFIIF- and HBx-binding. We found the double-strand DNA-binding ability of RPB5 and identified the critical and important residues including T111 and S113 for the DNA-binding. These results may imply that HBx interferes with DNA-binding of RPB5 to modulate transcription initiation or initiation to elongation.

We tried to evaluate biological roles of HBx in two aspects. Firstly the roles of HBx in HBV transcription and replication using a HBV replicon system were examined. The results demonstrate that HBx augments HBV transcription and replication though the coactivation ability since the HBx sequences critical for the coactivation and for the augmentation of HBV replication are the same, and HBV 3.5 kb RNA and DNA are affected at the similar extent by the ectopic expressed HBx. Secondly we evaluated whether HBx retains ability to immortalize human primary cells and/or to transform human immortalized primary cells *in vitro* since the oncogenic property of HBx has been all addressed with rodent systems, and it remains strongly controversial during last decades. We found that HBx overcomes active RAS-induced senescence and affords ability to the immortalized cells expressing RAS and HBx to make colonies in soft agar assay and tumors in nude mice. Such overcoming ability of HBx requires full-length HBx, indicating that the coactivation domain is not enough and that the N-terminal domain has some critical role(s) for the ability.

B) HCV NS5B, replicase, and HCV replication.

We previously identified the novel 5 residues of NS5B critical for RdRP activity and the

interaction between NS5B and NS5A, the HCV non-structural regulatory protein, using bacterial recombinant proteins *in vitro*. On the way to elucidate subcellular localization of NS5B, we found the tight interaction of NS5B and nucleolin, a RNA chaperon. In last several years, we concentrated to evaluate whether the findings obtained *in vitro* have biological relevance in HCV replication *in vivo* by introducing HCV RNA subreplicon system (provided by Dr. Shimotohno, Kyoto Univ.) and by establishing an improved system. We confirmed that the 5 residues of NS5B defined *in vitro* are also critical for HCV replication *in vivo*. By scanning with clustered alanine substitution libraries of NS5A in HCV replicon, the critical nature of the interaction of NS5A and NS5B was found to be crucial in HCV replication. We are now going to characterize the important role of nucleolin in HCV replication through the direct binding to NS5B.

C) Telomere and telomerase in yeast and human.

We characterize regulatory steps of telomere and telomerase in yeast and human.

In order to investigate relationships between life span and telomere function and to identify signal-transmitting pathway, which responds to ageing stress, we screened telomere length and silencing in the knock-out series of protein phosphatase (PPase) genes in budding yeast. Only the *sit4* mutant encoding the type 2A of PPase (PP2A) was selected. The mutant cells accumulated highly phosphorylated Sir3 and decreased amount of Sir3 on subtelomere region. Moreover, shortened life span comparable to *sgs1* cells, which lost RecQ type helicase, was observed in *sit4* cells. These suggest that PP2A regulates phosphorylation state of Sir3 responding to ageing stress.

In human system, we have tried to purify insect-expressed recombinant hTERT. During purification, we found the highly purified protein failed to exhibit reconstituted telomerase activity *in vitro*. Then, we established an insect coexpression system of the two-components, and are trying to purify the recombinant telomerase. We collaborated with Drs. Hahn and Weinberg to raise anti-hTERT mAbs that enabled to characterize telomerase in human primary fibroblasts. The results together with knock-down experiments of hTERT indicate that telomerase activity is important for cell proliferation and cell lifespan. We also found the interaction between hTERT and nucleolin that is important in regulating subcellular localization of telomerase.

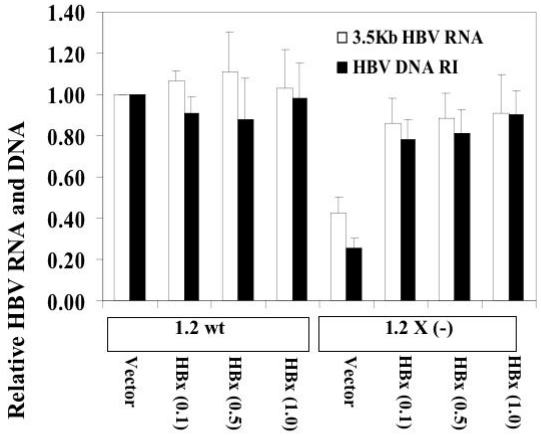
The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication[1].

Tang H*, Delgermaa L, Huang F*, Oishi N, Liu L*, He L*, Zhao L*, Murakami S.

The roles of HBx in regulating HBV transcription and replication were examined with a transient transfection system in HepG2 using wild-type or HBx-minus HBV genome constructs, and a series of mutation HBx expression plasmids. HBx has augmentation effects on HBV transcription and replication as HBV mutant genome with defective X gene led to decreased levels of 3.5-Kb HBV RNA and HBV replication intermediates, and these decreases can be complemented *in trans* by ectopic expression of HBx. The truncation mutant HBx-D1 (aa. 51-154) containing the coactivation domain is enough for this function. By alanine scan mutagenesis strategy, the regions between aa 52 - 65 and aa 88 -154 are important for the augmentation function of HBx in HBV replication. With reporter gene analysis, the transactivation and coactivation activities of HBx are well coincide with its augmentation function in HBV transcription and replication. Therefore HBx has important role in stimulating HBV transcription and replication, and the coactivation function of HBx may be critical for the augmentation effect on HBV replication. (*The project is based on the international collaboration between Sichuan Univ. and Kanazawa Univ,)

Reference 1: Tang H, et al., (2005) J. Virol., 79: 5548-5556.

Figure illustrates quantitative analysis of the 3.5-kb HBV RNA and HBV DNA replication intermediates of 1.2 genome-unit of wild and defective in X-ORF in the absence or in the presence of varying amount of the HBx-expression plasmid.



HBx contributes to the oncogenic conversion of human immortalized cells with active RAS by overcoming oncogene-induced senescence[1].

Oishi T, Masutomi K, Khurts S, Nakamoto T, Kaneko S, Murakami S.

HBx has been long suspected to be oncogenic although it remains still controversial. Pathological roles of HBx in the carcinogenic process have been previously examined only with rodent systems. Therefore we addressed effect of HBx on the immortalization and transformation abilities in human primary cells.

As HBx alone failed to immortalize human primary cells, BJ and TIG3 cells, nor to transform the hTERT-introduced immortalized BJ cells, we next examined HBx overcomes active RAS-induced cellular as oncogene-induced senescence (OIS) has been recently regarded as one of the antitumor processes of cells. The immortalized BJ cells expressing active RAS and HBx proliferate more than 80 population doublings and could form colonies in soft agar and tumors in nude mice (Figure) although RAS alone actually induced senescence of the immortalized BJ cells. A truncated mutant, HBx-D1 (aa 51-154), had the ability to overcome OIS in a population doubling analysis, but failed to exhibit colonigenic and tumorigenic abilities. These observations indicate that HBx-D1, which harbors the coactivation domain is not equivalent to full-length HBx in the ability to overcome active RAS-induced senescence, strongly suggesting that HBx can contribute to carcinogenesis by overcoming OIS in human cells.

Reference 1: Oishi N, et al., (2005) International Meeting on the Molecular Biology of Hepatitis B Viruses, Heidelberg, Germany, Sep. 18-21, 2005

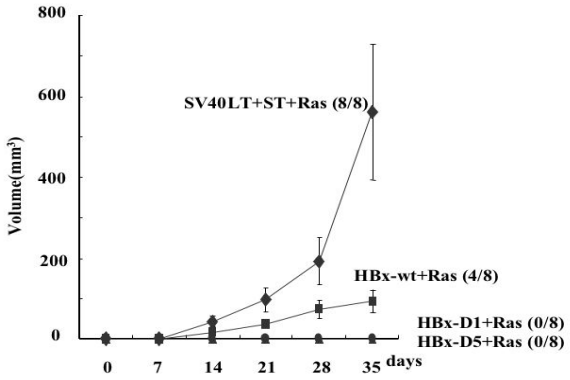


Figure illustrates tumor formation in nude mice. Each point on the graph represents the average volume of tumors. SV40 LT+ST+RAS is a positive control.

Mutational analysis of the central part of human RNA polymerase II subunit 5 (RPB5) by two-step alanine scanning: the residues critical for interactions with TFIIF subunit RAP30 and Hepatitis B Virus X protein and those for DNA-binding ability.

Le TTT, Zhang S, Hayashi N, Yasukawa M, Delgermaa L, Murakami S.

RPB5 is close to DNA downstream of initiation site and interacts with several regulators. HBx binds the central part of RPB5 to modulate activated transcription, and TFIIF subunit RAP30 interacts with the same part of RPB5 that is critical for the association between TFIIF and RNAPII. By introducing systematic mutagenesis of the central part of RPB5 using two-step alanine scanning libraries to pinpoint critical residues for its binding to RAP30 in the TFIIF complex and/or to HBx, and identified these residues in both mammalian cells and in an *in vitro* binding assay. Four residues, F76, I104, T111 and S113, are critical for both TFIIF- and HBx-binding, indicating the overlapping nature of the sites of interaction. In addition, V74 and N98 are required for HBx-binding, and T56 and L58 are needed for RAP30-binding. Interestingly the residues exposed to solvent, T111 and S113, are very close to the DNA, implying that two factors may modulate the interaction between DNA and RPB5[1].

Reference 1: Le TTT, Zhang S, Hayashi N, Yasukawa M, Delgermaa L, Murakami S. (2005) J. Biochem (Tokyo), 138(3): 215-224.

Summary of RPB5-binding abilities

Mutated humanRPB5 residue	HBx	RAP30 (TFIIF)	Mutated humanRPB5 residue	HBx	RAP30 (TFIIF)
T56A	++ [a]	-	I99A	++	++
D57A	++	++	T100A	++	++
L58A	++	-	R101A	++	++
D70A	++	++	L103A	++	++
Q71A	++	++	I104A	-	-
M72A	++	++	V105A	++	++
F73A	++	++	M110A	++	++
V74A	-	++	T111A	-	-
F75A	++	++	P112A	++	++
F76A	-	-	S113A	-	-
N98A	-	++			

[a] (++): binding positive; (-): binding negative

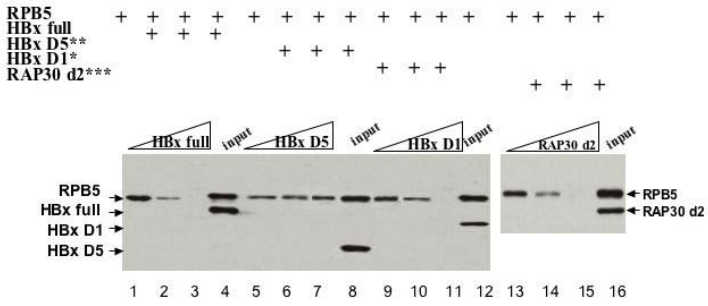
DNA-binding ability of RNA polymerase II subunit 5 (RPB5)

Zhang S, Le TTT, Tang H, Wei W, Hayashi N, Murakami S.

We found that RPB5 retains DNA-binding ability trapped by double-stranded DNA cellulose. The 6 residues critical for binding DNA were identified within the middle part of RPB5, by a three-step alanine scanning with clustered and point substitution libraries. Among them, T111 is solvent-exposed and the nearest neighbor of P112 that is the residue predicted to be closest to DNA. Three residues are important for the structural integrity of the mixed ß-sheet that may indirectly affect DNA-binding ability. We evaluated these residues conserved among human and yeast by introducing a point mutant in yeast in place of its wild-type counterpart. T117A, T117G (hT111) and the glycine substitution of the three residues in the ß-sheet affected cell growth at suboptimal temperatures. Interestingly, most of these residues are also indispensable for RPB5 to bind HBx and/or RAP30. Actually these factors inhibited DNA-binding of RPB5, strongly supporting the notion that these regulators may modulate transcription by inhibiting RPB5 from interacting with DNA.

Zhang S, Le TTT, Tang H, Wei W, Hayashi N, Murakami S. 2005 International Meeting on Molecular Biology of HBV viruses. Sept. 20 at Heidelberg Univ.

DNA-binding of RPB5 was inhibited by HBx and RAP30



*HBx-D1 harbors aa 51-154 including the coactivation domain and the RPB5-binding region that is responsible to inhibit DNA- binding of RPB5.
**HBx-D5 harbors aa 1-50 including the negative regulatory domain.
***RAP30 d2 harbors aa 1-176 missing the C-terminal region that retains DNA-binding ability.

Subcellular Localization of RPB5-Mediating Protein, RMP, and its putative functional partner[1].

Delgermaa L, Hayashi N, Dorjsuren D, Nomura T, Thuy LT, Murakami S.

We previously identified a novel cellular protein RMP that retains corepressor activity and functionally antagonizes transcriptional modulation by Hepatitis B virus X protein (Dorjsuren D. et al., Mol. Cell. Biol., 18: 7546-7555, 1998). Here, subcellular localization of RMP was examined with GFP-fused forms. We found that a nuclear localization signal (NLS) and a coiled-coil (CC) domain functioning as a cytoplasmic localization signal (CLS), are both important for the subcellular localization of RMP. The CLS apparently acts dominantly since RMP is mostly localized in cytoplasm with weak and diffuse signals in nuclei, and the NLS is indispensable for the nuclear localization of RMP only in the absence of the CLS. Using a yeast two-hybrid method, we isolated a putative corepressor, DNA methyltransferase 1 (DNMT1) associating protein, DMAP1, which was demonstrated to bind the CC domain of RMP. DMAP1 facilitates nuclear localization of RMP and the corepressor activity of RMP in a dose-dependent manner through the interacting with the CC domain of RMP. The results were discussed in light of the recent paper showing a novel evolutionary conserved role of RMP/URI in TOR signaling (Gstaiger, M. et al., Science, 302: 1208-1212, 2003).

Reference 1: Delgermaa L, Hayashi N, Dorjsuren D, Nomura T, Thuy LT, Murakami S. (2004) Mol. Cell. Biol., 24(19):8556-8566.

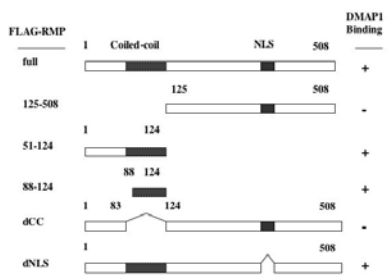


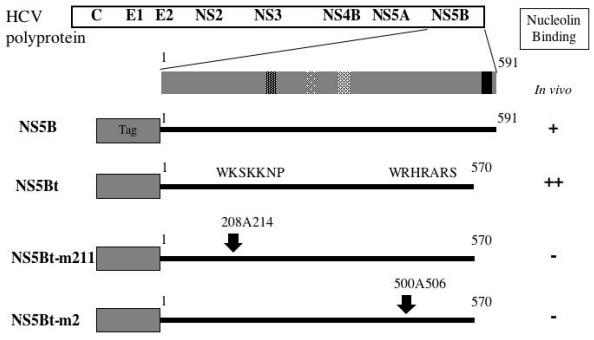
Figure illustrates the summary of the DMAP1-binding region of RMP that is within the coiled-coil domain by GST pull-down *in vitro* using the partially purified proteins. The result is consistent with that using extracts of cells transiently expressing two differentially tagged proteins[1].

Direct Interaction between nucleolin and HCV NS5B[1].

M. Hirano, S. Kaneko, T. Yamashita, H. Luo, W. Qin, Y. Shirota, T. Nomura, K. Kobayashi, S. Murakami.

HCV NS5B is an RNA-dependent RNA polymerase (RdRP), a central catalytic enzyme in HCV replication. While studying the subcellular localization of a NS5B mutant lacking the C-terminal membrane-anchoring domain, NS5Bt, we found that expression of the GFP-fused form was exclusively nucleolar. Interestingly, the distribution of endogenous nucleolin changed greatly in the cells expressing GFP-NS5B, with nucleolin colocalized with GFP-NS5B in perinuclear regions in addition to the nucleolus. The interaction between nucleolin and NS5B was demonstrated by GST pull-down assay. The results indicated that C-terminal region of nucleolin was important for its binding to NS5B. Scanning a clustered- alanine substitution mutant library of NS5B revealed that two sequences of NS5B, aa 208-214 and aa 500-506, were both found to be indispensable for the nucleolin binding. We reported that the latter sequence is essential for oligomerization of NS5B which is a prerequisite for the RdRP activity. C-terminal nucleolin inhibited the NS5B RdRP activity in a dose dependent manner. Taken together, the binding ability of nucleolin may be involved in NS5B functions.

Reference 1: M. Hirano, S. Kaneko, T. Yamashita, H. Luo, W. Qin, Y. Shirota, T. Nomura, K. Kobayashi, S. Murakami. (2003) J. Biol. Chem., 278: 5109-5115.



Two sequences of NS5B are critical for nucleolin-binding.

The critical sequences of NS5B shown at the top of NS5Bt which were clustered alanine-substituted as shown at the bottom constructs[1].

Effect of Interaction between Hepatitis C Virus NS5A and NS5B on the Hepatitis C Virus Replicon[1].

Shimakami T, Hijikata M*. Luo H, Ma Y, Kaneko S, Shimotohno K*, Murakami S.
*Virus Research Inst. Kyoto Univ.

HCV NS5A is important for the establishment of replication by adaptive mutations or localization, although its role in viral replication remains unclear. It was previously reported that NS5A interacts with NS5B via two regions of NS5A in the isolate JK-1 and modulates the activity of NS5B RdRp (Y. Shirota et al., J. Biol. Chem., 277:11149–11155, 2002), but the biological significance of this interaction has not been determined. We examined the effect of this interaction on HCV RNA replication with an HCV replicon system derived from the isolate M1LE (H. Kishine et al., Biochem. Biophys. Res. Commun., 293:993–999, 2002). We constructed three internal deletion mutants, M1LE/5Adel-1 and M1LE/5Adel-2, each encoding NS5A which cannot bind NS5B, and M1LE/5Adel-3, encoding NS5A that can bind NS5B. After transfection into Huh-7 cells, M1LE/5Adel-3 was replication competent, but both M1LE/5Adel-1 and M1LE/5Adel-2 were not. By scanning with 20 alanine-substituted clustered mutants within both NS5B-binding regions, only 5 of the 20 mutants were replication competent. Subsequently, we established more efficient replicon system by introducing a point mutation, S232I, into NS5A using cured Huh-7 cells as recipient cells. In this system, only the same five mutants were replication competent. These results strongly suggest that the interaction between NS5A and NS5B is critical for HCV RNA replication in the HCV replicon system. This improved system was applied to examine whether the 5 residues of NS5B indispensable for RdRP activity *in vitro* are critical in HCV replication. An alanine-substitution mutant of one of the 5 residues was replication incompetent in the HCV replicon system[2].

Reference 1: Shimakami, T., Hijikata, M. Luo, H., Ma, Y., Kaneko, S., Shimotohno, K., Murakami, S. (2004) J. Virol., 78: 2738-2748.
Reference 2: Ma Y, Shimakami T, Luo H, Hayashi N, Murakami S. (2004) J Biol Chem, 279(24):25474-25482.

Genetic studies of signal transduction pathway, responding to ageing stress and regulating telomere function.

N. Hayashi, S. Murakami

Cellular senescence is irreversible arrest of cell cycle. The probability to be into the senescence increases with the passage of cell division. Accumulation of many kinds of stress, such as ROS (reactive oxygen species), DNA lesion, shortened telomere, loss of heterochromatin structure and so on, would cause senescence. So, many signal pathways may work for the maintenance of its cellular life span. Budding yeast, *Saccharomyces cerevisiae*, has 32 protein phosphatases (PPase), and 30 in them are nonessential. We screened yeast knock-out series of PPases to investigate the regulatory pathway of cellular senescence. We examined telomere length of them by Southern blot analysis, and isolated the *sit4* cell that had slightly short telomere. *SIT4* gene encodes one of type 2A PPases (PP2A), and involved in cell cycle control. Silencing ability at telomere region in the *sit4* cells was also deficient, and phosphorylated Sir3 protein was accumulated. Life span in the *sit4* cells was as short as in the *sgs1* cells. Furthermore, we found altered structure of nucleoli and frequent recombination at rDNA region in them. These suggest that PP2A regulates phosphorylation of the Sir3 protein to maintain heterochromatin structure at telomere, and also these may suggest that formation of heterochromatin structure affects cellular life span.

Reference: Hayashi et al. The *SIT4* gene, which encodes protein phosphatase 2A, is required for telomere function in *Saccharomyces cerevisiae*. Curr Genet 47:359-367. 2005.

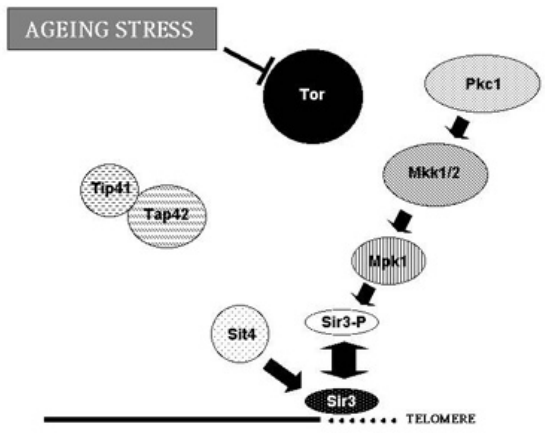


Figure. Model of regulatory system of heterochromatin status via Sir3 phosphorylation by PPase and MAP kinase.

Telomerase maintains telomere structure in normal human cells.

Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, Brooks MW, Kaneko S, Murakami S, DeCaprio JA, Weinberg RA, Stewart SA, Hahn WC.

In normal human cells, telomeres shorten with successive rounds of cell division, and immortalization correlates with stabilization of telomere length. These observations suggest that human cancer cells achieve immortalization in large part through the illegitimate activation of telomerase expression.

We found that the rate-limiting telomerase catalytic subunit hTERT is expressed in cycling primary presenescent human fibroblasts, previously believed to lack hTERT expression and telomerase activity. Disruption of telomerase activity in normal human cells by knock-down hTERT slows cell proliferation, restricts cell lifespan, and alters the maintenance of the 3' single-stranded telomeric overhang without changing the rate of overall telomere shortening. Together, these observations support the view that telomerase and telomere structure are dynamically regulated in normal human cells and that telomere length alone is unlikely to trigger entry into replicative senescence.

(This project was carried out by an international collaboration with Dr. Hahn WC, Dana-Farber Cancer Institute, Harvard Medical School, and Dr. Weinberg RA, Whitehead Inst. MIT.)

Reference 1: Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, Brooks MW, Kaneko S, Murakami S, DeCaprio JA, Weinberg RA, Stewart SA, Hahn WC. (2003) Cell, 114(2):241-253.

Nucleolin interacts with telomerase[1].

Khurts S, Masutomi K, Delgermaa L, Arai K, Oishi N, Mizuno H, Hayashi N, Hahn W, Murakami S.

Telomerase is a specialized reverse transcriptase composed of core RNA and protein subunits that plays essential roles in maintaining telomeres in actively dividing cells. Recent work indicates that telomerase shuttles between subcellular compartments during assembly and in response to specific stimuli. In particular, telomerase co-localizes with nucleoli in normal human fibroblasts. Here, we show that nucleolin, a major nucleolar phosphoprotein, interacts with telomerase and alters its subcellular localization. Nucleolin binds hTERT through interactions with its RNA-binding domain 4 and carboxyterminal RGG domain, and this binding also involves the telomerase RNA subunit *hTERC*. The protein-protein interaction between nucleolin and hTERT is critical for the nucleolar localization of hTERT. These findings indicate that interaction of hTERT and nucleolin participates in the dynamic intracellular localization of telomerase complex.

Reference 1: Khurts S, Masutomi K, Delgermaa L, Arai K, Oishi N, Mizuno H, Hayashi N, Hahn W,Murakami S. (2004) J Biol Chem, 279: 51508-51515.

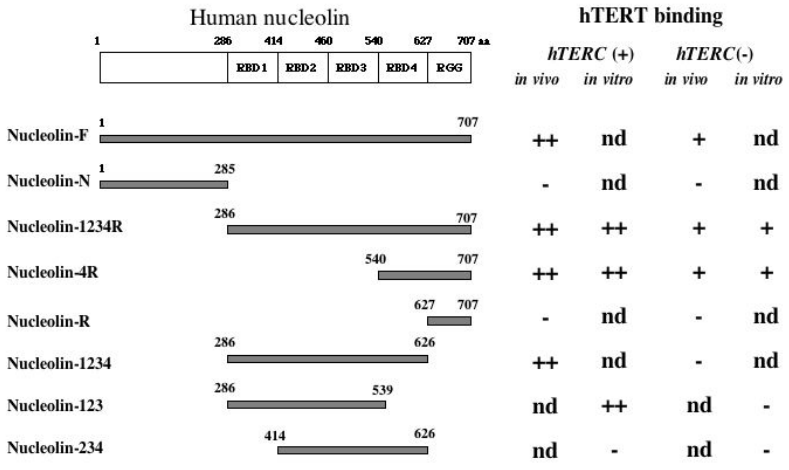


Figure illustrates the hTERT-binding region delineated by *in vivo* and *in vitro* with GST pull-down experiments[1].

Publications

1. Hirano M, Kaneko S, Yamashita T, Luo H, Qin W, Shirota Y, Nomura T, Kobayashi K, Murakami S. (2003) Direct interaction between nucleolin and Hepatitis C Virus NS5B. J Biol Chem. 278: 5109-5115.
2. Wei W, Gu JX. Zhu CQ, Sun FY, Dorjsuren D, Lin Y, Murakami S. (2003) Interaction with general transcription fator IIF (TFIIF) is required for the suppression on activated transcription by RPB5-mediating protein RMP. Cell Research. 13:111-120.
3. Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, Brooks MW, Kaneko S, Murakami S, DeCaprio JA, Weinberg RA, Stewart SA, Hahn WC. (2003) Telomerase maintains telomere structure in normal human cells. Cell. 114(2): 241-253.
4. Kyo S, Masutomi K, Maida Y, Kanaya T, Yatabe N, Nakamura M, Tanaka M, Takarada M, Sugawara I, Murakami S, Taira T, Inoue M. (2003) Significance of immunological detection of human telomerase reverse transcriptase: re-evaluation of expression and localization of human telomerase reverse transcriptase. Am J Pathol. 163(3): 859-867.
5. Shimakami T, Hijikata M, Luo H. Ma Y, Kaneko S, Shimotohno K, Murakami S. (2004) Effect of interaction between Hepatitis C Virus NS5A and NS5B on the Hepatitis C Virus replicon. J Virol. 78: 2738-2748.
6. Moon EJ, Jeong CH, Jeong JW, Kim KR, Yu DY, Murakami S, Kim CW, Kim KW. (2004) Hepatitis B virus X protein induces angiogenesis by stabilizing hypoxia -inducible factor-1alpha. FASEB J. 18(2): 382-384.
7. Luo H, Qin W, Ma Y, Shimakami T, Murakami S, He F, Zhao L.(2004) Purification and partial characterization of hepatitis C virus (HCV) non-structural protein 5A (NS5A) expressed in *Escherichia coli.* Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. Oct;21(5):795-799. [Article in Chinese]
8. Ma Y, Shimakami T, Luo H, Hayashi N, Murakami S. (2004) Mutational analysis of hepatitis C virus NS5B in the subgenomic replicon cell culture. J Biol Chem. 279 (24) : 25474-25482.
9. Delgermaa L, Hayashi N, Dorjsuren D, Nomura T, Le TTT, Murakami S. (2004) Subcellular localization of RPB5-mediating protein, RMP, and its putative functional partner. Mol Cell Biol. 24(19): 8556-8566.
10. Khurts S, Masutomi K, Delgermaa L, Arai K, Oishi N, Mizuno H, Hayashi N, Hahn WC, Murakami S. (2004) Nucleolin interacts with telomerase. J Biol Chem. 279: 51508-51515.
11. Tang H, Delgermaa L, Huang F, Oishi N, Liu L, He L, Zhao L, Murakami S. (2005) Transcriptional transactivation function of HBx protein is important for the augmentation role

in Hepatitis B Virus replication. J Virol. 79(9): 5548-5556.
12. Hayashi N, Nomura T, Sakumoto N, Mukai Y, Kaneko Y, Harashima S, Murakami S. (2005) The *SIT4* gene, which encodes protein phosphatase 2, is reqired for telomere function in *Sccharomyces cerevisiae.* Current genetics. 47: 359-367
13. Le TTT, Zhang S, Hayashi N, Yasukawa M, Delgermaa L, Murakami S.(2005) Mutational analysis of human RNA polymerase II subunit 5 (RPB5): the residues critical for interactions with TFIIF subunit RAP30 and Hepatitis B Virus X protein. J Biochem (Tokyo). 138(3): 215-224.
14. Shimakami T, Honda M, Kusakawa T, Murata T, Shimotohno K, Kaneko S, Murakami S. (2006) Effect of HCV NS5B-nucleolin interaction on HCV replication with HCV subgenomic replicon. J Virol., in press.

Other achievements

Murakami S. Hokkoku Bunka Prize by Hokkoku Shinbun. Nov. 3, 2004

Murakami S. Invited professor of Soochaw University, Souzhou, China
From April, 2005~

Murakami S. Head of Collaboration Research Center of Kanazawa University.
From April, 2004~March 2006.

Division of Molecular Virology and Oncology

General Summary of Division of Molecular Virology and Oncology

Degradation of extracellular matrix (ECM) is one of the first steps for tumor invasion and metastasis. Matrix metalloproteinases (MMPs) have been strongly implicated in this step. Membrane-type MMP-1 (MT1-MMP) was identified in this laboratory as an activator of MMP-2 expressed on the surface of tumor cells (Nature, 370, 61-65, 1994), and later not only ECM macromolecules but also various biologically important molecules were shown to serve as substrates for MT1-MMP. Accumulating evidences have demonstrated that MT1-MMP expression level is closely associated with invasiveness and malignancy of tumors, suggesting that MT1-MMP is one of the most critical factors for tumor invasion and metastasis. Despite enthusiasm for MMP inhibitors, phase III trials have not yet demonstrated significance in overall survival and side effects remain an issue. We believe that understanding of the functions of MT1-MMP could supply clues to develop novel therapeutic strategies targeting MT1-MMP. The goal of study in Molecular Virology and Oncology Division is to elucidate the molecular mechanism of tumor invasion and metastasis, and identify the molecular targets for diagnosis and therapy of malignant tumors.

A) Novel Functions of MT1-MMP:

MT1-MMP was first identified as an activator of pro-MMP-2, and later MT1-MMP was shown to degrade a various ECM components including collagens. While degradation of ECM is an important aspect of MMP biology, growing evidence has demonstrated specific processing/activation or degradation of cell surface receptors and ligands. To identify molecules which interact with MT1-MMP including its substrates, we have developed an expression cloning strategy and been screening cDNA libraries of human placenta, fetal brain and fetal kidney in which ECM turnover is active. So far we have identified claudin-5 and N-Tes (Testican-3), which regulates MT1-MMP activity. We also identified Kiss-1/metastin, syndecan-1 and lumican as substrates for MT1-MMP, and demonstrated that degradation of these substrates contributes to invasive growth of tumor cells.

B) Stimulation of Cell Migration by MT1-MMP:

Cell migration is a complex process that can be regulated by multiple mechanisms, including MAPK/ERK, phosphatidylinositol 3-kinase and ECM degrading proteinases such as MMPs. Adhesion-mediated ERK activation is dependent on integrin engagement. ERK activity has been implicated in ECM-dependent cell spreading and migration, concomitant with a role for ERK in the regulation of integrin-dependent adhesion/cytoskeletal organization. Although MMPs were known to be involved in cell migration, the molecular mechanism had remained elucidated. We examined the effect of MT1-MMP expression on ERK activation, and found that MT1-MMP positively regulates cell-collagen interactions which generate a sustained signal through the MEK/ERK pathway, and this sustained ERK activation in turn up-regulates MT1-MMP expression. Thus, MT1-MMP functions in a positive feedback loop to induce sustained ERK activation, which consequently stimulates cell migration on type I collagen.

C) JSAP1 Cooperates with FAK to Regulate JNK and Cell Migration

Focal adhesion kinase (FAK) plays a central role in adhesion, spreading and migration of cells. Previously we found that c-Jun N-terminal kinase (JNK)/stress activated protein kinase-associated protein 1 (JSAP1), a scaffold factor in the MAPK cascades forms a complex with FAK. Our recent finding showed that the JSAP1/FAK complex functions cooperatively as a scaffold for the JNK signaling pathway and regulator of cell migration on fibronectin, and that JSAP1 is also associated with malignancy in brain tumors.

Testican 2 Abrogates Inhibition of Membrane-Type Matrix Metalloproteinases by Other Testican Family Proteins.

M. Nakada*, H. Miyamori, J. Yamashita* and H. Sato. (*Dept. Neurosurgery, Med. Sch., Kanazawa Univ.)

Testican family protein are putative extracellular heparan/chondroitin sulfate proteoglycans of unknown function. Recently we identified N-Tes, which is a product of testican 3 splicing variant gene, as an inhibitor of membrane-type matrix metalloproteinases (MT-MMPs). The inhibitory function is common among testican family members except for testican 2, which was shown to uniquely abolish inhibition of MT1-MMP- or MT3-MMP-mediated pro-matrix metalloproteinase-2 activation by other testican family members. Testican 2 inactivates N-Tes by binding to the C-terminal extracellular calcium-binding (EC) domain of N-Tes through its N-terminal unique domain as demonstrated by co-immunoprecipitation analysis, and thus testican 2 was unable to inactivate an N-Tes deletion mutant lacking the EC domain (N-Tes-Δ122). Migration of U251 cells on collagen which was dependent on MT1-MMP activity under serum-free condition, was inhibited by N-Tes or N-Tes-Δ122 deposited on collagen. Testican 2 was not incorporated into collagen by itself, and was deposited only in the presence of N-Tes, suggesting that testican 2 bounds to N-Tes deposited on collagen. Binding of testican 2 to N-Tes deposited on collagen allowed migration of cells expressing MT1-MMP.
Unlike wild-type N-Tes, N-Tes-Δ122 did not bind to testican 2, and thus expression of testican 2 did not recover cell migration blocked by N-Tes-Δ122. *In situ* hybridization showed that neurons are major source of all testican family members in the normal brain. The quantitative reverse transcription-polymerase chain reaction analysis demonstrated that all testican family members are expressed prominently in normal brain, and their expression levels decrease as tumor grade increases. The expression level of testican 2 was the highest among testican family members regardless of histological grade of astrocytic tumors. These results suggest that abundant distribution of testican 2 may contribute to glioma invasion by inactivating other testican family members including N-Tes, which all inhibit MT-MMPs. We propose that N-Tes-Δ122, which is resistant to testican 2, may have therapeutic potential as a barrier against glioma invasion.

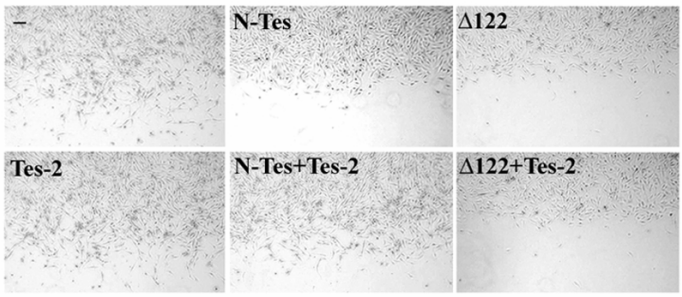


Fig. 1 Effects of testican on migration of U251 glioma cells expressing MT1-MMP. U251 cells transfected with MT1-MMP and indicated plasmids were subjected to wound-induced migration assay on plates coated with collagen.

Refference: Nakada, M., Miyamori, H., Yamashita, J. and Sato, H. (2003) Testican 2 abrogates inhibition of membrane-type matrix metalloproteinases by other testican family proteins. Cancer Res., 63, 3364-3369.

Cleavage of Syndecan-1 by Membrane-Type Matrix Metalloproteinase-1 Stimulates Cell Migration.

K. Endo*, T. Takino, H. Miyamori, H. Kinsen*, T. Yoshizaki*, M. Furukawa* and H. Sato
(*Dept. Otolaryngology, Med. Sch., Kanazawa Univ.)

The transmembrane heparan sulfate proteoglycan syndecan-1 was identified from a human placenta cDNA library by the expression cloning method as a gene product which interacts with membrane-type matrix metalloproteinase-1 (MT1-MMP). Co-expression of MT1-MMP with syndecan-1 in HEK293T cells promoted syndecan-1 shedding, and concentration of cell-associated syndecan-1 was reduced. Treatment of cells with MMP inhibitor BB-94 or tissue inhibitor of MMP (TIMP)-2 but not TIMP-1 interfered with the syndecan-1 shedding promoted by MT1-MMP expression. In contrast, syndecan-1 shedding induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment was inhibited by BB-94 but not by either TIMP-1 or TIMP-2. Shedding of syndecan-1 was also induced by MT3-MMP, but not by other MT-MMPs. Recombinant syndecan-1 core protein was shown to be cleaved by recombinant MT1-MMP or MT3-MMP at G^{82}-L^{83} and G^{245}-L^{246} peptide bonds. HT1080 fibrosarcoma cells stably transfected with the syndecan-1 cDNA (HT1080/SDC), which express endogenous MT1-MMP spontaneously, shed syndecan-1. Migration of HT1080/SDC cells on collagen-coated dishes was significantly slow compared with that of control HT1080 cells. Treatment of HT1080/SDC cells with BB-94 or TIMP-2 induced accumulation of syndecan-1 on cell surface, concomitant with further retardation of cell migration. These results suggest that the shedding of syndecan-1 promoted by MT1-MMP stimulates cell migration.

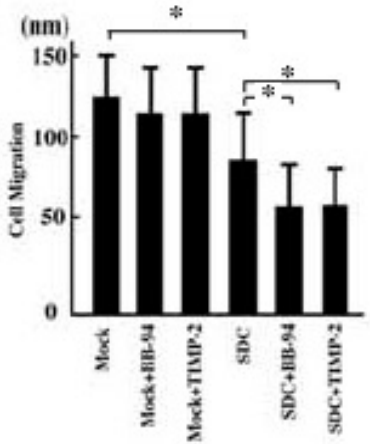


Fig. 1 Mock-transfected HT1080 (Mock lanes) or HT1080/syndecan-1 (SDC lanes) cells were subjected to wound-induced migration assay on collagen-coated dishes in medium containing 0.1 μM BB-94 or 2 μg/ml TIMP-2 protein. Note that migration of syndecan-1-expressing cells was severely inhibited by MMP inhibitors. *, $P< 0.01$.

Reference: Endo, K., Takino, T., Miyamori, H., Kinsen, H., Yoshizaki, T., Furukawa, M. and Sato, H. (2003) Cleavage of Syndecan-1 by Membrane-Type Matrix Metalloproteinase-1 Stimulates Cell Migration. J. Biol. Chem. 278, 40764-40770.

Membrane-Type 1 Matrix Metalloproteinase Regulates Collagen-dependent Mitogen-Activated Protein/Extracellular Signal-Related Kinase Activation and Cell Migration

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Mitogen-activated protein kinase (MAPK)-extracellular signal-related kinase (ERK) kinase 1 (MEK1)/ERK signaling has been implicated in regulation of tumor cell invasion and metastasis. Migration of HT1080 cells on type I collagen was suppressed by matrix metalloproteinase (MMP) inhibitors BB94 and tissue inhibitor of metalloproteinase (TIMP)-2 but not by TIMP-1. TIMP-2-specific inhibition suggests that membrane type-1 MMP (MT1-MMP) is involved in it. Activation of ERK was induced in HT1080 cells adhered on dishes coated with type I collagen, which was inhibited by BB94. MMP-2 processing in HT1080 cells, which was also stimulated by cultivation on type I collagen was inhibited by a MEK inhibitor PD98059. Expression of constitutively active form of MEK1 promoted MMP-2 processing concomitant with the increase of MT1-MMP level, suggesting that MT1-MMP is regulated by MEK/ERK signaling. In addition, expression of hemopexin-like domain of MT1-MMP in HT1080 cells interfered with MMP-2 processing, ERK activation and cell migration, implying that the enzymatic activity of MT1-MMP is involved in collagen-induced ERK activation which results in enhanced cell migration. Thus, adhesion of HT1080 cells to type I collagen induces MT1-MMP-dependent ERK activation, which in turn causes increase of MT1-MMP level and subsequent cell migration.

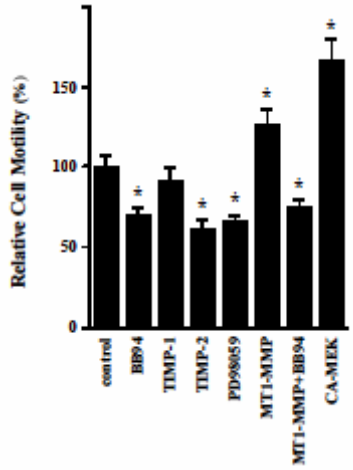


Fig. 1. MT1-MMP is involved in cell migration. HT1080 cells cultured in serum-free DMEM containing DMSO (Control), BB94 (1 μM), PD98059 (25 μM), recombinant TIMP-1 or TIMP-2 (10 μg/ml) and HT1080 cells transfected with MT1-MMP or CA-MEK were examined for migration on type I collagen. *Error bars* indicate S.D. for at least 30 cells per condition. *Asterisk*, $P < 0.01$ versus control.

Cleavage of Lumican by Membrane-Type Matrix Metalloproteinase-1 Abrogates This Proteoglycan-Mediated Suppression of Tumor Cell Colony Formation in Soft Agar

Y. Li, T. Aoki, Y. Mori, M. Ahmad, H.Miyamori, T. Takino and H. Sato

The small leucine-rich proteoglycan lumican was identified from a human placenta cDNA library by the expression cloning method as a gene product which interacts with membrane-type matrix metalloproteinase-1 (MT1-MMP). Co-expression of MT1-MMP with lumican in HEK293T cells reduced the concentration of lumican secreted into culture medium, and this reduction was abolished by addition of the MMP inhibitor BB94. Lumican protein from bovine cornea and recombinant lumican core protein fused to glutathione S-transferase were shown to be cleaved at multiple sites by recombinant MT1-MMP. Transient expression of lumican in HEK293 cells induced expression of tumor suppressor gene product p21/Waf-1, which was abrogated by the co-expression of MT1-MMP concomitant with a reduction in lumican concentration in culture medium. Stable expression of lumican in HeLa cells induced expression of p21 and reduction of colony formation in soft agar, which were both abolished by the expression of MT1-MMP. HT1080 fibrosarcoma cells stably transfected with the lumican cDNA (HT1080/Lum), which express endogenous MT1-MMP, secreted moderate levels of lumican, however, treatment of HT1080/Lum cells with BB94 resulted in accumulation of lumican in culture medium. The expression levels of p21 in HT1080/Lum were proportional to the concentration of secreted lumican, and showed reverse co-relation with colony formation in soft agar. These results suggest that MT1-MMP abrogates lumican-mediated suppression of tumor cell colony formation in soft agar by degrading this proteoglycan which down-regulates it through the induction of p21.

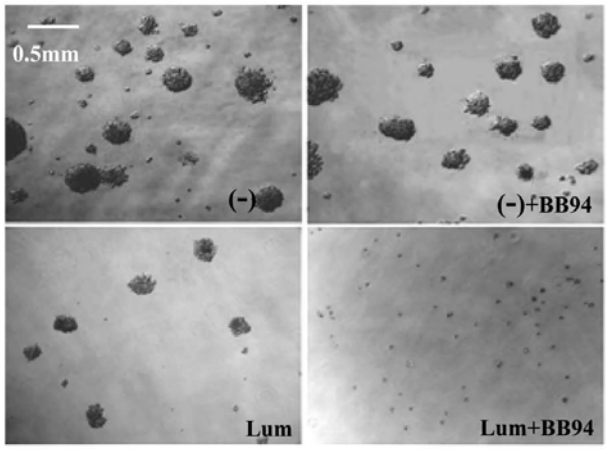


Fig. 1. Degradation of lumican by HT1080 cells enhances colony formation in soft agar. Control HT1080 (-) or HT1080 cells transfected with lumican plasmid (Lum) were plated into 0.3% agarose with or without 0.1 μM BB94, and colonies were observed under microscopy 2-weeks after incubation.

Cleavage of Apolipoprotein E by Membrane-Type Matrix Metalloproteinase-1 Abrogates Suppression of Cell Proliferation

T. Aoki, D. Sato, Y. Li, T. Takino, H. Miyamori and H. Sato

Apolipoprotein E (apoE) in a human fetal brain cDNA library was identified, using the expression cloning method, as a gene product that formed a complex with latent matrix metalloproteinase (MMP)-2. Co-expression of membrane-type MMP-1 (MT1-MMP) with apoE in HEK293T cells reduced the amount of apoE secreted into the culture medium, whereas cell-associated apoE core protein was not affected. Incubation of native apoE protein with recombinant MT1-MMP resulted in the cleavage of apoE. Recombinant apoE protein fused to glutathione S-transferase (apoE-GST) was cleaved by MT1-MMP at the following peptide bonds; T^{85}-M^{86}, K^{93}-S^{94}, R^{246}-L^{247}, A^{255}-E^{256} and G^{296}-L^{297}. HT1080 cells transfected with the apoE gene, which express endogenous MT1-MMP, secreted a low level of apoE protein and its cleaved fragments, and treatment with MMP inhibitor BB94 induced accumulation of apoE and retardation of cell proliferation. Addition of apoE-GST protein to the culture of HEK293T cells suppressed cell proliferation, and stable transfection of the MT1-MMP gene partly abrogated the suppression. These results suggest that cleavage of apoE protein by MT1-MMP abrogates apoE-mediated suppression of cell proliferation.

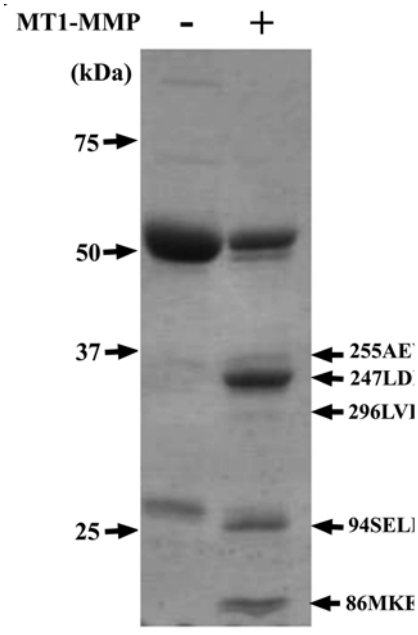


Fig. 1. Cleavage of apoE by MT1-MMP. ApoE-GST fusion protein (5 μg) was incubated with recombinant MT1-MMP catalytic domain (0.5 μg), separated on 15% SDS-polyacryamide gel, and then blotted onto PDF membrane. The N-terminal amino acid sequence of each fragment was determined with a Beckman Coulter LF300 amino acid sequencer.

JSAP1/JIP3 Cooperates with FAK to Regulate c-Jun N-terminal Kinase and Cell Migration

T. Takino, M. Nakada, K. Yoshioka[1], H. Miyamori, Y. Watanabe, K. M. Yamada[2] and H. Sato ([1]Dept. Cell Cycle Regulation, Cancer Res. Inst. Kanazawa Univ., [2]CDBRB, NIDCR, NIH, USA)

c-Jun N-terminal kinase (JNK)/stress-activated protein kinase-associated protein 1 (JSAP1) (also termed JNK interacting protein 3; JIP3) is a member of a family of scaffold factors for the mitogen-activated protein kinase (MAPK) cascades, and it also forms a complex with focal adhesion kinase (FAK). Here we demonstrate that JSAP1 serves as a cooperative scaffold for activation of JNK and regulation of cell migration in response to fibronectin (FN) stimulation. JSAP1 mediated an association between FAK and JNK, which was induced by either co-expression of Src or attachment of cells to FN. Complex formation of FAK with JSAP1 and p130 Crk-associated substrate ($p130^{Cas}$) resulted in augmentation of FAK activity and phosphorylation of both JSAP1 and $p130^{Cas}$, which required $p130^{Cas}$ hyperphosphorylation and was abolished by inhibition of Src. JNK activation by FN was enhanced by JSAP1, which was suppressed by disrupting the FAK/$p130^{Cas}$ pathway by expression of a dominant-negative form of $p130^{Cas}$ or by inhibiting Src. We also documented the co-localization of JSAP1 with JNK and phosphorylated FAK at the leading edge and stimulation of cell migration by JSAP1 expression, which depended on its JNK binding domain and was suppressed by inhibition of JNK. The level of JSAP1 mRNA correlated with advanced malignancy in brain tumors, unlike other JIPs. We propose that the JSAP1/FAK complex functions cooperatively as a scaffold for the JNK signaling pathway and regulator of cell migration on FN, and we suggest that JSAP1 is also associated with malignancy in brain tumors.

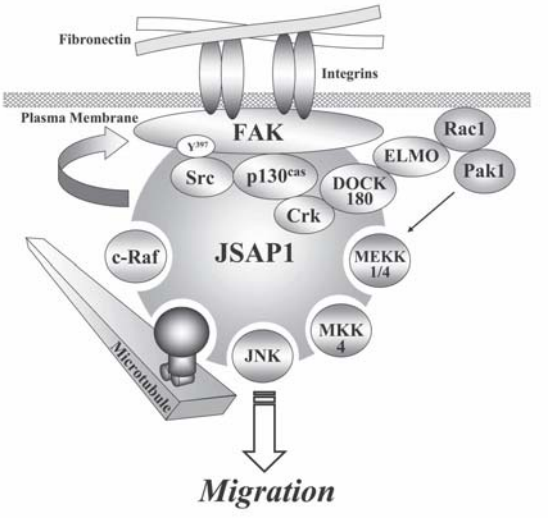


Fig. 1. Model depicting a FAK-JSAP1 scaffold for JNK activation and cell migration. JSAP1 seems to be a modulator of cell migration by associating with MAPK signaling pathways and possibly microtubules.

Publications

1. Takino, T., Miyamori, H., Kawaguchi, N., Uekita, T., Seiki, M., Sato, H. (2003) Tetraspanin CD63 promotes targeting and lysosomal proteolysis of membrane-type 1 matrix metalloproteinase. Biochem Biophys Res Commun., 304, 160-166.
2. Mori, N., Sato, H., Hayashibara, T., Senba, M., Geleziunas, R., Wada, A., Hirayama, T., Yamamoto, N. (2003) Helicobacter pylori induces matrix metalloproteinase-9 through activation of nuclear factor kappaB. Gastroenterology., 124, 983-992.
3. Takino, T., Koshikawa, N., Miyamori, H., Tanaka, M., Sasaki, T., Okada, Y., Seiki, M., and Sato. H. (2003) Cleavage of Metastasis Suppressor Gene Product KiSS-1 Protein/Metastin by Matrix Metalloproteinases. Oncogene, 22, 4617-4626, 2003.
4. Takino, T., Nakada, M., Miyamori, H., Yamashita, J., Yamada, K.M., Sato, H. (2003) CrkI adapter protein modulates cell migration and invasion in glioblastoma. Cancer Res., 63, 2335-2337.
5. Nakada, M., Miyamori, H., Yamashita, J. and Sato, H. (2003) Testican 2 abrogates inhibition of membrane-type matrix metalloproteinases by other testican family proteins. Cancer Res., 63, 3364-3369.
6. Endo, K., Takino, T., Miyamori, H., Kinsen, H., Yoshizaki, T., Furukawa, M., and Sato, H. (2003) Cleavage of Syndecan-1 by Membrane-Type Matrix Metalloproteinase-1 Stimulates Cell Migration. J. Biol. Chem. 278, 40764-40770.
7. Takino T, Tamura M, Miyamori H, Araki M, Matsumoto K, Sato H, Yamada KM. (2003) Tyrosine phosphorylation of the CrkII adaptor protein modulates cell migration. J Cell Sci., 116, 3145-3155.
8. Takahara, T., Zhang, L.P., Yata, Y., Xue, F., Minemura, M., Sato, H., Watanabe, A. (2003) Modulation of matrix metalloproteinase-9 in hepatic stellate cells by three-dimensional type I collagen: its activation and signaling pathway. Hepatol Res., 26, 318-326.
9. Takino, T., Miyamori, H., Watanabe, Y., Yoshioka, K., Sato, H. (2004) Membrane-Type 1 Matrix Metalloproteinase Regulates Collagen-dependent Mitogen-Activated Protein/Extracellular Signal-Related Kinase Activation and Cell Migration. Cancer Res., 64, 1044-1049.
10. Inaki, N., Tsunezuka, Y., Kawakami, K., Sato, H., Takino, T., Oda, M. and Watanabe, G. (2004) Increased matrix metalloproteinase-2 and membrane type 1 matrix metalloproteinase activity and expression in heterotopically transplanted murine tracheas. J. Heart and Lung Transplantation, 23, 218-227.
11. Li, Y., Aoki, T., Mori, Y., Ahmad, M., Miyamori, H., Takino T. and Sato, H. (2004)

Cleavage of lumican by membrane-type matrix metalloproteinase-1 abrogates this proteoglycan-mediated suppression of tumor cell colony formation in soft agar. Cancer Res., 64, 7058-7064.

12. Gum, R., Lengyel, E., Juarez, J., Chen, J.H., Sato, H., Seiki, M., Boyd, D. (2004) Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase -1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. J. Biol. Chem., 271, 10672-10680.
13. Uekita, T., Gotoh, I., Kinoshita, T., Itoh, Y., Sato, H., Shiomi, T., Okada, Y., Seiki, M. (2004) Membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 is a new member of the cupin superfamily: A possible multifunctional protein acting as an invasion suppressor down-regulated in tumors. J. Biol. Chem., 279, 12734-12743.
14. Kondo, S., Horikawa, T., Takeshita, H., Kanegane, C., Kasahara, Y., Sheen, T.-S., Sato, H., Furukawa, M., Yoshizaki, T. (2004) Diagnostic value of serum EBV-DNA quantification and antibody to viral capsid antigen in nasopharyngeal carcinoma patients. Cancer Science, 95, 508-513.
15. Ren, Q., Sato, H., Murono, S., Furukawa, M., Yoshizaki, T. (2004) Epstein-Barr Virus (EBV) Latent Membrane Protein 1 Induces Interleukin-8 through the Nuclear Factor-κB Signaling Pathway in EBV-Infected Nasopharyngeal Carcinoma Cell Line. Laryngoscope, 114, 855-859.
16. Uekita T, Yamanouchi K, Sato H, Tojo H, Seiki, M, Tachi C. (2004) Expression and localization of matrix metalloproteinases (MT1-MMP, MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) during synepitheliochorial placentation of goats (Capra hircus). Placenta, 10, 810-819.
17. Aoki, T., Sato, D., Li, Y., Takino, T., Miyamori, H. and Sato, H. (2005) Cleavage of Apolipoprotein E by Membrane-Type Matrix Metalloproteinase-1Abrogates Suppression of Cell Proliferation. J. Biochemistry, 137, 95-99.
18. Kondo S, Wakisaka N, Schell MJ, Horikawa T, Sheen TS, Sato H, Furukawa M, Pagano JS, Yoshizaki T. (2005) Epstein-Barr virus latent membrane protein 1 induces the matrix metalloproteinase-1 promoter via an Ets binding site formed by a single nucleotide polymorphism: enhanced susceptibility to nasopharyngeal carcinoma. Int J Cancer., 115, 368-376.
19. Takino, T., Nakada, M., Miyamori, H., Watanabe, Y., Sato, T., Gantulga, D., Yoshioka, K., Yamada, K.M., Sato, H. (in press) JSAP1/JIP3 cooperates with FAK to regulate c-Jun N-terminal kinase and cell migration. J. Biol. Chem.

20. Nakada, M., Miyamori, H., Kita, D., Takahashi, T., Yamashita, J., Sato, H., Miura, R., Yamaguchi, Y., Okada, Y. (in press) Human glioblastomas overexpress ADAMTS-5 that degrades brevican. Acta. Neuropathol. (Berl).

Division
of
Molecular Bioregulation

General Summary of Division of Molecular Bioregulation

Tumor cells and resident cells can produce various chemokines and pro-inflammatory cytokines, during carcinogenesis and metastasis processes. The produced chemokines and pro-inflammatory cytokines can modulate the microenvironment of tumor tissues, thereby affecting carcinogenic and/or metastatic processes. A major research object in our division is to elucidate the roles of these endogenously produced chemokines and pro-inflammatory cytokines in these processes, in order to use these molecules and/or their antagonists for the treatment of cancer.

We discovered recently that Pim-3, a proto-oncogene with serine/threonine kinase activity, was enhanced in chronic inflammation-mediated hepatoma tissues in mice and are investigating the roles of Pim-3 in the carcinogenesis processes of various types of cancers.

A) Chemokines and pro-inflammatory cytokines in carcinogenesis and metastasis processes

Tumor cells as well as resident normal cells can produce a wide variety of chemokines and pro-inflammatory cytokines, thereby affecting the course of carcinogenesis and metastasis processes. Until present, we have provided definitive evidence to indicate that tumor necrosis factor receptor p55 has crucial roles in inflammation-mediated colon carcinogenesis, liver metastasis, and lung metastasis. Moreover, we demonstrated that tumor cells and resident normal cells can produce various chemokines and that the produced chemokines have effects on tumor cells as well as inflammatory cells, thereby contributing to carcinogenesis and metastasis processes.

B) Pim-3, a proto-oncogene with serine/threonine kinase activity, in carcinogenesis

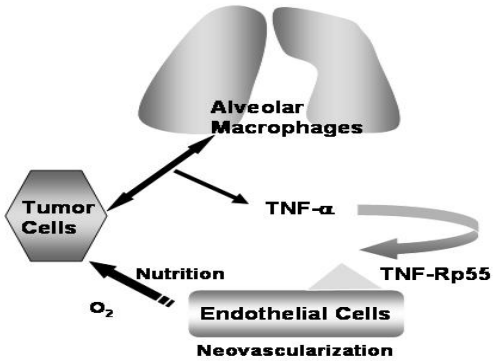
Pim-3 is aberrantly expressed in various types of tumor tissues and tumor cell lines, including hepatocellular carcinoma and pancreatic cancer. Cell proliferation was attenuated and apoptosis was enhanced in human hepatocellular carcinoma and pancreatic cancer cell lines by the ablation of Pim-3 gene with RNA interference. Therefore, Pim-3 may be a good molecular target for treating tumors, which exhibit the enhanced expression of Pim-3. We are now investigating the detailed molecular and pathological consequences of Pim-3-mediated phosphorylation of various substrates.

Essential contribution of tumor necrosis factor receptor (TNF-R) p55-mediated signals in metastasis and carcinogenesis

Y. Tomita, X. Yang, H. Kitakata, B.K. Popivanova, Y. Nemoto-Sasaki and N. Mukaida

We have previously demonstrated that TNF-Rp55-mediated signals could up-regulate the expression of an adhesion molecule, vascular adhesion molecule (VCAM)-1 in sinusoidal endothelial cells and eventually facilitate liver metastasis by intrasplenic injection of a colon carcinoma cell line into mice (1).

Intravenous injection of a mouse renal carcinoma cell line, Renca, caused a large number of lung metastasis foci in wild-type mice, with TNF-α protein at tumor sites. Metastasis foci expanded at similar rates in both wild-type and TNF-Rp55-deficient mice until 21 days after the injection. On the contrary, later than 21 days, metastasis foci regressed spontaneously in TNF-Rp55-deficient mice, but not wild-type mice. Concomitantly, the number of apoptotic tumor cells were greater in TNF-Rp55-deficient mice, whereas neovascularization was less evident in TNF-Rp55-deficient mice than wild-type mice, with depressed expression of a potent angiogenic factor, hepatocyte growth factor gene in TNF-Rp55-deficient mice (2) (see below Figure). These observations implied that TNF-Rp55-mediated signals have distinct but important roles in metastasis to liver and lung.



Mice developed multiple adenomatous lesions in colon after repeated oral intake of dextran sulfate sodium solution, following an intraperitoneal injection of a potent chemical carcinogen, azoxymethane. The same treatment caused few adenomatous lesions in TNF-Rp55-deficient mice. Because this model may recapitulate adenocarcinoma developed in patients with chronic ulcerative colitis, TNF-Rp55 may be a good molecular target for preventing this severe complication in chronic colitis.

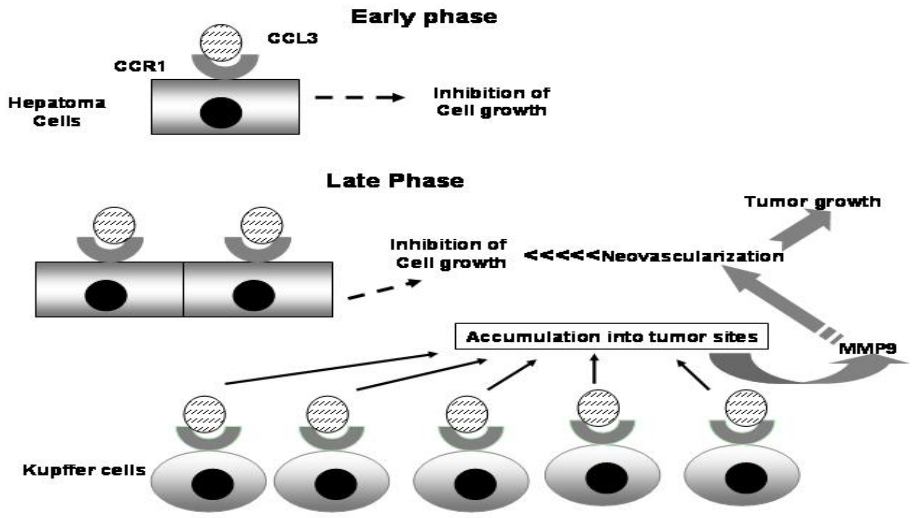
References

1. Kitakata H et al. Cancer Res.62: 6682, 2002.
2. Tomita Y et al. Intl. J. Cancer 112: 927, 2004.

Essential contribution of a chemokine, CCL3, and its receptor, CCR1, to hepatocarcinogenesis

P. Lu, X. Yang, C. Fujii, and N. Mukaida.

We previously observed that a chemokine, macrophage inflammatory protein-1 α/CCL3, and its receptor, CCR1, were aberrantly expressed in human hepatocellular carcinoma (HCC) tissues (1). We further demonstrated that CCL3 and CCR1 are also expressed in two different models of this cancer; *N*-nitrosodiethylamine (DEN)-induced HCC and HCC induced by hepatitis B virus surface (HBs) antigen-primed splenocyte transfer to myelo-ablated syngeneic HBs antigen transgenic mice (2). At 10 months after DEN treatment, tumor incidence was marginally but significantly higher in CCR1- and CCL3-deficient mice than WT mice, in line with the in vitro observation that CCL3 can inhibit the proliferation of hepatoma cell lines. On the contrary, foci numbers and sizes were remarkably reduced in CCR1- and CCL3-deficient mice, compared with wild-type mice. Of note is that tumor angiogenesis was also markedly diminished in CCL3- and CCR1-deficient mice, with a concomitant reduction in the number of intratumoral Kupffer cells, a rich source of growth factors and matrix metalloproteinases (MMPs). Among growth factors and MMPs that we examined, only MMP9 and MMP13 gene expression was augmented progressively in liver of WT mice after DEN treatment. Moreover, MMP9 but not MMP13 gene expression was attenuated in CCR1- and CCL3-deficient mice, compared with wild-type mice. Furthermore, MMP9 was expressed mainly by mononuclear cells, and MMP9-expressing cell numbers were decreased in CCR1- or CCL3-deficient mice, compared with wild-type mice (2). These observations suggest that the CCR1-CCL3 axis has different roles in HCC progression, depending on its phases (see below Figure).



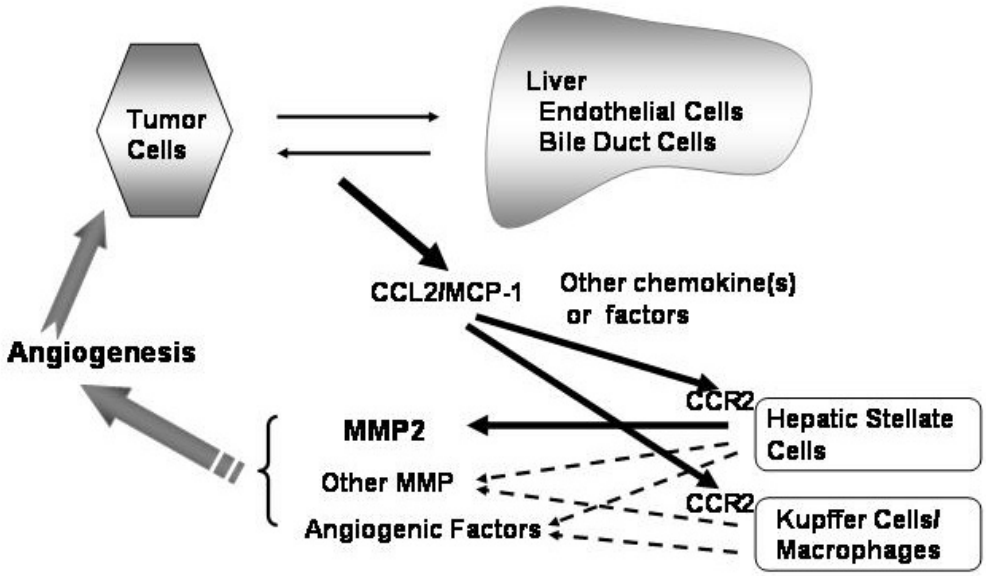
References

1) Lu P, et al. Am. J. Pathol. 162: 1249, 2003.
2) Yang X, et al., Intl. J. Cancer [Epub ahead of print] PMID: 16284949

Essential involvement of a chemokine receptor, CCR2, to liver metastasis

X. Yang, C. Fujii, and N. Mukaida.

The liver parenchyma is populated by hepatocytes and several non-parenchymal cell types including Kupffer cells (KC) and hepatic stellate cells (HSC). Both KC and HSC are responsive to the chemokine CCL2, but the precise roles of CCL2 and these cells in liver tumor formation remain undefined. Hence, we investigated the effects of the lack of the major CCL2 receptor, CCR2, on liver tumor formation induced by intraportal injection of the murine colon adenocarcinoma cell line, colon 26. Wild-type mice showed macroscopic tumor foci in the liver 10 days after injection of colon 26 cells. After 10 days, CCL2 proteins were detected predominantly in tumor cells, coincident with increased intratumoral KC and HSC numbers. Although tumor formation occurred at similar rates in wild-type and CCR2-deficient mice up to 10 days after tumor cell injection, the number and size of tumor foci were significantly attenuated in CCR2-deficient mice relative to wild-type mice thereafter. Moreover, neovascularization and matrix metalloproteinase (MMP) 2 expression were diminished in CCR2-deficient mice with a concomitant reduction in the accumulation of KC and HSC. MMP2 was detected predominantly in HSC but not in KC. Thus, CCR2-mediated signals can regulate the trafficking of HSC, a main source of MMP2, and consequently can promote neovascularization during liver tumor formation.

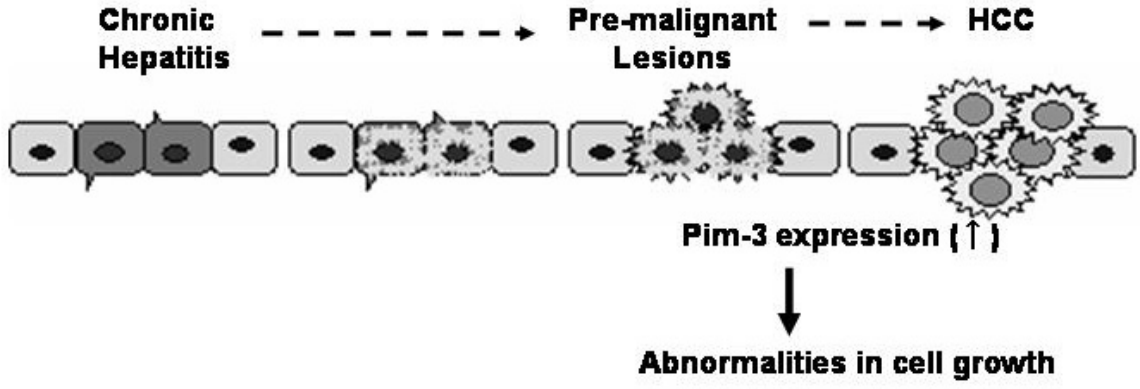


Reference
Yang X, et al. Intl. J. Cancer 118: 335, 2006.

Enhanced expression of a proto-oncogene, Pim-3, with serine/threonine kinase activity, in various types of tumors

C. Fujii, Y. Li, B. Popivanova, Y. Nakamoto, and N. Mukaida.

Most cases of human hepatocellular carcinoma develop after persistent chronic infection with human hepatitis B virus or hepatitis C virus, and host responses are presumed to have major roles in this process. To recapitulate this process, we have developed the mouse model of hepatocellular carcinoma using hepatitis B virus surface antigen transgenic mice. In order to identify the genes associated with hepatocarcinogenesis in this model, we compared the gene expression patterns between pre-malignant lesions surrounded by hepatocellular carcinoma tissues and control liver tissues by using a fluorescent differential display analysis. Among the genes which were expressed differentially in the pre-malignant lesions, we focused on Pim-3, a member of a proto-oncogene *Pim* family. Due to the unavailability of the nucleotide sequence of full-length human Pim-3 cDNA, we cloned full-length Pim-3 cDNA, consisting of 2,392 bp, which encodes a predicted open reading frame consisting of 326 amino acids. Pim-3 mRNA was selectively expressed in human hepatoma cell lines, but not in normal liver tissues. Moreover, Pim-3 protein was detected in human hepatocellular carcinoma tissues and cell lines but not in normal hepatocytes. Furthermore, cell proliferation was attenuated and apoptosis was enhanced in human hepatoma cell lines by the ablation of Pim-3 gene with RNA interference (reference). These observations suggest that aberrantly expressed Pim-3 can cause autonomous cell proliferation and/or prevent apoptosis in hepatoma cell lines.



We recently observed that Pim-3 expression was enhanced selectively also in human pancreas cancer tissues and pancreatic cancer cell lines, but not normal pancreas tissue. Moreover, similarly to human hepatoma cell lines, cell proliferation was attenuated and apoptosis was enhanced also in human pancreatic cancer cell lines by the ablation of Pim-3 gene with RNA interference. Thus, Pim-3 is aberrantly expressed in various types of tumors and can counteract apoptosis process, thereby contributing to carcinogenesis. Therefore, Pim-3 may be a good molecular target for treating tumors, which exhibit the enhanced expression of Pim-3.

Reference

Fujii C, et al. Intl. J. Cancer 114: 209, 2005.

Publications

1. Wang H, Nemoto-Sasaki Y, Kondo T, Akiyama M, and Mukaida N. (2003) Potential involvement of monocyte chemoattractant protein (MCP)-1/CCL2 in IL-4-mediated tumor immunity through inducing dendritic cell migration into the draining lymph nodes. Int. Immunopharmacol. 3: 627-642.
2. Kim H, Seo JY, Lim JW, Mukaida N, and Kim KH. (2003) Helicobacter pylori-induced IL-8 expression in AGS cells: mediation by NF-κB and AP-1. Dig. Dis. Sci. 48: 257-265.
3. Tsuchiyama T, Kaneko S, Nakamoto Y, Sakai Y, Honda M, Mukaida N, and Kobayashi K. (2003) Enhanced anti-tumor effects of a bicistronic adenovirus vector expressing both herpes simplex virus thymidine kinase and monocyte chemoattractant protein-1 against hepatocellular carcinoma. Cancer Gene Ther. 10: 260-269.
4. Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Hashimoto H, Ishiwata Y, Tomosugi N, Mukaida N, Matsushima K, Egashira K, and Yokoyama H. (2003) Gene therapy expressing amino-terminal truncated monocyte chemoattractant protein-1 prevents renal ischemia-reperfusion injury. J. Am. Soc. Nephrol. 14: 1066-1071.
5. Lu P, Nakamoto Y, Nemoto-Sasakai Y, Fujii C, Wang H, Hashii M, Ohmoto Y, Kaneko S, Kobayashi K, and Mukaida N. (2003) Potential interaction between CCR1 and its ligand, CCL3, induced by endogenously produced interleukin-1, in human hepatomas. Am. J. Pathol. 162: 1249-1258.
6. Popivanova B, Koike K, Tonchev AB, Ishida Y, Kondo T, Ogawa S, Mukaida N, Inoue M, and Yamashima T. (2003) Accumulation of microglial cells expressing ELR motif-positive CXC chemokines and their receptor CXCR2 in monkey hippocampus after ischemia-reperfusion. Brain Res. 970: 195-204.
7. Lin Z-Q, Kondo T, Ishida Y, Takayasu T, and Mukaida N. (2003) Essential involvement of IL-6 in skin wound healing process as evidenced by delayed wound healing in IL-6-deficient mice. J. Leukoc. Biol. 73: 713-721.
8. Inaoki M, Sato F, Shirasaki F, Mukaida N, and Takehara K. (2003) The frequency of type 2 CD8+ T cells is increased in peripheral blood from patients with psoriasis vulgaris. J. Clin. Immunol. 23: 269-278.
9. Kuwada Y, Sasaki T, Morinaka K, Kitadai Y, Mukaida N, and Chayama K. (2003) Potential involvement of IL-8 and its receptors in the invasiveness of pancreatic cancer cells. Int. J. Oncol. 22: 765-771.

10. Ohta M, Kitadai Y, Tanaka S, Yoshihara M, Yasui W, Mukaida N, Haruma K, and Chayama K. (2003) Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumor vascularity in human gastric carcinomas. Int. J. Oncol. 22: 773-778.
11. Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K-i, Hashimoto H, Ishiwata Y, Asano M, Wang H, Matsushima K, Takeya M, Kuziel WA, Mukaida N, and Yokoyama H. (2003) CCR2 signaling contributes to ischemia-reperfusion injury in kidney. J. Am. Soc. Nephrol. 14: 2503-2515.
12. Mukaida N. (2003) The pathophyiological roles of interleukin (IL)-8/CXCL8 in pulmonary diseases. Am. J. Physiol.Lung Cell. Molec. Physiol. 284: L566-L577, 2003.
13. Mukaida N, Ketlinsky SA, and Matsushima K. (2003) Interleukin-8 and other CXC chemokines. In The Cytokine Handbook (Thomson A and Lotze MT eds.), Elsevier Science Ltd, London, UK, pp. 1049-1081.
14. Nakamura M, Kyo S, Kanaya T, Yatabe N, Maida y, Tanaka M, Ishida Y, Fujii C, Kondo T, Inoue M, and Mukaida N. (2004) hTERT-promoter-based tumor-specific expression of MCP-1 effectively sensitizes cervical cancer cells to a low dose of cisplatin. Cancer Gene Ther. 11: 1-7.
15. Ishida Y, Kondo T, Tsuneyama K, Lu P, Takayasu T, and Mukaida, N. (2004) The pathogenic roles of tumor necrosis factor receptor p55 in acetaminophen-induced liver injury in mice. J. Leukocyte Biol. 75: 59-67.
16. Amano H, Morimoto K, Senba M, Wang H, Ishida Y, Kumatori A, Yoshimine H, Oishi K, Mukaida N, and Nagatake T. (2004) Essential contribution of monocyte chemoattractant protein (MCP)-1/C-C chemokine ligand-2 to resolution and repair process in acute bacterial pneumonia. J. Immunol. 172: 398-409.
17. Ishida Y, Kondo T, Takayasu T, Iwakura Y, and Mukaida N. (2004) The essential involvement of cross-talk between IFN-γ and TGF-β in the skin wound-healing process. J. Immunol. 172: 1848-1855.
18. Ishida Y, Maegawa T, Kondo T, Kimura A, Iwakura Y, Nakamura S, and Mukaida N. (2004) Essential involvement of IFN-γ in Clostridium difficile toxin A-induced enteritis. J. Immunol. 172: 3018-3025.
19. Wada T, Furuichi K, Sakai N, Iwata Y, Kitagawa K, Ishida Y, Kondo T, Hashimoto H, Ishikawa Y, Mukaida N, Tomosugi N, Matsushima K, Egashira K, and Yokoyama H. (2004) Gene therapy via blockade of monocyte chemoattractant protein-1 for renal fibrosis. J. Am. Soc. Nephrol. 15: 940-948.

20. Ryoo S, Won M, Kim DU, Kim L, Han G, Park SK, Mukaida N, Maeng P, Yoo HS, and Hoe KL. (2004) PPARα activation abolishes LDL-stimulated IL-8 production via AP-1 deactivation in human aortic smooth muscle cells. Biochem. Biophys. Res. Commun. 318: 329-334.
21. Hwang YS, Jeong M, Park JS, Kim MH, Lee DB, Shin BA, Mukaida N, Ellis LM, Kim HR, Ahn BW, and Jung YD. (2004) Interleukin-1β stimulates IL-8 expression through MAP kinase and ROS signaling in human gastric carcinoma cells. Oncogene 23: 6603-6611.
22. Kitagawa K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Tomosugi N, Matsushima K, Mukaida N, and Yokoyama H. (2004) Blockade of CCR2 ameliorates progressive fibrosis. Am. J. Pathol. 165: 237-246.
23. Qiu Z, Fujimura M, Kurashima K, Nakao S, and Mukaida N. (2004) Enhanced airway inflammation and decreased subepithelial fibrosis in interleukin 6-deficient mice following chronic exposure to aerosolized antigen. Clin. Exp. Allergy 34: 1321-1328.
24. Imamura R, Konaka K, Matsumoto N, Hasegawa M, Fukui M, Mukaida N, Kinoshita T, and Suda T. (2004) Fas ligand induces cell-autonomous NF-κ B activation and IL-8 production by a mechanism distinct from that of TNF-α. J. Biol. Chem. 279: 46415-46423.
25. Tomita Y, Yang X, Ishida Y, Nemoto-Sasaki Y, Kondo T, Oda M, Watanabe G, Chladakov GN, Fujii C, and Mukaida N. (2004) Spontaneous regression of lung metastasis in the absence of tumor necrosis factor receptor p55. Intl. J. Cancer 112: 927-933.
26. Kitamura K, Nakamoto Y, Kaneko S, and Mukaida, N. (2004) Pivotal roles of interleukin-6 in transmural inflammation in murine T cell transfer colitis. J. Leukocyte Biol. 76: 1111-1117.
27. Yanaba K, Mukaida, N, Matsushima K, Murphy PM, Takehara K, and Sato S. (2004) A role of C-C chemokine receptors 1 and 5 and macrophage inflammatory protein-1α on the cutaneous Arthus reaction: Possible attenuation of their inhibitory effects by compensatory production. Eur. J. Immunol. 34: 3553-3561.
28. Mukaida N. (2004) Chemokines. In The Encylopedia of Endocrine Diseases, Four-Volume Set (Martini L Editor-in-Chief), Elsevier Science Ltd, London, UK, vol. 1, pp.490-494.
29. Mukaida N. (2004) Cancer gene therapy using cytokine and chemokine genes. Ann. Cancer Res. Ther. 12: 33-51.
30. Kimura A, Ishida Y, Wada T, Yokoyama H, Mukaida N, and Kondo T. (2005) MRP-1 expression levels determine strain-specific susceptibility to arsenic-induced renal injury between C57BL/6 and BALB/c mice. Toxicol.Appl. Pharmacol. 203: 53-61.

31. Fujii C, Nakamoto Y, Lu P, Tsuneyama K, Popivanova B, Kaneko S, and Mukaida N. (2005) Aberrant expression of serine/threonine kinase Pim-3 in hepatocellular carcinoma development and its role in the proliferation of human hepatoma cell lines. Intl. J. Cancer 114: 209-218.
32. Iizasa H, Yoneyama H, Mukaida N, Kataoka Y, Naito M, Yoshida N, Nakashima E, and Matsushima K. (2005) Exacerbation of granuloma formation in IL-1 receptor antagonist-deficient mice with impaired dendritic cell maturation associated with Th2 cytokine production. J. Immunol. 174: 3273-3280.
33. Edwards MR, Mukaida N, JohnsonM,and Johnston SL. (2005) IL-1β induces IL-8 in bronchial cells via NF-κB and NF-IL6 transcription factors and can be suppressed by glucocorticoids. Pulmon. Ther. Pharmacol. 18: 337-345.
34. Sato K, Imai Y, Higashi N, Kumamoto Y, Mukaida N, and Irimura T. (2005) Redistributions of macrophages expressing the macrophage galactose-type C-type lectin (MGL) during antigen-induced chronic granulation tissue formation. Intl. Immunol. 17: 559-568.
35. Kurashiama K, Tamura J, Fujimura M, Qiu Z, Nakako S, and Mukaida N. (2005) Reduced serum antibody production and acute airway inflammation in interleukin-6-dericient mice challenged with ovalbumin. Allergology Intl. 54: 331-338.
36. Kuroda T, Kitadai Y, Tanaka S, Yang X, Mukaida N, Yoshihara M, and Chayama K. (2005) Monocyte chemoattractant protein-1 transfection induces angiogenesis and tumorigenesis of gastric carcinoma in nude mice via macrophage recruitment. Clin. Cancer Res. 11: 7629-7636.
37. Yang X, Lu P, Ishida Y, Kuziel WA, Fujii C, and Mukaida, N. (2006) Attenuated liver tumor formation in the absence of CCR2 with a concomitant reduction in the accumulation of hepatic stellate cells, macrophages, and neovascularization. Intl. J. Cancer 118: 335-345.
38. Kagaya T, Nakamoto Y, Sakai Y, Tsuchiyama T, Yagita H, Mukaida N, and Kaneko S. (2006) Monocyte chemoattractant protein-1 gene delivery enhances antitumor effects of herpes simplex virus thymidine kinase/ganciclovir system in a model of colon cancer. Cancer Gene Ther. [Epub ahead of print] PMID: 16224495.
39. Yang X, Lu P, Fujii C, Nakamoto Y, Gao J-L, Kaneko S, Murphy PM, and Mukaida N. (2006) Essential contribution of a chemokine, CCL3, and its receptor, CCR1, to hepatocellular carcinoma progression. Intl. J. Cancer [Epub ahead of print] PMID: 16284949.

Other achievements

Patent No. 2003-291060

Hepatoma-specific polypeptide, the polynucleotide encoding the polypeptide, and the inhibitory RNA for the polypeptide. (Pending on August 11, 2003)

Division
of
Molecular Genetics

General Summary of Division of Molecular Genetics

In 2005, Dr. Hirao was appointed to the professor and chairman of this division. Since then, the division has been dedicated mainly to basic researches on the mechanisms of self-renewal of stem cells. Stem cells are defined as cells that have the ability to perpetuate through self-renewal, and develop into mature cells of a particular tissue through differentiation. Appropriate controls of stem cell functions are critical for maintaining tissue homeostasis. It has been suggested that dysregulation of stem cell could lead to cell senescence or cancer. Most cancers comprise a heterogeneous population of cells with marked differences in their proliferative potential as well as the ability to reconstitute the tumor on transplantation. Cancer stem cells are a minor population of tumor cells that possess the stem cell property of self-renewal. To understand molecular mechanisms by which normal tissue stem cells and cancer stem cells are regulated, several approaches have been done as follows. In October 2005, the Japan Science and Technology Agency (JST) started to support the group as a part of Basic Technologies for Controlling Cell Functions Based on Metabolic Regulation Mechanism Analysis.

A) Identification of molecules essential for self-renewal of tissue stem cells.

Normal tissue stem cells are defined by three common properties: 1. Presence of an extensive capacity for self-renewal that allows maintenance of the undifferentiated stem cell pool over the life time of the host; 2. Strict regulation of stem-cell number; 3. Ability to undergo a broad range of differentiation events to clonally reconstitute all of the functional elements within the tissues. Since hematopoiesis provides the most well-characterized stem cell system, roles of several molecules on self-renewal of stem cells have been analyzed using hematopoietic system in this division. Further, for some molecules, other type of stem cells including germ or neural stem cells have been analyzed to address a question whether self-renewal of several tissue stem cells is regulated by common molecules or signaling pathways.

B) Development of identification system for cancer stem cells.

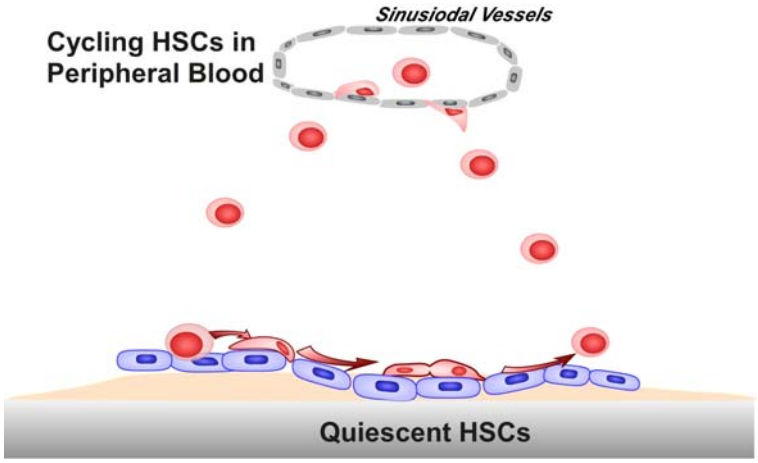
Recent evidence has demonstrated that in leukemia and solid tumors only a minority of cancer cells has the capacity to proliferate extensively and form new tumors. These tumorigenic or tumor-initiating cells, which are called cancer stem cells, have been identified and enriched on the basis of their expression of cell-surface markers. To identify the cancer stem cells, molecules that commonly express in several tissue stem cells were identified. Promoter DNA

fragments of the candidate molecules were used for a tool by which tissue stem cells, but not differentiated cells, can be labeled with EGFP in mice. Cancer stem cell has been analyzed by using the stem-cell marking system.

The identification of hematopoietic stem cell niche.

A. Hirao, F. Arai, T. Suda

Hematopietic stem cells (HSC) in adult mouse bone marrow are quiescent, while mobilized HSCs are cycling rapidly following depletion of mature and progenitor cells by bleeding, infection, cytotoxic reagents or irradiation. Although interaction between microenvironments and HSCs contributes to maintenance of quiescence of HSCs resulting in the long-term self-renewal, mechanisms are not understood. In this study, we demonstrate that side population (SP), based on FACS analysis with Hoechst33342, in c-kit+ Sca-1+ Lin-(KSL) cells represents a population of quiescent HSC. Cell cycle analysis with BrdU labeling showed that SP were slowly cycling in G0 phase. HSCs mobilized in peripheral blood from a bone marrow niche by G-CSF or 5-FU treatment were cycling and those cycling HSCs were not in SP. HSCs in developing mice, which were cycling and expanding, were also in main population (MP). SP cells in KSL fraction were resistant to myelosuppressive stress including X-ray and 5-FU treatment which depletes cycling hematopoietic cells. Histological examination revealed that the 5-FU resistant HSCs were surrounded by bone-lining osteoblast-like cells on the surface of the bone. These data indicate that the osteoblastic zone is a niche for quiescent HSCs in bone marrow. FACS, RT-PCR and histological examination showed that the expression of Tie2, a receptor tyrosine kinase, was limited to the HSCs in a niche. Angiopoietin-1 (Ang-1), a ligand for Tie2, increased the number of LTC-IC and mixed CFC, accompanied with enhanced adhesion of hematopoietic cells to stroma cells in vitro. Furthermore, Ang-1 induced SP phenotype in HSC in vivo. These data suggest that Ang-1/Tie2 plays a key role for recruitment of stem cells to a niche, and remains cells in quiescent state, resulting in stem cell maintenance.



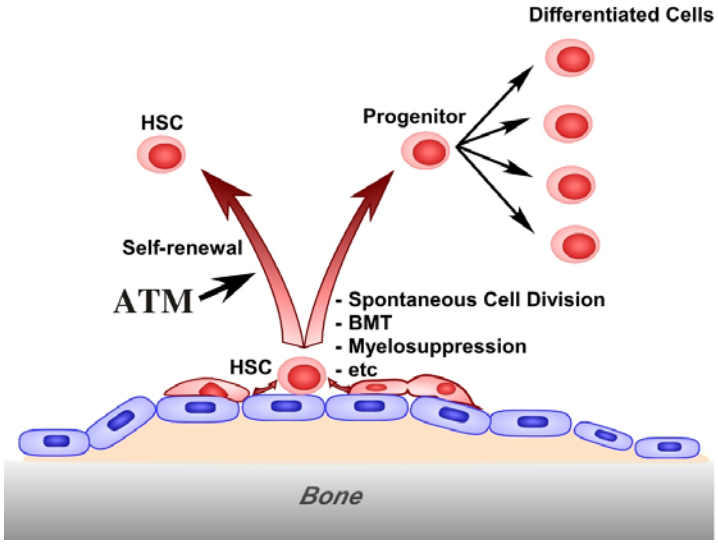
Role of ATM on self-renewal capacity of hematopoietic stem cells.

A. Hirao, K. Ito, T. Suda

The hematopoietic stem cells (HSC) are maintained in an undifferentiated quiescent state in a niche. Quiescent stem cells show resistance to various stresses, suggesting that mechanisms for protection of HSC from stress contribute to maintenance of self-renew through a whole life in animals. In this study, we demonstrate that cell-cycle checkpoint molecule, ATM (ataxia telangiectasia mutated), is essential for maintenance of capacity of regeneration in HSC.

Ataxia telangiectasia (A-T) is an autosomal recessive disorder caused by mutational inactivation of the ATM. ATM and its homologue have a central role in maintenance of genomic stability via regulating cell cycle checkpoint in response to DNA damage, stability of telomere and oxidative stress. A-T patients display variety of symptoms including premature aging. We hypothesized that a signaling pathway for regulating aging and longevity may be involved in stem cell functions. Hence we analyzed a role of ATM in self-renewal and repopulating capacity of HSCs.

In this study, we demonstrate that ATM has an essential role in the reconstitutive capacity of the haematopoietic stem cells (HSC), but is less required for the differentiation or proliferation into progenitors. $ATM^{-/-}$ mice over the age of 24 weeks showed progressive bone marrow failure due to a defect in HSC function that was associated with up-regulation of $p16^{INK4A}$ and $p19^{ARF}$ in response to elevated reactive oxygen species (ROS). Treatment of anti-oxidative agents restored the reconstitutive capacity of $ATM^{-/-}$ HSCs. These data demonstrate that the self-renewal capacity of HSC depends on ATM-mediated inhibition of oxidative stress.



Role of Chk2 on tumorigenesis

A. Hirao, K. Naka, N. Motoyama, R. Hakem, JP. McPherson, T. Mak,

In response to ionizing radiation (IR), the tumor suppressor p53 is stabilized and promotes either cell cycle arrest or apoptosis. Chk2 activated by IR contributes to this stabilization, possibly by direct phosphorylation. Like p53, Chk2 is mutated in patients with Li-Fraumeni syndrome. Since the ATM gene is required for IR-induced activation of Chk2, it has been assumed that ATM and Chk2 act in a linear pathway leading to p53 activation. To clarify the role of Chk2 in tumorigenesis, we generated gene-targeted Chk2-deficient mice. Unlike ATM-/- and p53-/- mice, Chk2-/- mice do not spontaneously develop tumors, although Chk2 does suppress DMBA-induced skin tumors. Tissues from Chk2-/- mice, including thymus, CNS, fibroblasts, epidermis and hair follicles, show significant defects in IR-induced apoptosis or impaired G1/S arrest. Quantitative comparison of the G1/S checkpoint, apoptosis, and expression of p53 proteins in Chk2-/- versus ATM-/- thymocytes suggested that Chk2 regulates p53-dependent apoptosis in an ATM-independent manner. These data indicate that distinct pathways regulate the activation of p53 leading to cell cycle arrest or apoptosis.

Disruption of Brca1 results in cellular demise or tumorigenesis depending on cellular context. Inactivation of p53 contributes to Brca1-associated tumor susceptibility. We show that Chk2 inactivation is partially equivalent to p53 inactivation, in that Chk2 deficiency facilitates the development, survival, and proliferation of Brca1-deficient T cells at the expense of genomic integrity. Brca1 deficiency was found to result in Chk2 phosphorylation and the Chk2-dependent accumulation and activation of p53. Furthermore, inactivation of Chk2 and Brca1 was cooperative in breast cancer. Our findings identify a critical role for Chk2 as a component of the DNA damage-signaling pathway activated in response to Brca1 deficiency.

Publications

1. Keramaris E, Hirao A, Slack RS, Mak TW, Park DS. (2003) ATM can regulate p53 and neuronal death independent of Chk2 in response to DNA damage. J Biol Chem. 278:37782-9
2. Kato N., Sugiyama K., Nanba K., Dansako H., Nakamura T., Takami M., Naka K., Nozaki A. and Shimotohno K. (2003) Establishment of a hepatitis C virus subgenomic replicon derived from human hepatocytes infected in vitro. Biochem. Biophys. Res. Commun. 306: 756-766
3. McPherson JP, Lemmers B, Hirao A, Hakem A, Abraham J, Migon E, Matysiak-Zablocki E, Tamblyn L, Sanchez-Sweatman O, Khokha R, Squire J, Hande MP, Mak TW, Hakem R. (2004) Collaboration of Brca1 and Chk2 in tumorigenesis. Genes Dev. 18:1144-53.
4. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY and Suda T. (2004) Tie2/Angiopoietin-1 Signaling Regulates Hematopoietic Stem Cell Quiescence in the Bone Marrow Niche. Cell, 118:149-61
5. Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, Nomiyama K, Hosokawa K, Sakurada K, Nakagata N, Ikeda Y, Mak TW, Suda T. (2004) Regulation of oxidative stress by ATM is required for the self-renewal of haematopoietic stem cells. Nature, 431:997-1002
6. Naka K., Tachibana A., Ikeda K. and Motoyama N. (2004) Stress-induced premature senescence in hTERT-expressing Ataxia Telangiectasia fibroblasts. J. Biol. Chem. 279 (3), 2030-2037
7. Kondo T., Oue N., Yoshida K., Mitani Y., Naka K., Nakayama H. and Yasui W. (2004) Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res. 64 (2): 523-529
8. Namba K., Naka K., Dansako H., Nozaki A., Ikeda M., Shiratori Y., Shimotohno K. and Kato N. (2004) Establishment of hepatitis C virus replicon cell lines possessing interferon-resistant phenotype. Biochem. Biophys. Res. Commun. 323(1): 299-309
9. Azuma M, Hirao A, Takubo K, Hamaguchi I, Kitamura T, Suda T. (2005) A quantitative matrigel assay for assessing repopulating capacity of prostate stem cells. Biochem Biophys Res Commun, 338(2):1164-70
10. Abe K., Ikeda M., Dansako H., Naka K., Shimotohno K. and Kato N. (2005) cDNA microarray analysis to compare HCVsubgenomic replicon cells with their cured cells, Virus Res. 107 (1): 73-81
11. Kato N., Nakamura T., Dansako H., Namba K., Abe K., Nozaki A., Naka K., Ikeda M. and Shimotohno K. (2005) Genetic variation and dynamics of hepatitis C virus replicons in

long-term cell culture. J. Gen. Virol. 86(3): 645-656.
12. Ikeda M., Abe K., Dansako H., Nakamura T., Naka K. and Kato N. (2005) Efficient replication of a full-length hepatitis C virus genome, strain O in cell culture, and development of a luciferase reporter system. Biochem. Biophys. Res. Commun 329(4), 1350-1359.
13. Tamura T., Nozaki A., Abe K., Dansako H., Naka K., Ikeda M., Tanaka K. and Kato N. (2005) cDNA microarray analysis of lactoferrin expression in non-neoplastic human hepatocyte PH5CH8 cells. Biochim. Biophys. Acta. 1721: 73-80
14. Tahara E., Tahara H., Kanno M., Naka K., Takeda Y., Ishihara H., Yasui W., Barrett J.C., Ide T. and Tahara E. (2005) G1P3, an interferon inducible gene 6-16, is expressed in gastric cancers and inhibits mitochondorial mediated apoptosis in gastric cancer cell line TMK-1 cell. Cancer Immunol. Immunother. 54 (8): 729-740.
15. Dansako H., Naka K., Ikeda M. and Kato N. (2005) Hepatitis C virus proteins exhibit conflicting effects on the interferon system in human hepatocyte cells. Biochem. Biophys. Res. Commun. 336(2): 458-468
16. Naka K., Ikeda M., Abe K., Dansako H. and Kato N. (2005) Mizoribine inhibits hepatitis C virus RNA replication: effect of combination with interferon-alpha. Biochem. Biophys. Res. Commun. 330(3): 871-879
17. Naka K., Takemoto K., Abe K., Dansako H., Ikeda M., Shimotohno K. and Kato N. (2005) Interferon resistance of hepatitis C virus replicon-harbouring cells is caused by functional disruption of type I interferon receptors. J. Gen. Virol., 86(10): 2787-2792.
18. Naka K., Dansako H., Kobayashi N., Ikeda M. and Kato N. (2006) Hepatitis C virus NS5B delays cell cycle progression by inducing interferon-beta via Toll-like receptor 3 signaling pathway without replicating viral genomes. Virology in press (VIRO-05-455R1).

Other achievements

The 41st Erwin von Bälz Prize: A. Hirao (2004)

Division
of
Cell Biology

General Summary of Division of Cell Biology

In the past three years the division has been dedicated to basic researches on new RNA functions, novel RNA polymerase II associating proteins and infection mechanism of hepadnaviruses.

A) Functional analysis of low molecular weight RNAs

Various low molecular weight RNAs function at many stages in gene expression and concern with maintenance and proliferation of the cells. Although functions of many kinds of low molecular weight RNAs were determined in the last decade, functions of several low molecular weight RNAs are still unknown. We are currently interested in the structure, function and biogenesis of low molecular weight RNAs. In this period we focused on the functional characterization of U13 small nucleolar RNA (snoRNA) and micro RNA (miRNA).

B) Novel proteins associated with phosphorylated RNA polymerase II

The carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II consists of multiple repeats of a highly conserved seven amino acid sequence YSPTSPS. The CTD can be extensively phosphorylated, especially at the serine 2 and 5 positions in the consensus repeat. CTD phosphorylation has been reported to act as an important signal not only for transcription but also for the recruitment of pre-mRNA processing factors to transcription sites. To approach to the molecular mechanism for coupling transcription with pre-mRNA processing, we have identified and characterized novel factor PCIF1 that can directly interact with the phosphorylated CTD.

C) Infection mechanism of hepatitis B viruses

Although much is known about genomic replication and gene expression of hepatitis B viruses (hepadnaviruses), little is known about the molecular mechanisms of the entry and the release pathways for this virus. To understand the nature of these pathways, we tried to search for the host proteins that interact to capsid protein of the duck hepatitis B virus (DHBV). We have obtained a 33-kDa protein (p33) as a DHBV-core binding protein. The human hepatitis B virus (HBV) core protein also binds to human p33. p33 was identified as complement component 1q receptor (gC1qR) by mass spectrometry. The binding mechanism between gC1qR and viral core protein was examined.

Functional analysis of microRNAs by the using of gene disruption

F. Harada, S. Ishii and Y. Kido

MicroRNAs (miRNAs) are small (approximately 22 nucleotides) RNAs that negatively regulate gene expression through interactions with 3'-untranslated regions of their target mRNAs, which leads to mRNA cleavage or translational repression. To date, 326 miRNA genes have been identified from human genome, the precise functions of these non-coding RNAs remains largely obscure. Interestingly, many miRNAs are found in close proximity to other miRNAs, and these clustered miRNAs are transcribed from a single polycistronic transcription unit by RNA polymerase II. Therefore clustered miRNAs might have very important roles in maintenance of the specific cell lineage. Human chromosome 13 has a one of the clustered miRNA gene, which encodes 6 miRNAs (miR-17-5P, 18a, 19a, 20a, 19b-1 and 92-1) in 800bp. To clarify the function of these miRNAs, we tried to obtain homologue of this clustered gene from chicken B cell line DT40 and disrupt it by targeted integration.

First of all, we obtained a chicken DNA fragment containing miRNA gene cluster homologous to human chromosome 13 by PCR amplification using human miR-17-5P and miR 92-1 sequences as primers. Screening of DT40 genomic library with this DNA fragment as a probe, we obtained several chicken genomic clones. These clones contained 6 miRNA genes in the same order as the human gene and the nucleotide sequence of each miRNA was completely identical with that of human. Therefore these clustered miRNAs are conserved between human and chicken cells and might have essential roles in these animals. To disrupt the clustered miRNA gene in DT40 cells, the targeting vectors containing blasticidin S and puromycin resistance genes were constructed and then introduced into DT40 cells by sequential homologous recombination. Three knockout cell lines on this clustered miRNA gene were obtained. The growth rate of these mutant cells was slightly slower than that of wild-type DT40 cells. The expression of each miRNA was analyzed by Northern hybridization of total RNA isolated from mutant and wild-type cells. In the mutant cells, miR-20a was undetectable while other five miRNAs still expressed about 50% of wild-type cells. In human genome, paralogous cluster in X chromosome, which encodes miR-106a, 18b, 20b, 19b-2 and 92-2 has been reported. Diminished expression of 5 miRNAs in mutant cells must be attributable to this miRNA cluster. We prepared DT40 genomic clone containing new miRNA cluster and construction of double knockout cells on miRNA clusters is now in progress.

Involvement of U13 small nucleolar RNA in cytidine modification of 18S rRNA.

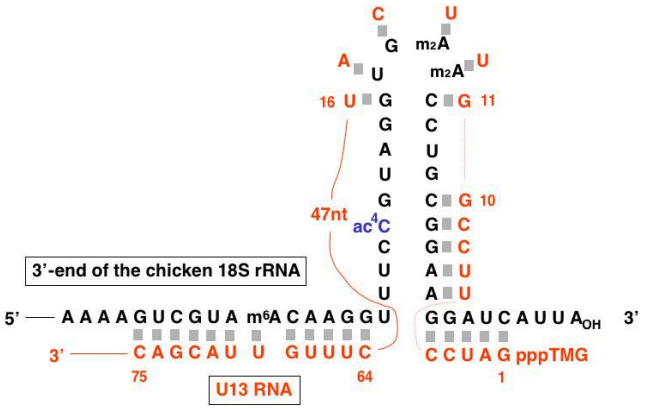
Y. Kido and F. Harada

Since 1990's, many small nucleolar RNAs (snoRNAs) are shown to be involved in 2'-O-methylation or pseudouridylation of nucleosides on rRNAs. U13 snoRNA was first identified in 1986 as a trimethylguanosine-capped, box C/D class snoRNA isolated from HeLa cell. According to the complementarity to 3'-end of 18S rRNA, U13 RNA has been hypothesized to be involved in maturation of 3'-end region of 18S rRNA. Cavaille et al. (1996) discussed the involvement of U13 snoRNA in cleavage of pre-rRNA at the 18S rRNA / ITS1 junction. There is, however, no direct evidence supporting the suggested function of U13 snoRNA so far.

In order to elucidate the function of U13 snoRNA we have established U13-knockout DT40 cell lines and have compared all the nucleoside (and its modification) of the 3'-end region of 18S rRNA between wild-type and knockout DT40 cells by 2-dimentional TLC. In all independent U13-knockout cell lines examined so far a modified cytidine (N4-acetylcytidine) on position 1796, which detected in wild-type cell, was not detected. By transfection of U13 RNA gene into a knockout strain, we have detected expression of U13 snoRNA at the same level as that of wild-type. In all independent transfectants examined so far, cytidine acetylation at the position 1796 was restored. These results strongly suggest that U13 snoRNA is involved in the modification of C1796 of 18S rRNA.

U13 snoRNA has a complementarity to the 3'-end of 18S rRNA (see Figure). To test the importance of the complementarity in acetylation activity, we are introducing several nucleotide substitutions on the complementary region of U13 snoRNA gene and are analyzing the modification status of 18S rRNA isolated from the individual transfectants of the mutant U13 snoRNA genes.

To study acetylation of the 3'-end of 18S rRNA biochemically, *in vitro* system of cytidine acetylation in a cell extract is required. So far, however, we have not been able to detect rRNA acetylation *in vitro*.



Functional characterization of the vertebrate phosphorylated RNA polymerase II interacting factor PCIF1

Y. Hirose, M. Yuda, I. Yunokuchi, K. Sakuraba, H. Fan and F. Harada

The carboxy-terminal domain of the largest subunit of RNA polymerase II (CTD) consists of multiple repeats of an evolutionally conserved seven amino acid sequence YSPTSPS. The CTD is subject to reversible phosphorylation at Ser2 and Ser5 of the heptapeptide during the transcription cycle. Ser5 phosphorylation occurs in the early stage of transcription cycle and promotes 5' capping of pre-mRNA. Transcription elongation is accompanied by Ser2 phosphorylation, which is also required for efficient 3' end formation of pre-mRNA. Thus CTD phosphorylation plays important role in coordinating transcription and pre-mRNA processing. To better understand the molecular mechanism by which transcription couples with pre-mRNA processing, we have identified and characterized novel human factors that can directly interact with the phosphorylated CTD (pCTD).

We recently identified a novel human nuclear WW domain protein, PCIF1 as a pCTD interacting factor. The WW domain of PCIF1 is responsible for specific binding to pCTD and exhibits the considerable homology to the WW domain of human peptidyl-prolyl isomerase Pin1 that has been shown to bind to a phophorylated Ser/Thr-Pro motif. An over expression of PCIF1 in human cells resulted in repressing trans-activation of the co-transfected reporter gene expression driven by various transcription activators. PCIF1 WW preferentially binds to a CTD peptide phosphorylated at only Ser5 position against a CTD peptide phosphorylated at only Ser2 position. We further examined the effect of PCIF1 on dephosphorylation of pCTD by a human CTD phosphatases SCP1 which have been shown to preferentially dephosphorylate pSer5 within the CTD repeat. We found that PCIF1 WW domains strongly inhibited CTD phosphatase activity of SCP1 in vitro. We speculate that PCIF1 play a role in early stage of transcription cycle and/or in regulating phosphorylation dynamics of the CTD.

To further investigate cellular functions of PCIF1, we have disrupted PCIF1 gene in the chicken B-cell line DT40. The growth curve for the PCIF1 deficient mutant DT40 cells was indistinguishable from that for wild-type DT40 cells. Thus chicken PCIF1 is not essential for cell growth. PCIF1 depletion in DT40 cells significantly affected neither global phosphorylation status of the CTD nor global methylation status of the histone H3. Interestingly, however, we observed that PCIF1 depletion resulted in augmentation of chicken Pin1 expression both at mRNA and protein level.

Hepatitis B virus core proteins associate with host cell protein gC1qR

K. Kuroki, Y. Yamano, T. Kawakami[1], Y. Fukushima[2], T. Nishimura[1], T. Ishikawa[2] and F. Harada ([1]Clinical Proteome Center, Tokyo Medical University, [2]Dept. of Internal Medicine, University of Tokyo)

The human hepatitis B virus (HBV) is a small and enveloped DNA virus of the prototype of a family of *Hepadnaviridae* that causes acute and chronic liver disease and increases the risk of developing hepatocellular carcinoma. Despite of considerable understanding of the details of hepadnaviral replication and gene expression, little is known about the nature of the entry and the release pathways for this virus.

To understand the nature of the uptake and the maturation pathways for the hepadnaviruses, we have begun the search for the host proteins that interacts to capsid proteins of HBV and the duck hepatitis B virus (DHBV) as a model of these viruses.

We have identified a 33-kDa protein as a DHBV-core binding protein in duck liver extracts using glutathione-S-transferase (GST)-DHBV core-CBD (chitin binding domain) fusion proteins. The same molecular sized protein was also identified with GST-HBV core-CBD fusion protein in HepG2 extracts, so human p33 was subjected to reversed-phase liquid chromatography (LC) coupled with electrospray-tandem mass spectrometry (MS/MS). p33 has turned out to be gC1qR.

To elucidate the function of gC1qR in hepadnavirus life cycle, we examined the binding domain on DHBV and HBV core proteins by constructing the DHBV and HBV core deletion mutants. Our data show 1) the cellular gC1qR protein binds to arginine-rich domain of carboxyl-terminal of core proteins 2) DHBV and HBV has a common binding motif with arginine repeated region, 3) gC1qR binds at least two domains in both DHBV and HBV core proteins (DHBV a.a.213-229, a.a.225-235, HBV a.a.157-169, a.a.164-177), 4) DHBV core proteins substituted two serine residues (a.a.230 and 232) with aspartic acid to mimic the phosphorylated form could not bind to gC1qR. These arginine-rich domains of the hepatitis B virus core protein have been shown to be required for pregenome encapsidation, productive viral DNA synthesis and sorting viral genome to the nuclei. gC1qR may regulate these processes by the interaction with nonphosphorylated core proteins.

Publications

1. Fan, H., Sakuraba, K., Komuro, A., Kato, S., Harada, F. and Hirose, Y. (2003) PCIF1, a novel human WW domain-containing protein, interacts with the phosphorylated RNA polymerase II. Biochem. Biophys. Res. Commun., 301:378-385.
2. Xu Y-X., Hirose Y., Zhou X.Z., Lu K.P. and Manley. J.L. (2003) Pin1 modulates the structure and function of human RNA polymerase II. Genes & Dev., 17:2765-2776
3. Kawakami, T., Tateishi, K., Yamano, Y., Ishikawa, T., Kuroki, K., Nishimura, T. (2005) Protein identification from product ion spectra of peptides validated by correlation between measured and predicted elution times in liquid chromatography/mass spectrometry. PROTEOMICS, 5:856-864.
4. Enomoto, M., Sawano, Y., Kosuge, S., Yamano, Y., Kuroki, K., Ohtsuki, K. High phosphorylation of HBV core protein by two a-type CK2-activated cAMP-dependent protein kinases in vitro. FEBS Lett. in press

Other achievements

Patent No. 3593482 (September 3, 2004)
Human nuclear proteins containing WW domain and cDNA encoding them.
(M. Kato, A. Komuro and Y. Hirose)

Division of Stem Cell Biology

General Summary of Division of Stem Cell Biology

In the past three years, we have been addressing the mechanism of developmental, physiologic and pathologic blood vessel formation and self-renewal of hematopoietic stem cells (HSCs). In the former, we analyzed interaction among endothelial cells (ECs) and hematopoietic cells (HCs) and mural cells (MCs) especially focusing on receptors, Flk-1, Tie2, EphB4, and nueropilin-1. In the later, we have been analyzing the function of Tie2 receptor tyrosine kinase. In both system, angiogenesis and self-renew of HSCs, Tie2 plays very important role, therefore we have cloned various genes that might be affected by Tie2 activation. The function of those genes have been studied by molecular biological technique and determined.

A) Role of hematopoietic cells in vascular development

Angiogenesis is an important event for embryonic organogenesis as well as for tissue repair in the adult. Among various kinds of HCs, we reported that HSCs play important roles for angiogenesis during embryogenesis. HSCs, which express angiopoietin-1 (Ang1), directly promoted migration of ECs and capillary formation. Moreover, we found that NP-1 expressed on HCs such as B lymphocyte, erythroblast, and monocyte binds VEGF and stimulates VEGF receptor on ECs strongly by an exogenous manner resulted in enhancement of angiogenesis.

1) In the past three years, we found that HSCs differentiate into MCs through CD11b positive monocyte lineage. This transdifferentiation is observed in physiological conditions as well as pathological conditions. Interestingly, we found that those CD11b positive cells produce Angiopoietin-1 and induce non-leaky vessel by the stabilization of blood vessel structure.
2) Based on the findings that HCs regulate angiogenesis by many ways, we evaluated the function of HCs for tumor angiogenesis. Bone marrow suppression by injection anti-c-Kit antibody suppressed the tumor angiogenesis resulted in delay of tumor growth. Therefore, we concluded that suppression of HC migration into tumor is one of promising approach for managing cancer.

B) Role of Tie2 for Self-renewal of HSCs

Tie2 is expressed on HSCs and plays a critical role for development of HSCs. In bone marrow, HSCs localize in trabecular bone and adhere to osteoblast. In such niche, it is suggested that osteoblasts produce angiopoietin-1 and stimulate Tie2 on HSCs, resulted in quiescent state of HSCs. In order to confirm this action of Tie2 on HSCs genetically, we constructed constitutive active form of Tie2 and generated conditional transgenic mice expressing constitutive active Tie2 in HSCs by cre-LoxP system. We previously reported the first site where HSCs proliferate is omphalomesenteric artery at E9.5 in mice. In this tg mice, we found that HSCs could not proliferate in this artery and died by severe anemia, suggesting that differentiation of HSCs is also impaired. Therefore, we confirm that Tie2 should be inactivated transiently for self-renewal of HSCs. Additonally, in this tg mice, severe defective angiogenesis was observed. Upon activation of Tie2 expressed on ECs, cell adhesion of MCs and ECs are induced. Therefore, we concluded that for the dissociation between MCs and ECs, inactivation of Tie2 is necessary for initiation of

sprouting angiogenesis.

C) Isolation of genes affected by Tie2 activation relating to stemness.

We and the other group have reported that Tie2 regulates cell adhesion of HSCs to osteoblasts and ECs to MCs, resulted in maintaining of dormant state and long term survival in both ECs and HSCs. Then, we have tried to isolate genes associating with such dormancy and anti-apoptotic effects regulated by Tie2.

1) Psf1: We isolated cell cycle related gene, psf1. Anaysis of this PSF1 has been well performed in yeast and it has been reported that PSF1 is critical for cell division by regulating the formation of DNA replication fork. We found that PSF1 expression is restricted in a certain stem /immature cell population. In knock out study of PSF1 gene in mice showed that lack of PSF1 lead deficiency of cells composing with inner cell mass (ES cells) proliferation.
2) Galectin-3: A member of galactose binding lectin, galectin-3 (Gal-3) is upregulated under Tie2 activation. We found that Gal-3 might induce anti-apoptotic effect under hypoxic condition. Niche is the place locating distant from vascular area, so HSCs in niche are exposed with hypoxia. Then, HSCs produce Gal-3 under Tie2 activation and might protect cell apoptosis under hypoxia.

D) Isolation of genes affected by Tie2 activation relating to blood vessel formation.

It is well known that Tie2 activation induces non-leaky and enlarged blood vessel. However, which molecules actually carry out such effects are not clearly known. Therefore, we isolated genes which regulate permeability or caliber change of blood vessel and analyzed the function of those molecules.

1) ACE (angiotensin converting enzye): It was previously reported that VEGF mediated hyperpermeability was suppressed by angiopoietin-1. We found that ACE is downregulated by Tie2 activation. ACE is well known molecule that converts angiotensin I to angitensin II and it is reported that angiotensin II induces vascular permeability in in vitro system. Therefore, we hypothesized that ACE is negatively regulated by Tie2 activation and inhibits leakiness. Using Miles assay, we confirmed that VEGF mediated hyperpermeability was inhibited by angiotensin type 1 receptor blocker, ARB. It has been reported that VEGF mediated leakiness was caused by the production of NO from ECs. We found that VEGF mediated NO production was inhibited by ARB.
2) Apelin: Apelin is a ligand for APJ, GPCR with seven transmembrane domain expressed on ECs and MCs. We found that Tie2 activation on ECs induces apelin production from ECs. To know the function of apelin on blood vessel formation, we induced apelin into tumor cells, inoculated them into mice and observed tumor angiogenesis. We found that apelin induced enlarged blood vessels compared with control. A variety of experiments suggested that apelin regulates cell adhesion in ECs and enhances assembly of ECs resulted in formation of enlarged vessels.

PSF1 Is Essential for Immature Cells Proliferation

M. Ueno and N. Takakura

Eukaryotic chromosomal replication is tightly regulated to maintain the integrity of genomic information. In yeast, Orc (origin recognition complex) is bound to replication origins throughout the cell cycle. From late M to G1 phase, MCM (minichromosome maintenance) protein is loaded onto the origin, marked by Orc, by Cdc6 and Cdt1, and forms the pre-replication complex (pre-RC). On activation and recruitment of additional factors, such as CDC45, the pre-RC is converted to the pre-initiation complex (pre-IC), which is the complex essential for the transition to DNA replication. In yeast, CDC45 is essential for the initiation and elongation of DNA replication.

Recently, a novel multi-protein complex "GINS" was identified. This GINS complex contains, Psf1 (partner of sld five 1), Psf2, Psf3, and Sld5 and forms a ring-like structure. During the S-phase, the GINS complex is loaded onto chromatin after the formation of pre-RCs, and then tightly associates with the replication origin. This binding is suppressed by p21 and geminin by inhibiting the loading of CDC45 onto chromatin and the pre-RC formation by binding to Cdt1. Moreover, the chromatin binding of GINS complex and of CDC45 are mutually dependent processes, but they do not associate with each other. The association of PSF1 and Dpb11/Cut5 with the origins is also mutually dependent. All genes encoding GINS components are evolutionarily conserved and are essential for cell growth. However, the functions of GINS complex in mammalian cells have not been reported.

We originally cloned the mouse ortholog of *PSF1* from a hematopoietic stem cell cDNA library and found that *PSF1* is expressed in blastocysts, adult bone marrow, and testis, in which the stem cell system is active. We used the gene-targeting technique to determine the physiological function of PSF1 *in vivo*. Mice homozygous for a non-functional mutant of *PSF1* died *in utero* around the time of implantation. *PSF1*$^{-/-}$ blastocysts failed to outgrowth in culture and exhibited a cell proliferation defect. Our data clearly indicate that *PSF1* is required for early embryogenesis.

Ref:
Ueno, M., et al. (2005) Mol Cell Biol. 25:10528-10532.
Kong, L., et al. (2006) Biochem. Biophys. Res. Commun. 339:1204-1207.

The identification of cardio-vascular stem cells and clarification its differentiation mechanism

Yoshihiro Yamada, Nobuyuki Takakura

Purpose: Ischemic disease is the main cause of death as well as cancer. To overcome it, we need to reveal the mechanism how cardio-vascular stem cells in tissue differentiate into cardiomyocytes or vascular cells. Furthermore, we would like to approach cancer stem cells, which have supposed as the cause of drug resistant cancer.

Results

1-1. In embryonic stage, vascular stem cells (VPCs) are originated from hematopoietic stem cells (HSCs) fraction and they differentiate into both ECs and SMCs (vascular cells) in vitro and in vivo.

1-2. HSCs($lin^-ckit^+CD45^+$), which differentiate into vascular cells, have tissue specific mechanism. HSCs in head region differentiate into vascular cells, but HSCs in fetal liver can not differentiate into vascular cells.

1-3. Tissue specificity in HSCs is due to the induction of Flk-1 expression by brain ECs.

1-4. In adult stage, especially in ischemic condition, vascular stem cells are exsited in monocyte/M-phage fraction, such as $CD45^+CD11b^+$ cells. Moreover, very interestingly, there are two populations, such as $CD45^+CD11b^{low+}$ and $CD45^+CD11b^{high+}$cells, and $CD45^+CD11b^{low+}$ cells mainly contribute to angiogenesis as vascular cells in both short term and long term.

2-1. We identified cardiac stem cells in brown adipose tissue. We named them BATCM (brown adipose tissue derived cardiomyocyte)

2-2. BATCMs expressed CD29, CD105, and CD133, such as mesenchymal stem cells marker, but not expressed linage marker, c-kit, and CD31, such as hematopoietic and endothelial cells marker.

2-3. In vitro, BATCMs differentiated into sarcomeric-actin, troponinT, MEF2C, and GATA-4 positive CMs, furthermore, they differentiated into CD31, VE-cadherin, Flk-1 positive ECs and SMA, PDGF-β and desmin-positive SMCs.

2-4. In vivo infarction model, BATCMs contributed as CM, ECs and SMCs in ischemic border zone, and improved the function of heart and mortality.

3-1. Cancer cells interacted with hematopoietic cells and performed as cancer stem like cells in vivo.

Future plan

1. To detect vascular stem cells in other condition, such as in tumorangiogenesis and brain ischemia. To detect the inducible factor for Flk-1 expression in HSCs.

2. To detect the regulating factor from stem cells into CMs in adipose tissue.

3. To analyze the features of cancer stem cell like cells precisely in vitro and in vivo.

Publication

1. Yamada. Y et al (2003) Blood 101:1801-1809.

2. Yamada. Y et al (inpress) Biochem. Biophys. Res. Commun.

Development of blood cells in the ascidian *Halocynthia roretzi*

S. Amano, N. Takakura and I. Hori* (*Dept. of Biol., Kanazawa Medical Univ., Uchinada, Ishikawa)

In recent years our understanding of the ontogenesis of mammalian hematopoietic cells has been improved greatly. However, we have only a limited knowledge about the ontogenesis of ascidian hemocytes. The trunk lateral cells (TLCs) of the tail bud embryo are the origin of hemocytes and body wall muscle in the ascidian *Halocynthia roretzi*. The TLCs migrate posteriorly and ventrally, and differentiate to hemocytes and muscle cells in the juveniles. Each ascidian species has nearly ten types of hemocytes in adulthood. It has been shown repeatedly that the hemocyte types of adults are very variable among ascidian species, and this fact made difficult to reveal the relationships of the hemocyte types between species. All ascidians ever studied, however, have a common type of hemocytes, the hemoblast. They are undifferentiated cells having typical morphology with a conspicuous nucleolated nucleus and scanty cytoplasm. The hemoblasts are thought to be hematopoietic stem cells (HSCs). Although the hemoblasts are found in the hematopoietic tissue and in circulation, it is not clear that both of them are truly HSCs.

In this study, the development of hematopoiesis was studied in detail with electron microscopy in the ascidian *H. roretzi*. We found only four types of hemocytes in the juveniles, and the scarcity of the hemocyte types allowed us to follow the process of their differentiation distinctly. In the swimming larvae and juveniles, nascent mesoderm cells infiltrate between the epidermis and endoderm. The mesoderm cells have yolk granules and compact masses of glycogen granules, and those nutritive granules are gradually consumed after metamorphosis. Consequently, in 5d juveniles, the mesoderm cells transform to hemoblasts with a scanty cytoplasm and a conspicuous nucleolated nucleus. Before the appearance of the hemoblasts, hematopoiesis initiates soon after metamorphosis. This fact means that the mesoderm cells directly differentiate to giant cells, vacuolated cells, and granular cells. About 5 days after metamorphosis the hemoblasts begin to differentiate to the three types of hemocytes instead of the mesoderm cells. The proportion of hemocyte types in the juveniles is very different from that in adults, for example the hemoblasts and giant cells are rare in the adults but they are major hemocyte types in the juveniles. The hemoblasts seem to be rapidly dividing cells because we found their mitotic figures in the hemocoel of the juveniles. We concluded that the circulating hemoblasts are HSCs that self-renew and replenish multiple types of hemocytes in the ascidian *H. roretzi*.

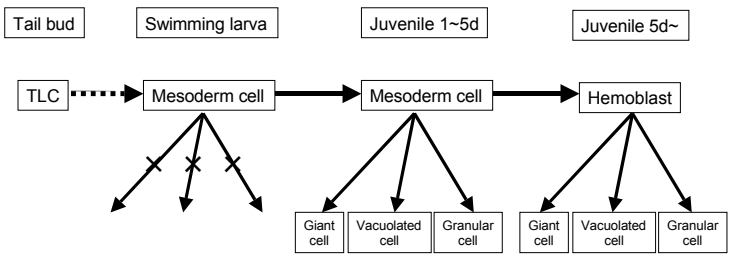


Figure shows the ontogenesis of hemocytes revealed in this study in the ascidian *H. roretzi*. Nascent mesoderm cells never differentiate to hemocytes in the swimming larvae. They begin to differentiate to three types of hemocytes in the juveniles after metamorphosis. Prior to the 5th day after metamorphosis, the nascent mesoderm cells transform to hemoblasts. Thereafter the hemoblasts differentiate to the three types of hemocytes, that is, giant cells, vacuolated cells, and granular cells. All these results distinctly show that the nascent mesoderm cells in the juveniles are multipotential cells. In addition, it is evident that the hemoblasts, derived from the nascent mesoderm cells, are HSCs.

Publications

1. Yamada, Y., Oike, Y., Ogawa, H., Ito, Y., Fujisawa, H., Suda, T., Takakura, N. (2003) Neuropilin-1 on hematopoietic cells as a source of vascular development. Blood 101:1801-1809.
2. Nakajima, M., Yuasa, S., Ueno, M., Takakura, N., Koseki, H., Shirasawa, T. (2003) Abnormal blood vessel development in mice lacking presenilin-1. Mech Dev. 120: 657-667.
3. Li, Z., Takakura, N., Oike, Y., Imanaka, T., Araki, K., Suda, T., Kaname, T., Abe, K. and Yamamura, K. (2003) Defective smooth muscle development in *qkI* deficient mice. Development Growth. Differ. 45:449-461.
4. Takakura, N. (2003) Hematopoietic stem cells and angiogenesis. Cardiomyopathies and Heart Failure. Kluwer Academic Publishers. 469-480.
5. Contribution of hematopoietic stem cells in blood vessel formation. Okamoto R, Yamada Y, Sano H, Ueno M, Takakura N. Biomed Rev. 14: 1-8, 2003(accepted &published in 2004).
6. Ueno, M., Itoh, M., Kong, L., Sugihara, K., Asano, M., and Takakura, N. *PSF1* is essential for Early Embryogenesis in mice. Mol. Cell. Biol. In press
7. Fukuhara, S., Sakurai, A., Sano, H., Yamagishi, A., Somekawa, S., Takakura, N., Saito, Y., Kangawa, K., and Mochizuki, N. (2005) Cyclic AMP potentiates VE-cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway. Mol. Cell. Biol. 25:136-146.
8. Okamoto R, Ueno M, Yamada Y, Takahashi N, Sano H, and Takakura N: Hematopoietic cells regulate the angiogenic switch during tumorigenesis. *Blood* 105: 2757-2763, 2005
9. Lingyu Kong, Masaya Ueno, Machiko Itoh, Katsuji Yoshioka, and Nobuyuki Takakura: Identification and Characterization of Mouse PSF1 Binding Protein, SLD5. BBRC 339:1204-1207.
10. Yamada Y. et al Cardiac progenitor cells in brown adipose tissue repaired damaged myocardium. BBRC in press

Other achievements

Patent

1. Title: Myocardial cell induction utilizing adipose tissue in mammal. No.2003-429088,PCT/JP2004/19666
2. Title: Methods and machine for expansion of organ specific stem cells. No. 2004-119179,PCT/JP2005/006424
3. Title: Methods for usage of PSF1deficient mice. No. 2004-242022
4. Title: anti-ephrinB2 antibody. No. 2004-244433
5. Title: drug for blood vessel enlargement. No. 2004-300653
6. Title: Method for generation of cancer stem cells. No. 2005-126266

Division
of
Molecular Pathology

General Summary of Division of Molecular Pathology

Our main research interest is to clarify how cells recognize DNA damage and transduce signals to cell cycle control, DNA repair and apoptotic machineries. To achieve this goal, we are currently using a gene knockout approach in chicken DT40 cell lines.

A) Direct ATM Activation by Sulfhydryl-Reactive Inflammatory Cyclopentenone Prostaglandins

It is not clear whether or not ATM responds to oxidative DNA damage or rather responds to a change in intracellular redox state independent of DNA damage. We found that ATM is directly activated by protein sulfhydril-group-modifying agents, N-methyl-N'-nitro-nitrosoguanidine and 15-deoxy-Δ12,14-prostaglandin J_2, in chicken DT40 cells deficient for DNA damage sensors, such as *NBS1* or *MSH6*.

B) c-Abl tyrosine kinase family is involved in the formation of stable chromatin-associated Rad51 complex

It is unclear how these Abl tyrosine kinase members mechanistically regulate Rad51 functions during homologous recombination repair (HRR). We found that the c-Abl tyrosine kinase family enhances the formation of stable chromatin-associated Rad51 complexes through phosphorylation of Rad51 mainly on Tyr-315 during HRR.

C) Involvement of the ATR- and ATM-dependent checkpoint responses in cell cycle arrest evoked by pierisin-1

Piersin-1 identified from the cabbage butterfly, *Pieris rapae*, is a novel mono ADP-ribosylating toxin that transfers the ADP-ribose moiety of NAD at N^2 of dG in DNA. We found that mono ADP-ribosylation of DNA causes a specific type of fork blockage which induces checkpoint activation and signaling.

D) Msh6 and Nbs1 are essential for ATR-mediated Chk1 activation induced by DNA replication stalling in higher vertebrate cells

We found that ATR-mediated Chk1 and Chk2 phosphorylation linked to methylated DNA damage requires DNA damage sensors, Msh6 and Nbs1, in addition to early acting checkpoint proteins, such as Rad17 and Rad9, and ATRIP, the DNA binding subunit of ATR.

We are very grateful to investigators outside of this institute for their contributions to our projects; Kenshi Komatsu, Radiation Biology Center, Kyoto University; Koji Uchida, Graduate School of Bioagricultural Science, Nagoya University; Hiroshi Tauchi, Department of Environmental Science, Faculty of Science, Ibaraki University; Masayuki Takahashi, UMR 6204 Centre National de la Recherche Scientifique & Universite de Nantes; Hitoshi Kurumisaka, Graduate School of Science and Engineering, Waseda University; Shunichi Takeda, Graduate School of Medicine, Kyoto University; Minoru Takata, Kawasaki Medical School; Keiji Wakabayashi, National Cancer Center Research Institute; Ashok R. Venkitaraman, Cancer Research UK Department of Oncology, University of Cambridge; Yosef Shiloh, Sackler School of Medicine, Tel Aviv University; Steve Jackson, The Wellcom Trust and Cancer Research UK Gurdon Institute, Cambridge University.

Direct ATM Activation by Sulfhydryl-Reactive Inflammatory Cyclopentenone Prostaglandins

Masahiko Kobayashi, Hiroto Ono, Keiko Mihara, Hiroshi Tauchi, Kenshi Komatsu, Hiroko Shimizu, Koji Uchida, and Ken-ichi Yamamoto

ATM (ataxia-telangiectasia mutated) is essential for cellular response to double strand breaks in vertebrate cells. However, ATM is activated by a variety of noxious agents, including oxidative stress, and ATM deficiency results in anomalous cellular response to oxidative stress. While this defective response to oxidative stress may underlie the pathogenesis of cerebellar ataxia, premature aging and cancer predisposition in ataxia-telangiectasia, mechanisms for ATM activation by oxidative stress remain to be established. Furthermore, it is not clear whether or not ATM responds to oxidative DNA damage or rather responds to a change in intracellular redox state independent of DNA damage. To address these questions, we studied ATM activation by protein sulfhydril (SH)-group-modifying agents, N-methyl-N'-nitro-nitrosoguanidine (MNNG) and 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2), in chicken DT40 cells deficient for *NBS1* or *MSH6* generated by targeted disruption. We found that ATM is effectively activated with MNNG and 15d-PGJ_2 in *NBS1*$^{-/-}$ and *MSH6*$^{-/-}$ cells. We further found that ATM is directly activated by treatment of chromatin-free ATM immunoprecipitates with MNNG or 15d-PGJ_2 through modification of SH groups and that 15d-PGJ_2 covalently binds to ATM. Interestingly, 15d-PGJ_2-induced ATM activation leads to p53 phosphorylation and apoptosis but not Chk2 phosphorylation in human tumor cells. These results indicate that ATM is activated through direct modification by free SH-group-modifying reagents independently of DNA damage, resulting in apoptotic downstream response. However, it remains to be established how ATM regulates intracellular oxidative stress and how ATM abnormality leads to various defective manifestations in oxidative stress response. More extensive proteomics analysis is, thus, required for establishing ATM functions in oxidative stress response. For such analysis, 15d-PGJ_2 might be a useful agent, since ATM activation by 15d-PGJ_2 does not result in the activation of DNA damage-linked Chk1 and Chk2 phosphorylation.

c-Abl tyrosine kinase family is involved in the formation of stable chromatin-associated Rad51 complex

Hiroko Shimizu, Satoko Kitayama, Masahiko Kobayashi, Hitoshi Kurumisaka, Masayuki Takahashi, and Ken-ichi Yamamoto

c-Abl tyrosine kinase is activated by DNA damage, such as ionizing radiation (IR), in an ATM-dependent manner, and plays important roles in growth arrest and cell death. Several recent studies also indicate that c-Abl, Arg (the only other known member of the c-Abl family) and Bcr-Abl (the oncogenic form of c-Abl fusion kinase) are involved in DNA repair through the phosphorylation of Rad51, a key molecule in homologous recombination repair (HRR). However, it is unclear how these Abl tyrosine kinase members mechanistically regulate Rad51 functions. We found that Rad51 with the mutation of Arg-167 to Gly (Rad51-R167G) is defective in IR-induced nuclear focus formation, chromatin association and in vivo self-association, though this Rad51 mutant retains the capacity to bind BRCA2 in vivo. Interestingly, we also found that this Rad51 mutant is not effectively phosphorylated by c-Abl and Arg. However, co-transfection of wild-type but not kinase-dead c-Abl or Arg with Rad51-R167G enhanced the chromatin association of Rad51-R167G, suggesting that phosphorylation of tyrosine residues, probably Tyr-315, enhances the chromatin association of Rad51-R167G. We subsequently confirmed the phosphorylation of Tyr-315 by antibodies specific to tyrosine phosphorylated Tyr-315. To study further roles for c-Abl or Arg in the chromatin association of Rad51, we studied effects of Glivec, a relatively specific inhibitor for the c-Abl tyrosine kinase, on the chromatin association of transiently expressed Rad51. We first examined effects of Glivec on Rad51 tyrosine phosphorylation and on phosphorylation-mediated interactions between Rad51 and Arg, and confirmed that Glivec inhibits Rad51 tyrosine phosphorylation mediated by c-Abl or Arg and the interaction between Rad51 and Arg when wild-type Rad51 is transiently co-expressed with Arg or c-Abl. We subsequently found that Glivec effectively inhibits the enhancement of chromatin association of Rad51-R167G mediated by c-Abl or Arg. These results therefore indicate that the c-Abl tyrosine kinase family enhances the formation of stable chromatin-associated Rad51 complexes through phosphorylation of Rad51 mainly on Tyr-54 or Tyr-315 during HRR.

Involvement of the ATR- and ATM-dependent checkpoint responses in cell cycle arrest evoked by pierisin-1

Bunsyo Shiotani, Masahiko Kobayashi, Masahiko Watanabe, Ken-ichi Yamamoto, Takashi Sugimura and Keiji Wakabayashi

Piersin-1 identified from the cabbage butterfly, *Pieris rapae*, is a novel mono ADP-ribosylating toxin that transfers the ADP-ribose moiety of NAD at N^2 of dG in DNA. Resulting mono ADP-ribosylated DNA adducts cause mutations and the induction of apoptosis. However, little is known about checkpoint responses elicited in mammalian cells by the formation of such bulky DNA adducts. In the present study, it was demonstrated that DNA polymerases were blocked at the specific site of mono ADP-ribosylated dG which might lead to the replication stress. Pierisin-1-treatment of Bcl-2-overexpressing HeLa cells was found to induce the S-phase arrest and the G2/M-phase delay. In the colony survival assays, Rad17$^{-/-}$ DT40 cells showed greater sensitivity to pierisin-1-induced cytotoxicity than wild type and ATM$^{-/-}$ DT40 cells, possibly due to defects of checkpoint responses, such as the Chk1 activation. Further investigation of HeLa cells confirmed that Chk1 was activated ATR- and Rad17-dependently and that mitotic delay was inhibited in ATR- and Rad17-knockdown HeLa cells, but not in ATM-knockdown cells. The results thus suggest ATR-Rad17-Chk1 pathway mainly contributes to the S-phase arrest and G2/M-phase delay induced by pierisin-1. Simultaneously, pierisin-1-treatment activated Chk2 pathway ATM-dependently and ATM-independently. Furthermore characteristic 50 kbp DNA fragmentation, known to be blocked by Bcl-2 overexpression was observed and activation of ATM and Chk2 was only partially inhibited. Thus, the roles of ATM-Chk2 or Chk2 pathways appear minor in cell cycle arrest, but may be involved in the induction of apoptosis. From these findings, it is suggested that mono ADP-ribosylation of DNA causes a specific type of fork blockage which induces checkpoint activation and signaling.

Msh6 and Nbs1 are required for ATR-mediated Chk1 activation induced by DNA replication stalling in higher vertebrate cells

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MutSα, which consists of Msh2 and Msh6, is known to recognize O^6-MeG generated by MNNG and to bind to this DNA lesion. This is consistent with the recent findings that Msh2 and Msh6 interact with ATR and is involved in ATR-mediated Chk1 phosphorylation induced by MNNG. We confirmed the requirement of Msh6 for MNNG-induced and ATR-mediated Chk1 phosphorylation. However, MNNG-induced Chk2 phosphorylation did not require Msh6, and was rather enhanced in *MSH6*$^{-/-}$ DT40 cells. Nbs1, in addition to its role in DSB-induced ATM activation, is involved in ATR-mediated Chk1 phosphorylation induced by HU and UV. Thus, consistent with these findings, we found that MNNG-induced Chk1 phosphorylation on Ser-345 was severely diminished in *NBS1*$^{-/-/-}$ cells. We also observed MNNG-induced Chk2 phosphorylation, which was previously shown to be independent of human ATM and Mlh1 mismatch repair proteins, particularly with higher MNNG concentrations such as those used in the present study. However, we observed greatly reduced MNNG-induced Chk2 phosphorylation in *NBS1*$^{-/-/-}$ cells, indicating that ATM and mismatch repair proteins are dispensable but Nbs1 is required for Chk2 phosphorylation induced by high concentrations of MNNG. Many recent studies have provided evidence for a role for Nbs1 as a damage sensor or activator acting upstream of ATM in cellular response to DSB. There is also evidence for a direct physical interaction between ATM and Nbs1. In addition, both of Nbs1 and ATM are involved in DSB repair by the homologous recombinational DNA repair system. These findings explain shared clinical features of A-T and NBS (Nijmegen breakage syndrome caused by a hypomorphism in *NBS1*), such as immunodeficiency, radiosensitivity, chromosome instability and cancer predisposition. However, the results presented in the present study as well as those reported recently by other investigators indicate that Nbs1 is also involved in ATR-mediated Chk1 phosphorylation, which is induced by MNNG, HU, UV or cisplatin, which cause replication stalling. It is also likely that a kinase involved in Msh6/ATM- independent but Nbs1-dependent Chk2 phosphorylation induced by high concentrations of MNNG is ATR. This functional relationship between Nbs1 and ATR may explain the embryonic lethality of *NBS1* knockout in mice, which is distinct from the non-essential feature of ATM in mice and human.

Publications

1. M. Hashimoto, S. Rao, K. Yamamoto, M. Takata, S. Takeda, and H. Utsumi. DNA-PK: the major target for wortmannin-mediated radiosensitization by the inhibition of DSB repair via NHEJ pathway. J. Radiat. Res., 44, 155-163, 2003.
2. Wang, W., Seki, M., Otsuki, M., Tada, S., Takao, N., Yamamoto, K., Hayashi, M., Honma, M., and Enomoto, T. The absence of a functional relationship between ATM and BLM, the components of BASC, in DT40 cells. Biochem. Biophys. Acta, 1688, 137-144, 2004.
3. Kobayashi, M., Hirano, A., Kumano, T., Shuang-Lin Xiang, S-L., Mihara, K., Haseda, Y., Matsui, O., Shimizu, H., and Yamamoto, K. Critical role for vertebrate Rad17 and Rad9 in the cellular response to DNA damage and stalled DNA replication. Genes to Cells, 9, 291-303, 2004.
4. Barzilai, A., and Yamamoto, K. DNA damage responses to oxidative stress. DNA Repair, 3, 1109-1115, 2004.
5. Fukao, T., Chen, P., Ren, J., Kaneko, H., Yamamoto, K., Takeda, S., Kondo, N., and Lavin, M. F. Disruption of the BLM gene in ATM – null DT40 cells does not exacerbate either phenotype. Oncogene, 23, 1498-1506, 2004.
6. He, J., Tohyama, Y., Yamamoto, K., Kobayashi, M., Shi, Y., Takano, T., Noda, C., Tohyama, K., and Yamamura, H. Lysosome is a primary organelle in B cell receptor-mediated apoptosis: an indispensable role of Syk in lysosomal function. Genes to Cells, 79, 2597-2603, 2005
7. Yamamoto, K., Kobayashi, M., and Shimizu, H. ATM, a paradigm for a stress-responsive signal transducer in higher vertebrate cells. In DT40 Handbook, in press, 2006.
8. Shibata, T., Iio, K., Kawai, Y., Shibata, N., Kawaguchi, M., Toi, S., Kobayashi, M., Kobayashi, M., Yamamoto, K., and Uchida, K. Identification of a lipid peroxidation product as a potential trigger of the p53 pathway. J. Biol. Chem., in press, 2006
9. Shiotani, B., Kobayashi, M., Watanabe, M., Yamamoto, K., Sugimura, T., and Wakabayashi, K. Involvement of the ATR- and ATM-dependent checkpoint responses in cell cycle arrest evoked by pierisin-1. Mol. Cancer Res., in press, 2006

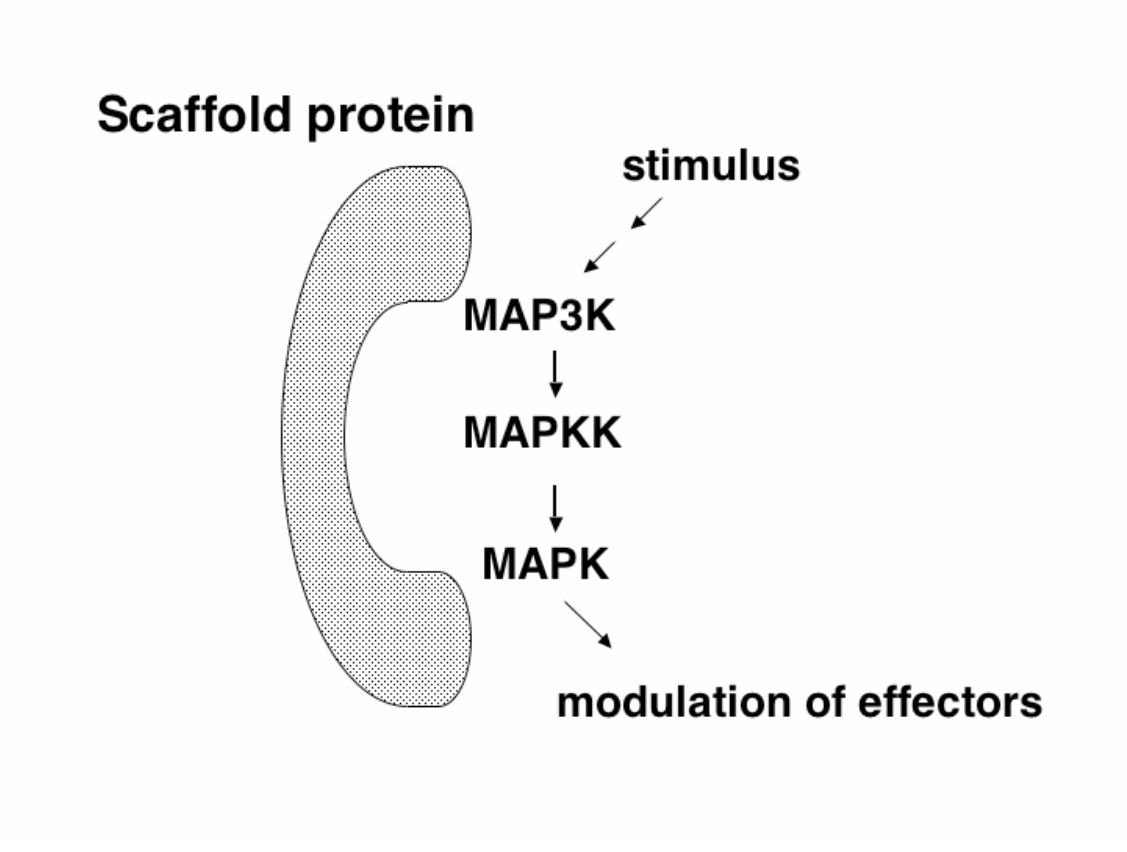
Division
of
Cell Cycle Regulation

General Summary of Division of Cell Cycle Regulation

Since 1998 the division has been dedicated to basic researches on MAP kinase (MAPK) signaling pathways, which play key roles in a wide array of cellular processes, including proliferation, differentiation, and apoptosis.

The MAPK pathway is an intracellular cascade consisting of MAPK, MAPK kinase (MAPKK) and MAPKK kinase (MAP3K). Recent rapid progress in identifying members of MAPK cascades suggests that there are a number of these distinct signaling pathways in cells. However, how the specificity and efficiency of the MAPK cascades is maintained is poorly understood. Furthermore, the function of each MAPK cascade *in vivo* is largely unknown.

We have identified two novel JNK MAPK-binding proteins, termed JSAP1 and JNKBP1, respectively. The subsequent biochemical analyses revealed that the proteins function as scaffold proteins in the JNK MAPK cascades. The goal of our projects is to demonstrate the functions of the scaffold proteins *in vivo*, and furthermore, to clarify the molecular mechanisms of how these scaffolds ensure the specific and efficient activation of the MAPK cascades *in vivo*.



Scaffold protein JSAP1 is transported to growth cones of neurites independently from JNK signaling pathways in PC12h cells

S. Sato, M. Ito, T. Ito and K. Yoshioka

The c-Jun NH_2-terminal kinase (JNK)/stress-activated protein kinase-associated protein 1 (JSAP1; also known as JNK-interacting protein 3) has been identified as a scaffold protein for JNK mitogen-activated protein kinase signal transduction pathways and as a cargo adapter in conventional kinesin-mediated transport system. Furthermore, the functional relationship between UNC-16, *C. elegans* ortholog of JSAP1, and JNK signaling has been genetically established. In this study, we first investigated expression properties of endogenous JSAP1 in differentiating PC12h cells, and demonstrated the requirement of kinesin light chain for the targeting and localization of JSAP1 to the tips of the neurites. Furthermore, to understand whether JNK signaling is involved in the kinesin-mediated JSAP1 trafficking, we established PC12h rat pheochromocytoma stable cell lines that express wild type and mutant JSAP1 lacking JNK-binding domain, respectively. Immunocytochemical studies of the cell lines indicated that the mutant JSAP1 is localized to growth cones of differentiating PC12h cells in a similar manner to the wild type JSAP1. Taken together, these results suggest that the proper subcellular localization of JSAP1 along microtublues does not require JNK signaling.

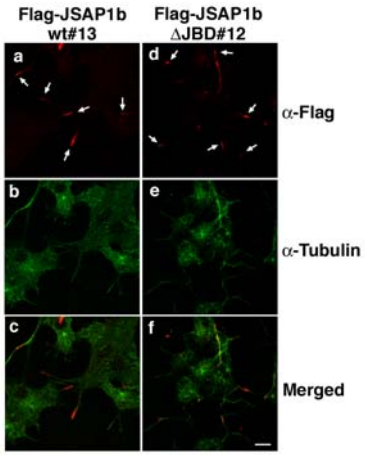


Figure Expression and subcellular distribution of JSAP1b in established PC12h cell lines. Cells of established PC12h lines (wt#13 and ΔJBD#12) that differentiated in response to treatment with NGF were fixed and processed for double-label indirect immunofluorescence microscopy. Arrows (in a and d) indicate signals over the tips of neurites.

Reference:
S. Sato, M. Ito, T. Ito and K. Yoshioka (2004) Gene 329, 51-60.

Selective expression of the scaffold protein JSAP1 in spermatogonia and spermatocytes

M. Bayarsaikhan, A. Shiratsuchi, D. Gantulga, Y. Nakanishi and K. Yoshioka

Scaffold proteins of mitogen-activated protein kinase (MAPK) intracellular signal transduction pathways mediate the efficient and specific activation of the relevant MAPK signaling modules. Our group and others previously identified c-Jun NH_2-terminal kinase (JNK)/stress-activated protein kinase-associated protein 1 (JSAP1, also known as JNK-interacting protein 3) as a scaffold protein for JNK MAPK pathways. Although JSAP1 is expressed in the testis in adults, its expression during development has not been investigated. In addition, it is unknown which types of cells in the testis express the scaffold protein. Here, we examined the expression of JSAP1 in the testis of mice aged 14 days, 20 days, 6 weeks, and 12 weeks by immunohistochemistry and Western blotting. The specificity of the anti-JSAP1 antibody was evaluated from its reactivity to exogenously expressed JSAP1 and a structurally related protein, and by antigen-absorption experiments. The immunohistochemical analyses with the specific antibody showed that the JSAP1 protein was selectively expressed in the spermatogonia and spermatocytes, but not in other cell types, including spermatids and somatic cells, during development. However, not all spermatogonia and spermatocytes were immunopositive either, especially in the 12-week-old mouse testis. Furthermore, we found by Western blotting that the expression levels of JSAP1 protein vary during development; there is high expression until 6 weeks after birth, which approximately corresponds to the end of the first wave of spermatogenesis. Collectively, these results suggest that JSAP1 function may be important in spermatogenic cells during early postnatal development.

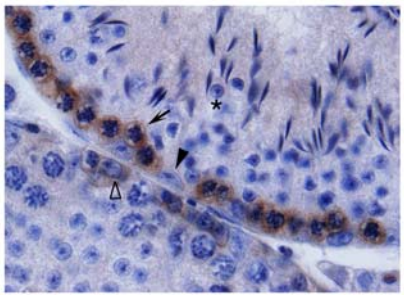


Figure JSAP1 immunoreactivity in the testis of a 12-week-old mouse. The positive signals were seen in the spermatogonia (openarrowhead) and spermatocytes (arrow), but not in Sertoli cells (arrowhead) or spermatids (*).

Reference: M. Bayarsaikhan, A. Shiratsuchi, D. Gantulga, Y. Nakanishi and K. Yoshioka (2005) Reproduction (in press)

Impairment of cardiomyogenesis in embryonic stem cells lacking scaffold protein JSAP

T. Sato, K. Hidaka, A. Iwanaga, M. Ito, M. Asano, Y. Nakabeppu, T. Morisaki and K. Yoshioka

We previously reported that c-Jun NH_2-terminal kinase (JNK)/stress-activated protein kinase-associated protein 1 (JSAP1), a scaffold protein for JNK signaling pathways, is important in embryonic stem (ES) cells during neurogenesis. In that study, we also observed altered expression of mesodermal marker genes, implying that JSAP1 is involved in differentiation during mesodermal lineages. In the present study, we investigated the function of JSAP1 focusing on cardiomyocyte development using JSAP1-null ES cells, and found that cardiomyogenesis is impaired in the JSAP1-null mutant. We also found that JSAP1-deficiency results in lower expression of the cardiac transcription factor Nkx2.5 and contractile proteins, and contrarily, in significantly higher expression of mesoderm-related markers other than the cardiomyocyte lineage. Taken together, these results suggest that JSAP1 may play an important role in differentiation during mesodermal lineages, where JSAP1 functions as a positive factor for cardiomyocyte differentiation, and as an inhibitory factor for differentiation into the other lineages.

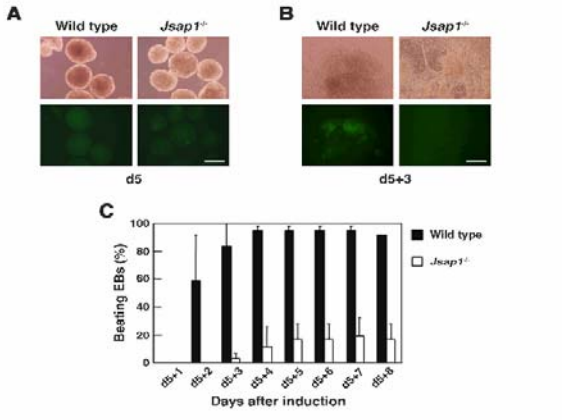


Figure Impairment of cardiomyogenesis in *Jsap1*$^{-/-}$ Nkx2.5GFP ES cells. (A) EBs derived from wild-type and *Jsap1*$^{-/-}$ ES cells were almost identical morphologically at d5. Phase-contrast photomicrographs of the EBs (upper panels) and the corresponding fluorescent micrographs (lower panels) are shown. Scale bar represents 250 μm. (B) Cardiomyogenesis of *Jsap1*$^{-/-}$ ES cells was largely impaired at d5+3. Micrographs are as in (A). Scale bar represents 250 μm. (C) Quantification of contracting EBs derived from wild-type and *Jsap1*$^{-/-}$ ES cells.

Reference: T. Sato, K. Hidaka, A. Iwanaga, M. Ito, M. Asano, Y. Nakabeppu, T. Morisaki and K. Yoshioka (2005) BBRC, 338, 1152-1157.

Publications

1. Ito, M., and Yoshioka, K. (2003) Scaffold proteins of the c-Jun NH_2-terminal kinase (JNK) signaling pathways in neuronal cells. *In* Pandalai, S.G. ed., Recent Res. Devel. Biochem. 4:271-282
2. Enomoto, A., Suzuki, N., Morita, A., Ito, M., Liu, C.Q., Matsumoto, Y., Yoshioka, K., and Shiba, T., Hosoi, Y. (2003) Caspase-mediated cleavage of JNK during stress-induced apoptosis. Biochem. Biophys. Res. Commun. 306(4):837-842
3. Xu, P., Yoshioka, K., Yoshimura, D., Tominaga, Y., Nishioka, T., Ito, M., and Nakabeppu, Y. (2003) In vitro development of mouse embryonic stem cells lacking JNK/stress-activated protein kinase-associated protein (JSAP1) scaffold protein revealed its requirement during early embryonic neurogenesis. J. Biol. Chem. 278(48):48422-48433
4. Takino, T., Miyamori, H., Watanabe, Y., Yoshioka, K., Seiki, M., and Sato, H. (2004) Membrane-type 1 matrix metalloproteinase regulates collage-dependent mitogen-activated protein/extracellular signal-regulated kinase activation and cell migration. Cancer Res. 64(3):1044-1049
5. Ikeda, F., Nishimura, R., Matsubara, T., Tanaka, S., Inoue, J., Reddy, S.V., Hata, K., Yamashita, K., Hiraga, T., Watanabe, T., Kukita, T., Yoshioka, K., Rao, A., and Yoneda, T. (2004) Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation. J. Clin. Invest. 114(4):475-484
6. Akiyama, S., Yonezawa, T., Kudo, T., Li, M.G., Wang, H., Ito, M., Yoshioka, K., Ninomiya-Tsuji, J., Matsumoto, K., Kanamaru, R., Tamura, S., and Kobayashi, T. (2004) Activation mechanism of c-Jun amino-terminal kinase in the course of neural differentiation of P19 embryonic carcinoma cells. J. Biol. Chem. 279(35):36616-36620
7. Yoshioka, K. (2004) Scaffold proteins in mammalian MAP kinase cascades. J. Biochem. 13(6)5:657-661
8. Tsuruta, F., Sunayama, J., Mori, Y., Hattori, S., Shimizu, S., Tsujimoto, Y., Yoshioka, K., Masuyama, N., and Gotoh, Y. (2004) JNK promotes Bax translocation to mitochondria through phosphorylation of 14-3-3 proteins. EMBO J. 23(8):1889-1899
9. Sato, S., Ito, M., Ito, T., and Yoshioka, K. (2004) Scaffold protein JSAP1 is transported to growth cones of neurites independent of JNK signaling pathways in PC12h cells. Gene 329:51-60
10. Yoshimura, S., Yoshioka, K., Barr, F.A., Lowe, M., Nakayama, K., Ohkuma, S., and Nakamura, N. (2005) Convergence of cell cycle regulation and growth factor signals on GRASP65. J. Biol. Chem. 280(24):23048-23056

11. Takino, T., Nakada, M., Miyamori, H., Watanabe, Y., Sato, T., Gantulga D., Yoshioka, K., Yamada, K.M., and Sato, H. (2005) JSAP1/JIP3 cooperates with FAK to regulate c-Jun N-terminal kinase and cell migration. J. Biol. Chem. 280(45): 377772-377781
12. Sato, T., Hidaka, K., Iwanaga, A., Ito, M., Asano, M., Nakabeppu, Y., Morisaki, T. and Yoshioka, K. (2005) Impairment of cardiomyogenesis in embryonic stem cells lacking scaffold protein JSAP1. Biochem. Biophys. Res. Commun. 338(2): 1152-1157
13. Bayarsaikhan, M., Shiratsuchi, A., Gantulga, D., Nakanishi, Y. and K. Yoshioka Reproduction (in press)
14. Kong, L., Ueno, M., Itoh, M., Yoshioka, K. and Takakura, N. Identification and characterization of mouse PSF1-binding protein, SLD5. Biochem. Biophys. Res. Commun. (in press)
15. Yoshioka, K. Control of granule cell precursor proliferation in the developing cerebellum and medulloblastoma Biomed. Rev. (in press)

Division of Genetics

General Summary of Division of Genetics

Division of Genetics has been started at June 2005 as a laboratory of "Cancer Stem Cell Research Center" that will be launched at April 2006. In the past decade, we have been studying molecular pathogenesis and prevention of gastrointestinal tumors by constructing genetically engineered mouse models (*i.e.*, knockout mice for *Apc, Tgfbr2* or COX-2; and transgenic mice for COX-2, mPGES-1 or *Wnt1*). Using these mouse models, we demonstrated that host reactions through COX-2 pathway in addition to oncogenic mutations are required for gastrointestinal tumor formation. It has been suggested that tissue injury stimulates replication of tissue stem cells to repair the damaged tissues. Importantly, COX-2 pathway is also induced in the injured tissues, resulting in elevated prostaglandin production. Accordingly, it is possible that induction of COX-2 is required for maintenance and replication of the cancer stem cells as well as those of normal tissue stem cells. For the future cancer stem cell research, we need to use genetically engineered mouse models because host responses through COX-2 pathway must be critical for stem-cell maintenance in the tumor tissues. In the past 6 months, we have been concentrating to the following two projects to establish the rationale and mouse models for future approach.

A) Host inflammatory responses and gastrointestinal tumorigenesis

We have demonstrated using mouse models that COX-2 and downstream PGE_2 play a key role in gastrointestinal tumorigenesis. To investigate the molecular mechanism, we constructed transgenic mice (*K19-C2mE*) expressing two enzymes for PGE_2 synthesis, COX-2 and mPGES-1. *K19-C2mE* mice developed hyperplastic tumors, suggesting that PGE_2 signaling enhances proliferation of normal progenitor cells of the gastric gland. Moreover, we have shown that TNF-α-dependent inflammatory responses are responsible for suppression of gastric epithelial differentiation. These results suggest that COX-2 and PGE_2 play a pivotal role in tumor development through keeping progenitor cells undifferentiated.

B) Construction of transgenic mouse model for gastric cancer

It has been shown that activation of Wnt pathway is important for tumor formation. We have investigated 80 samples of human gastric cancer and found that Wnt signaling is activated in more than 50% of cases. To investigate whether Wnt activation induces gastric tumor development, we constructed transgenic mice (*K19-Wnt1*) expressing *Wnt1* gene in the gastric mucosa. *K19-Wnt1* mice showed limited preneoplastic lesions, indicating that Wnt signaling triggers gastric tumorigenesis.

Host inflammatory responses and gastrointestinal tumorigenesis

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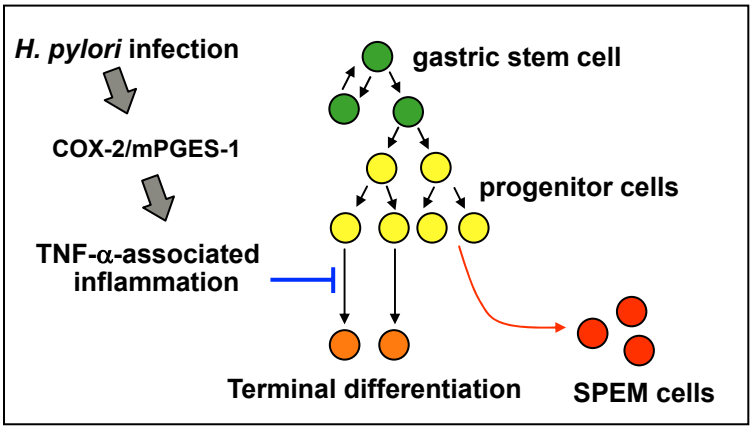
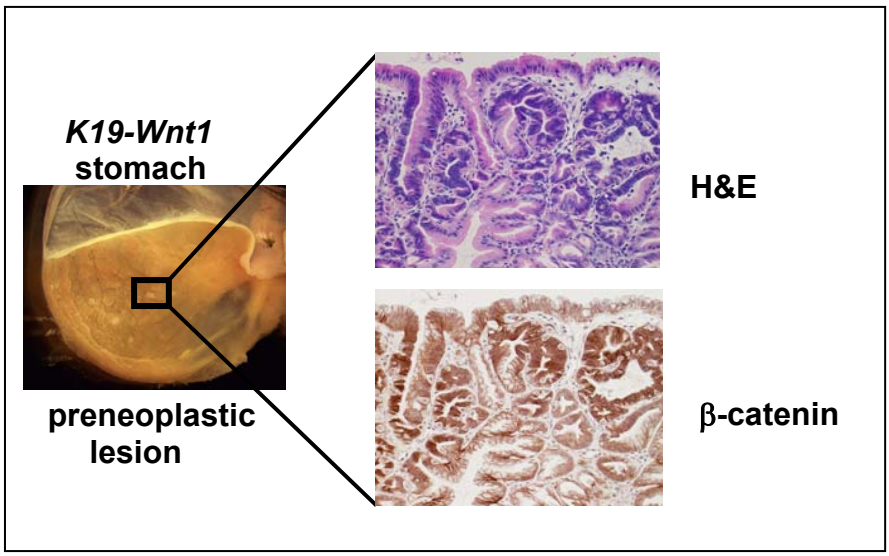
Cyclooxygenase-2 (COX-2), a rate-limiting enzyme for prostanoid biosynthesis, plays a key role in gastrointestinal carcinogenesis. Among various prostanoids, prostaglandin E_2 (PGE_2) appears to be most responsible for cancer development. It has been shown that *Helicobacter pylori* infection induces expression of COX-2 and microsomal prostaglandin E synthase (mPGES)-1 in the gastric mucosa, resulting in increased level of PGE_2 in the stomach. To investigate the role of PGE_2 in gastric tumorigenesis, we constructed transgenic mice (*K19-C2mE*) expressing COX-2 and mPGES-1 in the gastric mucosa. The transgenic mice developed hyperplastic gastric tumors associated with inflammatory responses. To further investigate the roles of PGE_2-dependent host inflammatory and immune responses in gastric tumorigenesis, we introduced knockout mutations for tumor necrosis factor (TNF)-α, interleukin (IL)-1 receptor α chain and Rag 2 genes, respectively, into *K19-C2mE* mice. Among the compound mutants, only TNF-α (–/–) *K19-C2mE* mice showed significant suppression of hyperplastic tumors with reduced epithelial cell proliferation (Figure). Importantly, spasmolytic polypeptide-expressing metaplasia (SPEM) in the *K19-C2mE* stomach was also suppressed in the TNF-α (–/–) *K19-C2mE* mice. Gastric metaplasia to the SPEM lineage has been considered as a preneoplastic lesion of gastric cancer. These results indicate that TNF-α-dependent inflammation caused by increased PGE_2 is responsible for development of hyperplastic tumors and SPEM (Figure). Therefore, it is possible that inhibition of TNF-α-dependent inflammation together with eradication of *Helicobacter* is an effective prevention strategy for gastric cancer.



Construction of transgenic mouse model for gastric cancer

H. Oshima, T. Fujimura*, M.M. Taketo[+] and M. Oshima
(*Gastroenterologic Surgery, Kanazawa University Hospital, [+]Department of Pharmacology, Kyoto University Graduate School of Medicine)

It has been demonstrated that nuclear translocation of β-catenin, a hallmark of Wnt activation, is found in human gastric cancer, suggesting the causal role of Wnt pathway in gastric carcinogenesis. We examined 80 cases of gastric cancer by immunohistochemistry, and found nuclear β-catenin in 54% (51% and 58% for intestinal- and diffuse types, respectively). Accordingly, it is conceivable that Wnt pathway activation is one of the major causes of gastric cancer development regardless of the histological type. To investigate the genetic mechanism further, we constructed transgenic mice (*K19-Wnt1*) expressing *Wnt1*, one of the ligands that activate the canonical Wnt signaling, in the gastric epithelial cells. Importantly, trefoil factor 2 (TFF2)-expressing cell population was expanded in the gastric gland of the *K19-Wnt1* mice. TFF2 is a marker for undifferentiated gastric epithelium which expresses in the small isthmal cells where β-catenin accumulated. Moreover, we found dysplastic preneoplastic lesions consisted of β-catenin-accumulated epithelial cells in the *K19-Wnt1* mice (Figure). These results, taken together, suggest that Wnt signaling keeps gastric stem cells and progenitors undifferentiated and that activation of Wnt pathway leads to development of preneoplastic lesions in the stomach. For gastric cancer development, activation of an additional pathway (e.g., COX-2) appears to be required. It is of important to investigate compound mutants of *K19-Wnt1* and *K19-C2mE*.



Publications

1. Thumkeo, D., Keel, J., Ishizaki, T., Hirose, M., Nonomura, K., Oshima, H., Oshima, M., Taketo, M.M. and Narumiya, S. (2003) Targeted disruption of the mouse rho-associated kinase 2 gene results in intrauterine growth retardation and fetal death. Mol. Cell. Biol. 23:5043-5055.
2. Takeda, H., Sonoshita, M., Oshima, H., Sugihara, K., Chulada, P.C., Langenbach, R., Oshima, M. and Taketo, M.M. (2003) Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis. Cancer Res. 63:4872-4877.
3. Aoki, K., Tamai, Y., Horiike, S., Oshima, M. and Takto, M.M. (2003) Colonic polyposis caused by mTOR-mediated chromosomal instability in $Apc^{\Delta 716}$ $Cdx2^{+/-}$ compound mutant mice. Nat. Genet. 35:323-330.
4. Harada, N., Oshima, H., Katoh, M., Tamai, Y., Oshima, M. and Taketo, M.M. (2004) Hepatocarcinogenesis in mice with β-catenin and Ha-ras gene mutations. Cancer Res. 64:48-54.
5. Takeda, H., Miyoshi, H., Tamai, Y., Oshima, M. and Taketo, M.M. (2004) Simultaneous expression of COX-2 and mPGES-1 in mouse gastrointestinal hamartomas. Br. J. Cancer 90:701-704.
6. Oshima, H., Oshima, M., Inaba, K., and Taketo, M.M. (2004) Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. EMBO J. 23:1669-1678.
7. Kawada, K., Sonoshita, M., Sakashita, H., Takabayashi, A., Yamaoka, Y., Manabe, T., Inaba, K., Minato, N., Oshima, M. and Taketo, M.M. (2004) Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res. 64:4010-4017.
8. Fujishita, T., Doi, M., Sonoshita, M., Hiai, H., Oshima, M., Huebner, K., Croce, C.M. and Taketo, M.M. (2004) Development of spontaneous tumors and intestinal lesions in Fhit knockout mice. Br. J. Cancer 91:1571-1574.
9. Li, Q., Ishikawa, T., Miyoshi, H., Oshima, M. and Taketo, M.M. (2005) A targeted mutation of *Nkd1* impairs mouse spermatogenesis. J. Biol. Chem. 280:2831-2839.
10. Shimizu, Y., Thumkeo, D., Keel, J., Ishizaki, T., Oshima, H., Oshima, M., Noda, Y., Matsumura, F., Taketo, M.M. and Narumiya, S. (2005) ROCK-I regulates closure of the eyelids and ventral body wall by inducing assembly of actomyosin bundles. J. Cell Biol. 168:941-953.
11. Oshima, M., Oshima, H. and Taketo, M.M. (2005) Hypergravity induces expression of

cyclooxygenase-2 in the heart vessels. Biochem. Biophys. Res. Commun. 330:928-933.

12. Li, Q., Ishikawa, T., Oshima, M. and Taketo, M.M. (2005) The threshold level of APC protein for mouse intestinal tumorigenesis. Cancer Res. 65:8622-8627.
13. Oshima, M., Oshima, H., Matsunaga, A. and Taketo, M.M. (2005) Hyperplastic gastric tumors with spasmolytic polypeptide-expressing metaplasia (SPEM) caused by TNF-α-dependent inflammation in COX-2/mPGES-1transgenic mice. Cancer Res. 65:9147-9151.
14. Takeda, H., Miyoshi, H., Kojima, Y., Oshima, M. and Taketo, M.M. (2005) Accelerated onsets of gastric hamartomas and hepatic adenomas/carcinomas in *Lkb1*$^{+/-}$*p53*$^{+/-}$ compound mutant mice. Oncogene [advance online 07 Nov, doi:10.1038/sj.onc.1209207]

Division
of
Medical Oncology

General Summary of Division of Medical Oncology

We have have been basically and clinically analyzing the conditions of digestive cancers from the precancerous state to onset to determine the groups at high risk for each kind of cancer, and have also been evaluating the biological characters and clinical usefulness of tumor-related genes such as oncogenes, oncosuppressive genes, apoptosis-related genes, and so on in various digestive cancers. Through these researches, we aim to develop new tumor markers that are useful for early diagnosis, selection of treatment methods, and prediction and evaluation of outcomes. In addition, pancreatic disease such as pancreatic cancer (PCa) and pancreatitis has been our major target of our research. We have been working on research projects focused on cancer-related substances, and biological factors associated with pancreatitis.

We already reported that *K-ras* mutations were detectable in more than 90% of endoscopically aspirated pancreatic juice (PJ) from patients with PCa,using a highly sensiteve method such as MASA. However, the frequent presence of *K-ras* mutation was shown in PJ from noncancerous patients, rasing quenstions as to the cancer specificity of this marker. On the other hand, the prevalence of *p53* mutations in PJ was about 50%, but not as high as that of *K-ras* mutations. However, *p53* mutations were specically found only in PCa, suggesting that it has high specificity for PCa. Recent studies have demonstrated that not only genetic changes but also epigenetic changes in various factors have an important role in cancinogenesis. It is increasingly apparent that many important genes can be inactivated in association with promoter hypermethylation in carcinogenesis of each kind of cancer. Using methylation-specific PCR or real-time quantitative MSP, we have been analyzing the methylation status of 6 CpG islands of *ppENK*, *SARP2*, *TFPI-2*, *NPTX-2*, *CLDN5* and *RASSFIA* in PJ samples from pancreatic diseases. Aberrant methylation of *ppENK* in the PJ was found in 50% of PCa patients and only in 5% of CP patients, and that of *TFPI-2* was detectable in 62% of PCa patients and in 14% of CP patients, suggesting that aberrant methylation of *ppENK* or *TFPI-2* has high specificity for cancer, although its incidence is not so high. Furthermore, we have detected aberrant methylation of *SARP2* in 85% of PCa, in 70% of intraductal papillary mucinous neoplasm of the pancreas and in 26% of CP. Although cancer specificity of aberrant methylation of *SARP2* in PJ is not sufficient, its prevalence in PJ from PCa is strikingly high, indicating usefulness for the detection of pancreatic neoplasm. In addition, we have investigated some overexpressed genes such as mesothelin and midkine. *Mesothelim* mRNA expression in PJ has been discovered using RT-PCR in 52% of PCa patients, but also in 14% of CP patients. Serum levels of midkine measured by ELISA elevated in a varity of digestive cancer, especially in gastric and colonic cancer patients in whom the positive rate of serum midkine was relatively high and has no relation to the stage of cancer.

On the assumption that in pancreatic diseases, substances derived from the pancreas reflect abnormalities more sensitively in the PJ than in the blood, lactoferrin, reg-protein identical to pancreatic stone protein and tumor carbohydrate andtigens in the PJ were measured, and interesting results were

already reported. We have been also analyzing cancer-related genes in the PJ as above mentioned, and studing the pathophysiological and clinical significance of pancreatitis-associated protein (PAP) belonging to *reg*-gene family,which is almost absent in mormal pancreas and is markdly induced in pancreatitis. Furthermore, the monitoring of serum PAP levels in acute pancreatitis has been shown the most useful for estimating the severity and clinical healing of the disease. In an attempt to elucidate the role of PAP in the pathogenesis of pancreatitis, we have analyzed expression kinetics of PAP, various cytokines/chemokines, and apoptosis-related factors in spontaneous CP of the WBN/Kob rat *in vivo*, and in arginine-induced rat pancreatic acinar AR4-2J cell injury *in vitro*. TNF-alpha peaked at 8 weeks prior to the onset of CP, PAP, IL-6, IL-8, TGF-beta, and vacuole membrane protein 1 peaked at 12 weeks, and IFN-gamma peaked at 16 weeks in the pancreas of WBN/Kob rats. Thus, PAP is strongly induced in pancreatic acinar cells at the onset of CP in the cascade of cytokines/chemokines. Acinar cell apoptosis as well as the expressions of pro-apoptotic factors such as Fas/FasL, c-*myc* and stress-induced protein (TP53INP1) showed two peaks at 12 and 20 weeks, suggesting different roles of apoptosis in the onset and progression of CP. The first peak at 12 weeks is both for pro- and anti-apoptotic factors (PAP, *bcl*-2, clusterin), whereas the second peak at 20 weeks is only for pro-apoptosis. The first peak of apoptosis indicates the acute-phase reaction of the pancreas in response to various stimuli such as oxidative stress, and the second peak suggests the remodeling and pancreatic regeneration in the course of CP. Therapeutic drugs such as camostat mesilate, TJ-10 and IS-741, suppressed the expressions of these factors, suggesting molecular action mechanisms of the drugs.

The use of endoscopy has been extended from diagnosis to treatment. We have been enthusiastically performing endoscopy to treat cancer such as EMR (endoscopic mucosal resection) or ESD (endoscopic submucosal dissection), although the use of this method requires accurate evaluation of the depth of cancerous invasion. In this respect, endoscopic ultrasonography (EUS) that allows three-dimensional evaluation is of diagnostic value, and we have been conducting fundamental and clinical studies on the diagnostic usefulness of this metbod in gastrointestinal cancers. Furthermore, EUS has been expected to detect mild lesions or small tumor in pancreas where other tests including endoscopic retrograde pancreatocholangiography (ERCP) cannot show diagnostic findings, since EUS is capable of providing not only ductal but also parenchymal features in pancreas. We have intended to elucidate the diagnostic ability of EUS in detecting small pancreatic cancer, applying EUS examination before ERCP. Additionally, we are currently attempting to develop the ability of early diagnosis for PCa in which biopsy specimens cannot be easily obtained in combination with imaging test by EUS and molcular biological analysis of tumor-related substances in endoscopically aspirated PJ as above mentioned. Autoimmune pancreatitis (AIP) is a newly proposed clinical entity in which autoimmune mechanisms are involved in the pathogensis. However, it is sometimes difficult to differentiate AIP from PCa. We have reviewed more than 40 patients with AIP in cooperative study with the affiliated medical facilities, and reported the clinical and imaging features of AIP, differential diagnosis of AIP from PCa, its long-term prognosis, outcome of steroid therapy in AIP, and so on.

Aberrant methylation of *secreted apoptosis-related protein 2 (SARP2)* in pure pancreatic juice in diagnosis of pancreatic neoplasms

H. Watanabe, G. Okada, K. Ohtsubo, F. Yao, PH. Jiang and N. Sawabu.

Secreted apoptosis-related protein (SARP) families are considered to counteract the oncogenic Wnt signaling pathway and inactivation of this gene may aid cancer development and progression. Recently, the aberrant methylation of *SARP2* was detected frequently in pancreatic carcinoma (PCa) tissues, but not in normal pancreatic tissues.

We evaluated the hypermethylation of *SARP2* in pure pancreatic juices (PPJ) aspirated endoscopically from patients with PCa, intraductal papillary mucinous neoplasm of the pancreas (IPMN), chronic pancreatitis (CP), and a control group (C) who was consequently free of pancreatic disease by methylation-specific PCR (MSP) and real-time MSP.

The incidence of the aberrant methylation of *SARP2* using MSP was 79% (26/33) in the PPJ with PCa, and 85% (17/20) with IPMN. However, it was only 5% (1/19) in the PPJ with CP and 0% (0/10) in the PPJ of C, respectively. The incidences of aberrant methylation of *SARP2* in the PPJ with PCa and IPMN were significantly higher than that in the PPJ with CP ($p< 0.001$, $p< 0.001$). Melting curve analysis by real-time MSP as shown in Figure revealed that the incidence of aberrant methylation of *SARP2* in PPJ was 85% (28/33) with PCa, 82% (9/11) with the malignant group of IPMN, 56% (5/9) with the benign group of IPMN and 26% (5/19) with CP. In this analysis, there were significant differences between PCa and CP ($p<0.001$), and between the malignant group of IPMN and CP ($p<0.005$). In the quantitative analysis by real-time MSP with a suitable cut-off value, the incidences of aberrant methylation of *SARP2* in the PPJ with PCa, the malignant group of IPMN, the benign group of IPMN and CP were 58 % (19/33), 55% (6/11), 33% (3/9) and 11% (2/19), respectively. The incidence of the aberrant methylation of *SARP2* in the PPJ was significantly different between PCa and CP, and between the malignant group of IPMN and CP ($p<0.005$, $p<0.05$).

These results suggest that promoter methylation of *SARP2* in the PPJ may be a highly sensitive and useful marker for the detection of pancreatic neoplasms, including PCa and the malignant group of IPMN.

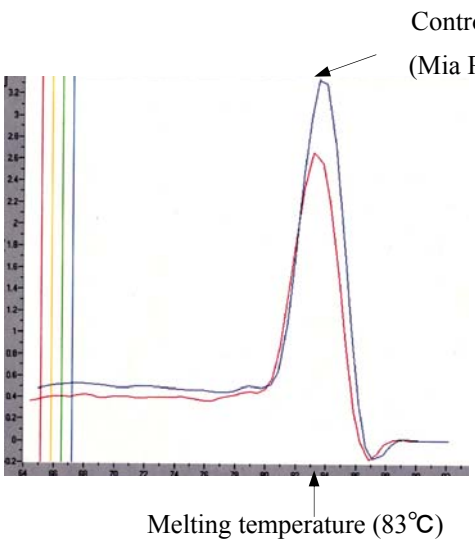


Figure Melting curve analysis of the quantitative MSP products by real-time PCR

Representative case of aberrant methylation of *SARP2* in the PPJ sample from patients with PCa revealed a similar melting curve (red curve) and the same melting temperature (83℃), compared with methylation profiles of the human PCa celll line Mia PaCa-2 as a methylated control of *SARP2*.

Usefulness of aberrant methylation of *tissue factor pathway inhibitor 2 (TFPI-2)* in pure pancreatic juice in diagnosis for pancreatic carcinoma

H. Watanabe, PH. Jiang, G. Okada, F. Yao, K. Ohtsubo and N. Sawabu.

Tissue factor pathway inhibitor 2 (TFPI-2) is a Kunitz-type serine proteinase inhibitor and is thought to protect the matrix from degradation therefore counteracting tumor invasion and metastasis. Recently, aberrant methylation of *TFPI-2* was detected frequently in pancreatic carcinoma (PCa) tissues and not in normal pancreatic tissues. We analyzed the aberrant methyaltion of *TFPI-2* in the pure pancreatic juice (PPJ) from patients with various pancreatic diseases and evaluated its clinical usefulness in the diagnosis of PCa.

We evaluated the hypermethylation of *TFPI-2* in PCa cell lines and PPJ aspirated endoscopically from patients with PCa, benign and malignant intraductal papillary mucinous neoplasm of the pancreas (IPMN), chronic pancreatitis (CP) by methylation-specific PCR (MSP) and real-time quantitative MSP (Q-MSP).

The incidence of the aberrant methylation of *TFPI-2* using MSP was 7 (77.8%) of 9 PCa cell lines by Q-MSP and the expression of *TFPI-2* mRNA by quantitative RT-PCR showed inverse correlation to the aberrant methylation of *TFPI-2*. Moreover, the incidence of the aberrant methylation of *TFPI-2* in the PPJ was 21 (58.3%) of 36 PCa patients, 2 (20%) of 10 malignant IPMN and 1 (14.3%) of 7 benign IPMN and 1 (4.8%) of 21 CP by MSP assay. The incidence of the aberrant methylation of *TFPI-2* in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.05$) or CP ($p< 0.001$) as shown in Table. Using Q-MSP analysis, the ratio of hypermethylation *TFPI-2/MyoD1* in the PPJ from patients with PCa, IPMN and CP was 6.62 ± 7.20, 0.58 ± 1.02 and 1.28 ± 1.73, respectively. The ratio of hypermethylation*TFPI-2/MyoD1* in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.01$) or CP ($p< 0.05$). Using the suitable cut-off value set as 2.5 according to the ROC curve, the incidence of the aberrant methylation of *TFPI-2* in the PPJ by real-time MSP was 18 (62.1%) of 29 PCa patients, 1 (5.9%) of 17 IPMN and 3 (14.3%) of 21 CP, respectively. The incidence of quantitative *TFPI-2* hypermethylation in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.001$) or CP ($p< 0.001$).

These results suggest that promoter methylation of *TFPI-2* in the PPJ may be specific to PCa and a useful marker in the diagnosis of PCa.

Table The incidence of aberrant methylation of *TFPI-2* in the pure pancreatic juice from patients with various pancreatic diseases by methylation-specific PCR

Pancreatic disease	Incidence of aberrant methylation of *TFPI-2*	
Pancreatic carcinoma	58.3% (21/36)	
Malignant IPMN	20.0% (2/10)	*
Benign IPMN	14.2% (1/7)	*
Chronic pancreatitis	4.8% (1/21)	**

IPMN: intraductal papillary mucinous neoplasm, * : $p< 0.05$, ** : $p< 0.001$.

Detection of *preproenkephalin (ppENK)* hypermethylation in the pancreatic juice from patients with pancreatic carcinomas:compared with *p53* mutations

K. Ohtsubo, H. Watanabe, F. Yao, G. Okada, H. Mouri, Y. Yamaguchi, and N. Sawabu

Aberrant methylation of CpG islands is a common mechanism for tumor suppressor genes in a variety of human malignancies. The *preproenkephalin (ppENK)* gene encodes a native opioid peptide, met-enkephalin, which is known to be a potent regulator of development, cell proliferation and angiogenesis. *ppENK* hypermethylation is recognized in 90% in pancreatic carcinoma (PCa), but not normal pancreas tissues. We analyzed the *ppENK* hypermethylation in pure pancreatic juice (PPJ) in patients with PCa and chronic pancreatitis (CP), and elucidated the usefulness for diagnosis of PCa. Furthermore, *p53* mutations were also studied in the same PCa patients.

PPJ was collected endoscopically from 28 patients with PCa and 20 patients with CP. DNA was extracted from PPJ. Methylation specific PCR was performed for hypermethylation analyses with the DNA extracted from these samples. In addition, single-strand conformation polymorphism and sequencing were performed simultaneously for the analyses of *p53* mutations.

The incidence of *ppENK* hypermethylation in PPJ was 50% (14 of 28) in patients with PCa. In contrast, only one of 20 cases presented hypermethylation in patients with CP ($p < 0.002$). *p53* mutations were observed in 12 of 28 PCa cases (42.9%), but not in 20 CP cases. In combination analyses of *ppENK* hypermethylation and *p53* mutations both were positive in 7 and both were negative in 9 of 28 patients. *ppENK* hypermethylation alone was positive in 7 and *p53* mutations alone in 5 patients. *ppENK* hypermethylation or *p53* mutations were observed in 19 of 28 shown in Table, so that positivity improved to 67.9% upon their combination.

These results suggest that *ppENK* hypermethylation in PPJ would be specific for cancer, and the combination assay with *p53* could enhance the genetic diagnosis of PCa.

Table. Comparison of *ppENK* hypermethylation and *p53* mutations from patients with pancreatic cancer

		p53 mutations +	-	Total
ppENK hypermethylation	+	7	7	14
	-	5	9	14
Total		12	16	28

Quantitative measurement of *hTERT* mRNA in sera of the patients with pancreatic carcinoma by real-time RT-PCR

G. Okada, H. Watanabe, H. Mouri, K. Ohtsubo and N. Sawabu

Human telomerase reverse transcriptase (hTERT) is regarded as a catalytic component of telomerase and a rate-limiting determinant of the enzymatic activity of human telomerase. Its expression reflects the telomerase activity. Usually, telomerase is inactivated or its activity repressed in the majority of normal somatic cells but is activated in germ cells, embryonal cells, proliferating cells of renewable tissues, and activated lymphocytes as well as in most malignant tumor cells. We examined whether the detection of *hTERT* mRNA in sera by real-time RT-PCR is useful or not for the diagnosis of pancreatic carcinoma (PCa).

The serum samples were obtained from 16 patients with PCa, 4 with chronic pancreatitis (CP), and 23 healthy subjects and the RNA was extracted from the serum immediately and stored at –80℃ until the assay. The RNA samples were reverse-transcribed after DNase I treatment, and measured by the real-time RT-PCR.

The *hTERT* mRNA in sera was detected in 13 (57%) of the 23 control subjects and 16 (100%) of 16 PCa patients by the real-time RT-PCR and the agarose gel electrophoresis. Based upon the standard curve using *hTERT* cDNA as a positive control, a linear relationship between PCR products and cycle number of *hTERT* cDNA was confirmed in the range from 0.03pg to 30ng. A linear relationship about 18S rRNA was also confirmed by real-time RT-PCR. The mean ±SD of the ratio of *hTERT* mRNA X 10^{-2}/18S in sera from the control subjects, patients with PCa, and patients with CP was 0.07±0.18, 0.13±0.25, and 0.002±0.05, respectively. With a standard value of 0.07 as the mean value of normal subjects, 3 (13%) of 23 normal subjects, 6 (38%) of 16 patients with PCa, and none (0%) of 4 with CP showed elevated values. The PCa patients with the high values of *hTERT* mRNA/18S in sera showed advanced clinical stages such as liver metastasis and peritoneal dissemination, whereas some of the PCa patients with its low value less than 0.07 in sera underwent surgical resection and obtained longer survival. However, the values of *hTERT* mRNA/18S in sera were elevated in a part of normal subjects as well as PCa patients. Moreover, the values of *hTERT* mRNA/18S with PCa patients considerably overlapped with those with normal subjects even in the high range. It is probably because the *hTERT* mRNA derived from proliferating cells of renewal tissues or activated lymphocytes may be included in the serum. Therefore, assay of *hTERT* mRNA in the serum may be troublesome and not useful for the clinical application from the results of our study although *hTERT* mRNA has been reportedly supposed to be a useful tumor marker.

Elevated levels of serum midkine as a tumor marker in patients with digestive cancer.

H. Mouri, K. Ohtsubo, Y. Yamaguchi, H, Watanabe, and N. Sawabu

Midkine (MK) is one of a family of heparin-binding growth factors whose gene was identified in embrypnal carcinoma. MK messenger RNA (mRNA) is highly expressed in various tissues during the midgestation period of mouse embryogenesis, and it is considered to be involved in regulation of organogenesis. Increased MK mRNA and protein expression are exhibited in many human carcinomas such as gastric, pancreas, bile duct, colorectal, hepatocellular cancers as well as in Wilms' tumor and neuroblastoma. Serum level of MK is reportedly elevated in patients with esophageal cancer or neuroblastoma However, little is unknown about usefulness of determination of serum MK as a tumor marker in patients with digestive cancers. In the present study, we measured serum levels of MK in various digestive cancers and benign diseases, and evaluate its clinical significance as a tumor marker.

Serum levels of MK were measured by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human MK antibody as a first antibody, and biotinylated mouse anti-MK antibody as a second one in the sera from digestive malignant diseases (n=139) and benign diseases (n=87). The cut-off value of serum MK was set at 450pg/ml chiefly based on the ROC curve. The positive rate of MK in each cancer was as follows: 40% (14/35) in pancreatic cancer, 64% (23/36) in gastric cancer, 67% (16/24) in colorectal cancer, 38% (17/45) in hepatocellular cancer, and 71% (5/7) in bile duct cancer. Among patients with gastric or colorectal cancer, the positive rate of serum MK was relatively high and had no relation to the stage of cancer shown in Fig. High positivity of MK of gastric or colorectal cancer in stage I is worthy of notice. On the other hand, positivity of serum MK in benign digestive disease was as follows: 31% (5/16) in chronic hepatitis, 33% (4/12) in chronic pancreatitis, 16% (2/12) in acute pancreatitis, and 67% (6/9) in cholangitis., indicating that false positivity is high in inflammatory diseases.

In conclusion , serum MK level increased in a variety of digestive cancers, especially in gastric and colorectal cancer. These results suggest that determination of serum MK may be useful as an aid in initial screening of gastrointestinal cancers, although false positivity is high in inflammatory diseases.

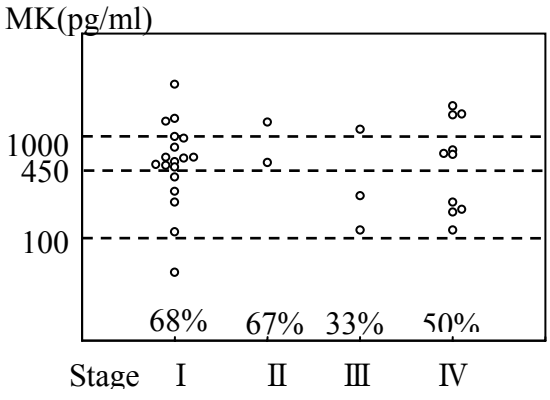


Fig. Relationships between serum MK levels and tumor stage in gastric cancer

Prolonged, NK cell-mediated antitumor effects of suicide gene therapy combined with monocyte chemoattractant protein-1 against hepatocellular carcinoma.

T. Tsuchiyama, Y. Nakamoto, Y. Sakai, N. Mukaida, N Sawabu and S. Kaneko

Tumor recurrence rates remain high after curative treatments for hepatocellular carcinoma (HCC). Immunomodulatory agents, including chemokines, are believed to enhance the antitumor effects of tumor cell apoptosis induced by suicide gene therapy. Recently, the antitumor effects of the herpes simplex virus thymidine kinase / ganciclovir (HSV-tk / GCV) system were observed to be enhanced by codelivery of monocyte chemoattractant protein-1 (MCP-1). The current study was designed to evaluate the immunomodulatory effects of a bicistronic adenovirus vector expressing both HSV-tk and MCP-1 on HCC cells.

Using an athymic nude mouse model (BALB/c-nu/nu), subcutaneous tumors were completely eradicated by rAd followed by treatment with ganciclovir, subsequently these mice were re-challenged with HCC cells and tumor development was monitored. In addition, after the administration of re-challenged tumors, the serum levels of interleukin (IL)-12 and IL-18 were measured, and the recruitment or activation of natural killer (NK) cells was analyzed immunohistochemically or by measuring interferon (IFN)-γ mRNA expression. Finally, we evaluated the antitumor response in immunocompetent wild type mice (BALB/c-jcl) using the same experimental protocol.

Tumor growth was markedly suppressed compared with mice treated with rAd expressing HSV-tk gene alone ($P<0.001$). Suppression of tumor growth was associated with elevation of serum IL-12 and IL-18. During suppression, NK cells were recruited exclusively, and T helper 1 cytokine gene expression was enhanced in tumor tissues. The antitumor activity, however, was abolished when the NK cells were inactivated with anti-asialo GM1 antibody. Moreover, the effects of the anti-tumor response in wild type mice were comparable to them in nude mice.

In conclusion, these results indicate that suicide gene therapy, together with delivery of MCP-1, eradicates HCC cells and exerts prolonged NK cell-mediated antitumor effects in a model of HCC, suggesting a plausible strategy to prevent tumor recurrence.

Tumor protein p53-induced nuclear protein 1 (TP53INP1) in spontaneous chronic pancreatitis in the WBN/Kob rat: drug effects on its expression in the pancreas

Pei-Hong Jiang, Yoshiharu Motoo, Gensaku Okada, and Norio Sawabu

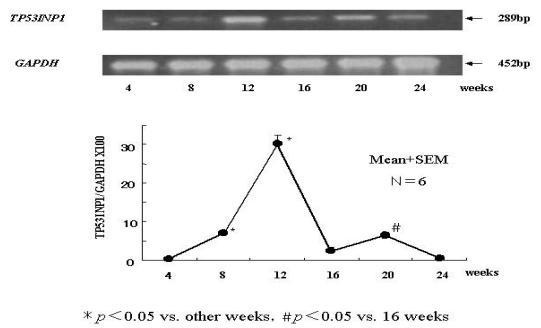
TP53INP1 is a nuclear factor that is rapidly and strongly induced by various stresses. TP53INP1 is overexpressed in acinar cells during acute pancreatitis in the mouse. TP53INP1 overexpression promotes cellular apoptosis. It is known acinar cell apoptosis is involved in acinar destruction/degeneration in the course of chronic pancreatitis. Therefore, examining TP53INP1 gene expression in CP and related to acinar cell apoptosis will contribute to a better understanding of the molecular mechanisms of the onset and progression of chronic pancreatitis.

Four-week-old male WBN/Kob rats were fed a special breeding diet MB-3 to induce chronic pancreatitis. Anti-inflammatory drug such as camostat mesilate (0.01%) and Saiko-keishi-to (0.08%) were mixed in the diet MB-3 fed to the rats from 4 weeks. Sections were stained with hematoxylin and eosin for histological evaluation. TP53INP1 mRNA expression was determined by RT-PCR with a semi-quantitative analysis and *in situ* hybridization. TUNEL was used to detect cell apoptosis. *In vitro*, 5 mg /ml L-arginine was dissolved in the medium of AR4-2J cell.

TP53INP1 mRNA expression began at 4 weeks, reached its first peak at 12 weeks, after decreased at 16 weeks, TP53INP1 mRNA reached its second peak at 20 weeks, finally decreased at 24 weeks. By *in situ* hybridization analysis, TP53INP1 mRNA was strong in the acinar cells but absent in duct or islet cells. The apoptotic index was significantly increased at 12 and 20 weeks showing two peaks, so that, the kinetics of TP53INP1 mRNA expression was paralleled to apoptotic index. TP53INP1 mRNA expression was significantly suppressed in the camostat mesilate group at 8, 12, and 20 weeks, and it was also significantly suppressed in the Saiko-keishi-to treated group at 8 and 12 weeks. TP53INP1 mRNA was expressed in AR4-2J cells from 2 to 24 hours. The expression level was significantly higher at 6 hr than those at other time points.

In the present study, TP53INP1 expression showed two peaks in the course of WBN/Kob rat. The first peak of TP53INP1 would reflect the concomitant induction of pro-, and anti-apoptotic factors at the onset of chronic pancreatitis. Around this time point, the histopathology of the pancreas resembles acute edematous pancreatitis, although there is already pancreatic fibrosis. Acinar cell apoptosis would attenuate the severity of pancreatitis, preventing the excessive release of harmful pancreatic enzymes. The second peak of acinar cell apoptosis and pro-apoptotic factors including TP53INP1 could reflect acinar cell loss and acinar remodeling or regeneration during the progression of chronic pancreatitis. Suppression of TP53INP1 mRNA expression by therapeutic drugs may confirm that TP53INP1 expression is deeply involved in the onset and progression of chronic pancreatitis in this model.

In conclusion, we have shown that TP53INP1 mRNA expression is induced in the onset and progression of chronic pancreatitis in WBN/Kob rats. TP53INP1 may be involved in the pathogenesis of chronic pancreatitis as a pro-apoptotic factor.



*$p<0.05$ vs. other weeks, #$p<0.05$ vs. 16 weeks

Figure: TP53INP1 mRNA was expressed from 4 to 24 weeks. It showed the first peak at 12 weeks and the second peak at 20 weeks.

Expression of vacuole membrane protein 1 (VMP1) in spontaneous chronic pancreatitis in the WBN/Kob rat

Pei-Hong Jiang, Yoshiharu Motoo, Gensaku Okada, and Norio Sawabu

VMP1 is a transmembrane protein located in the Golgi and endoplasmic reticulum. It is strongly and rapidly induced in pancreas during acute pancreatitis whereas it is hardly detectable in normal rats pancreas. Its overexpression promotes formation of intracellular vacuoles and cell death through apoptosis. Intracellular vacuolation is a cellular response to injury in the pancreas during pancreatitis to maintain function under stress. The vacuoles have been shown to contain both digestive and lysosomal enzymes suggesting an abnormal intracellular processing of newly synthesized enzymes in acute pancreatitis. Physiologically, digestive and lysosomal enzymes are synthesized in ribosomes attached to the rough endoplasmic reticulum. Vacuoles represent early morphological changes during apoptosis. The aim of this work was to examine the kinetics of VMP1 gene expression in spontaneous chronic pancreatitis in WBN/Kob rat.

Four-week-old male WBN/Kob rats were fed a special breeding diet MB-3 to induce chronic pancreatitis. Sections were stained with hematoxylin and eosin for histological evaluation and vacuoles examination. VMP1 mRNA expression was determined by RT-PCR with a semi-quantitative analysis and *in situ* hybridization. TUNEL was used to detect cell apoptosis. Cytoplasmic vacuoles with different sizes were observed in the pancreas of the WBN/Kob rat from 4 to 24 weeks. The vacuoles increased in size and number at the onset of CP at 12 weeks. VMP1 mRNA expression began at 8 weeks, reached a peak at 12 weeks, decreased at 16 and 20 weeks, then disappeared at 24 weeks. It was showed VMP1 mRNA was located in acinar cells and absent in the islets of Langerhans, duct cells, inflammatory infiltrates, and other stromal cells by *in situ* hybridization analysis. The vacuoles formation was paralleled to VMP1 mRNA expression. The kinetics of VMP1 mRNA expression also corresponded to the apoptosis of acinar cells.

Vacuolation is an early phenomenon proceeding cell death, and is related to apoptosis. We have shown, for the first time, that cytoplasmic vacuoles in acinar cells are increased during the course of CP in the WBN/Kob rat and that the number of vacuoles was most increased at the onset of the CP. Acinar cell vacuolation is possibly a general feature of the development of acute pancreatitis and therefore, may also have important pathophysiological implications for CP. VMP1 gene activation in chronic pancreatitis is part of the acinar cell response to aggression. In conclusion, we have revealed cytoplasmic vacuolization in CP of the WBN/Kob rat, and demonstrated that VMP1 mRNA expression is strongly induced at the onset of CP. VMP1 mRNA expression, relating to acinar cell apoptosis, may reflect important pathophysislogical changes in CP.

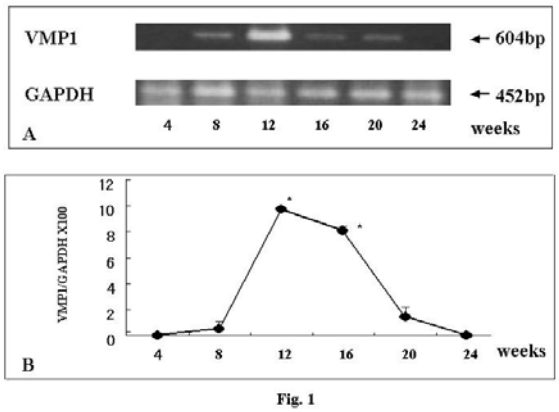


Fig. 1

Figure: A: VMP1 mRNA of 604 bp was expressed from 8 to 20 weeks**.** **B**: VMP1 mRNA expression began at 8 weeks reached its peak at 12 weeks decreased at 16 and 20 weeks.

Duct-narrowing chronic pancreatitis without immunoserological abnormality: comparison with duct-narrowing chronic pancreatitis with positive serological evidence and its clinical management.

N. Sawabu, T. Wakabayashi, Y. Satomura, H. Watanabe, Y. Motoo

We reviewed the clinical features and clinical course of patients with duct-narrowing chronic pancreatitis who were negative for immunoserological test results (n=16) in comparison with the findings for serological test-positive patients (n=20) in order to determine an adequate treatment for those who had typical morphology of autoimmune pancreatitis in the absence of immunoserological abnormality. No significant differences were found between the two groups of patients in clinical profiles including associated autoimmune-related diseases, pancreatic histology, and response to steroid therapy. Of the seronegative patients, eight patients who showed an improvement in narrowing of the main pancreatic duct with steroid therapy and three who did no show an improvement or who relapsed after surgical resection without this therapy had stenosis of the common bile duct with increased levels of serum hepatobiliary enzymes, except for two patients with affected sites limited to the body or tail of the gland. For the remaining five patients, who showed an improvement in pancreatic duct changes or long-term remission after surgery without steroid administration, normal biochemistry test results for liver functions were obtained, with no abnormal cholangiographic findings in the 3 patients examined.

Duct-narrowing chronic pancreatitis without immunoserological abnormality overlaps in clinical features with that fulfilling the immunoserological criteria for a diagnosis of autoimmune pancreatitis. In particular, the disease with bile duct involvement should be treated clinically as autoimmune pancreatitis, for which steroid therapy is recommended, even if an autoimmune mechanism is not demonstrated serologically.

Table. Comparison of clinicopathologic findings for seropositive and seronegative DNCP patients

Clinicopathologic finding	seropositive (n=20)	seronegative (n=16)	P value
Sex (male/female)	17/3	10/6	p＞0.10
Age (mean±SD; yr)	62.9±10.0	57.6±13.9	p=0.20
Location (diffuse/focal)	17/3	12/4	p＞0.90
DM	12 (60%)	6 (38%)	p＞0.25
Extrabilopancreatic autoimmune-related disease	6 (30%)	3 (19%)	p＞0.50
Jaundice	11 (55%)	5 (31%)	p＞0.25
Upper abdominal pain	4 (20%)	7 (44%)	p＞0.10
Increased cholestatic enzymes	15 (75%)	9 (56%)	p＞0.25
Increased pancreatic enzymes	10 (50%)	13 (81%)	p＞0.10
CBD stenosis	18/19 (95%)	9/12 (75%)	p＞0.25
Dense inflammatory cell infiltration	7/8 (88%)	4/6 (67%)	p＞0.75
Effective steroid therapy	14/15 (93%)	8/8 (100%)	p＞0.50

DNCP, duct-narrowing chronic pancreatitis; DM, diabetes mellitus; CBD, common bile duct

Long-term prognosis of duct-narrowing chronic pancreatitis: strategy for steroid treatment

N. Sawabu, T. Wakabayashi, Y. Satomura, H. Watanabe and Y. Motoo.

The purpose of this study was to assess the long-term prognosis in patients with duct-narrowing pancreatitis (DNCP) and determine the indications for steroid therapy. We evaluated clinical and imaging outcomes in patients with DNCP classified into three groups (A, B, and C) according to the treatment given. Group A included 6 patients who underwent surgical resection. Groups B and C included 21 and 10 patients treated medically with and without steroid therapy, respectively.

In group A, 2 patients relapsed in the remnant pancreas. In group B, pancreatic swelling was resolved in all the patients, and moreover, both the irregular narrowing of the main pancreatic duct (MPD) and the strictures of the common bile duct (CBD) improved after initiation of corticosteroid therapy in all but 1 of the patients, including 5 without immunoserological abnormality. However, clinical recurrences occurred in 4 patients (19%) during or after the maintenance therapy. In group C, all the patients showed an improvement in swelling of the gland, while only 5 showed improvement of pancreatic duct involvement. Four of these 5 patients did not show any serological data suggesting autoimmune abnormality or CBD involvement. In all of the other 5 patients in whom MPD irregularities did not improve, bile duct strictures or positive immunoserological test results were noted.

Steroid therapy is effective for improving pancreatic duct and bile duct lesions in patients with DNCP, and is indicated particularly in those who show CBD strictures or immunoserological abnormality, although some patients have recurrences.

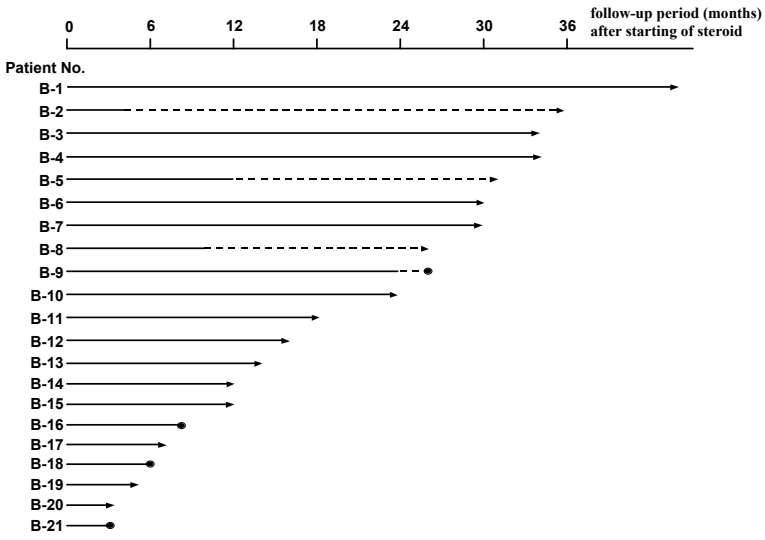


Figure. Prognosis after clinical remission in 21 patients of group B. ——●, relapsed during maintenance therapy; ----● , relapsed after the cessation of maintenance therapy; ——► , continued to be in clinical remission with maintenance therapy; ------► , continued to be in clinical remission after the cessation of maintenance therapy.

Publications

1. Sabet EA, Okai T, Minamoto T, and Sawabu N: Visualizing the gastric wall with a 30-MHz ultrasonic miniprobe: *ex vivo* imaging of normal gastric sites and sites of early gastric cancer. Abdom Imaging 28：252-256, 2003.
2. Okai T, Minamoto T, Ohtsubo K, Minato H, Kurumaya H, Oda Y, Mai M, and Sawabu N: Endosonographic evaluation of c-kit-positiv gastrointestinal stromal tumor. Abdom Imaging 28: 301-307, 2003.
3. Xie M-J, Motoo Y, Iovanna JL, Su S-B, Ohtsubo K, Matsubara F, and Sawabu N: Overexpression of pancreatitis-associated protein (PAP) in human pancreatic ductal adenocarcinoma. Dig Dis Sci 48(3): 459-464, 2003.
4. Ohtsubo K, Watanabe H, Yamaguchi Y, Hu Y-X, Motoo Y, Okai T, and Sawabu N: Abnormalities of tumor suppressor gene *p16* in pancreatic carcinoma: immunohistochemical and genetic findings compared with clinicopathological parameters. J Gastroenterol 38: 663-671, 2003.
5. Okai T, Shirasaki F, and Sawabu N: Erythematous maculopapular eruption due to ribavirin administration in a patient with chronic hepatitis C. J Clin Gastroenterol 36(3): 283-284, 2003.
6. Motoo Y, Xie M-J, Su S-B, and Sawabu N: Molecular mechanisms of therapeutic effects of Saiko-keishi-to on spontaneous chronic pancreatitis in the WBN/Kob rat. J Trad Med 20(4): 132-138, 2003.
7. Ito Y, Yoshida H, Motoo Y, Miyoshi E, Iovanna JL., Tomoda C, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Matsuura N, Kuma K, and Miyauchi A: Expression and cellular localization of p8 protein in thyroid neoplasms. Cancer Letters 201: 237-244, 2003.
8. Wakabayashi T, Kawaura Y, Satomura Y, Watanabe H, Motoo Y, Okai T, and Sawabu N: Clinical and imaging features of autoimmune pancreatitis with focal pancreatic swelling or mass formation: comparison with so-called tumor-forming pancreatitis and pancreatic carcinoma. Am J Gastroenterol 98(12): 2679-2687, 2003.
9. Motoo Y, and Uchide K: Regulations of ephedra in the United States. Am J Chin Med 31: 1003-1004, 2003.
10. Wang Y, Yamaguchi Y, Watanabe H, Ohtsubo K, Motoo Y, and Sawabu N: Detection of *p53* gene mutations in the supernatant of pancreatic juice and plasma from patients with pancreatic carcinomas. Pancreas 28(1): 13-19, 2004.
11. Feng S-Y, Ohta K, Yamada Y, Sawabu N and Ito T: A yeast one-hybrid system to detect methylation-dependent DNA-protein interactions. Biochem Biophy Res Commun 313: 919-922, 2004.
12. Sawabu N, Watanabe H, Yamaguchi Y, Ohtsubo K, and Motoo Y: Serum tumor markers and molecular biological diagnosis in pancreatic cancer. Pancreas 28(3): 263-267, 2004.
13. Motoo Y, Xie MJ, Mouri H, and Sawabu N. Expression of interleukin-8 in human obstructive pancreatitis. Journal of the Pancreas (JOP), 5(3): 138-144, 2004.
14. Jiang PH, Motoo Y, Iovanna JL, Pebusque MJ, Xie MJ, Okada G, and Sawabu N. Tumor

protein p53-induced nuclear protein 1(TP53INP1) in spontaneous chronic pancreatitis in the WBN/Kob rat : drug effects on its expression in the pancreas. Journal of the Pancreas (JOP), 5(4): 205-216, 2004.
15. Jiang PH, Motoo Y, Vaccaro MI, Iovanna JL, Okada G, and Sawabu N. Expression of vacuole-membrane protein-1 (VMP1) in spontaneous chronic pancreatitis in the WBN/Kob rat. Pancreas, 29(3): 225-230, 2004.
16. Watanabe H, Yamaguchi Y, Ohtsubo K, Mouri H, Motoo Y, Yamashita K, Minamoto T, Gabata T, and Sawabu N. Epidermoid cyst of the intrapancreatic accessory spleen producing CA 19-9. Dig Endosc, 16(3): 244-248, 2004.
17. Wakabayashi T, Kawaura Y, Satomura Y, Watanabe H, Motoo Y, and Sawabu N. Long-term prognosis of duct-narrowing chronic pancreatitis: strategy for steroid treatment. Pancreas, 30(1): 31-39, 2005.
18. Motoo Y, Mouri H, Ohtsubo K, Yamaguchi Y, Watanabe H, and Sawabu N. The herbal medicine Ninjinyoeito ameliorates ribavirin-induced anemia in chronic hepatitis C: a randomized controlled trial. World J Gastroenterol, 11(26): 4013-4017, 2005.
19. Takahashi Y, Mai M, Sawabu N, and Nishioka K. A pilot study of individualized maximum repeatable dose (iMRD), a new dose finding system, of weekly gemcitabine for patients with metastatic pancreas cancer. Pancreas, 30(3): 206-210, 2005.
20. Wakabayashi T, Kawaura Y, Satomura Y, Urabe T, Watanabe H, Motoo Y, and Sawabu N. Duct-narrowing chronic pancreatitis without immunoserological abnormality: comparison with duct-narrowing chronic pancreatitis with positive serological evidence and its clinical management. Dig Dis Sci, 50(8): 1414-1421, 2005.
21. Sawabu N, and Watanabe H. Altered expression of p27 protein in pancreatic carcinomas (Molecular pathology). Elsevier Science/ Academic Press, Hagat M.A. (eds); 331-333, 2005.
22. Ito Y, Yoshida H, Motoo Y, Iovanna JL, Nakamura Y, Nakudo K, Uruno T, Takamura Y, Miya A, Noguchi S, Kuma K, and Miyauchi A. Expression of P8 protein in breast carcinoma; an inverse relationship with apotosis. Anticancer Res 25: 833-838, 2005.
23. Watanabe H, Okada G, Ohtsubo K, Yamaguchi Y, Mouri H, Motoo Y, Wakabayashi T, and Sawabu N. Expression of mesothelin mRNA in pure pancreatic juice from patients with pancreatic carcinoma, intraductal papillary mucinous neoplasm of the pancreas, and chronic pancreatitis. Pancreas 30(4):349-354,2005.
24. Jiang PH, Motoo Y, Iovanna JL, Garcia S, Pebusque MJ, and Sawabu N. The down-expreesion of tumor protein 53-induced nuclear protein 1 (TP53INP1) in human gastric cancer. World J Gastroenterol, (in press).
25. Watanabe H, Ohtsubo K, Yamaguchi Y, Mouri H, Motoo Y, Noto M, Kitagawa H, Kayahara M, Ohta T, Gabata T, Sakamoto S, and Sawabu N. Successful cyotic fenestration for a macrocyotic serous cyotadenoma of the pancreas causing obstructive jaundice: report of a case. Surg Today 36: 89-93, 2006.
26. Jiang PH, Motoo Y, Minamoto T, and Sawabu N. Gemcitabine changes the expression of apoptosis-related genes in human pancreatic cancer cells. World J, Gastroenterol (in press).
27. Kamisawa T, Wakabayashi T, and Sawabu N. Autoimnune pancreatitis in young patients. J. Gastroenterol, (in press).

28. Watanabe H, Okada G, Ohtsubo K, Yao F, Jiang PH, Mouri H, Wakabayashi T, and Sawabu N. Aberrant methylation of secreted apoptosis-related protin 2(SARP2) in pure pancreatic juice in diagnosis of pancreatic neoplasms. Pancreas, (in press).

Division
of
Surgical Oncology

General Summary of Division of Surgical Oncology

Since Cancer Institute Hospital had unified into University Hospital at October 2001, we have very busily occupied with hospital work and they had no time to spare for research work. Under this circumstance we continued several research work on cancer metastasis, molecular pathology, and tumor immunology. Recent projects may be summarized as follows.

Project on cancer metastasis

To clarify molecular mechanism of cancer metastasis, especially matrix metalloproteinase and angiogenesis using clinical materials, and to inhibit metastasis. After we have firstly reported that vessel density and expression of VEGF and its receptor, KDR, correlates with metastasis, and proliferation of human colon cancer, many researchers have reported VEGF is one of important angiogenic factor in patients with colon cancer. Moreover, many clinical trials using these VEGF targeting drugs were done in several cancers such as breast, lung and colon cancer. Among them, phase II and III of recombinant human monocolonal antibody to VEGF (Avastin, Genentech) showed positive data, and accepted a new molecular targeting drug for metastatic colon cancer by FDA at February 2004. Therefore we continued this project for some new indication of this drug.

We have shown that mRNA upregulation of metastasis-related genes such as angiogenic factors, metalloproteinases, growth factors, and adhesion molecules, occur just prior to metastasis in human colon cancer in an orthotopic nude mice model of colon cancer liver metastasis. We have also shown that upregulation of angiogenesis, i.e. angiogenic switch may occur between Tis and T1, simultaneous to initiation of invasion, in the early development of human colon cancer. Finally, we showed that VEGF inhibit angiogenic switch and liver metastasis of the orthotopic xenograft model in site-dependent tumor of VEGF. These results suggest that VEGF antibody have another possibility as a drug for chemoprevention of colon cancer, especially premalignant lesions such as polyposis and long-termed ulcerative colitis.

We have shown that VEGF expression was higher in patients with liver metastasis of gastric cancer. Recently, we showed the correlation between alpha-fetroprotein (AFP) and VEGF expression in AFP producing gastric cancer, which is well known to have a high incedence of liver metastasis. From these results, we are planning to indicate the Avastin in patients with liver metastasis of gastric cancer.

Project on chemotherapy including tumor dormancy therapy

The goal of chemotherapy in patients with far advanced cancer should be aimed to prolong the survival of cancer patients, tumor dormancy in other words, rather than tumor shrinkage (killing cancer cells), with fairly good QOL. In order to induce tumor dormancy two approaches are considered: one is a molecular targeting therapy to prolong the dormant period itself and

other is obtain prolonged NC with chemotherapy. We developed and established a new dose-finding system, the individualized maximum repeatable dose (iMRD), suitable to induce prolonged TTP rather than tumor shrinkage. And we started a randomized Phase II clinical trial of tailored CPT-11 + S-1 vs S-1 in patients with advanced or recurrent gastric carcinoma as the first line chemotherapy to study the significance of iMRD. Moreover we started and planned some similar clinical studies for colon, breast, ovary, pancreas cancers.

Project on minimally invasive surgery (MIS)

Laparoscopic surgery in patients with early GI cancer allows minimally invasive surgery to be carried out to maintain the patient's quality of life in future. Dr. Omote has conducted this project and extended the indication of MIS in advanced gastric cancer and colorectal cancer. He showed that the laparoscopic surgery was associated with less bleeding, rapid recovery of bowel movement, faster initiation of oral intake and shorter hospital stay. He also showed that the recovery after laparoscopy assisted partial gastrectomy is as successful as after laparoscopic local resection.

Project on cytokines

Dr. Yasumoto studied the significance of CSCL12 in peritoneal caricinomatosis of gastric cancer. Finally, he showed that three out of seven human gastric carcinoma cell lines selectively expressed CXCR4 mRNA and protein at high levels, and which were all highly efficient in generating malignant ascites in nude mice upon intraperitoneal inoculation. NUGC4 cells expressed CXCR4 mRNA at high levels and showed vigorous migratory responses to its ligand CXCL12 (also called stromal-derived factor-1a, SDF-1a). Furthermore, CXCL12-treated NUGC4 cells showed enhanced proliferation and rapid increases in phosphorylation of protein kinase B/Akt and extracellular signal-regulated kinase (ERK). He also CXCR4-positivity of primary gastric carcinomas significantly correlated with the development of peritoneal carcinomatosis, and concluded that CXCR4/CXC12 axis plays an important role in the development of peritoneal carcinomatosis from gastric carcinoma.

Project on gene expression in human gastric cancer

Dr. Yamashita analyzed gene expression in human gastric cancer by serial analysis of gene expression (SAGE). SAGE is a method for comprehensive analysis of gene expression patterns. A short sequence tag (10-14bp) contains sufficient information to uniquely identify a transcript. He showed that total of 60621 transcripts were identified, and higher expression of SIAT6, APOC1, galectin-1 and COL1A1 in lymph node metastasis versus primaly tumor among them.

A pilot study of individualized maximum repeatable dose (iMRD), a new dose finding system, of weekly gemcitabine for patients with metastatic pancreas cancer

Y. Takahashi and K. Yamashita

We developed and established a new dose-finding system, the individualized maximum repeatable dose (iMRD), suitable to induce prolonged TTP rather than tumor shrinkage.

We applied this system in weekly gemcitabine therapy for 18 metastatic pancreas cancer patients. We determined the iMRD at the 5th week, after weekly dose adjustments. We started at 500 mg/m^2 (1/2 MTD (maximum tolerated dose)) of gemcitabine and repeated the treatment with an increase or a decrease of 100 mg/m^2 each week, if toxicity was 0 or more than grade 1, respectively (Figure 1).

The iMRD of weekly gemcitabine was 300 mg/m^2 in 2 patients, 400 mg/m^2 in 3 patients, 500 mg/m^2 in 5 patients, 600 mg/m^2 in 6 patients, and 700 mg/m^2 in 2 patients, demonstrating significant differences among individual patients. Grade 3 marrow depression occurred in only 1 patient (5.6%). Of these 18 patients, 3 (16.7%), 13 (72.2%) and 2 (11.1%) patients showed PR, SD and PD, respectively. The median of TTP and survival was 4.5 months and 9.5 months, respectively. There were no significant differences in 1 year survival time and more than 50 % reduction rate of serum CA19-9, a tumor marker for pancreatic cancer, between patients with lower (500 mg/m^2 or less) and higher (600 mg/m^2 or more) iMRD.

These results suggest that iMRD is a simple method to determine an individual's tailored dose for chemotherapy and could be the optimal dose for patients with non-curable cancers such as metastatic pancreas cancer.

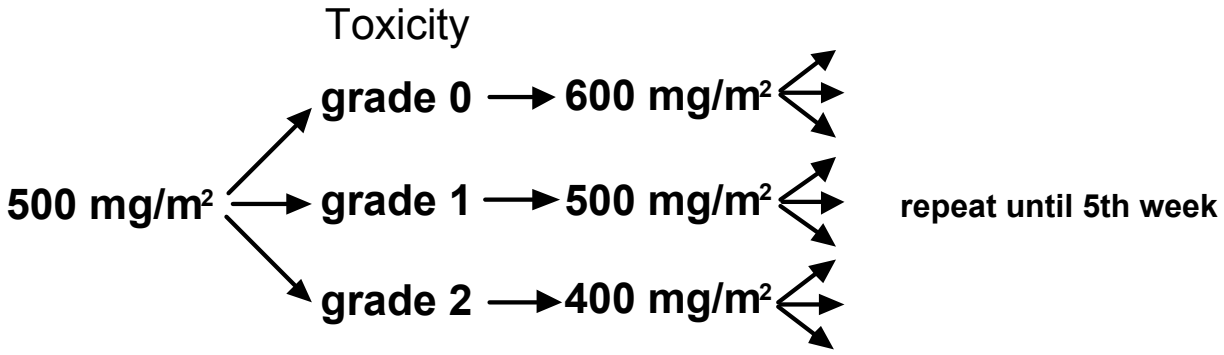


Figure 1. The method of determination of iMRD

Chemotherapy under cachectic conditions and the possibility of cachexia-controlled chemotherapy

Y. Takahashi and K. Yasumoto

We studied the role of chemotherapy in cancer cachexia, which is well known to lower QOL as well as responsiveness to chemotherapy. In BALB/c mice we used two clones derived from the murine colon 26 adenocarcinoma cell line, clone 5, a non-cachexigenic tumor and clone 20, a cachexigenic tumor, in which IL-6 mRNA was selectively detected. While maximum tolerated dose (MTD) of CPM and CPT-11 showed significantly smaller tumor weight and higher survival than 1/2 MTD in both drug groups with clone 5, the results were reversed with clone 20 (Fig 1). The tumor weights with MTD of CPM or CPT-11 in combination with anti-IL-6 antibody treatment, which decreases serum IL-6 level and improves cachexia status, were significantly smaller than those in the MTD treatment-alone group with clone 20, but not with clone 5 (Fig 2).

From these results, we suggest that lower dose chemotherapy or cachexia-controlled chemotherapy such as some chemotherapeutic agents with neutralizing cachexia-related cytokine effects, elicits superior antitumor effects in cachectic individuals than conventional MTD chemotherapy.

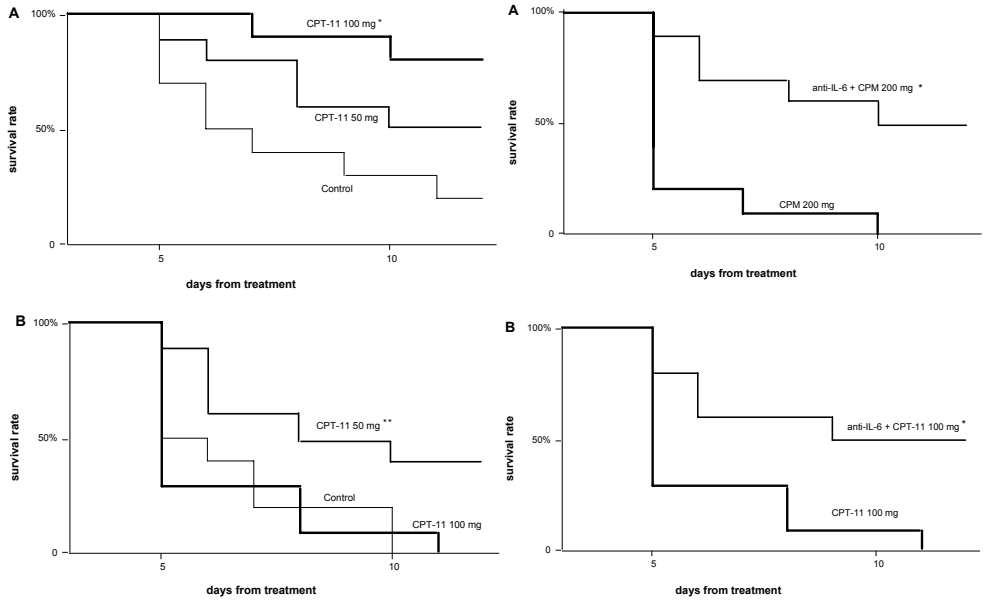


Fig 1. Survival curves of CPT-11 therapy

Fig. 2. The effect of anti-IL-6 antibody

Laparoscopic Surgery for Early Gastric Cancer

K. Omote, H. Kitakata, K. Yamashita, K. Yasumoto and Y. Takahashi

Resent advancement of endoscopy and its technique lead to early detection of gastric cancer, and enable us to perform an entirely new surgical approach to gastric cancer. Laparoscopic surgery for early gastric cancer is minimally invasive and offers patients the advantages of reduced pain, shorter hospital stay and quicker convalescence, compared with open surgery. In Japan, laparoscopic local (wedge) resection of the stomach (lesion lifting method) and laparoscopic mucosal resection (intra-gastric surgery) were first performed in 1992 and in 1993 respectively, and laparoscopy assisted partial resection of the stomach with lymph node dissection in 1994. Laparoscopic surgery for early gastric cancer has been introduced in our department since 1995. Since then, we have experienced 20 cases with laparoscopic local resection of the stomach and 24 cases with laparoscopy assisted partial gastrectomy. Recently, the opportunity to select laparoscopy assisted partial gastrectomy has been increased. Compared with open surgery, the laparoscopic surgery is associated with less bleeding, rapid recovery of bowel movement, faster initiation of oral intake and shorter hospital stay. Laparoscopic surgery for early gastric cancer is feasible, with favorable short-term outcome. Although procedure of laparoscopy assisted partial gastrectomy is much different in that of laparoscopic local resection, the recovery after laparoscopy assisted partial gastrectomy is as successful as after laparoscopic local resection.

Intra- and early post-operative outcomes

	Laparoscopic local resection (n=20)	Laparoscopy assisted partial gastrectomy (n=24)	Open surgery (n=124)	P-value
Operative time (min)	191	304*	187*	*<0.05
Blood loss (ml)	55†	186†‡	386‡	†‡<0.001
Complication rate	0.15	0.13	0.18	NS
	Perforation 1	Bleeding 1	Bleeding 3	
	Wound infection 2	Stomal ulcer 1	Anastomotic leak 6	
		Anastomotic stricture 1	Anastomotic stricture 4	
			Others 8	
First flatus (day)	2.0	2.1¶	4.2¶	¶<0.05
First defecation (day)	2.9	3.6	4.7	NS
Initiation of oral intake (day)	2.8	3.2#	5.4#	#<0.05
Hospital stay (day)	12	16§	25§	§<0.01

Open surgery : Conventional open distal partial gastrectomy and open pylorus preserving partial gastrectomy for the case with preoperative diagnosis of early gastric cancer in 2000 to 2004.

Role of the CXCL12/CXCR4 axis in Peritoneal Carcinomatosis of Gastric Cancer

K. Yasumoto[1], K. Koizumi[2], I. Saiki[2], and Y. Takahashi[1]
[1]Division of Surgical Oncology, Cancer Research Institute, Kanazawa University
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Peritoneal carcinomatosis is a frequent cause of death in patients with advanced gastric carcinoma. Since chemokines are now considered to play an important role in the metastasis of various malignancies, we hypothesized that they may also be involved in the development of peritoneal carcinomatosis by gastric carcinoma. We found that three out of seven human gastric carcinoma cell lines selectively expressed CXCR4 mRNA and protein at high levels. These three cell lines were those that were all highly efficient in generating malignant ascites in nude mice upon intraperitoneal inoculation. In particular, NUGC4 cells expressed CXCR4 mRNA at high levels and showed vigorous migratory responses to its ligand CXCL12 (also called stromal-derived factor-1a, SDF-1a). Furthermore, CXCL12-treated NUGC4 cells showed enhanced proliferation and rapid increases in phosphorylation of protein kinase B/Akt and extracellular signal-regulated kinase (ERK). We also demonstrated that AMD3100 (a specific CXCR4 antagonist) effectively reduced tumor growth and ascitic fluid formation in nude mice inoculated with NUGC4 cells. We next examined human clinical samples. We found that malignant ascites fluids from patients with peritoneal carcinomatosis contained CXCL12 (average, 4.67 ng/mL) enough to exhibit biological effects on NUGC4 cells. Moreover, immunohistochemical analysis revealed that 22 out of 33 primary gastric tumors with peritoneal metastasis were scored positive for CXCR4 expression (67%), and only 4 out of 16 with other distant metastasis were positive (25%). Notably, 22 out of 26 CXCR4-expressing primary tumors developed peritoneal metastases (85%). CXCR4-positivity of primary gastric carcinomas significantly correlated with the development of peritoneal carcinomatosis. Collectively, our results strongly suggest that the CXCR4/CXC12 axis plays an important role in the development of peritoneal carcinomatosis from gastric carcinoma. Thus, CXCR4 may be a potential therapeutic target for peritoneal carcinomatosis of gastric carcinoma.

Serial Analysis of Gene Expression in Human Gastric Cancer

Kaname Yamashita[1)], Hidekazu Kitakata[1)], Kazuo Yasumoto[1)], Kazuhiko Omote[1)], Yutaka Takahashi[1)] Toshinari Minamoto[2)]
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It is important to identify genes responsible for metastasis of gastric cancer, as this disease is one of the leading causes of cancer death in the world. Here we analyzed gene expression in human gastric cancer by serial analysis of gene expression (SAGE). SAGE is a method for comprehensive analysis of gene expression patterns. A short sequence tag (10-14bp) contains sufficient information to uniquely identify a transcript. The tag is obtained from a unique position within each transcript. Sequence tags can be linked together to from long serial molecules that can be cloned and sequenced and quantitation of the number of times a particular tag is observed provides the expression level of the corresponding transcript. We obtained primaly tissue, normal mucosa, and lymph node metastasis from a advanced gastric cancer patient. Total RNA was isolated and SAGE was performed. Tag sequences were analyzed using SAGE software. A total of 60621 transcripts were identified. We show tags which highly expressed, in primaly tumor, normal mucosa, and lymph node metastasis. The lymph node metastasis library was compared with primaly tumor library, we could identify certain set of genes up-regulated in lymph node metastasis in tag ratio of two fold or more. Validation by RT-PCR on RNA from another patients shows higher expression of SIAT6, APOC1, galectin-1 and COL1A1 in lymph node metastasis versus primaly tumor. These results together with currently accumulating data would provide basic knowledge toward understanding molecular mechanism underlying progression and metastasis of gastric cancer.

Summary of SAGE tag libraries

Library	Sequences	Total Tags	Unique Tags
Normal	731	14843	5243
Tumor	933	22681	8337
LN meta	1030	23097	7976

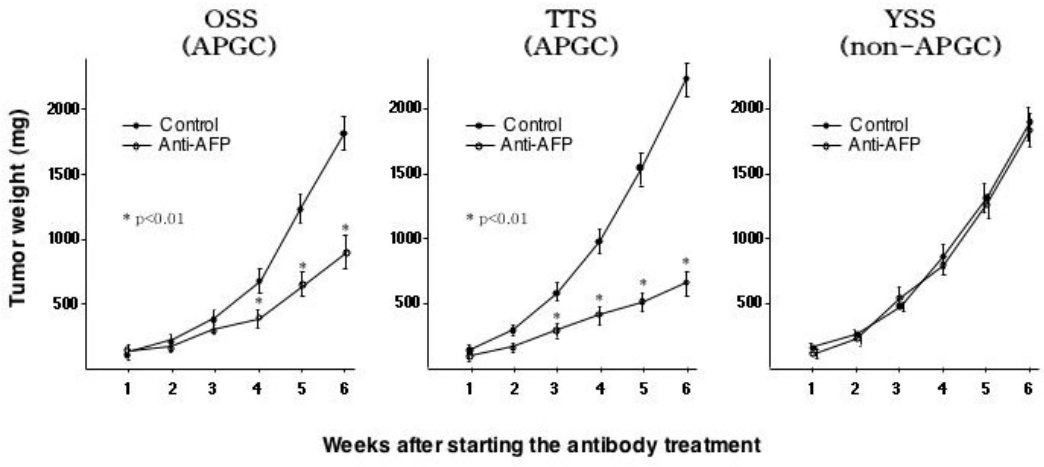
Up-regulation of VEGF in Alpha-fetoprotein-producing gastric cancer

H.Kitakata, Y.Takahashi

Alpha-fetoprotein (AFP)-producing gastric carcinoma (APGC) is well known to have a poor prognosis and high incidence of lymph node and liver metastases compared to non-producing carcinoma. Although the reason why APGC frequently metastasizes to the liver is not well known. We hypothesizes that angiogenesis is one of the important reasons for frequent liver metastases from APGC. In order to clarify this hypothesis, we examined the vessel density and vascular endothelial growth factor (VEGF) expression in APGC. Furthermore, we studied the effects of anti-AFP antibody for angiogenesis using APGC xenotransplanted mouse model.

Archival specimens of APGC (n=25) and non-APGC (n=68) were studied. Expressions of vessel density and VEGF were significantly higher in APGC than those in non-APGC ($p<0.001$). Immunohistochemical analysis of patients with gastric cancer shows that expression of VEGF and factor VIII are significantly higher in APGC than non-APGC. There is correlation among the AFP expression, the vessel density and the VEGF expression in APGC ($p<0.001$).

Next, we studied the effects of anti-AFP antibody on APGC xenotransplanted in nude mice. There is significant inhibition of tumor growth in the treatment groups compared to the control groups in two APGC lines ($p<0.01$). But, there is no difference in non-APGC cell line. Moreover, vessel densities of the treatment groups were significantly lower than those of the control groups in these two lines. These findings thus suggest that the biological behavior of APGC is angiogenesis-dependent. Down regulation of angiogenesis by anti-AFP antibody suggest that AFP itself may upregulate the angiogenesis, and the treatment by antibody could have anti-angiogenic effects, inhibiting metastasis, especially liver metastasis in APGC.



Publications

1. Takahashi, Y., Ellis, LM., Mai, M. (2003) The angiogenic switch of human colon cancer occurs simultaneous to initiation of invasion. Oncology Report. 10: 9-13.
2. Takahashi, Y., Takeuchi, T., Sakamoto, J., Touge, T., Mai, M., Ohkura, H., Kodaira, S., Okajima, K., Nakazato, H. (Tumor Marker Committee, Japanese Foundation for Multidisciplinary Treatment of Cancer) (2003) The Significance of CEA and/or CA19-9 in Monitoring for Recurrence in Gastric Cancer Cases. A Prospective Clinical Study. Gastric Cancer. 6: 142-145.
3. Ougolkov, A., Yamashita, K., Bilim, V., Takahashi, Y., Mai, M., Minamoto, T. (2003) Abnormal expression of E-cadherin, β-catenin and c-erbB-2 in gastric cancer with liver metastasis. Int J Colorect Dis. (Clin Mol Gastroenterol Surg) 18: 160-166.
4. Zhang, B., Ougolkov, A., Yamashita, K., Takahashi, Y., Mai, M., Minamoto, T. (2003) β-catenin and ras oncogenes detect most human colorectal cancer. Clin. Cancer Res. 9: 3073-3079.
5. Zhang, B., Fujimoto, T., Kaneda, A., Mai, M. (2003) Irinotecan Enhances IL-12 Production by OK-432-activated Murine Macrophages. Anticancer Res. 23(3B): 2499-2504.
6. Sakamoto, J., Hamashima, H., Suzuki, H., Ito, K., Mai, M., Saji, S., Fukushima, M., Matsushima, Y., Nakazato, H., For the CIP Genetic Marker Committee. (2003) Thymidylate synthase expression as a predictor of the prognosis of curatively resected colon carcinoma in patients registered in an adjuvant immunochemotherapy clinical trial. Oncology Report. 10: 1081-1090.
7. Sabet, EA., Okai, T., Minamoto, T., Mai, M., Sawabu, N. (2003) Visualizing the gastric wall with a 30-MHz ultrasonic miniprobe: ex vivo imaging of normal gastric sites and sites of early gastric cancer. Abdom Imaging. 28: 252-256.
8. Okai, T., Minamoto, T., Ohtsubo, K., Minato, H., Kurumaya, H., Oda, Y., Mai, M., Sawabu, N. (2003) Endosonographic evaluation of c-kit-positive gastrointestinal stromal tumor. Abdom Imaging. 28: 301-307.
9. Takahashi, Y., Kitakata, H., Hirano, K., Yamashita, K., Yasumoto, K., Omote, K., Minamoto, T., Mai, M. (2004) Pilot study of low dose, divided MTD of CPT-11 on 21 consecutive patients with metastatic colorectal or gastric cancer. Surgery Today. 34 :246-250.
10. Takahashi, Y., Ohta, T., Mai, M. (2004) Angiogenesis of AFP producing gastric carcinoma: Correlation with frequent liver metastasis and its inhibition by anti-AFP antibody. Oncology Reports. 11: 809-813.

11. Takahashi, Y., Sakamoto, J., Takeuchi, T., Mai, M., Kubota, T., Kitajima, M., Tanigawara ,Y., Komatsu, Y., Toge,T., Saji, S. (2004) A randomized phase II clinical trial of tailored CPT-11 + S-1 vs S-1 in patients with advanced or recurrent gastric carcinoma as the first line chemotherapy. Japanese Journal of Clinical Oncology. 34: 342-345.
12. Takahashi, Y., Yamashita, K., Endo, Y., Sasaki, T., Mai, M. (2004) Oral administration of uracil-tegarfur (UFT) inhibits liver micrometastasis of human colon cancer in an orthotopic model of nude mice and its early detection system. Surgery Today. 34: 855-859.
13. Takahashi, Y., Mai, M., Sawabu, N., Nishioka, K. (2005) A pilot study of individualized maximum repeatable dose (iMRD), a new dose finding system, of weekly gemcitabine for patients with metastatic pancreas cancer. Pancreas. 30: 206-210.
14. Takahashi, Y., Mai, M., Nakazato, H. (2005) Preoperative CEA and PPD values as prognostic factors for immunochemotherapy using PSK and 5-FU. Anticancer Res. 25: 1377-1384.
15. Takahashi, Y., Yasumoto, K., Mai, M. (2005) Chemotherapy under cachectic conditions and the possibility of cachexia-controlled chemotherapy. Oncology Reports. 14: 135-140.
16. Takahashi, Y., Mai, M. (2005) Antibody against Vascular Endothelial Growth Factor (VEGF) Inhibits Angiogenic Switch and Liver Metastasis in Orthotopic Xenograft Model with Site-dependent Expression of VEGF. Journal of Experiment and Clinical Research. 24: 237-243.
17. Yasumoto, K., Takahashi, Y., Mai, M. and Kawashima, A. (2005) Meningeal carcinomatosis preceded by a rapid increase in serum CA19-9 levels in a patient with breast cancer. Int J Clin Oncol. 10:276-280.
18. Yasumoto, K., Koizumi, K., Kawashima, A., Saitoh, Y., Arita, Y., Shinohara, K., Minami, T., Nakayama, T., Sakurai, H., Takahashi, Y., Yoshie, O., and Saiki, I. Role of CXCL12/CXCR4 axis in Peritoneal Carcinomatosis of Gastric Cancer. Cancer Res. in press.
19. Shakoori, A., Ougolkov, A., Yu, XW., Zhang, B., Modarresi, MH., Biladeau, DD., Mai, M., Takahashi, Y., Minamoto, T. (2005) Deregulated GSK3beta activity in colorectal cancer: Its association with tumor cell survival and proliferation. Biochem Bioph Res Co. 334: 1365-1373.
20. Kaibara, N., Otani, Y., Takahashi, Y., et al. (2004) Meeting report of the 76th Japanese gastric cancer congress. Gastric Cancer. 7: 185-195.

Division of Diagnostic Molecular Oncology

General Summary of the Division of Diagnostic Molecular Oncology

To strengthen its capabilities for responding to the rapid pace of advance in basic and clinical cancer research, and to better meet its evolving responsibilities, the Cancer Research Institute of Kanazawa University was restructured in April of 1997. At the time the Division of Diagnostic Molecular Oncology was established as a clinical oncology division. Following a semester of four years for building up research activity and organizing laboratory, the division assumed an active role in July of 2001, when Dr. T. Minamoto was elected Professor and Chief of the division. Through twice movements, the division has been enlarged having more research members and two exclusive laboratories in the Institute.

The mission of the division, broadly, centers on basic and clinical research to develop the novel strategies and methods for diagnosis and treatment of cancer on the basis of molecular and cellular assessment of tumor aggressiveness and biological characteristics of individual tumor types relevant to metastatic potential, recurrence and outcome.

Table. Research directions and activities in the Division of Diagnostic Molecular Oncology

I. Activities toward molecular diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC)
1. Clinical and genetic analysis of the patients and their families
2. Genetic testing for DNA mismatch repair enzymes' genes
3. Surveillance and (surgical) intervention

II. Molecular diagnosis of pathology and malignant potential of cancers
1. Oncogenic signaling in colorectal cancers, targeting Wnt pathway and its regulators
2. Molecular pathology of clinical gastrointestinal cancers
3. Development of new modalities for diagnosis and treatment of cancer on the basis of molecular and cellular characteristics of cancer

III. Genome-wide analysis of gene expression in gastrointestinal cancers
1. Genome level: restriction landmark genomic scanning (RLGS)
2. Transcriptome level: serial analysis of gene expression (SAGE)
3. Epigenetic level: development of a new strategy covering all epigenetic alterations spanning through the genome

Current efforts in this division are focused on 1) clinical and basic activities toward molecular diagnosis of HNPCC; 2) the molecular characterization of gastrointestinal cancer by an interdisciplinary approach bringing together clinical oncology, molecular and cellular pathology and biology; and 3) comprehensive (genome-wide) analysis of gene expression and epigenetic alterations in stomach and colorectal cancers by the highly sophisticated methods including RLGS and SAGE, to cover the post-genome research fields. The most important achievements in this semester have been (1) discovery of previously unrecognized tumor supportive properties of GSK3β; and (2) identification of the genes responsible for lymph node metastasis in stomach cancer. We will intend to translate as much the results of these studies as possible to those responsible for diagnosis and treatment of cancer patients in clinical setting.

β-catenin and ras oncogenes detect most human colorectal cancer *

B. Zhang, A. Ougolkov, K. Yamashita, Y. Takahashi, M. Mai, T. Minamoto

PURPOSE AND STUDY DESIGN: Recent studies have shown that β-catenin translocated into the cell nucleus functions like an oncogene. Accumulating evidence suggests that activation of the β-catenin oncogenic signaling cascade along with its twin, the K-ras cascade, may exert syngeneic or synergistic effects on tumor development and progression. In the study reported here, we analyzed oncogenic β-catenin activation on the basis of its nuclear accumulation (NA) and compared the results with those of mutational activation of K-ras in 74 patients with colorectal cancer to determine whether the two oncogene-mediated signaling cascades interact.

RESULTS: We found two distinct patterns of β-catenin activation, i.e., diffuse NA in 20 cases (27%) and selective NA at the tumor invasion front (NAinv) in 19 cases (26%). The presence of the NAinv pattern was significantly correlated with advanced Dukes' stage tumor (P = 0.0005) and the presence of distant metastases (P = 0.0064). K-ras proto-oncogene was mutated in the tumors of 31 cases (42%). Activated β-catenin or K-ras was detected in most (78%) colorectal cancers analyzed, although a weak inverse correlation was found between the activities of the two oncogenes in the tumors. Importantly, most (7 of 8) patients with tumor showing both K-ras activation and the NAinv pattern of β-catenin activation were in Dukes' stage C at surgery, and half of them developed distant metastases to the liver and lungs.

CONCLUSION: The results suggest that although oncogenic activation of β-catenin and K-ras is independent in the process of clinical cancer development, combined analysis of the two major oncogenes can detect most colorectal cancers and identify a subset of patients with poorer outcomes. Consequently, activation of either or both of these oncogenes may serve as a genetic marker for molecular diagnosis.

*Reference:

Zhang B, Ougolkov A, Yamashita K, Takahashi Y, Mai M, Minamoto T. *β-catenin* and *ras* oncogenes detect most human colorectal cancers. Clin Cancer Res 9: 3073-3079, 2003.

Associations among β-TrCP, an E3 ubiquitin ligase receptor, β-catenin, and NF-κB in colorectal cancer *

A. Ougolkov, B. Zhang, K. Yamashita, V. Bilim, M. Mai, S.Y. Fuchs, T. Minamoto

BACKGROUND: The ubiquitin-proteasome pathway is important in regulating protein signaling pathways that are involved in tumorigenesis. β-transducin repeat-containing proteins (β-TrCP) are components of the ubiquitin ligase complex targeting β-catenin and IκBα for proteasomal degradation and are thus a negative regulator of Wnt/β-catenin signaling and a positive regulator of NF-κB signaling. We analyzed expression of β-TrCP in colorectal cancers and its association with types of β-catenin subcellular localization, an indirect measure of activation. **METHODS:** Levels of β-TrCP1 mRNA and protein were measured by quantitative reverse transcription-polymerase chain reaction and immunoblotting, respectively, in samples of tumor and normal tissues from 45 patients with colorectal cancer. Types of β-catenin activation (diffuse or invasion edge) and NF-κB activation were examined by immunohistochemistry. Apoptosis was determined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) assay. All statistical tests were two-sided. **RESULTS:** Compared with the β-TrCP1 levels in normal tissues, 25 (56%) of 45 tumors had increased β-TrCP1 mRNA and protein levels. Of the 22 (49%) tumors with β-catenin activation, 12 had the diffuse type (i.e., nuclear accumulation throughout the tumor) and 10 had the invasion edge type (i.e., nuclear accumulation predominantly in the tumor cells that formed the invasion edge). Increased β-TrCP1 levels were statistically significantly associated with β-catenin activation (P =.023) and decreased apoptosis (P =.035). β-TrCP accumulated in the nuclei of tumor cells that contained increased levels of β-TrCP1 mRNA and the active form of NF-κB. Higher levels of β-TrCP1 mRNA were detected in primary tumors of patients who had metastases (0.960 arbitrary units, 95% confidence interval = 0.878 to 1.042) than in the tumors of patients who did not (0.722 arbitrary units, 95% confidence interval = 0.600 to 0.844; P =.016). **CONCLUSION:** In colorectal cancer, increased expression of β-TrCP1 is associated with activation of both β-catenin and NF-κB, suggesting that the integration of these signaling pathways by increased β-TrCP expression may contribute to an inhibition of apoptosis and tumor metastasis.

*References: Ougolkov A, Zhang B, Yamashita K, Bilim V, Mai M, Fuchs SY, Minamoto T. Associations among β-TrCP, an E3 ubiquitin ligase receptor, β-catenin and NF-κB in colorectal cancer. J Natl Cancer Inst 96:1161-1170, 2004.

Deregulated GSK3β activity in colorectal cancer: its association with tumor cell survival and proliferation *

A. Shakoori, A. Ougolkov, Z.W. Yu, B. Zhang, M.H. Modaressi, D.D. Billadeau, M. Mai, Y. Takahashi, T. Minamoto

GSK3β is a multifunctional serine/threonine kinase that regulates various cellular pathways, depending on its substrates for phosphorylation. It is evident that regulation of Wnt/β-catenin signaling is only one of its diverse functions. Since oncogenic transcription factors (e.g., c-Jun, c-Myc) and proto-oncoproteins (i.e., β-catenin, Gli proteins) are putative GSK3β substrates for phosphorylation-dependent inactivation, it is hypothesized that GSK3β interferes with cellular neoplastic transformation and tumor development, as exemplified by its activity in Wnt/β-catenin signaling. However, only a few studies have addressed its role(s) in human cancer, and these studies have reported differing effects of GSK3β on cancer cells. Using GSK3β deficient mouse embryonic fibroblasts, it was shown that GSK3β plays a crucial role in cell survival mediated by the nuclear factor-kappaB (NF-κB) pathway (Nature 2000; 406:86-90). Interestingly, we have recently shown that the Wnt/β-catenin and NF-κB pathways were co-activated in colorectal cancer by dysregulation in the ubiquitin system (J Natl Cancer Inst 2004; 96:1161-70). Thus, these observations bring forward apparently opposing notions regarding the functions of GSK3β in neoplastic cells on the one hand, removing a neoplastic trigger by phosphorylation-dependent degradation of β-catenin oncoprotein in the ubiquitin system, and on the other, contributing to a cell proliferation and survival pathways by regulating NF-κB. The present study was therefore undertaken to clarify the role of GSK3β in cancer by analyzing expression and activity of this kinase in colon cancer cells and clinical colorectal cancers and by investigating its effects on cancer cells. In colon cancer cell lines and colorectal cancer patients, levels of GSK3β expression and its active form were higher in tumor cells than in their normal counterparts; these findings were independent of nuclear accumulation of β-catenin oncoprotein in the tumor cells. Inhibition of GSK3β activity by its Ser9 phosphorylation was defective in colorectal cancers but preserved in non-neoplastic cells and tissues. Strikingly, inhibition of GSK3β activity by chemical inhibitors and its expression by RNA interference targeting GSK3β induced apoptosis and attenuated proliferation of colon cancer cells *in vitro*. Our findings demonstrate an unrecognized role of GSK3β in tumor cell survival and proliferation and warrant proposing this kinase as a novel and potential therapeutic target in colorectal cancer.

*Reference: Shakoori A, Ougolkov A, Yu ZW, Zhang B, Modarressi MH, Billadeau DD, Mai M, Takahashi Y, Minamoto T. Deregulated GSK3β activity in colorectal cancer: its association with tumor cell survival and proliferation. Biochem Biophys Res Commun 334: 1365-1373, 2005.

Oncogenic β-catenin signaling networks in colorectal cancer *

S.Y. Fuchs, A.V. Ougolkov, V.S. Spiegelman, T. Minamoto

β-catenin has two distinct functions, namely, maintaining cell-to-cell adhesion and mediating the Wnt/β-catenin signal transduction pathway, which plays pivotal roles in embryogenesis and in malignant transformation of cells. The oncogenic properties of Wnt/β-catenin signaling stem from alteration in phosphorylation-dependent protein degradation and subcellular localization of β-catenin from cell membrane to the nucleus, where it binds to T-cell factor (Tcf) to form a bipartite transcription factor. The β-catenin/Tcf complex facilitates transcription of target genes that encode effectors for activation of cell proliferation and invasion and inhibition of apoptosis, leading to colorectal cancer development. In addition, in the tumor invasion front, stabilized and activated β-catenin interacts with other molecular pathways to facilitate tumor progression. This review highlighted the β-catenin-dependent oncogenic signaling network involved in the multi-step process of colorectal tumorigenesis. Wnt signaling evidently regulates stem cells, leading them to differentiate or self-renew. We are addressing roles of oncogenic β-catenin signaling in the microenvironment of the tumor-host interface that determine the individual tumor's malignant potential and in regulation of putative cancer stem or progenitor cells that represent plausible targets for cancer eradication.

*Reference:

Fuchs SY, Ougolkov AV, Spiegelman VS, Minamoto T. Oncogenic β-catenin signaling networks in colorectal cancer. Cell Cycle 4:1522-1539, 2005 (review).

Detection and characterization of oncogene mutations in preneoplastic and early neoplastic lesions *

T. Minamoto

Although it has been more than 20 yr since its discovery, the *ras* family of genes has not yet lost its impact on basic and clinical oncology. These genes remain central to the field of molecular oncology as tools for investigating carcinogenesis and oncogenic signaling, as powerful biomarkers for the identification of those who have or are at high risk of developing cancer, and as oncogene targets for the design and development of new chemotherapeutic drugs. Mutational activation of the K-*ras* proto-oncogene is an early event in the development and progression of the colorectal, pancreatic, and lung cancers that are the major causes of cancer death in the world. The presence of point mutational "hot spots" at sites necessary for the activation of this proto-oncogene has led to the development of a number of highly sensitive PCR-based methods that are feasible for the early detection of K-*ras* oncogene mutations in the clinical setting. In light of these facts, mutation at the K-*ras* oncogene has the potential to serve as a useful biomarker in the early diagnosis and risk assessment of cancers with oncogenic Ras signaling. This chapter described a highly sensitive method for detecting mutant K-*ras*, enriched PCR, and its application to early detection of this oncogene in preneoplastic and early neoplastic lesions of the colon and rectum.

*Reference:

Minamoto T. Detection and characterization of oncogene mutations in preneoplastic and early neoplastic lesions. Methods Mol Biol 291:263-78, 2005.

Publications

1. Ougolkov, A., Yamashita, K., Bilim, V., Takahashi, Y., Mai, M., Minamoto, T. (2003) Abnormal expression of E-cadherin, β-catenin and c-erbB-2 in gastric cancer with liver metastasis. Int J Colorect Dis (Clin Mol Gastroenterol Surg) 18:160-166.
2. Sabet, E.A., Okai, T., Minamoto, T., Ma,i M., Sawabu, N. (2003) Visualizing the gastric wall with a 30-MHz ultrasonic miniprobe: ex vivo imaging of normal gastric sites and sites of early gastric cancer. Abdom Imaging 28:252-256.
3. Morita, H., Hasegawa, T., Minamoto, T., Oda, Y., Inui, K., Tayama, H., Nakao, N., Ideura, T., Yoshimura, A. (2003) Collagenofibrotic glomerulopathy with a widespread expression of type V collagen. Virchow's Archiv 442:163-168.
4. Okai, T., Minamoto, T., Ohtsubo, K., Minato, H., Kurumaya, H., Oda, Y., Mai, M., Sawabu, N. (2003) Endosonographic evaluation of c-kit-positive gastrointestinal stromal tumor. Abdom Imaging 28:301-307.
5. Kawashima, A., Tsugawa, S., Boku, A., Kobayashi, M., Minamoto, T., Nakanishi, I., Oda, Y. (2003) Expression of αv integrin family in gastric carcinomas: increased αvβ6 is associated with lymph node metastasis. Pathol Res Prac 199:57-64.
6. Zhang, B., Ougolkov, A., Yamashita, K., Takahashi, Y., Mai, M., Minamoto, T. (2003) *β-catenin* and *ras* oncogenes detect most human colorectal cancers. Clin Cancer Res 9: 3073-3079.
7. Takahashi, Y., Kitakata, H., Yamashita, K., Yasumoto, K., Omote, K., Minamoto, T., Mai, M. (2004) Pilot study of low dose, divided maximum tolerated dose of CPT-11 in 21 consecutive patients with metastatic colorectal or gastric cancer. Surg Today 34:246-250.
8. Watanabe, H., Yamaguchi, Y., Ohtsubo, K., Mouri, H., Motoo, Y., Yamashita, K., Minamoto, T., Gabata, T., Sawabu, N. (2004) Epidermoid cyst of the intrapancreatic accessory spleen producing CA19-9. Digest Endosc 16:244-248.
9. Ougolkov, A., Zhang, B., Yamashita, K., Bilim, V., Mai, M., Fuchs, S.Y., Minamoto, T. (2004) Associations among β-TrCP, an E3 ubiquitin ligase receptor, β-catenin and NF-κB in colorectal cancer. J Natl Cancer Inst 96:1161-1170.
10. Minamoto, T. (2005) Detection and characterization of oncogene mutations in preneoplastic and early neoplastic lesions. In; Keohavong P and Grant S, eds., Molecular Toxicology Protocols/Methods of Molecular Biology Vol. 291, Humana Press, Totawa, NJ, pp. 263-278.
11. Fuchs, S.Y., Ougolkov, A.V., Spiegelman, V.S., Minamoto, T. (2005) Oncogenic β-catenin signaling networks in colorectal cancer. Cell Cycle 4:1522-1539 (review).

12. Aoki, M., Yamamura, Y., Noshiro, H., Sakai, K., Yokota, J., Kohno, T., Tokino, T., Ishida, S., Ohyama, S., Ninomiya, I., Uesaka, K., Kitajima, M., Shimada, S., Matsuno, S., Yano, M., Hiratsuka, M., Sugimura, H., Itoh, F., Minamoto, T., Maehara, Y., Takenoshita, S., Aikou, T., Katai, H., Yoshimura, K., Takahashi, T., Akagi, K., Sairenji, M., Yamamoto, K., Sasazuki, T. (2005) A full genome scan for gastric cancer. J Med Genet 42:83-87.
13. Misaki, K., Marukawa, K., Hayashi, Y., Fukusato, T., Minamoto, T., Hasegawa, M., Yamashita, J., Fujisawa, H. (2005) Correlation of γ-catenin expression with good prognosis in medulloblastomas. J Neurosurg (Pediatrics 2) 102:197-206.
14. Kawahara, E., Saito, A., Kobayashi, J., Maenaka, S., Minamoto, T., Akasofu-Imai, M., Oda, Y. (2005) Adhesiveness of β5 integrin variant lacking $FNK^{767-769}$ is similar to that of the prototype containing $FNKFNK^{767-769}$. Cell Biol Int 29:521-528.
15. Shakoori, A., Ougolkov, A., Yu, Z.W., Zhang, B., Modarressi, M.H., Billadeau, D.D., Mai, M., Takahashi, Y., Minamoto, T. (2005) Deregulated GSK3β activity in colorectal cancer: its association with tumor cell survival and proliferation. Biochem Biophys Res Commun 334: 1365-1373.
16. Aoki, M., Yamamoto, K., Ohyama, S., Yamamura, Y., Takenoshita, S., Sugano, K., Minamoto, T., Kitajima, M., Sugimura, H., Shimada, S., Noshiro, H., Hiratsuka, M., Sairenji, M., Ninomiya, I., Yano, M., Uesaka, K., Matsuno, S., Maehara, Y., Aikou, T., Sasazuki, T. (2005) A genetic variant in the gene encoding the stress70 protein chaperone family member STCH is associated with gastric cancer in the Japanese population. Biochem Biophys Res Commun, 335:566-574.
17. Kamakura, Y., Hasegawa, M., Kida, S., Tachibana, O., Okamoto, Y., Minamoto, T., Yamashita, J., Fujisawa, H. (2006) c-kit gene mutation is common and widely distributed in intracranial germinomas. J Neurosurg, in press.
18. Maenaka, S., Hikichi, T., Imai-Akasofu, M., Minamoto, T., Kawahara, E. (2006) Loss of imprinting in IGF2 in colorectal carcinoma assessed by microdissection. Oncol Rep, in press.
19. Noubissi, F., Elcheva, I., Bhatia, N., Shakoori, A., Ougolkov, A., Liu, J., Minamoto, T., Ross, J., Fuchs, S.Y., Spiegelman, V.S. CRD-BP mediates stabilization of βTrCP1 and c-myc mRNA in response to β-catenin signaling, in submission with revision.
20. Yamashita, K., Nakazato, H., Ito, K., Ougolkov, A., Takahashi, Y., Mai, M., Minamoto, T. Effect of adjuvant immunochemotherapy with PSK for colon cancer patients showing oncogenic β-catenin activation in primary tumor. J Clin Oncol, in submission.

21. Yamashita, K., Zhang, B., Tamano, Y., Yu, Z.W., Takahashi, Y., Minamoto, T. Serial analysis of gene expression identifies genes associated with nodal metastasis in stomach cancer. Cancer Res, in submission.

Other achievements

1. Minamoto T. A Research Fellow as Scientist Exchange under The U.S.-Japan Cooperative Cancer Research Program, supported by National Cancer Institute (NCI) and Japan Society for the Promotion of Science (JSPS).

   Sponsor and Host Institution: Dr. Ze'ev Ronai, Ph.D., Derald H. Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, NY

   Subject of research: Alterations in transcription factors in human cancers: implication in molecular diagnosis and molecular targeting therapy

   Dates of visit: from November 5th, 2003 to December 18th, 2003

2. Patent application

   Minamoto T.

   Application number: 2005-000133 Country : Japan

   Subject: Development of anti-cancer agents (drugs) inhibiting GSK3β (glycogen synthase kinase 3β)

   Applicant: Kanazawa University

   Date of application: January 4th, 2005

Center for the Development of Molecular Target Drugs

General Summary Center for the Development of Molecular Target Drugs

The purpose of this center is to perform basic research by the full-time researchers of the center (described below) and to organize collaborative research projects based on creative research products of our institute or other research groups, aiming the development of clinically truly effective molecular targeted drugs. We have invited and selected 15 collaborative projects in the last three years, and have shared 10,000,000 JPY for the selected projects. To spread our research outcomes promptly, we have held an annual symposium, and have published an annual report every year. The former director of this center, Prof. Takuma Sasaki retired in March 2005, and Prof. Takashi Suda succeeded to the post.

Research Projects of Suda's Group

A) Molecular mechanism of Fas ligand-induced inflammation

Fas ligand (FasL) is a prototype of death factors that induces apoptosis by binding to its receptor, Fas. We previously demonstrated that anti-Fas ligand antibody administration ameliorates various inflammatory diseases, and prevent chronic-hepatitis-induced hepatic cancer development using animal models. We also demonstrated that Fas ligand-induced caspase activation in neutrophils causes not only apoptosis but also proteolytic maturation and release of IL-1β, which in turn induces massive neutrophil infiltration in vivo. In the last three years, we further demonstrated that Fas ligand induces production of various cytokines including IL-1β, IL-6, IL-17, IL-18, IL-23, KC, and Mip2 in mice. Fas ligand induces IL-23 production in dendritic cells at the mRNA level in a cell-autonomous manner. The IL-23 then induces IL-17 production in T cells synergistically with IL-1β. We also found that Fas ligand induces IL-8 production in human embryonic kidney (HEK) 293 cells in a manner dependent on the NF-κB activation. The Fas ligand-induced NF-κB activation is a cell-autonomous response and requires FADD and caspase-8.

B) Molecular mechanisms of activation and regulation of ASC-mediated inflammation.

ASC is an adaptor molecule that mediates apoptotic and inflammatory signals from several Apaf-1-like molecules, including CARD12/Ipaf, PYPAF1/cryopyrin, PYPAF5, PYPAF7, and NALP1. ASC is also implicated in tumor suppression, because the ASC gene expression is suppressed in various cancer cells. We have established an experimental system in which muramyl dipeptide, the bacterial component recognized by another Apaf-1-like molecule, Nod2, induced an interaction between a CARD12-Nod2 chimeric protein and ASC, and elicited cell-autonomous NF-κB activation leading to IL-8 production. Using this system, we demonstrated that caspase-8 plays an essential role in ASC-mediated NF-κB activation. On the other hand, we have found that some members of Apaf-1-like proteins such as PYNOD, PYPAF2 and PYPAF3 inhibit ASC-mediated NF-κB activation and or caspase-1-mediated

proteolytic maturation of IL-1β.

Research Projects of Dr. Endo

Antitumor Mechanisms of 3'-Ethynyl Nucleosides: Cellular Metabolic Enzyme and Drug Resistance

The antitumor 3'-ethynyl nucleosides, ECyd and EUrd, are potent inhibitors of RNA polymerases and show excellent antitumor activity against various human solid tumors in xenograft models. ECyd and EUrd require the activity of uridine/cytidine kinase (UCK) to produce the corresponding active metabolite. We established cell lines that are highly resistant to the 3'-ethynyl nucleosides using human tumor cell lines. All the resistant cell lines showed a high cross-resistance to ECyd and EUrd and had mutations in the UCK2 gene. UCK2 protein was markedly decreased in the resistant cells. In contrast, no mutation in the mRNA or differences in protein expression levels of UCK1 were shown in the resistant cells. These results suggest that UCK2 is exclusively responsible for the phosphorylation and activation of the antitumor 3'-ethynyl nucleosides. UCK2 may be a useful marker to predict chemosensitivity to antitumor nucleoside analogs including EUrd and ECyd.

Research Projects of Dr. Kuno

A) Effects of ADAMTS-1 on tumor growth and metastasis

In 1997, we identified ADAMTS-1, the first member of the ADAMTS family. We have reported that ADAMTS-1 is an extracellular matrix-anchored metalloproteinase and is able to cleave a cartilage proteoglycan, aggrecan. As ADAMTS-1 has anti-angiogenic activity, we examined the effects of ADAMTS-1 on *in vivo* tumor growth and tumor metastasis. Unexpectedly, full-length ADAMTS-1 was found to promote tumorigenicity. We also found that ADAMTS-1 significantly suppresses the experimental metastasis of CHO cells.

B) Roles of ADAMTS-1 in ovarian function

We have reported that ADAMTS-1 null mice display renal anomalies that resemble human ureteropelvic junction (UPJ) obstruction. As ADAMTS-1 null female mice are infertile, ADAMTS-1 is also important for the function of female reproductive organs. We found that ADAMTS-1 null females showed a reduced ovulation rate of mature follicles in the superovulatory condition, impairment of follicular growth at the late preantral stage, and the appearance of a number of unusual atretic follicles without the granulosa cell layers. These results demonstrate that ADAMTS-1 is involved in both follicular development and ovulation.

Molecular mechanisms of Fas ligand-induced inflammation.

M. Umemura, T. Kawabe, H. Kidoya, K. Shudo, M. Fukui, M. Asano, Y. Iwakura, G. Matsuzaki,A. Yahagi, R. Imamura, and T. Suda

Fas ligand (FasL) has been well characterized as a death factor. However, recent studies revealed that ectopic expression of FasL induces inflammation associated with massive neutrophil infiltration. We previously demonstrated that the neutrophil infiltration-inducing activity of FasL is partly dependent on but partly independent of IL-1β. Therefore, we investigated the cytokine profile of peritoneal lavage fluid obtained from mice that received intraperitoneal injections of FFL, a FasL-expressing tumor cell line. We found that FFL injection caused a marked increase of not only IL-1β but also IL-6, IL-17, IL-18, KC/chemokine CXC ligand 1, and macrophage inflammatory protein (MIP)-2, but not of IL-1α, IFN-γ, TGF-β, or TNF-α. Among cells transfected to express individually IL-1β, IL-6, IL-17, or IL-18, only those expressing IL-1β and IL-17 induced neutrophil infiltration. Co-administration of the anti-IL-17 antibody with FFL diminished the peritoneal KC levels and neutrophil infiltration in IL-1-deficient mice. In addition, the expression of IL-17 by the tumor cells inhibited tumor growth in wild-type and nude mice. These results suggest that IL-17 is involved in FasL-induced inflammation and tumor rejection in the absence of IL-1β.

Then, we investigated the mechanism of the FasL-induced IL-17 production. We found that the culture supernatant of mouse resident peritoneal exudate cells (PEC) cocultured with FFL cells induced IL-17 production in freshly isolated resident PEC. Anti-IL-1β Ab strongly inhibited the IL-17-inducing activity. However, recombinant IL-1β by itself induced only weak IL-17 production. Intriguingly, anti-IL-12 Ab but not an IL-15 neutralizing agent, IL15R-Fc, strongly inhibited the FasL-induced IL-17-inducing activity. IL-23, which shares the p40 subunit with IL-12, but not IL-12 itself, induced IL-17 production synergistically with IL-1β in resident PEC. FasL induced the production of IL-23 in PEC *in vivo* and *in vitro*, and IL-17 production following the i.p. injection of FFL cells was severely impaired in p40-/- mice, indicating that IL-23 plays an important role in the FasL-induced IL-17 production. FFL also induced the production of IL-23 in bone marrow- or PEC-derived dendritic cells. Finally, FasL induced only weak p40 production in a mixture of p40-/- and Fas-/- dendritic cells, indicating that FasL induces IL-23 production in dendritic cells mainly in a cell-autonomous manner.

1. Umemura, M., et al., Int. Immunol., 16:1099-108, 2004

2. Kidoya, H., et al., J. Immunol., 175:8024-31, 2005

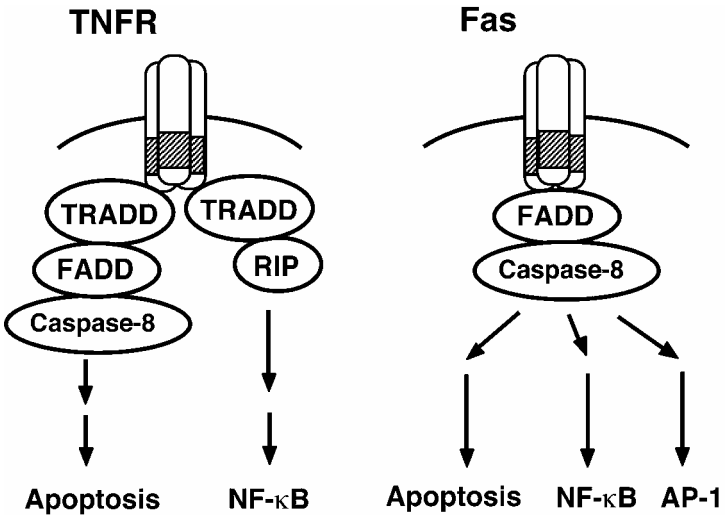
Molecular Mechanism of Fas ligand-induced IL-8 Production

R. Imamura, N. Matsumoto, M. Hasegawa, K. Konaka, and T. Suda

It has been believed that apoptosis does not induce inflammation. However, there are remarkable similarities between the molecular mechanisms of apoptosis and inflammation. Fas (CD95) is not an exception and recent studies revealed that Fas ligand (FasL)-Fas system possesses inflammatory activity. We recently found that FasL induces production of the inflammatory chemokine IL-8 in human cell lines and FasL-induced NF-κB and AP-1 activation is required for this IL-8 production. Our further analyses revealed that the death domain of Fas, FADD, and caspase-8, which are essential for the apoptosis signaling, are required for both NF-κB and AP-1 activation by FasL. However, rsponses of NF-κB and AP-1 activation are independent of each other. In the NF-κB signaling pathway, we also showed that TRADD and RIP, which are essential for the TNF-α-induced NF-κB activation, were not involved in the FasL-induced NF-κB activation and CLARP/FLIP inhibited the FasL- but not the TNF-α-induced NF-κB activation. More interestingly, our results revealed that enzymatic activity of caspase-8 is required for both NF-κB and AP-1 activation induced by FasL. Further characterization of these pathways will help us to understand and, hopefully, to control the FasL-induced inflammation. (Imamura, R. et al., J. Biol. Chem., 279:46415-46423, 2004)

Figure

In the case of FasL-Fas system, contrary to TNF-α-TNFR system, signaling pathway of apoptosis and transcription factor activation separates downstream of caspase-8 and enzymatic activity of caspase-8 is required for both pathways. Current our goal is identification of targets (substrates) of caspase-8, which are important for FasL-induced NF-κB and/or AP-1 activation.

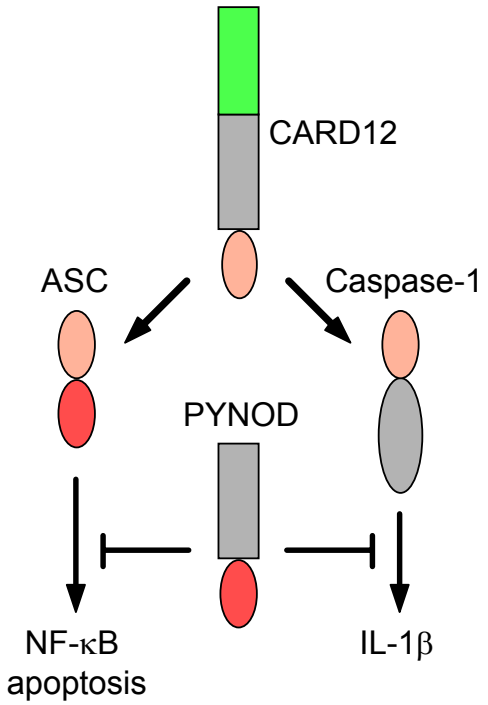


PYNOD, a Novel Apaf-1/CED4-like Protein is an Inhibitor of ASC and Caspase-1

Y. Wang, M. Hasegawa, R. Imamura, T. Kinoshita, C. Kondo, K. Konaka, and T. Suda

Recently, a large subfamily of nucleotide-binding and oligomerization domain-containing proteins that have an N-terminal pyrin-like domain and C-terminal leucine-rich repeats has been described. In this study, we identified PYNOD, a novel member of this family that lacks the leucine-rich repeats. We found that human PYNOD mRNA is expressed in various tissues and at high levels in heart, skeletal muscle, and brain. It is also expressed in various cell lines, including haematopoietic cell lines. PYNOD oligomerizes and binds to ASC, an adaptor protein that plays a role in apoptotic and inflammatory signal transduction, and to caspase-1 and IL-1β. PYNOD inhibits ASC-mediated NF-κB activation and apoptosis, and caspase-1-mediated IL-1β maturation, and it does so in the presence and absence of constitutively active mutants of CARD12 and PYPAF1, which are enhancers of these processes. Thus, PYNOD is a novel regulator of apoptosis and inflammation. (Wang, Y. et al., Int. Immunol. 16:777-86, 2004)

Figure
PYNOD is a member of Apaf-1-like proteins and inhibits ASC-mediated NF-κB activation and apoptosis, and caspase-1-mediated IL-1β activation.



PYPAF3, a PYRIN-containing Apaf-1-like protein, is a feedback regulator of caspase-1-dependent interleukin-1β secretion

T. Kinoshita, Y. Wang, M. Hasegawa, R. Imamura, T. Suda

PYPAF3 is a member of the PYRIN-containing apoptotic protease-activating factor-1-like proteins (PYPAFs) that are thought to function in inflammatory signaling pathways. Among the members of this family, PYPAF1, PYPAF5, PYPAF7, and NALP1 have been shown to induce caspase-1-dependent interleukin-1β secretion and NF-κB activation in the presence of the adaptor molecule ASC. On the other hand, we recently identified PYNOD, another member of this family, as a suppressor of these responses. In this study, we showed that PYPAF3 is the second member that inhibits caspase-1-dependent interleukin-1β secretion (Fig. 1) and that PYPAF2 does not inhibit this response, but rather inhibits the ASC-mediated NF-κB activation (Fig. 2). Both PYPAF2 and PYPAF3 mRNAs are broadly expressed in a variety of tissues; however, neither is expressed in skeletal muscle, and only PYPAF2 mRNA is expressed in heart and brain. They are also expressed in many cell lines of both haematopoietic and non-haematopoietic lineages. Stimulation of monocytic THP-1 cells with lipopolysaccharide or interleukin-1β induced PYPAF3 mRNA expression (Fig. 3). Furthermore, the stable expression of PYPAF3 in THP-1 cells abrogated the cells' ability to produce interleukin-1β in response to lipopolysaccharide. These results suggest that PYPAF3 is a feedback regulator of interleukin-1β secretion. Thus, PYPAF2 and PYPAF3, together with PYNOD, may constitute an anti-inflammatory subgroup of PYPAFs. (Kinoshita, T. et al., J. Biol. Chem. 280:21720-5, 2005)

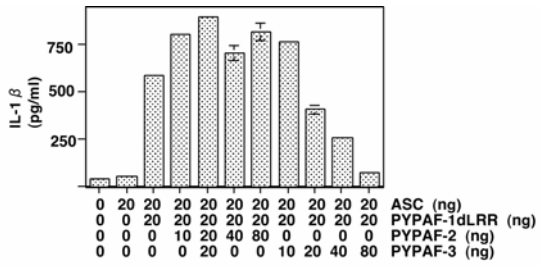


Fig. 1. PYPAF3 inhibits caspase-1-dependent IL-1 β secretion.

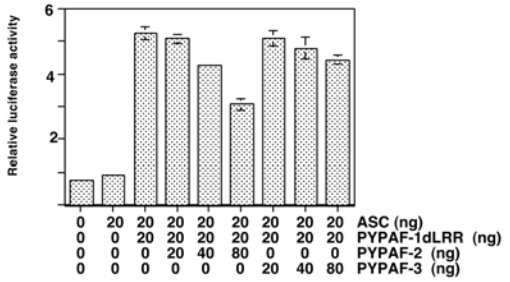


Fig. 2. PYPAF2 inhibits ASC-mediated NF- κ B activation.

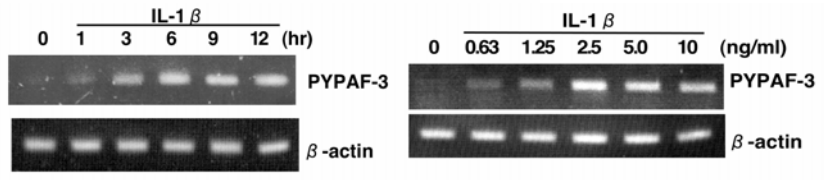


Fig. 3. IL-1 β induces expression of PYPAF3 mRNA in THP-1 cells.

ASC-mediated NF-κB Activation Leading to IL-8 Production Requires Caspase-8 and Is Inhibited by CLARP

M. Hasegawa, R. Imamura, T. Kinoshita, N. Matsumoto, J. Masumoto, N. Inohara, and T. Suda

ASC is an adaptor molecule that mediates apoptotic and inflammatory signals from several Apaf-1-like molecules, including CARD12/Ipaf. ASC is also implicated in tumor suppression, because the ASC gene expression is suppressed in various cancer cells. To characterize the signaling pathway mediated by ASC, we established cell lines in which muramyl dipeptide, the bacterial component recognized by another Apaf-1-like molecule, Nod2, induced an interaction between a CARD12-Nod2 chimeric protein and ASC, and elicited cell-autonomous NF-κB activation. This response required caspase-8, and was suppressed by CLARP/FLIP, an inhibitor of caspase-8. The catalytic activity of caspase-8 was required for the ASC-mediated NF-κB activation when caspase-8 was expressed at an endogenous level, although it was not essential when caspase-8 was overexpressed. In contrast, FADD, the adaptor protein linking Fas and caspase-8, was not required for this response. Consistently, ASC recruited Caspase-8 and CLARP but not FADD and Nod2 to its speck-like aggregates in cells. Finally, muramyl dipeptide induced IL-8 production in MAIL8 cells. These results are the first to indicate that caspase-8 plays an important role in the ASC-mediated NF-κB activation, and that the ASC-mediated NF-κB activation actually induces physiologically relevant gene expression. (Hasegawa M, et al., J. Biol. Chem. 280: 15122-30, 2005.)

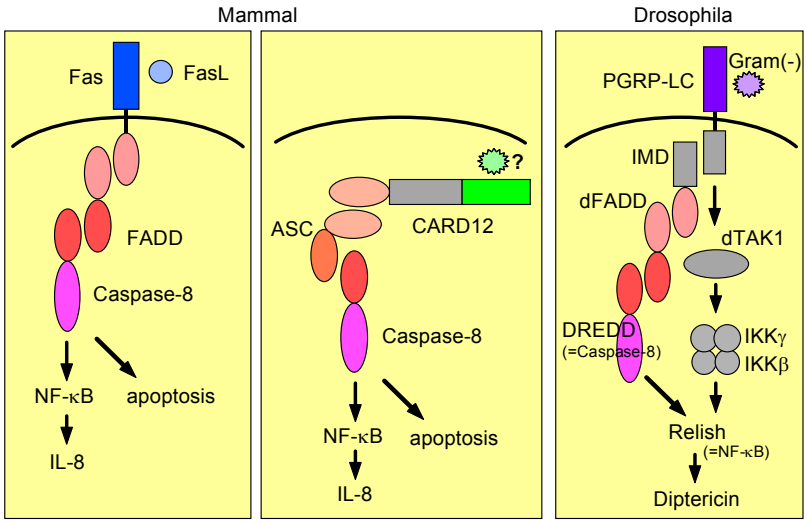


Figure
DREDD, the drosophila homolog of caspase-8 has been shown to play an important role in NF-κB activation. In mammals, caspase-8 also plays an important role in Fas ligand-induced and ASC-mediated NF-κB activation.

A CRUCIAL ROLE OF URIDINE/CYTIDINE KINASE 2 IN ANTITUMOR ACTIVITY OF 3'-ETHYNYL NUCLEOSIDES

Y. Endo, T. Obata, D. Murata, M. Ito, M. Kadohira and T. Sasaki

The antitumor 3'-ethynyl nucleosides, 1-(3-C-ethynyl-β-D-ribopentofuranosyl)cytosine (ECyd) and 1-(3-C-ethynyl-β-D-ribopentofuranosyl)uracil (EUrd), are potent inhibitors of RNA polymerases and show excellent antitumor activity against various human solid tumors in xenograft models. ECyd is being investigated in phase I clinical trials as a novel anticancer drug possessing a unique antitumor action. ECyd and EUrd require the activity of uridine/cytidine kinase (UCK) to produce the corresponding active metabolite (ECTP and EUTP). The UCK family consists of two members, UCK1 and UCK2, and both UCKs are expressed in many tumor cells. It was unclear, however, whether UCK1 or UCK2 is responsible for the phosphorylation of the 3'-ethynyl nucleosides. We therefore established cell lines that are highly resistant to the 3'-ethynyl nucleosides from human fibrosarcoma HT-1080 and gastric carcinoma NUGC-3. All the resistant cell lines showed a high cross-resistance to ECyd and EUrd. As a result of cDNA sequence analysis, we found that UCK2 mRNA expressed in EUrd-resistant HT-1080 cells has a 98-base pair deletion of exon 5, whereas EUrd-resistant NUGC-3 cells were harboring the point mutation at nucleotide position 484 (C to T) within exon 4 of UCK2 mRNA. This mutation was confirmed by genome sequence analysis of the UCK2 gene. In ECyd-resistant NUGC-3 cells, the substitution of G to A at 391 nt of cording region of UCK2 cDNA (Val to Met at codon 131) was found. Moreover, the expression of UCK2 protein and the enzymatic activity were decreased in these resistant cells. In contrast, no mutation in the mRNA or differences in protein expression levels of UCK1 were shown in all the resistant cells. These results suggest that UCK2 is responsible for the phosphorylation and activation of the antitumor 3'-ethynyl nucleosides. Although UCK2 is expressed in many types of tissues and tumor cells, the expression level of UCK2 appears to be very diverse in cells. UCK2 may be a useful marker to predict chemosensitivity to antitumor uridine and cytidine analogs including EUrd and ECyd. In addition, UCK2 may be a novel molecular target for biochemical modulation to potentiate antitumor effects of such nucleoside analogs.

References

1. Murata D, Endo Y, Obata T, Sakamoto K, Syouji Y, Kadohira M, Matsuda A, Sasaki T. (2004) A crucial role of uridine/cytidine kinase 2 in antitumor activity of 3'-ethynyl nucleosides. Drug Metab Dispos. 32(10):1178-82.
2. Tabata S, Tanaka M, Endo Y, Obata T, Matsuda A, Sasaki T. (1997) Anti-tumor mechanisms of 3'-ethynyluridine and 3'-ethynylcytidine as RNA synthesis inhibitors: development and characterization of 3'-ethynyluridine-resistant cells. Cancer Lett. 116(2):225-31.

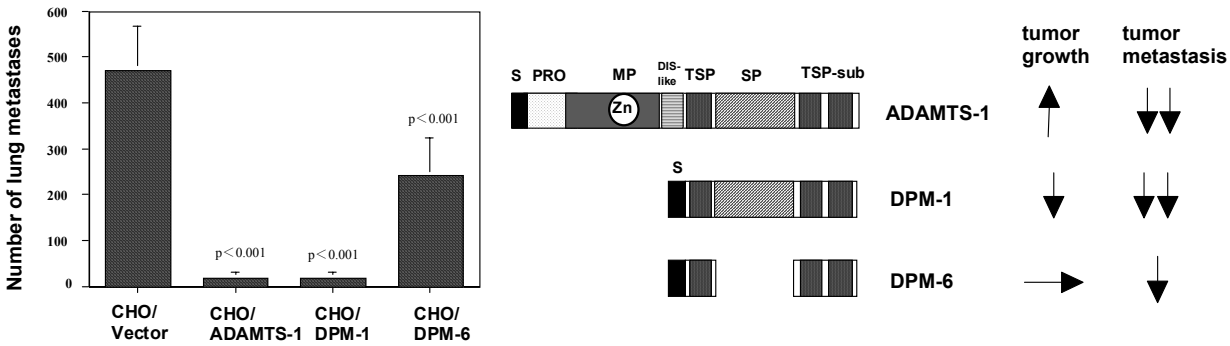
Enhancement of the tumorigenicity of both Lewis lung carcinoma and Chinese hamster ovary cells but suppression of experimental tumor metastatic potential by ADAMTS-1

K. Kuno

ADAMTS-1 is an extracellular matrix (ECM)-anchored metalloproteinase with proteoglycan-degrading activity. As ADAMTS-1 also has an angiogenesis-inhibiting activity, we examined the effects of ADAMTS-1 overexpression on *in vivo* tumor growth and metastasis.

Unexpectedly, we found that overexpression of full-length ADAMTS-1 in both Chinese hamster ovary (CHO) cells (CHO/ADAMTS-1) and Lewis lung carcinoma (LLC) cells (LLC/ADAMTS1) resulted in enhancement of *in vivo* tumor growth, suggesting that ADAMTS-1 can promote tumorigenicity. In contrast, overexpression of only the C-terminal half region of ADAMTS-1 (DPM-1), which lacks the metalloproteinase domain, suppressed CHO tumor growth in mice, suggesting that that the metalloproteinase activity of ADAMTS-1 is required for increased tumorigenicity. We presume that the increased tumorigenicity by ADAMTS-1 results from its potential action to promote degradation of the ECM barriers surrounding tumors or to release growth factors from the ECM.

When the CHO/ADAMTS1 was inoculated intravenously into mice, the number of visible surface lung metastatic nodules markedly decreased in comparison with that of mice implanted with the control vector clone (CHO/vector). Deletional analyses revealed that the C-terminal half region of ADAMTS-1 is responsible for its experimental metastasis-inhibitory activity. Tumor vascular density in the microscopic lung metastases formed by CHO/ADAMTS-1 or CHO/DPM-1 cells were reduced in comparison with that of the control CHO/vector cells. Therefore, one possibility is that ADAMTS-1 may suppress experimental metastasis by inhibiting tumor angiogenesis in the lung metastatic lesion. Our present study suggests that the C-terminal half region of ADAMTS-1 has therapeutic potential as an inhibitor of tumor growth and metastasis when ADAMTS-1 is applied to anti-cancer therapy.

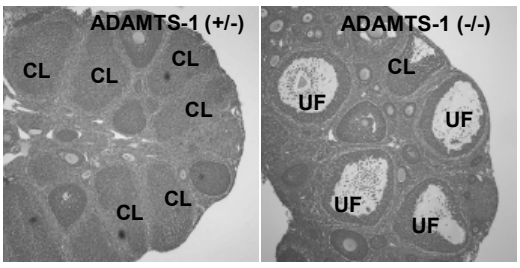


Involvement of ADAMTS-1 in folliculogenesis, ovulation, and organization of the medullary vascular network in the ovary

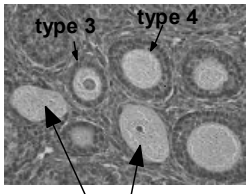
M. Shozu, K. Kuno

ADAMTS-1 null mice displayed renal anomalies involving enlarged calices and atrophic renal papillae, which resemble ureteropelvic junction (UPJ) obstruction in humans. We also found that ADAMTS-1 null female mice were infertile, indicating that ADAMTS-1 is important for the function of female reproductive organs. To clarify the role of ADAMTS-1 in ovarian function, we examined abnormalities in the ovulatory processes, folliculogenesis, and the vascular system of ADAMTS-1 null ovaries.

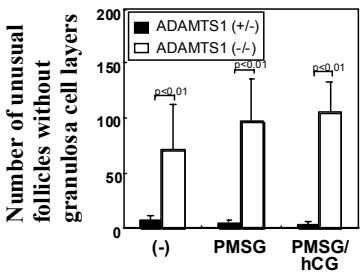
First, when immature female mice were treated with pregnant mare serum gonadotropin (PMSG)/human chorionic gonadotropin (hCG), the number of ovulated oocytes was markedly decreased in ADAMTS-1 null mice when compared with ADAMTS-1 (+/-) controls. The proportion of anovulated follicles to total mature follicles was significantly higher in ADAMTS-1 null females when compared with controls. The numbers of growing follicles at each stage were counted. The number of follicles at type 5b (late preantral) and later stages was markedly reduced in ADAMTS-1 null mice, irrespective of gonadotropin treatment. These data demonstrate that impairment of ovarian function to ovulate oocytes in ADAMTS-1 null mice occurs at two different levels: in the development of growing follicles and in ovulatory processes. Furthermore, ADAMTS-1 null ovaries was found to include a number of unusual atretic follicles that showed no sign of oocyte degeneration but that had lost the surrounding granulosa cell layers and were considered to be derived from type 4 or 5a follicles. These results suggest that ADAMTS-1 is important for follicular development beyond the type 4 and/or 5a and for maintaining normal granulosa cell layers in follicles. Finally, the number of large blood vessels in the medullar zone was significantly decreased in ADAMTS-1 null mice ovaries, suggesting that ADAMTS-1 is also involved in the organization of the medullary vascular network in the ovary.



CL, Corpus luteum UF, Unruptured follicles



Unusual follicles without granulosa cell layers



Publications

1. Kidoya, H., Umemura, U., Kawabe, T., Matsuzaki, G., Yahagi, A., Imamura, R., and Suda, T. (2005) Fas ligand induces cell-autonomous IL-23 production in dendritic cells, a mechanism for Fas ligand-induced IL-17 production. J. Immunol. 175:8024-31.
2. Kinoshita, T., Wang, Y., Hasegawa, M., Imamura, R., and Suda, T. (2005) PYPAF3, a PYRIN-containing APAF-1-like protein, is a feedback regulator of caspase-1-dependent interleukin-1β secretion. J. Biol. Chem. 280:21720-5
3. Hasegawa M, Imamura R, Kinoshita T, Matsumoto N, Masumoto J, Inohara N, and Suda T. (2005) ASC-mediated NF-κB activation leading to interleukin-8 production requires caspase-8 and is inhibited by CLARP. J. Biol. Chem. 280: 15122-30.
4. Suda, T. (2005) Physiological and pathological roles of apoptosis. Nippon Rinsho. Suppl 4:395-400. Review. (Japanese)
5. Moriyama-Kita M, Endo Y, Yonemura Y, Heizmann CW, Miyamori H, Sato H, Yamamoto E, Sasaki T. (2005) S100A4 regulates E-cadherin expression in oral squamous cell carcinoma. Cancer Lett. 230:211-218.
6. Nozaki S, Endo Y, Nakahara H, Yoshizawa K, Hashiba Y, Kawashiri S, Tanaka A, Nakagawa K, Matsuoka Y, Kogo M, Yamamoto E. (2005) Inhibition of invasion and metastasis in oral cancer by targeting urokinase-type plasminogen activator receptor. Oral Oncol. 41:971-7.
7. Yonemura Y, Endo Y, Tochiori S, Bando E, Kawamura T, Shimada T, Miyamoto K, Tanaka M, Sasaki T. (2005) Effect of intraperitoneal chemotherapy on experimental peritoneal dissemination of gastric cancer. Gan To Kagaku Ryoho. 32:1635-9. [in Japanese]
8. Shimada T, Nomura M, Yokogawa K, Endo Y, Sasaki T, Miyamoto K, Yonemura Y. (2005) Pharmacokinetic advantage of intraperitoneal injection of docetaxel in the treatment for peritoneal dissemination of cancer in mice. J Pharm Pharmacol. 57:177-81.
9. Koshida K, Egawa M, Imao T, Mizokami A, Namiki M, Endo Y, Sasaki T. (2005) In vitro chemosensitivity test for human genito-urinary tumors using collagen gel matrix. Int J Urol. 12:67-72.
10. Shozu M, Minami N, Yokoyama H, Inoue M, Kurihara H, Matsushima K, Kuno K (2005) ADAMTS-1 is involved in follicular development, ovulatory process and organization of the medullary vascular network in the ovary. J. Mol. Endocrinol. 35: 343-355.
11. Imamura, R., Konaka, K., Matsumoto, N., Hasegawa, M., Fukui, M., Mukaida, N., Kinoshita, T., and Suda, T. (2004) Fas ligand induces cell-autonomous NF-κB activation and IL-8 production by a mechanism distinct from that of TNF-α. J. Biol. Chem. 279:46415-23.

12. Umemura, M., Kawabe, T., Shudo, K., Kidoya, H., Fukui, M., Asano, M., Iwakura, Y., Matsuzaki, G., Imamura, R., and Suda, T. (2004) Involvement of IL-17 in Fas ligand-induced inflammation. Int. Immunol. 16:1099-108.
13. Wang, Y., Hasegawa, M., Imamura, R., Kinoshita, T., Kondo, C., Konaka, K., Suda, T. (2004) PYNOD, a novel Apaf-1/CED4-like protein is an inhibitor of ASC and caspase-1. Int. Immunol. 16:777-86.
14. Nakamoto Y, Suda T, Momoi T, and Kaneko S. (2004) Different procarcinogenic potentials of lymphocyte subsets in a transgenic mouse model of chronic hepatitis B. Cancer Res. 64:3326-3333
15. Uekita, T., Gotoh, I., Kinoshita, T., Itoh, Y., Sato, H., Shiomi, T., Okada, Y., and Seiki, M. (2004). Membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 is a new member of the Cupin superfamily. A possible multifunctional protein acting as an invasion suppressor down-regulated in tumors. J Biol Chem 279, 12734-43.
16. Takahashi Y, Yamashita K, Endo Y, Sasaki T, Mai M. (2004) Oral administration of uracil-tegafur (UFT) inhibits liver micrometastasis of human colon cancer in an orthotopic nude mouse model and its early detection system. Surg Today. 34:855-9.
17. Kaneko M, Kawakami K, Oyama K, Endo Y, Tanaka M, Sasaki T, Watanabe G. (2004) The association of expression and gene polymorphism of the thymidylate synthase gene with 5-fluoro-5'-deoxyuridine sensitivity in gastric cancer cell lines. Gan To Kagaku Ryoho. 31:1347-50. [in Japanese]
18. Murata D, Endo Y, Obata T, Sakamoto K, Syouji Y, Kadohira M, Matsuda A, Sasaki T. (2004) A crucial role of uridine/cytidine kinase 2 in antitumor activity of 3'-ethynyl nucleosides. Drug Metab Dispos. 32:1178-82.
19. Moriyama-Kita M, Endo Y, Yonemura Y, Heizmann CW, Schafer BW, Sasaki T, Yamamoto E. (2004) Correlation of S100A4 expression with invasion and metastasis in oral squamous cell carcinoma. Oral Oncol. 40:496-500.
20. Yonemura Y, Endo Y, Bando E, Kuno K, Kawamura T, Kimura M, Shimada T, Miyamoto K, Sasaki T, Sugarbaker PH. (2004) Effect of intraperitoneal administration of docetaxel on peritoneal dissemination of gastric cancer. Cancer Lett. 210(2): 189-96.
21. Kuno K., Bannai K., Hakozaki M., Matsushima K., Hirose K. (2004) The carboxyl-terminal half region of ADAMTS-1 suppresses both tumorigenicity and experimental metastatic potential. Biochem. Biophys. Res. Comm. 319: 1327-1333.
22. Kuno K (2004) ADAMTS-1. in Handbook of Proteolytic Enzymes (Second Edition) (Barrett A.J., Rawlings N.D., and Woessner J. F., eds.), London, Academic Press 735-737.

23. Fukui, M., Imamura, R., Umemura, M., Kawabe, T., Suda, T. (2003) Pathogen-associated molecular patterns sensitize macrophages to Fas ligand-induced apoptosis and IL-1beta release. J. Immunol.. 171:1868-74.
24. Tachiiri, A., Imamura, R., Wang, Y., Fukui, M., Umemura, M., and Suda, T. (2003) Genomic structure and inducible expression of the IL-22 receptor α chain in mice. Gene. Immun., 4:153-159
25. Endo Y, Tanaka M, Obata T, Sasaki T（2003）Experimental model for studying metastasis using the developing embryonic chick. Nippon Rinsho. 61(Suppl. 8): 9-54. [in Japanese]
26. Yonemura Y, Sakurai S, Yamamoto H, Endo Y, Kawamura T, Bandou E, Elnemr A, Sugiyama K, Sasaki T, Akiyama T, Takasawa S, Okamoto H. (2003) REG gene expression is associated with the infiltrating growth of gastric carcinoma. Cancer. 98:1394-400.
27. Yonemura Y, Bandou E, Kinoshita K, Kawamura T, Takahashi S, Endo Y, Sasaki T. (2003) Effective therapy for peritoneal dissemination in gastric cancer. Surg Oncol Clin N Am. 12:635-48. Review.
28. Elnemr A, Yonemura Y, Bandou E, Kinoshita K, Kawamura T, Takahashi S, Tochiori S, Endo Y, Sasaki T. (2003) Expression of collagenase-3 (matrix metalloproteinase-13) in human gastric cancer. Gastric Cancer. 6:30-8.

Meetings and Seminars

第 24 回 がん研究所セミナープログラム

2003(平成 15).2.26～27
金沢大学医学部記念館 2 階ホール

◆ 腫瘍外科研究分野

○サマリー	磨伊正義
○新しい抗癌剤の濃度設定法，individualized maximum repeatable dose(iMRD)の開発とそれによるテイラード化学療法	高橋　豊
○早期胃癌に対する Minimally Invasive Surgery	表　和彦
○ヒト大腸癌細胞株同所性移植自然転移モデルを用いた転移関連因子群の解析	安本和生

◆ 遺伝子診断研究分野

○サマリー	源　利成
○β-カテニンがん化シグナルの特異的活性化・制御機構の個別的，網羅的解析	源　利成

◆ 腫瘍内科研究分野

○サマリー	澤武紀雄
○超音波内視鏡研究２０年の軌跡	岡井　高
○ヒト膵癌における clusterin の発現とその臨床的意義	元雄良治
○膵癌患者血漿中 p53 遺伝子変異の検出	山口泰志
○膵癌組織ならびに膵液における mesothelin mRNA 発現の検討	渡邊弘之

◆ 細胞分子病態研究分野

○サマリー	山本健一
○DNA 損傷修復経路における c-Abl チロシンキナーゼファミリー分子の機能	清水弘子
○の解析	
○DNA 損傷，酸化ストレスに対する ATM 依存的シグナル伝達の解析	小林昌彦

◆ 細胞情報調節研究分野

○サマリー	原田文夫
○Ｂ型肝炎ウィルスの構造蛋白質と結合する細胞蛋白質	黒木和之
○リン酸化 RNA ポリメラーゼⅡ結合因子の機能検索	廣瀬　豊
○rRNA 生合成における U13 snoRNA の機能	木戸敬治

◆ 細胞周期制御研究分野

○サマリー	善岡克次

◆ 細胞分化研究分野

○サマリー	髙倉伸幸
○世界各地に生息する野生ハツカネズミにこれまで発見された４種類のアロタイプの分布と環境との関係	坂井俊之助
○線虫で RNAi 法による新規遺伝子 ell の機能解析	天野重豊
○血管内皮細胞特異的受容体型チロシンキナーゼ TIE2 により活性化される	上野将也

遺伝子の同定とその機能解析

◆ 遺伝子発現研究分野

◆ 細胞機能統御研究分野

◆ 組織分子構築研究分野

◆ 分子薬理学研究分野

◆ 化学療法研究分野

◆ 分子標的薬剤開発センター

◆ 遺伝子・染色体構築研究分野

第 25 回　がん研究所セミナープログラム

2004(平成 16).2.5～6
金沢大学医学部記念館 2 階ホール

◆腫瘍外科研究分野
○サマリー　磨伊正義
○Tumor dormancy therapy に基づく Tailored dose 化学療法 -根拠，意義，実際-　高橋豊
○視線追従式自動腹腔鏡操作システムの開発 -腹腔鏡手術において術者の負担を軽減する Robotic Assistance-　表和彦
○胃癌腹膜播種形成における SDF-1a/CXCR4 リガンド・レセプターシステムの関与　安本和生

◆遺伝子診断研究分野
○サマリー　源利成
○大腸癌のがん化シグナルの特異的活性化・制御機構の解析　源利成

◆腫瘍内科研究分野
○サマリー　澤武紀雄
○ラット自然発症慢性膵炎モデルにおけるアポトーシス関連遺伝子の発現動態　元雄良治
○Real-time PCR 法による膵癌患者の血清中 hTERTmRNA の定量的測定　渡邊弘之
○各種消化器癌患者における血清 Midkine 値の測定　山口泰志
○膵癌診断を目指した膵液中 ppENK のメチル化異常に関する検討　大坪公士郎

◆分子標的薬剤開発センター
○サマリー　須田貴司
○ADAMTS-1 の卵胞成熟，排卵過程への関与と ADAMTS-1 による実験的肺転移抑制　久野耕嗣
○血管新生阻害性 ANO 化合物の水溶化による bioavailabit の改善　遠藤良夫
○Fas リガンドによる NF-kB 活性化機構の解析　今村龍
○アポトーシス促進因子 Bid の制御機構　木下健

◆化学療法研究分野
○サマリー　佐々木琢磨
○抗腫瘍性ヌクレオシドの感受性規定因子の解明：CDA の関与と網羅的遺伝子発現解析法による探索　小幡徹

◆遺伝子・染色体構築研究分野
○サマリー　伊藤隆司
○パラレルアフィニティタグ精製法によるユビキチン化蛋白質の網羅的同定　太田一寿

◆組織分子構築研究分野
○サマリー　向田直史
○セリン／スレオニンキナーゼ Pim-3 の肝細胞がんにおける役割　藤井千文

第 26 回　がん研究所セミナープログラム

2005(平成 17).3.3～4
金沢大学医学部臨床第一講義室

◆腫瘍外科研究分野
- ○サマリー　磨伊正義
- ○VEGF を標的とした Tumor dormancy therapy　高橋豊
- ○早期胃大腸癌に対する腹腔鏡手術　表和彦
- ○胃癌腹膜播種形成における SDF-1a / CXCR4 リガンド・レセプターシステムの関与　安本和生

◆遺伝子診断研究分野
- ○サマリー　源利成
- ○大腸癌における b-TrCP(E3 ユビキチンリガーゼ受容体)の発現とがん化シグナル活性化　源利成

◆腫瘍内科研究分野
- ○サマリー　澤武紀雄
- ○胃癌における stress-induced protein (SIP)の発現低下について　元雄良治
- ○膵液中 SARP2 のメチル化測定による膵癌診断へのアプローチ　渡邊弘之
- ○膵管内乳頭腫瘍(IPMN)における膵液中遺伝子のメチル化異常に関する検討　大坪公士郎
- ○血清 Midkine 値の腫瘍マーカーとしての意義　毛利久継

◆分子標的薬剤開発センター
- ○サマリー　須田貴司
- ○ADAMTS-1 の卵巣機能における役割と実験的肺転移抑制機構の解析　久野耕嗣
- ○Fas リガンド刺激による IL-8 産生の分子機構　今村龍
- ○PYPAF ファミリーによる炎症抑制機構　木下健

◆化学療法研究分野
- ○サマリー　佐々木琢磨
- ○抗腫瘍性エチニルヌクレオシドの耐性機序と感受性規定因子　遠藤良夫
- ○ヌクレオシドトランスポーターと抗腫瘍性ヌクレオシドに対する感受性との関連性　小幡徹

◆組織分子構築研究分野
- ○サマリー　向田直史
- ○セリン／スレオニンキナーゼ Pim-3 の肝細胞がんにおける役割　藤井千文

◆遺伝子発現研究分野
- ○サマリー　村上清史
- ○細胞老化を制御する信号伝達系の探索　林直之

◆細胞機能統御研究分野
- ○サマリー　佐藤博

International Symposium on Tumor Biology in Kanazawa 2003

Program Schedule

March 12,Wed.

Opening Remarks : **Ken-ichi Arai** (IMS, University of Tokyo)

Session A. Search for Novel Targets for Cancer Treatment

Chairperson: **Takashi Ito** (Cancer Res. Inst., Kanazawa Univ.)

Masa-aski Muramatsu (Tokyo Medical and Dental University)

Application of single nucleotide polymorphism (SNP) to clinical and epidemiological studies

Satoshi Inoue (University of Tokyo)

Estrogen responsive genes and breast cancer

John N. Weinstein (NIH, USA)

Integromics for cancer drug discovery: integrating genomics, proteomics, and bioinformatics

Chairperson: **Tadashi Yamamoto** (IMS, University of Tokyo)

Masafumi Shibuya (IMS, University of Tokyo)

A unique signal transduction of VEGF receptors involved in tumor angiogenesis and metastasis

Kyu-Won Kim (Seoul National University)

Novel HIF-1α interacting proteins and their roles in hypoxia-induced tumor angiogenesis

Chairperson: **Hiroshi Sato** (Cancer Res. Inst., Kanazawa Univ.)

Motoharu Seiki (IMS, University of Tokyo)

Membrane-type 1 matrix metalloproteinase (MT1-MMP) in cancer invasion and angiogenesis

Toshinari Minamoto (Cancer Res. Inst., Kanazawa Univ.)

Distinct pattern of oncogenic β-catenin activation in colorectal cancer

Makoto Taketo (Kyoto University)

Mouse models for colon cancer: studies on chemoprevention and chemotherapy

March 13,Thu.

Session B. Novel Approaches to Cancer Treatment

Chairperson: **Masafumi Shibuya** (IMS, University of Tokyo)

Michael O'Reilly (MD Anderson Cancer Center, USA)

Angiogenesis : from the laboratory to the Clinic

Yutaka Takahashi (Kanazawa University)

Tumor Dormancy Therapy-paradigm shift of cancer therapy

Chairperson: **Naofumi Mukaida** (Cancer Res. Inst., Kanazawa Univ.)

Mark Smyth (Peter MacCallum Cancer Institute)

Effectors and regulators of cancer immune surveillance

Akira Yamada (Kurume University)

Development of peptide-based vaccine for epithelial cancer

Chairperson: **Nobuyuki Takakura** (Cancer Res. Inst., Kanazawa Univ.)

Tatsutoshi Nakahata (Kyoto University)

Ex vivo expansion of human hematopoietic stem cells for a variety of clinical applications

Richard Childs (NIH)

Using nonmeyloablative conditioning to expand the role of allogeneic stem cell transplantation

Closing Remarks : **Ken-ichi Yamamoto** (Cancer Research Institute, Kanazawa University)

International Symposium on Tumor Biology in Kanazawa 2004

Program Schedule

February 12, Thu.

Opening Remarks : **Ken-ichi Yamamoto** (Cancer Res. Inst. Kanazawa Univ.)

Session A.

Chairperson: **Naofumi Mukaida** (Cancer Res. Inst., Kanazawa Univ.)

Kenkichi Masutomi (Dana-Farber Cancer Institute)

Functional role of telomerase in normal human cells

Satoru Kyo (Graduate School of Medicine, Kanazawa Univ.)

Recent advances in telomerase-based medicine for targeting cancers

R.Dalla-Favera (Institute for Cancer Genetics, Columbia University)

Molecular pathogenesis of B cell lymphoma

Tadashi Yamamoto (IMS, University of Tokyo)

The tumor suppressor protein Tob functions as a transcriptional coregulator

Chairperson: **Tadaomi Takenawa** (IMS, University of Tokyo)

Yoshinori Murakami (National Cancer Center Research Institute)

Involvement of a cell adhesion molecule, TSLC1, in human oncogenesis

Yoh Takuwa (Kanazawa Univ. Graduate School of Medicine)

Bimodal regulation by the S1P-Edg signaling system of Rho family GTPase and invasion/metastasis in cancer cells

Yoshimi Takai (Osaka University Graduate School of Medicine)

Nectins and necls: Roles in cell migration, adhesion, and proliferation

February 13, Fri.

Session B.

Chairperson: **Hiroshi Sato** (Cancer Research Institute, Kanazawa Univ.)

Seishi Murakami (Cancer Research Institute, Kanazawa University)

Hepatitis C Virus (HCV) NS5B, a central catalytic enzyme for HCV replication

Claus Scheidereit (Max-Delbruck-Center for Molecular Medicine , Germany)

NF-κB/IKK and AP-1 pathways as molecular targets in human lymphomas

Tomohisa Kato (Biosignal Research Center, Kobe University)

NF-κB signaling in the stress response: Cellular response to environmental carcinogens

Jun-ichiro Inoue (IMS, University of Tokyo)

TRAF6-NFκB pathway in cell growth and differentiation

Chairperson: **Ken-ichi Yamamoto**

Makoto Nakanishi (Nagoya City University)

Chk1, an essential key player that regulates S/M phase transition

Junjie Chen (Mayo Clinic)

Regulation of DNA damage signaling pathways

Closing Remarks : **Tadashi Yamamoto** (IMS, University of Tokyo)

International Symposium on Tumor Biology in Kanazawa 2005

Program Schedule

January 20, Thu.

Opening Remarks : **Ken-ichi Yamamoto** (Cancer Res. Inst., Kanazawa Univ.)

Session A. Stem Cell Biology in Cancer and Normal Stem Cell

Chairperson: **Yoh Takuwa** (Kanazawa Univ. School of Medicine)

Hiroshi Koide (Kanazawa Univ. School of Medicine)

Regulation of proliferation and differentiation in embryonic stem cells

Yumi Matsuzaki (Keio Univ. School of Medicine)

Blood specific differentiation potency of purified HSC

Chairperson: **Naofumi Mukaida** (Cancer Res. Inst., Kanazawa Univ.)

Atsushi Hirao (Keio University School of Medicine)

Essential role of ATM (ataxia telangiectasia mutated) on stem cell self-renewal and tumor suppression

In-Kyung Park (University of Michigan, USA)

Regulation of self-renewal in normal and cancer stem cells

Session B. New Targets for Cancer Treatment

Chairperson: **Takashi Yokota** (Kanazawa Univ. School of Medicine)

JaeHun Cheong (Pusan National Univ., Korea)

Molecular mechanism of hepatic carcinogenesis by HBx

Shigeki Higashiyama (Ehime University School of Medicine)

Ectodomain shedding of HB-EGF and novel cell signaling

Chairperson: **Takashi Suda** (Cancer Res. Inst., Kanazawa Univ.)

Margaret Quinlan (MGH Cancer Center, USA)

Tumorigenesis from primary epithelial cells

Osamu Hori (Kanazawa Univ. School of Medicine)

Regulation of ER stress and ER stress-induced cell death in F9 Herp KO cells

Session C. Molecular Mechanism of Tumor Angiogenesis

Chairperson: **Hiroshi Sato** (Cancer Res. Inst., Kanazawa Univ.)

Nobuyuki Takakura (Cancer Res. Inst., Kanazawa Univ.)

Molecular regulation of blood vessel maturation

Hajime Kubo (Kyoto University School of Medicine)

Lymphangiogenesis and cancer

Closing Remarks : **Ken-ichi Yamamoto** (Cancer Res. Inst., Kanazawa Univ.)

Organization and Personnel

as of February, 2006

Cancer Research Institute
Successive Directors and Acting Directors of the Institute

Dr. Hajime Okamoto	(岡本　肇)	June	1, 1967	～	March	31, 1968
Dr. Tachiomaru Ishikawa	(石川太刀雄丸)	April	1, 1968	～	March	31, 1971
Dr. Ryo Itoh	(伊藤　亮)	April	1, 1971	～	April	1, 1978
Dr. Saburo Koshimura	(越村　三郎)	April	2, 1978	～	April	1, 1982
Dr. Yoriaki Kurata	(倉田　自章)	April	2, 1982	～	April	1, 1984
Dr. Motoichi Hatano	(波田野基一)	April	2, 1984	～	March	31, 1988
Dr. Shunsuke Migita	(右田　俊介)	April	1, 1988	～	March	31, 1990
Dr. Tadanori Kameyama	(亀山　忠典)	April	1, 1990	～	March	31, 1993
Dr. Morinobu Takahashi	(高橋　守信)	April	1, 1993	～	March	31, 1997
Dr. Masayoshi Mai	(磨伊　正義)	April	1, 1997	～	March	31, 2001
Dr. Ken-ichi Yamamoto	(山本　健一)	April	1, 2001	～	March	31, 2005
Dr. Hiroshi Sato	(佐藤　博)	April	1, 2005	～	Present	

Department of Molecular Oncology

Division of Molecular Biology

Division Chief,Professor	Seishi Murakami	(村上　清史)
Research Associate	Naoyuki Hayashi	(林　直之)
Research Technician	Kazuko Kuwabara	(桑原　和子)
Technician	Mami Yasukawa	(安川　麻美)
Graduate Student	Takashi Kusakawa	(草川　貴史)
Graduate Student	Hideki Mizuno	(水野　秀城)
Graduate Student	Shijun Zhang	(張　世俊)
Research Fellow	Hong Tang	(唐　紅)

Division of Virology

Division Chief,Professor	Hiroshi Sato	(佐藤　博)
Associate Professor	Takahisa Takino	(滝野　隆久)
Research Associate	Hisashi Miyamori	(宮森　久志)
Technician	Sayuri Yamagishi	(山岸小百合)
Graduate Student	Munirah Ahmad	(ムニラ　アハマド)
Graduate Student	Abdelaziz Abdalla	(アブデエルアジス　アブダラ)
Graduate Student	Keattikunpairoj Sunisa	(ケアティクンパイロ　スニサ)
Graduate Student	Tomoya Kudo	(工藤　知也)
Graduate Student	Yumi Saeki	(佐伯　裕美)
Graduate Student	Miyuki Matsui	(松井　美雪)

Division of Molecular Bioregulation

Division Chief,Professor	Naofumi Mukaida	(向田　直史)
Research Associate	Chifumi Fujii	(藤井　千文)
Research Technician	Kuniko Minami	(南　　邦子)
Graduate Student	Xiaoqin Yang	(羊　　暁勤)
Graduate Student	Boryana Popivanova	(ボリアナ　ポピバノーバ)
Graduate Student	Wu Yu	(呉　　侯)
Graduate Student	Jorge Espinoza	(ホルヘ　エスピノーザ)
Research Fellow	Mariko Akiyama	(秋山萬里子)
Research Fellow	Li Ying Yi	(李　　影奕)

Division of Molecular Genetics

Division Chief,Professor	Atsushi Hirao	(平尾　　敦)
Research Associate	Kazuhito Naka	(仲　　一仁)
Technician	Miyako Takegami	(竹上美也子)
Technician	Chiharu Minami	(南　　千晴)
Research Fellow	Masako Omura	(大村　昌子)

Division of Macromolecular Biochemistry

Visiting Professor	Wei Wenxiang	(魏　　文祥)

Department of Molecular and Cellular Biology

Division of Cell Biology

Division Chief,Professor	Fumio Harada	(原田　文夫)
Associate Professor	Kazuyuki Kuroki	(黒木　和之)
Research Associate	Yutaka Hirose	(廣瀬　　豊)
Research Associate	Yukiharu Kido	(木戸　敬治)
Technician	Toyoko Kikukawa	(菊川　豊子)
Graduate Student	Shunji Ishii	(石井　俊二)
Graduate Student	Masamichi Yuda	(湯田　昌道)

Division of Stem Cell Biology

Division Chief,Professor	Nobuyuki Takakura	(髙倉　伸幸)
Research Associate	Shigetoyo Amano	(天野　重豊)
Research Associate	Masaya Ueno	(上野　将也)
Research Associate	Yoshihiro Yamada	(山田　賢裕)
Technician	Yoriko Nakano	(中野　頼子)
Graduate Student	Wen-Ling Li	(李　　文玲)
Graduate Student	Kong Lingyu	(孔　　令宇)
Graduate Student	Huang Xiaoyong	(黄　　暁勇)
Graduate Student	Shinya Katoh	(加藤　真也)

Graduate Student	Hiroyasu Kidoya	(木戸屋浩康)
Graduate Student	Ivan Hristov Vachkov	(イバン　フリストフ　ヴァチ)
Graduate Student	Han Yonglu	(韓　　瑛路)
Graduate Student	Hisamichi Naitoh	(内藤　尚道)
Graduate Student	Sanae Akiba	(秋場　早苗)
Graduate Student	Machiko Itoh	(伊藤真知子)
Graduate Student	Naoko Takahashi	(高橋　直子)
Research Fellow	Rika Okamoto	(岡本　里香)
JSPS Research Fellow	Hideto Sano	(佐野　秀人)
JST Technical Assistant	Yuri Shimizu	(清水　友里)
JST Technical Assistant	Kumi Ishida	(石田　久美)
JST Technical Assistant	Miho Sato	(佐藤　美帆)

Division of Molecular Pathology

Division Chief,Professor	Ken-ichi Yamamoto	(山本　健一)
Research Associate	Hiroko Shimizu	(清水　弘子)
Research Associate	Masahiko Kobayashi	(小林　昌彦)
Technician	Kiyoko Take	(武　紀代子)
Graduate Student	Aki Takahashi	(高橋　亜紀)
Graduate Student	Makiko Ikei	(池井　牧子)
Graduate Student	Satoko Kitayama	(北山　聡子)
Research Fellow	Keiichi Muraoka	(村岡　恵一)

Division of Cell Cycle Regulation

Division Chief,Professor	Katsuji Yoshioka	(善岡　克次)
Research Associate	Hiroshi Tanahashi	(棚橋　　浩)
Research Associate	Naoko Taniura	(谷浦　直子)
Technician	Tomoe Ohashi	(大橋　智江)
Graduate Student	Bayarsaikhan Munkhuu	(バイヤルサハン　ムンフォー)
Graduate Student	Davaakhuu Gantulga	(ダバーク　ガントウルガ)
Graduate Student	Asuka Iwanaga	(岩永　飛鳥)
Graduate Student	Tokiharu Sato	(佐藤　時春)
Graduate Student	Masashi Shimizu	(清水　雅司)
Graduate Student	Guangmin Wang	(王　　光敏)
Research Fellow	Youichi Yamada	(山田　洋一)
Research Fellow	Jun Nishida	(西田　　純)

Department of Basic and Clinical Oncology

Division of Genetics

Division Chief,Professor	Masanobu Oshima	(大島　正伸)
Research Associate	Hiroko Oshima	(大島　浩子)
Technician	Manami Watanabe	(渡邊真奈美)

Division of Experimental Therapeutics

Research Associate	Tohru Obata	(小幡　徹)
Technician	Michiyo Aikawa	(相川みち代)
Graduate Student	Norimichi Ito	(伊藤　教道)
Graduate Student	Daisuke Matano	(俣野　乃輔)

Division of Medical Oncology

Division Chief,Professor	Norio Sawabu	(澤武　紀雄)
Associate Professor	Hiroyuki Watanabe	(渡邊　弘之)
Lecturer	Koushiro Ohtsubo	(大坪公士郎)
Research Associate	Hisatsugu Mouri	(毛利　久継)
Research Associate	Tomoya Tsuchiyama	(土山　智也)
Technician	Tomoyo Irie	(入江　朋代)
Technician	Keiko Okamoto	(岡本　恵子)
Research Technician	Gensaku Okada	(岡田　源作)
Graduate Student	Peihong Jiang	(姜　培紅)
Research Fellow	Yoshitake Satomura	(里村　吉威)
Research Fellow	Tokio Wakabayashi	(若林　時夫)
Research Fellow	Osamu Yamakawa	(山川　治)
Research Fellow	Hideki Ohta	(太田　英樹)
Research Fellow	Yasushi Yamaguchi	(山口　泰志)
Research Fellow	Yao Fan	(姚　凡)

Division of Surgical Oncology

Associate Professor	Yutaka Takahashi	(高橋　豊)
Lecturer	Kazuhiko Omote	(表　和彦)
Research Associate	Kazuo Yasumoto	(安本　和生)
Resident	Kaname Yamashita	(山下　要)
Resident	Hidekazu Kitagata	(北方　秀一)
Technician	Toshie Tanaki	(棚木　敏恵)
Technician	Hiroko Horita	(堀田　博子)
Research Fellow	Tohru Itoh	(伊藤　透)
Research Fellow	Takahito Ohta	(太田　孝仁)
Research Fellow	Michio Watanabe	(渡辺美智夫)
Research Fellow	Yasushi Deguchi	(出口　康)
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Center for the Development of Molecular Target Drugs

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Dr. Takashi Suda　(須田　貴司)	April 1, 2005 ～	Present

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