

# Cancer Science

Open Access

The official journal of the Japanese Cancer Association

## Issue Highlights

Phosphorylation-mediated regulation of  
GSK3 $\beta$

Inhibition of Hedgehog pathway inhibits  
clonogenic growth of primary AML cells

Mechanisms of action of oncolytic virus therapy

# Cancer Science

The official journal of the Japanese Cancer Association

**Editor-in-Chief/Editor for Review Articles**

Yusuke Nakamura *The University of Chicago*

**Deputy Editor-in-Chief**

Hiroyuki Mano *The University of Tokyo*

**Editors**

Yutaka Kawakami  
*Keio University*

Kohei Miyazono  
*The University of Tokyo*

Hitoshi Nakagama  
*National Cancer Center*

Tomoki Naoe  
*Nagoya Medical Center*

Masahide Takahashi  
*Nagoya University*

**Editor Emeritus**

Takashi Sugimura

**Associate Editors**

Richard J. Ablin  
Hiroyuki Aburatani  
Koichi Akashi  
Hideyuki Akaza  
Tetsuo Akimoto  
Hirotoshi Akita  
Shin-ichi Akiyama  
Tetsu Akiyama  
Yuichi Ando  
Daisuke Aoki  
Masahiro Aoki  
Hirofumi Arakawa  
Suk-Chul Bae  
Yung-Jue Bang  
William T. Beck  
Maarten C. Bosland  
Xuetao Cao  
Anthony T.C. Chan  
Ceshi Chen  
Yao-Tseng Chen  
Shigeru Chiba  
Toshihiko Doi  
Tommaso A. Dragani  
Keigo Endo  
Hiroyasu Esumi  
Soldano Ferrone  
Naoya Fujita  
Toshiyoshi Fujiwara  
Yasuhiro Fujiwara  
Kiyoko Fukami  
Masashi Fukayama  
Yoichi Furukawa  
Koichi Goto  
Noriko Goto  
Michael M. Gottesman  
Parry Guilford  
Mingzhou Guo  
Koichi Hagiwara  
Nobuyuki Hamajima  
Eiji Hara  
Hideyoshi Harashima  
Curtis C. Harris  
Kiyohiko Hatake  
Masanori Hatakeyama  
Carl-Henrik Heldin  
Shigeki Higashiyama  
Okio Hino  
Atsushi Hirao  
Masahiro Hiraoka  
Seiichi Hirota  
Akira Hori  
Chung-cheng Hsieh  
Kay Huebner  
Hidenori Ichijo  
Shinsuke Iida  
Hiroaki Ikeda

Katsumi Imaida  
Takeshi Imamura  
Issei Imoto  
Masaya Imoto  
Masaki Inagaki  
Johji Inazawa  
Jun-ichiro Inoue  
Manami Inoue  
Masahiro Inoue  
Satoshi Inoue  
Takashi Ishida  
Genichiro Ishii  
Hideshi Ishii  
Fuyuki Ishikawa  
Yuichi Ishikawa  
Chikashi Ishioka  
Kyogo Itoh  
Hirotaka Iwase  
David H. Johnson  
Kenji Kadomatsu  
Takehiko Kamijo  
Yae Kanai  
Yuzuru Kanakura  
Atsushi Kaneda  
Yasufumi Kaneda  
Shuichi Kaneko  
Mari Kannagi  
Reiji Kannagi  
Masanori Kasahara  
Toyomasa Katagiri  
Kazunori Kataoka  
Mitsuyasu Kato  
Elliott Kieff  
Akira Kikuchi  
Chul-Woo Kim  
Kyu-Won Kim  
Seong-Jin Kim  
Shinya Kimura  
Tomohiro Kinoshita  
Issay Kitabayashi  
Yuko Kitagawa  
Toshio Kitamura  
Chifumi Kitanaka  
Hitoshi Kiyoi  
Tohru Kiyono  
Shinae Kizaka-Kondoh  
Alexander Knuth  
Kimitoshi Kohno  
Takashi Kohno  
Kazuhiko Koike  
Yutaka Kondo  
Koichi Kono  
Mineo Kurokawa  
Michinori Kuwano  
Kiyotaka Kuzushima  
Jin Li

Jincai Luo  
Yoshihiko Maehara  
Taira Maekawa  
Yoshiro Maru  
Kenkichi Masutomi  
Michiyuki Matsuda  
Yasuhiro Matsumura  
Keitaro Matsuo  
Masao Matsuo  
Yoshio Miki  
Hironobu Minami  
Kinuko Mitani  
Tetsuya Mitsudomi  
Kiyoshi Miyagawa  
Satoru Miyano  
Keiji Miyazawa  
Eiji Miyoshi  
Morito Monden  
Masaki Mori  
Eiichi Morii  
Hozumi Motohashi  
Yoshinori Murakami  
Yohji Nagashima  
Chisato Nagata  
Mikihiko Naito  
Yusaku Nakabeppu  
Kei Nakachi  
Hidewaki Nakagawa  
Kazuhiko Nakagawa  
Masayuki Nakagawa  
Akira Nakagawara  
Shigeo Nakamura  
Takuro Nakamura  
Makoto Nakanishi  
Ichiro Nakano  
Mitsuyoshi Nakao  
Tetsuya Nakatsura  
Eiichi Nakayama  
Keiichi Nakayama  
Toshinori Nakayama  
Yoshiro Niitsu  
Toshiro Niki  
Hiroyoshi Nishikawa  
Yasuhiro Nishimura  
Kazuto Nishio  
Tetsuo Noda  
Masayuki Noguchi  
Shinzaburo Noguchi  
Norio Nonomura  
Atsushi Ochiai  
Takashi Ochiya  
Tatsuya Oda  
Yoshinao Oda  
Katsuhiko Ogawa  
Osamu Ogawa  
Seishi Ogawa

Yuichiro Ohe  
Hiroko Ohgaki  
Miki Ohira  
Koichi Ohshima  
Atsushi Ohtsu  
Futoshi Okada  
Yasunori Okada  
Koichi Okamoto  
Hiroyuki Osada  
Masanobu Oshima  
Mototsugu Oya  
Keiya Ozawa  
Pier Paolo Pandolfi  
Jae-Gahb Park  
Manuel Peruchio  
You-Lin Qiao  
Roger Reddel  
Shigehira Saji  
Ryuichi Sakai  
Toshiyuki Sakai  
Michiie Sakamoto  
Jacques Samarut  
Yasutsuna Sasaki  
Hironobu Sasano  
Noriyuki Sato  
Yasufumi Sato  
Hideyuki Saya  
Motoharu Seiki  
Hiroyuki Seimiya  
Yoshitaka Sekido  
Manuel Serrano  
Masao Seto  
Hiroyuki Shibata  
Tatsuhiko Shibata  
Masabumi Shibuya  
Hiroshi Shiku  
Shigeomi Shimizu  
Kunitada Shimotohno  
Hai-Rim Shin  
Hiroki Shirato  
Robert H. Shoemaker  
Rashmi Sinha  
Tomotaka Sobue  
Tomoyoshi Soga  
Erwei Song  
Eric Stanbridge  
Ih-Jen Su  
Yoshikazu Sugimoto  
Haruhiko Sugimura  
Akira Suzuki  
Hiromu Suzuki  
Hidetoshi Tahara  
Chiaki Takahashi  
Satoru Takahashi  
Takashi Takahashi  
Nobuyuki Takakura

Mutsuhiro Takekawa  
Junko Takita  
Koji Tamada  
Tomohide Tamura  
Hideo Tanaka  
Takui Tanaka  
Kenzaburo Tani  
Yusuke Tanigawara  
Snorri S. Thorgeirsson  
Tomoki Todo  
Junya Toguchida  
Masakazu Toi  
Takashi Tokino  
Yutaka Tokuda  
Akihiro Tomida  
Shinya Toyokuni  
Hitoshi Tsuda  
Shoichiro Tsugane  
Tatsuhiko Tsunoda  
Heichiro Udonon  
Koji Ueda  
Yoshimasa Uehara  
Hiroyuki Ueno  
Kazuo Umezawa  
Toshikazu Ushijima  
Toshihiko Wakabayashi  
Kenji Wakai  
Tetsuro Watabe  
Paul Workman  
Hideo Yagita  
Tesshi Yamada  
Yasuhiro Yamada  
Masayuki Yamamoto  
Takao Yamori  
Seiji Yano  
Wataru Yasui  
Masaki Yasukawa  
Yasushi Yatabe  
Jun Yokota  
Minoru Yoshida  
Teruhiko Yoshida  
Naoki Yoshimi  
Akihiko Yoshimura  
Tadashi Yoshino  
Yasuhito Yuasa  
Stuart H. Yuspa  
Hitoshi Zenbutsu  
Zhiqian Zhang

**Secretaries for Editor-in-Chief**

Yataro Daigo | Koichi Matsuda

For submission instructions and all other information visit [wileyonlinelibrary.com/journal/cas](http://wileyonlinelibrary.com/journal/cas)

This journal is available online at [Wiley Online Library](http://Wiley Online Library). Visit [wileyonlinelibrary.com/journal/cas](http://wileyonlinelibrary.com/journal/cas) to search the articles and register for table of contents email alerts.



*Cancer Science* (formerly *Japanese Journal of Cancer Research*) is the official journal of the Japanese Cancer Association, and is published monthly. The journal was founded in 1907 as *Gann*, meaning cancer, by the late Dr. Katsusaburo Yamagiwa, who first produced skin cancer in experimental animals by painting tar on their skin. *Cancer Science* publishes original articles, editorials, letters to the editor, and reports describing original research in the fields of basic, translational and clinical cancer research. The following subject categories are covered: basic and clinical immunology, carcinogenesis, cell, molecular, and stem cell biology, clinical research, drug discovery and delivery, epidemiology and prevention, genetics, genomics, and proteomics, inflammation and virology, and pathology. The Journal also publishes review articles which may be solicited or submitted.



*Cancer Science* is now part of the Wiley Open Access publishing program of fully open access journals published by Wiley. For further information visit the Wiley Open Access website at <http://www.wileyopenaccess.com>.

#### JCA Board of Directors 2016:

President:	Kohei Miyazono
Vice Presidents:	Yoshio Miki, Masaki Mori, Hitoshi Nakagama
Board:	Masanori Hatakeyama, Okio Hino, Fuyuki Ishikawa, Chikashi Ishioka, Yutaka Kawakami, Hiroyuki Mano, Yasuhiro Matsumura, Yoshinori Murakami, Takuro Nakamura, Tetsuo Noda, Atsushi Ochiai, Atsushi Ohtsu, Hideyuki Saya, Takashi Takahashi, Wataru Yasui
President 2016 Annual Meeting	Kohei Miyazono
President 2017 Annual Meeting	Hitoshi Nakagama
Auditors:	Takeshi Imamura, Masanobu Oshima
Secretary:	Mami Sano, Miho Hirai, Rie Yamaguchi, Satomi Onose

#### Address for Editorial Correspondence:

*Cancer Science* Editorial Office  
c/o Wiley Japan  
Frontier Koishikawa Building, 4F,  
1-28-1 Koishikawa, Bunkyo-ku, Tokyo 112-0002, Japan  
E-mail: [cancerscience@wiley.com](mailto:cancerscience@wiley.com)  
Tel: +81 3 3830 1256  
Fax: +81 3 5689 7278

#### Address for Membership Queries:

The Japanese Cancer Association  
c/o International Medical Information Center  
Shinanomachi Rengakan  
35 Shinanomachi, Shinjuku-ku, Tokyo 160-0016, Japan  
E-mail: [office@jca.gr.jp](mailto:office@jca.gr.jp)  
Tel: +81 3 5361 7156  
Fax: +81 3 3358 1633

© 2016 (Japanese Cancer Association).

The Japanese Cancer Association thanks Foundation of Japan Cancer Society for the financial help. *Cancer Science* is also supported in part by a Grant-in-Aid for Publication of Scientific Research Results from the Japan Society for the Promotion of Science (JSPS), Japan.

ISSN 1349-7006 (Online)

# WILEY

#### PUBLISHER

*Cancer Science* is published by John Wiley & Sons Australia, Ltd  
42 McDougall Street,  
Milton, QLD 4064 Australia  
(PO Box 1226, Milton, QLD 4064 Australia)  
Tel: +61 (0)7 3859 9755  
Fax: +61 3 9274 3101

#### Production Editor

Monaliza Castaneda (email: [CASproduction@wiley.com](mailto:CASproduction@wiley.com))

#### Abstracting and Indexing Services

The Journal is indexed by AgBiotech News & Information (CABI), BIOBASE: Current Awareness in Biological Sciences (Elsevier), Biological Abstracts (Thomson Reuters), BIOSIS Previews (Thomson Reuters), CAS: Chemical Abstracts Service (ACS), CSA Biological Sciences Database (ProQuest), CSA Oncogenes & Growth Factors Abstracts (ProQuest), Current Contents: Clinical Medicine (Thomson Reuters), Embase (Elsevier), HEED: Health Economic Evaluations Database (Wiley-Blackwell), Index Medicus/MEDLINE (NLM), InfoTrac, Journal Citation Reports/ Science Edition (Thomson Reuters), MEDLINE (NLM), PubMed Dietary Supplement Subset (NLM), Science Citation Index (Thomson Reuters), Science Citation Index Expanded (Thomson Reuters), SCOPUS (Elsevier).

#### Open Access and Copyright

All articles published by *Cancer Science* are fully open access: immediately freely available to read, download and share. All *Cancer Science* articles accepted from 1 September 2013 are published under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) license, which permits use, distribution and reproduction in any medium, provided the original work is properly cited. Articles accepted before this date were published under the agreement as stated in the final article. Copyright on any *Cancer Science* article accepted after 30 August 2013, the date on which *Cancer Science* became an open access journal, is retained by the author(s). Authors grant to *Cancer*

*Science* and Wiley a license to publish the article and for Wiley to identify itself as the original publisher. Authors also grant any third party the right to use the article freely as long as its integrity is maintained and its original authors, citation details and publisher are identified.

#### Use by commercial “for-profit” organisations (not applicable to CC BY 3.0 licensed articles)

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley ([corporatesales@wiley.com](mailto:corporatesales@wiley.com)) and will be subjected to a fee. Commercial purposes include:

- Copying or downloading of articles, or linking to such articles for further redistribution, sale or licensing;
- Copying, downloading or posting by a site or service that incorporates advertising with such content;
- The inclusion or incorporation of article content in other works or services (other than normal quotations with an appropriate citation) that is then available for sale or licensing, for a fee (for example, a compilation produced for marketing purposes, inclusion in a sales pack);
- Use of article content (other than normal quotations with appropriate citation) by for-profit organisations for promotional purposes;
- Linking to article content in e-mails redistributed for promotional, marketing or educational purposes;
- Use for the purposes of monetary reward by means of sale, resale, licence, loan, transfer or other form of commercial exploitation such as marketing products.

Print reprints of Wiley Open Access articles can be purchased from [corporatesales@wiley.com](mailto:corporatesales@wiley.com).

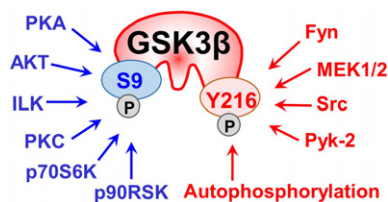
**Disclaimer:** The Publisher, The Japanese Cancer Association and Editors cannot be held responsible for errors or any consequences arising from the use of information contained in this journal; the views and opinions expressed do not necessarily reflect those of the Publisher, The Japanese Cancer Association and Editors, neither does the publication of advertisements constitute any endorsement by the Publisher, The Japanese Cancer Association and Editors of the products advertised.

Wiley Open Access articles posted to repositories or websites are without warranty from Wiley of any kind, either express or implied, including, but not limited to, warranties of merchantability, fitness for a particular purpose, or non-infringement. To the fullest extent permitted by law Wiley disclaims all liability for any loss or damage arising out of, or in connection with, the use of or inability to use the content.

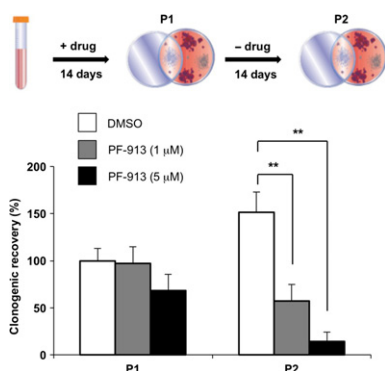
# Cancer Science

## Volume 107, Issue 10, October 2016

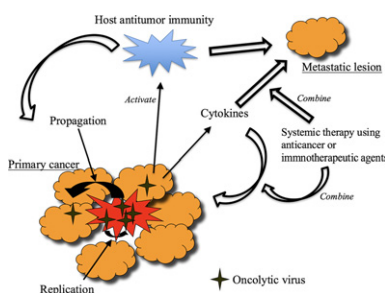
# Science In This Issue



Page 1363–72



Page 1422–29



Page 1373–79

### Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy

Page 1363–72

Many types of human malignancies frustrate both clinicians and patients with their resistance to multimodal therapy and high rate of recurrence in spite of aggressive treatment. Research efforts have focused on identifying genetic and epigenetic patterns that may lead to resistance, and identifying a common link that defines resilience across cancer subtypes would be a major step forward in therapy. In this issue of *Cancer Science*, Domoto and colleagues provide a comprehensive review on glycogen synthase kinase-3β (GSK-3β) and its potential role as a driver of cancer invasiveness and resistance to therapy. The authors first provide the reader with a fundamental understanding of how cancers become invasive and then detail how GSK-3β functions in cells under normal and malignant conditions. The reader is left with a prospective on what next steps can be taken to better understand GSK-3β and how this may lead to novel anti-cancer agents.

doi: 10.1111/cas.13028

### Small-molecule Hedgehog inhibitor attenuates the leukemia-initiation potential of acute myeloid leukemia cells

Page 1422–29

Aberrant activation of the Hedgehog (Hh) pathway is thought to be linked to a variety of cancers, including acute myeloid leukemia (AML), particularly in the role of maintaining the leukemic stem cell population; however, the particulars are not known. In this proof-of-concept study, Fukushima *et al.* attempted to determine how PF-04449913 (PF-913) works on the G-protein inhibitor of Hh called Smoothened in

both *in vivo* and *in vitro* models of AML. They show that *in vivo* PF-913 works primarily on the quiescent cell population, causing minimal cell death, whereas *in vitro* reduced the tumor burden in a xenotransplant system, likely by working through inhibition self-renewal signals and cell cycle progression. In addition, PF-913 served to sensitize leukemic cells to cytosine arabinoside. This exciting work serves to deepen the understanding of this novel drug and will hopefully guide treatment to improve outcomes for AML patients.

doi: 10.1111/cas.13019

### Oncolytic virus therapy: a new era of cancer treatment at dawn

Page 1373–79

Cancer immunotherapy is an emerging field that has already revolutionized the way clinicians treat patients and researchers focus their efforts. In this timely review, Fukuhara *et al.* provide a summary of oncolytic virus therapy, a new cancer therapy that also utilizes host immunity, which seizes naturally occurring viruses and targets them to destroy cancer cells. The unique function of this therapy is that these viruses do not function as transgene carriers, but instead themselves are targeted anti-neoplastic agents. Local treatment with the viruses not only causes a robust viral replication and killing of cancer cells but also induces systemic antitumor immunity. The authors outline the long history of oncolytic virus therapy and describe how the field has been reborn, which has led to several recent approvals for novel cancer treatments. This review provides excellent groundwork for those interested in oncolytic virus therapy and simultaneously updates those in the field of the latest developments and emerging concepts.

doi: 10.1111/cas.13027

Review Article

# Glycogen synthase kinase-3 $\beta$ is a pivotal mediator of cancer invasion and resistance to therapy

Takahiro Domoto,<sup>1</sup> Ilya V. Pyko,<sup>1,2</sup> Takuya Furuta,<sup>2,3</sup> Katsuyoshi Miyashita,<sup>1,2</sup> Masahiro Uehara,<sup>1</sup> Takeo Shimasaki,<sup>1,4</sup> Mitsutoshi Nakada<sup>2</sup> and Toshinari Minamoto<sup>1</sup>

<sup>1</sup>Division of Translational and Clinical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa; <sup>2</sup>Department of Neurosurgery, Graduate School of Medical Science, Kanazawa University, Kanazawa; <sup>3</sup>Department of Pathology, Kurume University School of Medicine, Kurume; <sup>4</sup>Medical Research Institute, Kanazawa Medical University, Uchinada, Ishikawa, Japan

## Key words

Cancer, GSK3 $\beta$ , invasion, therapeutic target, therapy resistance

## Correspondence

Toshinari Minamoto, Division of Translational and Clinical Oncology, Cancer Research Institute and Cancer Center, Kanazawa University and Hospital, 13-1 Takara-machi, Kanazawa 920-0934, Japan.  
Tel: +81-76-265-2792; Fax: 81-76-234-4529;  
E-mail: minamoto@staff.kanazawa-u.ac.jp

## Funding Information

Japanese Ministry of Education, Science, Sports, Technology and Culture; Ministry of Health, Labour and Welfare; Japan Society for the Promotion of Science, Extramural Collaborative Research Grant of Cancer Research Institute, Kanazawa University.

Received April 23, 2016; Revised July 26, 2016; Accepted August 1, 2016

Cancer Sci 107 (2016) 1363–1372

doi: 10.1111/cas.13028

Tumor cell invasion and resistance to therapy are the most intractable biological characteristics of cancer and, therefore, the most challenging for current cancer research and treatment paradigms. Refractory cancers, including pancreatic cancer and glioblastoma, show an inextricable association between the highly invasive behavior of tumor cells and their resistance to chemotherapy, radiotherapy and targeted therapies. These aggressive properties of cancer share distinct cellular pathways that are connected to each other by several molecular hubs. There is increasing evidence to show that glycogen synthase kinase (GSK)-3 $\beta$  is aberrantly activated in various cancer types and this has emerged as a potential therapeutic target. In many but not all cancer types, aberrant GSK3 $\beta$  sustains the survival, immortalization, proliferation and invasion of tumor cells, while also rendering them insensitive or resistant to chemotherapeutic agents and radiation. Here we review studies that describe associations between therapeutic stimuli/resistance and the induction of pro-invasive phenotypes in various cancer types. Such cancers are largely responsive to treatment that targets GSK3 $\beta$ . This review focuses on the role of GSK3 $\beta$  as a molecular hub that connects pathways responsible for tumor invasion and resistance to therapy, thus highlighting its potential as a major cancer therapeutic target. We also discuss the putative involvement of GSK3 $\beta$  in determining tumor cell stemness that underpins both tumor invasion and therapy resistance, leading to intractable and refractory cancer with dismal patient outcomes.

One of the well-recognized but still poorly understood characteristics of cancer is its ability to adapt and survive in a harsh microenvironment. Tumor cells survive the hypoxic and starved conditions by changing their morphological and biological properties and by interacting with tumor-associated stromal components.<sup>(1)</sup> Another striking characteristic of cancers is their ability to invade host organs and to resist therapeutic insults, thus limiting the success of curative tumor resection and leading to tumor metastasis and therapy failure. These pathological behaviors of cancer are associated with acquisition of the hallmark biological traits of cancer.<sup>(2–4)</sup> Despite recent advances in cancer treatments, the existence of unresectable and recurrent cancers that share the persistent capacity for invasion, metastasis and therapy resistance remains a challenge for current medical therapies.<sup>(5,6)</sup> Although our knowledge of the molecular and biological mechanisms by which cancer evolves to the refractory stage is increasing,<sup>(2)</sup> few strategies have been established to attenuate the ability of tumors to invade or to prevent the failure of therapy.

Modern options for cancer treatment consist of surgery, cytotoxic or cytostatic chemotherapeutics, radiation, molecular-

targeted and immunomodulating agents, and their multidisciplinary combination.<sup>(7,8)</sup> These treatments aim primarily to reduce tumor cell survival and proliferation, but not to eliminate invasive ability or resistance to therapy. Therefore, understanding the pathways by which cancer cells acquire both invasive and therapy-resistant phenotypes is critical for the development of more efficient therapeutic strategies against refractory cancers and, therefore, improvements in patient survival. Apart from the known mediators of invasion and therapy resistance [reviewed in Alexander and Friedl<sup>(9)</sup>], one emerging candidate is glycogen synthase kinase (GSK)-3 $\beta$ . This molecule has been extensively implicated in critical cell biology processes and has causal roles in common diseases including glucose intolerance, neurodegenerative disorders and cancer.<sup>(10–12)</sup> Here we review previous studies that have reported on the association between therapeutic stimuli/resistance and induction of pro-invasive phenotypes in various cancer types. Many of these cancers have proven to be responsive to experimental treatment which targets GSK3 $\beta$ . This review focuses on the role of GSK3 $\beta$  as a molecular hub that connects pathways responsible for tumor invasion and resistance to

therapy, thus highlighting this kinase as a promising multipurpose cancer therapeutic target. It also discusses a putative role for GSK3 $\beta$  in sustaining the tumor cell “stemness” that is central to both tumor invasion and therapy resistance, thereby leading to intractable cancer and resulting in treatment failure.<sup>(13–15)</sup>

### Invasion and therapy resistance co-segregate in refractory cancer

High invasiveness and resistance to therapy are common biological and clinical characteristics of refractory cancer that represent major challenges for research and treatment. The mechanisms involved in tumor invasion include cell motility and migration, degradation of the extracellular matrix and interaction with stromal and inflammatory cells. These are orchestrated in a way that enables the tumor to invade the host organ and often beyond.<sup>(3,4)</sup> An early morphological and functional change for tumor cells of epithelial origin to acquire a proinvasive phenotype is epithelial to mesenchymal transition (EMT), altering cell behavior to resemble a mesenchymal type.<sup>(16,17)</sup> The major modes by which cancers evade the effect of anti-cancer drugs include intrinsic (or constitutive) resistance and acquired resistance. Constitutive resistance to therapy may be due to mutational activation of signaling for cell survival, cytoprotective alterations in the cell cycle and in DNA repair ability, differences in the efficiency of drug uptake and efflux by cancer cells, and insufficient drug delivery [reviewed in Alexander and Friedl<sup>(9)</sup>]. Acquired resistance to chemotherapeutic and molecular-targeted agents involves distinct mechanisms, including genetic alterations to the drug targets, activation of surrogate prosurvival pathways, and interactions between tumor cells and components of the tumor environment [reviewed in Holohan *et al.*<sup>(5)</sup>, Ramos and Bentes-Alj<sup>(6)</sup> and Alexander and Friedl<sup>(9)</sup>]. Intra-tumor heterogeneity emerges in many tumors and often underlies their resistance to therapeutic agents.<sup>(18,19)</sup>

The processes of tumor invasion and therapy resistance and their underlying mechanisms are often investigated as independent pathological events in cancer. However, recent studies (shown in Table 1) have demonstrated that tumor cells acquire morphological and functional proinvasive phenotypes with the ability to migrate and invade following the development of resistance to anti-cancer therapeutics (Suppl. References [SR] 1–24) (Data S1).<sup>(SR1–24)</sup> The therapeutic modalities included conventional chemotherapeutic agents<sup>(SR1–15)</sup>, different types of radiation therapy<sup>(SR16–20)</sup> as well as bioactive compounds targeting epidermal growth factor (EGF) receptor and vascular endothelial growth factor (VEGF).<sup>(SR21–24)</sup> These studies indicate the therapeutic insult and/or resistance elicits proinvasive phenotypes and evokes the invasive capability of tumor cells in a broad spectrum of cancer types, including breast, colorectal, pancreatic, ovarian and prostate cancers, as well as rare intractable tumors (e.g. glioblastoma and osteosarcoma). A large number of preclinical studies have demonstrated that all available cancer treatments, including surgery, facilitate metastatic tumor spread. Such treatment often results in therapeutic benefit, but occasionally also results in resistance, leading to the paradoxical concept of “treatment-induced metastasis” [reviewed in Ebos<sup>(20)</sup>]. These experimental and preclinical studies suggest that the invasive behavior of cancer cells and their acquired resistance to therapy may not be separate pathological properties and could, instead, represent interconnected processes [reviewed in Alexander and Friedl<sup>(9)</sup>].

Regardless of the tumor type and therapeutic agent, the proposed mechanisms for invasion and resistance are attributable to molecular pathways that participate in tumor cell survival, transition to mesenchymal (EMT) and cancer stem cell (CSC) phenotypes, migration and invasion with extracellular matrix degradation, and drug efflux (Table 1). The reported molecules that mediate and interconnect these pathways include growth and transcription factors, chemokines, RAS and Rho family members (e.g. Ras, Rac1) and cell-matrix adhesion molecules (e.g. integrin family and focal adhesion kinase [FAK]) (Table 1).<sup>(8)</sup> Bevacizumab is a humanized monoclonal antibody to VEGF used clinically as an anti-angiogenic agent. Acquired resistance to bevacizumab leads to enhanced tumor cell invasion due to the metabolic shift to glycolysis and degradation and remodeling of tumor stromal tissues.<sup>(SR21–24)</sup> A better understanding of the biological mechanisms underlying the tight association between tumor invasion and therapy resistance should provide a solid rationale for the development of innovative cancer treatments.<sup>(9)</sup>

### Aberrant GSK3 $\beta$ in cancer

GSK3 is a family of serine/threonine protein kinases comprising two highly conserved isoforms, GSK3 $\alpha$  and GSK3 $\beta$ , that show approximately 85% overall homology and 98% homology in their kinase domains. GSK3 is constitutively active in normal cells and its activity is regulated by the differential phosphorylation at serine (S) residues 21 (pGSK3 $\alpha$ <sup>S21</sup>) and 9 (pGSK3 $\beta$ <sup>S9</sup>) (both inactive forms), and tyrosine (Y) residues 279 (pGSK3 $\alpha$ <sup>Y279</sup>) and 216 (pGSK3 $\beta$ <sup>Y216</sup>) (both active forms) (Fig. 1a,b). GSK3 regulates a diverse array of physiological cellular functions via the phosphorylation of and interaction with various proteins (Fig. 1c). Although the two GSK3 isoforms share many substrates, they are not functionally identical and show some differences in their substrate specificity [reviewed in Beurel *et al.*<sup>(10)</sup>]. Negative regulation of GSK3 activity is desirable to maintain physiological cellular functions. A growing number of studies have demonstrated that deregulated GSK3 activity contributes to the pathogenesis and progression of various diseases, including glucose intolerance, neurodegenerative disorders, and chronic inflammatory and immunological diseases.<sup>(10,21)</sup> This points to GSK3 being an attractive and druggable target for a broad spectrum of diseases.<sup>(22)</sup> Consequently, many GSK3 inhibitor compounds have been developed in academic and pharmaceutical institutes over recent years. Some are reported to inhibit both the  $\alpha$  and  $\beta$  isoforms with different affinities,<sup>(SR25–28)</sup> while others are specific for GSK3 $\beta$  (reviewed in SR25,26). Among the latter compounds, it was reported that AR-A014418 does not inhibit the activity of 26 closely related kinases and is, therefore, considered to be highly specific for GSK3 $\beta$ .<sup>(SR29)</sup>

Of the two GSK3 isoforms, most studies in the field of oncology have focused on GSK3 $\beta$ . This is partly because of the functional redundancy of the two isoforms in regulating the canonical Wnt/ $\beta$ -catenin pathway, responsible for generating the most prevalent oncogenic signaling.<sup>(23,24)</sup> Based on its known effects on several proto-oncoproteins (e.g.  $\beta$ -catenin, cyclin D1 and c-Myc), cell cycle regulators (e.g. p27<sup>Kip1</sup>) and mediators of EMT (e.g. snail) (Fig. 1c), it has long been recognized that GSK3 $\beta$  suppresses tumor development [reviewed in Clevers and Nusse,<sup>(24)</sup> Jope *et al.*,<sup>(25)</sup> Luo<sup>(26)</sup> and McCubrey *et al.*<sup>(27)</sup>], as discussed later (Table S1).<sup>(SR30–58)</sup> Paradoxically, our earlier studies found increased expression and deregulated activity of GSK3 $\beta$  due to changes in the differential



**Table 1. Representative previous studies showing interrelationship between therapeutic stimuli/resistance and pro-invasive phenotype in cancer**

Tumor type	Therapeutic insults	Biological mechanism (Suppl. Reference number)	Therapeutic effect of GSK3 $\beta$ inhibition and underlying mechanism (Reference number)
Breast cancer	Tamoxifen	EGFR pathway, enhanced tumor cell motility and invasion <sup>(SR1)</sup>	Suppression of invasion through dysregulation of actin-reorganization via down-regulation of WAVE2 <sup>(68)</sup>
	Tamoxifen	EMT induction with EGFR pathway-dependent $\beta$ -catenin activation <sup>(SR2)</sup>	
	Adriamycin	Twist 1-mediated EMT induction and P-gp up-regulation <sup>(SR3)</sup>	
	Doxorubicin and cyclophosphamide alone or in combination	TNF- $\alpha$ /NF- $\kappa$ B-mediated amplification of CXCL1 paracrine network between carcinoma, myeloid, and endothelial cells <sup>(SR4)</sup>	
Colorectal cancer	Oxaliplatin	NF- $\kappa$ B-mediated EMT induction with enhanced cell migration and invasion <sup>(SR5)</sup>	Suppression of tumor cell survival and proliferation by inhibition of hTERT/telomerase and promoting p53-dependent apoptosis <sup>(28–30,32,33)</sup> and invasion by down-regulation of WAVE2, <sup>(68)</sup> and enhancing 5-FU effect via PARP1-dependent and AIF-mediated necroptosis <sup>(73)</sup>
	Erlotinib	EMT induction <sup>(SR21)</sup>	
Pancreatic cancer	Gemcitabine	EMT induction with activation of $\beta$ -catenin and c-Met and acquisition of CSC phenotype <sup>(SR6)</sup>	Suppression of tumor cell survival and proliferation <sup>(30)</sup> by inhibition of NF- $\kappa$ B transcriptional activity, <sup>(34,35)</sup> synergistic with gemcitabine by restoration of Rb function <sup>(60)</sup> and inhibition of TP53INP1-mediated DNA repair, <sup>(70)</sup> increase in radiosensitivity and suppression of invasion via FAK/Rac1/MMP-2 and CXCR4/MMP-2 pathways <sup>(60,67)</sup>
	Gemcitabine	Acquisition of EMT and CSC phenotypes with activation of Notch pathway <sup>(SR7)</sup>	
	Gemcitabine, 5-FU, cisplatin	Gene expression profile responsible for EMT phenotype <sup>(SR8)</sup>	
	Gemcitabine	NF- $\kappa$ B-mediated acquisition of EMT and CSC phenotypes <sup>(SR9)</sup>	
	$\gamma$ -irradiation	Tumor cell migration and invasion with enhanced MMP-2 activity <sup>(SR16)</sup>	
	Erlotinib	EMT induction <sup>(SR21)</sup>	
Ovarian cancer	Taxol, vincristine	Increased expression of twist <sup>(SR10)</sup>	Suppression of tumor cell proliferation by decrease in cyclin D1 expression <sup>(39)</sup>
	Paclitaxel	Acquisition of EMT and metastatic potential <sup>(SR11)</sup>	
Prostate cancer	Cisplatin, taxol	Acquisition of EMT via endothelin A receptor-mediated pathway <sup>(SR12)</sup>	Suppression of tumor cell survival and proliferation by eliminating TRAIL resistance, repressing AR activity, <sup>(41–44)</sup> and attenuation of metastasis by depleting CSC population <sup>(78)</sup>
	Taxol, vincristine	Increased expression of twist <sup>(SR10)</sup>	
Other epithelial cancer	Docetaxel	Gene expression profile responsible for EMT phenotype <sup>(SR13)</sup>	Suppression of tumor cell survival and proliferation by inhibiting ERK-mediated prosurvival pathway <sup>(40)</sup>
	Docetaxel	Gene expression profile responsible for EMT phenotype <sup>(SR13)</sup>	
Endometrial	Ionizing radiation	EMT induction with enhanced cell migration <sup>(SR17)</sup>	Suppression of tumor cell survival by interrupting ERK-mediated prosurvival pathway <sup>(40)</sup>
Bladder	Taxol, vincristine	Increased expression of twist <sup>(SR10)</sup>	Suppression of tumor cell survival and proliferation by inhibition of NF- $\kappa$ B transcriptional activity <sup>(45)</sup>
Tongue	Cisplatin	EMT induction and enhanced cell migration and invasion in association with increased BMI1 by down-regulation of miR-200b and miR-15b <sup>(SR14)</sup>	Not shown
Nasopharyngeal	Taxol, vincristine	Increased expression of twist <sup>(SR10)</sup>	Not shown

Table 1 (Continued)

Tumor type	Therapeutic insults	Biological mechanism (Suppl. Reference number)	Therapeutic effect of GSK3 $\beta$ inhibition and underlying mechanism (Reference number)
Glioblastoma	Doxorubicin	Therapeutic effect and enhancement of doxorubicin effect by a new anti-invasive small molecule (IB) <sup>(SR15)</sup>	Suppression of tumor cell survival and proliferation by restoring p53/p21 pathway and inhibition of c-Myc, NF- $\kappa$ B and abnormal glycolysis, <sup>(31,36)</sup> synergistic with temozolomide and increase in radiosensitivity via
	Sublethal irradiation	Enhanced tumor cell migration and invasion involving $\alpha$ v $\beta$ 3 integrin, MMP-2, MMP-9, MT1-MMP, TIMP-2 and BCL-2/BAX rheostat <sup>(SR18)</sup>	Rb-mediated and c-Myc/DMNT3A/MGMT pathways, <sup>(31,69)</sup> suppression of invasion <sup>(58,59,61)</sup> by inhibition of FAK/Rac1/JNK pathway <sup>(61)</sup> and induction of CSC differentiation <sup>(37,77)</sup>
	Ionizing radiation	Enhanced tumor cell migration with increased expression of $\beta$ 3 and $\beta$ 1 integrins <sup>(SR19)</sup>	
	Bevacizumab	Resistance to the therapy is associated with up-regulation of MMP-2, MMP-9, MMP-12, TIMP1, SPARC and HIF-2 $\alpha$ , and with activation of bFGF-mediated alternate angiogenesis pathway <sup>(SR22)</sup>	
	Bevacizumab	Treatment of tumor xenograft is associated with decrease of mitochondria, induction of glycolytic metabolites (lactate and alanine) and HIF-1 $\alpha$ , and activation of PI3K pathway <sup>(SR23)</sup>	
	Bevacizumab	Resistance to the therapy induced genes associated with a mesenchymal origin, cellular migration/invasion, and inflammation. <sup>(SR24)</sup>	
Osteosarcoma	Low-dose photon irradiation	Enhanced cell migration and invasion concomitant with up-regulation of $\alpha$ v $\beta$ 3 integrin <sup>(SR20)</sup>	Suppression of tumor cell survival and proliferation by inhibition of NF- $\kappa$ B transcriptional activity <sup>(38)</sup> and restoring $\beta$ -catenin osteosarcoma suppressor (S. Shimozaki <i>et al.</i> ) <sup>†</sup>

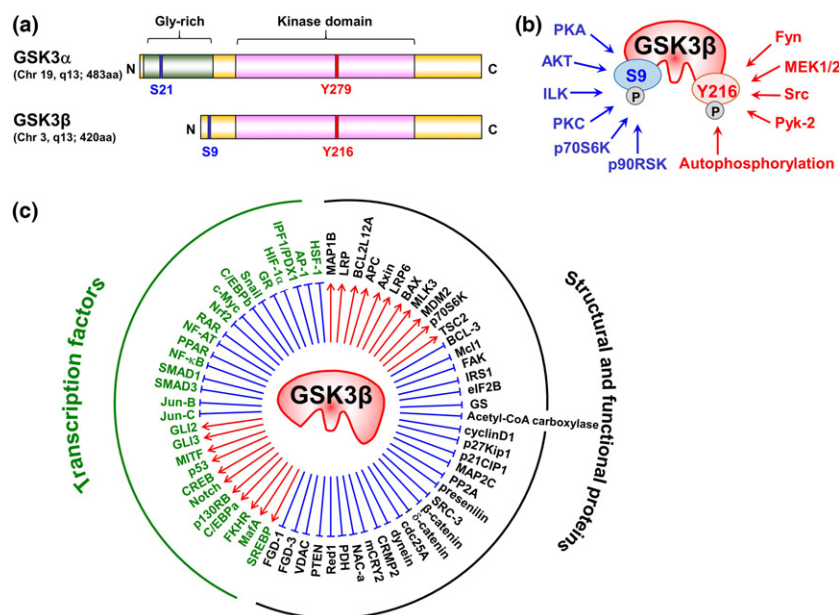
<sup>†</sup>S. Shimozaki *et al.*, unpublished observation. AIF, apoptosis-inducing factor; AR, androgen receptor; BAX, Bcl-2-associated X protein; bFGF, basic fibroblast growth factor; BMI1, B lymphoma Mo-MLV insertion region 1 homolog; CSC(s), cancer stem-like cell(s); CXCL1, chemokine (C-X-C motif) ligand 1; CXCR4, CXC receptor type 4; DNMT3A, DNA (cytosine-5)-methyltransferase 3A; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; 5-FU, 5-fluorouracil; HIF, hypoxia inducible factor; hTERT, human telomerase reverse transcriptase; JNK, c-Jun N-terminal kinase; MGMT, O<sup>6</sup>-methylguanine DNA methyltransferase; miR, micro-RNA; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1-MMP; NF- $\kappa$ B, nuclear factor- $\kappa$ B; PARP1, poly [ADP-ribose] polymerase 1; P-gp, P-glycoprotein (multidrug resistance); PI3K, phosphatidylinositol-3-kinase; Rb, retinoblastoma; SPARC, secreted protein, acidic, cysteine rich; SR, supplementary reference No.; TIMP, tissue inhibitor of MMP; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TP53INP1, tumor protein p53-inducible nuclear protein 1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WAVE2, WAS (Wiskott-Aldrich syndrome) protein family member 2.

phosphorylation of S9 and Y216 residues in gastrointestinal cancers, glioblastoma and osteosarcoma compared to respective normal cells and tissues.<sup>(28–31)</sup> These observations suggested that GSK3 $\beta$  could participate in cancer development and progression, despite the general belief that it has tumor-suppressive functions.<sup>(25–27)</sup> We and other research groups have shown that inhibition of GSK3 $\beta$  activity with specific pharmacological inhibitors, or inhibition of its expression by RNA interference, can preferentially attenuate the survival and proliferation of tumor cells and induce them to undergo apoptosis. This effect has been observed not only in gastrointestinal and pancreatic cancer cells<sup>(28–30,32–35)</sup> but also in glioblastoma,<sup>(31,36,37)</sup> osteosarcoma (S. Shimozaki, N. Yamamoto, T. Domoto, H. Nishida, K. Hayashi, H. Kimura, A. Takeuchi, S. Miwa, K. Igarashi, T. Kato, Y. Aoki, T. Higuchi, M. Hirose,

R.M. Hoffman, T. Minamoto, H. Tsuchiya, unpublished data, 2016)<sup>(38)</sup> and other malignant neoplasms such as gynecological and urogenital cancers,<sup>(39–47)</sup> soft tissue sarcomas,<sup>(48,49)</sup> hematological malignancies<sup>(50,51)</sup> and lung cancers.<sup>(52,53)</sup> This accumulating evidence firmly establishes GSK3 $\beta$  as a valuable target in cancer treatment.<sup>(11,12)</sup>

There has been substantial interest in the molecular mechanisms by which GSK3 $\beta$  favors tumor progression and by which inhibition of its activity or expression attenuates tumor cell survival, immortalization and proliferation. The reported mechanisms for tumor cell survival include the nuclear factor (NF)- $\kappa$ B-mediated prosurvival pathway,<sup>(34–36,38)</sup> tumor cell resistance to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL),<sup>(41)</sup> and failure of the p53-mediated tumor suppressor pathway or of the Rb-mediated cell





**Fig. 1.** (a) Comparison of the structural and functional domains of the two GSK3 isoforms, (b) the sites (S9 and Y216) of phosphorylation of GSK3 $\beta$  by different kinases regulating GSK3 $\beta$  activity, and (c) the substrates of GSK3 $\beta$  and proteins that interact with it. (a) GSK3 $\alpha$  (51 kD) and GSK3 $\beta$  (47 kD) are products of their respective genes located in chromosomes 19q13 and 3q13. The isoforms share high (98%) homology of the catalytic domains, and GSK3 $\alpha$  has a glycine-rich extension at the N-terminal side. Blue and red narrow columns indicate the sites of serine (S) and tyrosine (Y) phosphorylation, respectively. (b) The kinases indicated in blue phosphorylate GSK3 $\beta$ -S9 resulting in its inactivation, while those indicated in red phosphorylate GSK3 $\beta$ -Y216 resulting in its activation. (c) GSK3 $\beta$  stabilizes/activates (red arrows) and destabilizes/inactivates (blue lines) various transcription factors as well as structural and functional proteins. AP-1, activator protein 1; APC, adenomatous polyposis coli; BAX, BCL2-associated X protein; BCL, B-cell lymphoma; C, C-terminal of protein; C/EBP, CCAAT (cytosine-cytosine-adenosine-adenosine-thymidine)-enhancer-binding protein; cdc25A, cell division cycle 25 homolog A; CREB, cAMP (cyclic adenosine monophosphate) response element binding protein; CRMP2, collapsin response mediator protein 2; eIF2B, eukaryotic initiation factor 2B; FAK, focal adhesion kinase; FGD-1/3, FYVE RhoGEF (guanine nucleotide exchange factor) and PH domain-containing protein 1/3; FKHR, forkhead in rhabdomyosarcoma; Gly, glycine; GR, glucocorticoid receptor; GS, glycogen synthase; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; HIF-1 $\alpha$ , hypoxia inducible factor-1 $\alpha$ ; HSF-1, heat shock transcription factor-1; ILK, integrin-linked kinase; IPF1/PDX1, insulin promoter factor 1/pancreatic and duodenal homeobox 1; IRS1, insulin receptor substrate 1; LRP5/6, lipoprotein receptor-related protein 5/6; MafA, musculoaponeurotic fibrosarcoma oncogene homolog A; MAP1B/2C, microtubule associated protein 1B/2C; Mcl1, myeloid cell leukemia 1; mCRY2, mouse cryptochrom 2; MDM2, mouse double minute 2 homolog; MEK, MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) kinase; MITF, microphthalmia-associated transcription factor; MLK3, mixed lineage kinase 3; N, N-terminal of protein; NAC- $\alpha$ , nascent polypeptide-associated complex subunit- $\alpha$ ; NF-AT, nuclear factor of activated T-cells; NF- $\kappa$ B, nuclear factor- $\kappa$ B; Nrf2, nuclear factor erythroid 2-related factor 2; p130RB, p130 retinoblastoma; p21CIP1, p21 CDK (cyclin-dependent kinase)-interacting protein 1; p90RSK, p90 ribosomal protein S6 kinase; PDH, pyruvate dehydrogenase; PKA, protein kinase A; PKC, protein kinase C; PP2A, protein phosphatase 2A; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; Pyk-2, proline-rich tyrosine kinase 2; RAR, retinoic acid receptor; Red1, RNA-editing deaminase 1; S, serine; SRC-3, steroid receptor coactivator-3; SREBP, sterol regulatory element-binding protein; TSC2, tuberous sclerosis complex 2; VDAC, voltage-dependent anion channel; Y, tyrosine

cycle regulation.<sup>(31,32)</sup> Preservation of telomere length by maintaining activity of human telomerase reverse transcriptase (hTERT) and telomerase also immortalizes tumor cells in response to the aberrant activation of GSK3 $\beta$ .<sup>(30)</sup> Cell proliferation pathways mediated by c-Myc and cyclin D1 sustain tumor cell proliferation that is dependent on GSK3 $\beta$ .<sup>(36,39)</sup> In osteosarcoma and rhabdomyosarcoma, the induction of  $\beta$ -catenin signaling, a well-known tumor suppressive mechanism in these cancers, has been linked to decreased cell proliferation following GSK3 $\beta$  inhibition (S. Shimozaki *et al.*, unpublished data).<sup>(49)</sup> Another recent study reported that mitotic catastrophe caused by disruption of centrosome biodynamics was associated with attenuated tumor cell survival and proliferation following GSK3 $\beta$  inhibition.<sup>(54)</sup> Importantly, GSK3 $\beta$  has little impact on the cell survival, immortalization and growth of normal cells, where the differential phosphorylation of S9 and Y216 residues functions to fine tune GSK3 $\beta$  activity.<sup>(28–31,34)</sup> The growing body of evidence on the role of aberrant GSK3 $\beta$  in sustaining and promoting cancer cell survival, immortalization and proliferation, together with the differential effects of GSK3 $\beta$  inhibition on cancer and normal cells, underpins the targeting of GSK3 $\beta$  as a novel cancer treatment.<sup>(11,12)</sup>

### GSK3 $\beta$ mediates both the invasive phenotype and therapy resistance in refractory cancer

The dependency of cancer cells on GSK3 $\beta$  for their survival and proliferation has encouraged further studies on whether aberrant GSK3 $\beta$  participates in tumor cell invasion and therapy resistance, the two major determinants of patient outcome.<sup>(3–6)</sup> As shown in Table 1, most tumors that acquire pro-invasive phenotypes as they evade therapy are susceptible to experimental treatment involving inhibition of GSK3 $\beta$ . Here we review what is known about the involvement of GSK3 $\beta$  in tumor invasion and resistance to therapy based on our previous studies in pancreatic cancer and glioblastoma. These are representative of lethal tumors and are characterized by high invasive capacity and resistance to available therapies.<sup>(55,56)</sup>

**GSK3 $\beta$  and cancer invasion.** While GSK3 $\beta$  plays pivotal roles in cytoskeletal organization, cell polarity and migration in the physiological processes of organogenesis and wound healing,<sup>(57)</sup> little is known about its role in cancer cell motility, migration and invasion. Earlier studies showed that lithium chloride and some indirubins, both classical GSK3-inhibiting agents, inhibited the migration and invasion of glioblastoma

cells, but the mechanism underlying this effect was not clear.<sup>(58,59)</sup> Subsequently, we demonstrated that inhibition of GSK3 $\beta$  by a specific pharmacological inhibitor and by RNA interference decreases the capacity for migration and invasion of pancreatic cancer and glioblastoma cells and of their tumors in animal models.<sup>(60,61)</sup> Morphologically, these effects were associated with reduced formation of lamellipodia and invadopodia, the horizontal and vertical cellular surface microarchitectures, respectively, that drive cell migration, stromal degradation and invasion.<sup>(62,63)</sup> Inhibition of GSK3 $\beta$  disrupted the functional subcellular colocalization of Rac1<sup>(64)</sup> and cortactin<sup>(63)</sup> with the cytoskeletal molecule F-actin in lamellipodia and invadopodia, respectively. These changes in the pro-invasive phenotype of tumor cells following GSK3 $\beta$  inhibition coincide with the suppression of molecular pathways mediated by focal adhesion kinase (FAK),<sup>(65)</sup> guanine nucleotide exchange factors (GEF)/Rac1<sup>(64,66)</sup> and c-Jun N-terminal kinase (JNK). This results in decreased expression of matrix metalloproteinase (MMP)-2 and membrane type (MT) 1-MMP. Our observations therefore suggest that GSK3 $\beta$  sustains tumor invasion via the promotion of morphological and functional pro-invasive cellular phenotypes and that the molecular axis involves FAK, GEFs/Rac1 and JNK (Fig. 2).<sup>(60,61)</sup>

Subsequent to our studies,<sup>(60,61)</sup> other workers have reported the proinvasive effect of GSK3 $\beta$  in pancreatic, colorectal and breast cancer cells in association with modulation of cytoskeletal organization and cytokine-mediated matrix degradation.<sup>(67,68)</sup> These studies highlight the pivotal role of GSK3 $\beta$  in tumor invasion and probably also in metastatic spread.

**GSK3 $\beta$  and cancer therapy resistance.** The combination of multiple agents having different targets and mechanisms of actions is important in the treatment of cancer in order to optimize the therapeutic effects and minimize the adverse effects. This is particularly relevant for the treatment of refractory cancer, and, hence, molecular-targeted therapy is often combined with conventional chemotherapeutics and/or radiation therapy, as well as with other targeted agents.<sup>(7,8)</sup> As discussed above, accumulating evidence on the effects of GSK3 $\beta$  inhibition against cancer cell survival and proliferation has led us to

address whether this could be used in combination with chemotherapy and irradiation.

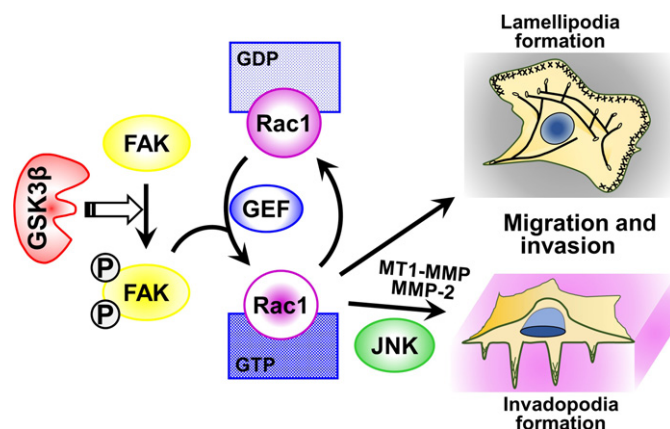
The standard treatment modalities prescribed are often ineffective in patients with refractory tumors such as pancreatic cancer and glioblastoma.<sup>(55,56)</sup> We have shown that administration of a GSK3 $\beta$ -specific pharmacological inhibitor (e.g. AR-A014418)<sup>(SR29)</sup> at a dose below that which results in a therapeutic effect when given on its own can significantly enhance the cytotoxic effects of temozolomide and gemcitabine.<sup>(31,60,69,70)</sup> These are the standard chemotherapeutic agents prescribed for patients with glioblastoma and pancreatic cancer, respectively.<sup>(55,56)</sup> Low-dose GSK3 $\beta$  inhibitor given in combination with chemotherapeutic agents had additive and synergistic effects. Investigation of the underlying molecular mechanism is crucial to justify treatment combinations of GSK3 $\beta$  inhibitor with other anti-cancer therapeutics (Fig. 3). In glioblastoma, the combined effect was attributed to modulation of the mouse double minute (Mdm)2/p53 and c-Myc-mediated pathways resulting in epigenetic silencing of O<sup>6</sup>-methylguanine DNA methyltransferase (MGMT), a clinically proven biomarker of temozolomide efficacy<sup>(56)</sup> in tumor cells (Fig. 3b).<sup>(31,69)</sup> In pancreatic cancer, inhibition of tumor GSK3 $\beta$  activity decreased cyclin D1/cyclin-dependent kinase (CDK)4/6 complex-dependent phosphorylation of Rb tumor suppressor protein.<sup>(60)</sup> One of the important functions of Rb is to bind the oncogenic transcription factor E2F1 and inhibit its transcriptional activity.<sup>(71,72)</sup> GSK3 $\beta$  may, therefore, sensitize pancreatic cancer to gemcitabine by downregulating the expression of ribonucleotide reductase, a well-known pharmacological biomarker of gemcitabine<sup>(55)</sup> that is regulated by the Rb/E2F pathway (Fig. 3a).<sup>(71,72)</sup> In addition to modulation of this pathway, we found that downregulation of the *TP53INP1* gene (encoding tumor p53-induced-nuclear-protein 1) involved in DNA repair is associated with the chemosensitizing effect of GSK3 $\beta$  inhibitor.<sup>(70)</sup> The GSK3 $\beta$  inhibitor used in these studies also sensitized both pancreatic cancer and glioblastoma to ionizing radiation.<sup>(31,60)</sup> This radio-sensitizing effect may depend on the restoration of Rb function following GSK3 $\beta$  inhibition, resulting in the inability of E2F1 to induce the transcription of thymidylate synthase and thymidine kinase (Fig. 3a).

Other recent studies have also reported that GSK3 $\beta$  inhibition allows therapy-resistant colon and pancreatic cancer cells to become susceptible to 5-fluorouracil (5-FU),<sup>(73)</sup> and renal cell carcinoma cells to become susceptible to a synthetic multikinase inhibitor (sorafenib) that targets growth signaling and angiogenesis.<sup>(74)</sup> Together, these studies suggest that GSK3 $\beta$  participates in multiple molecular pathways used by various cancer types to evade chemotherapy, radiotherapy and targeted therapies.

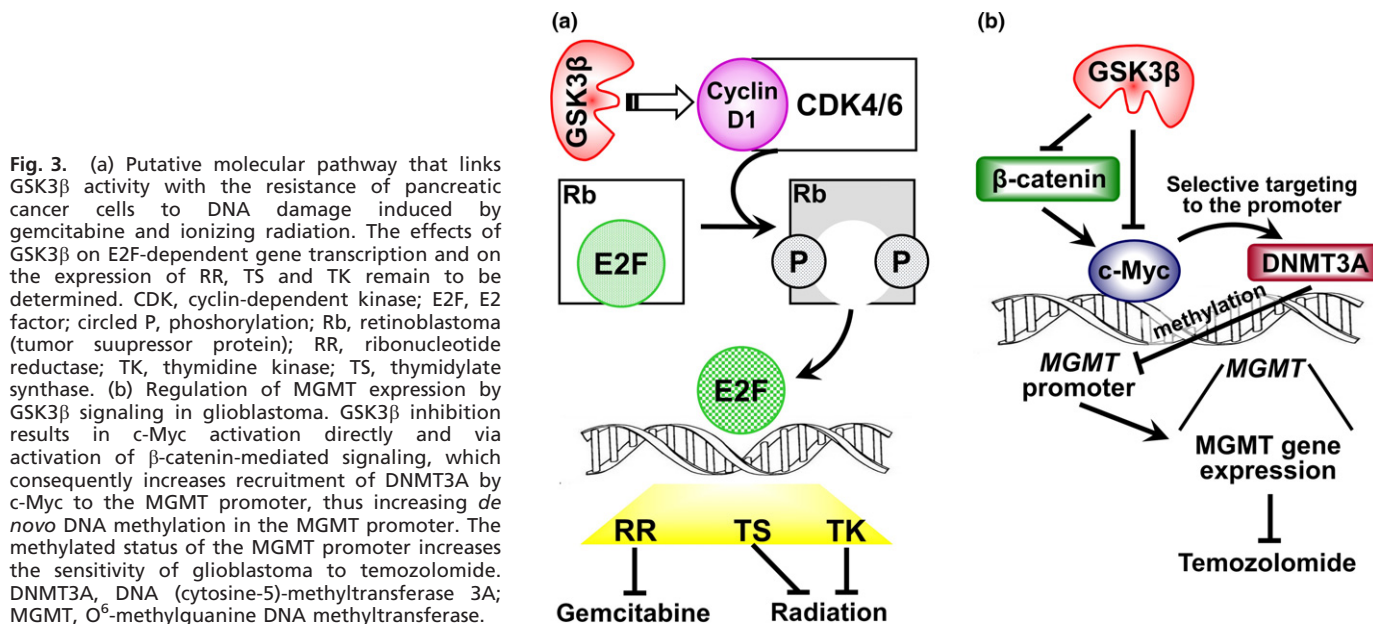
Collectively, a growing body of experimental evidence supports the notion that GSK3 $\beta$  is a molecular "hub" that mediates and connects various pathways responsible for tumor invasion and resistance to therapy. Together with the role of GSK3 $\beta$  in promoting tumor cell survival and proliferation and its differential functions between tumor and normal cells [reviewed in Miyashita *et al.*<sup>(11)</sup> and McCubrey *et al.*<sup>(12)</sup>], this evidence reinforces the promise of novel cancer therapeutic strategies that target GSK3 $\beta$ .

## Future perspectives

Targeting of the biological pathways that link tumor invasion and therapy resistance is clearly an attractive prospect for the



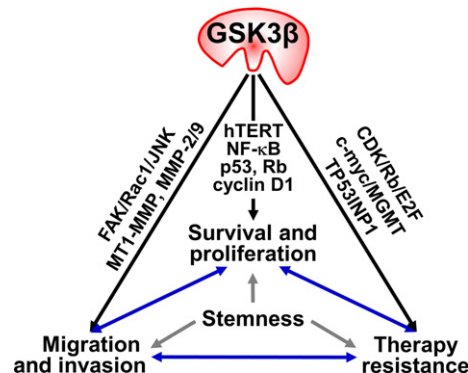
**Fig. 2.** Putative molecular pathway through which deregulated GSK3 $\beta$  promotes tumor cell migration and invasion. The exact molecular pathway by which GSK3 $\beta$  mediates the activation of FAK (open arrow) remains to be determined. FAK, focal adhesion kinase; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; GTP, guanosine triphosphate; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1-MMP; circled P, phosphorylation.



development of innovative cancer treatments. Another important challenge is to understand the biology of cancer initiating or stem-like cells (CSC) so that “stemness” phenotypes can also be targeted. Based on the clonal evolution model of tumorigenesis and on the normal stem cell concept, CSC are conceptually defined as a population of tumor cells with self-renewing and pluripotent capacity.<sup>(13)</sup> CSC have been identified in a wide range of hematopoietic malignancies and solid tumors and are assumed to be progenitors of the entire neoplastic cell population. One of the most striking characteristics of CSC is their influence on tumor cell plasticity, intra-tumor heterogeneity, invasion and metastasis, therapy resistance and tumor relapse after surgery and adjuvant treatment.<sup>(14,75)</sup> Eradication of CSC is, therefore, of paramount clinical importance for combatting refractory cancer.<sup>(15,76)</sup>

GSK3 $\beta$  has pivotal functions in normal cell biology<sup>(10,21,22)</sup> and is also central to the processes of cancer cell survival, proliferation, invasion and therapy resistance, as discussed above. A number of studies have reported that CSC phenotypes share unique molecular pathways and tumor–environment interactions that are also associated with tumor invasion and therapy resistance.<sup>(14,75)</sup> This leads us to propose a working hypothesis whereby GSK3 $\beta$  underlies the basal mechanism for sustaining the CSC phenotype (Fig. 4). Recent studies have shown that GSK3 $\beta$  negatively controls the differentiation of malignant glioma cells,<sup>(77)</sup> and that GSK3 $\beta$  inhibition results in depletion of cancer stem-like or progenitor-like cells and attenuation of metastatic spread in prostate cancer.<sup>(78)</sup> Consistent with the physiological roles of GSK3 $\beta$  in negatively regulating canonical Wnt/ $\beta$ -catenin and hedgehog signaling,<sup>(10,23,24)</sup> inhibition of GSK3 $\beta$  is a prerequisite for the maintenance of “stemness” phenotypes in embryonic and hematopoietic stem cells.<sup>(79,80)</sup> Therefore, future work should aim to understand the influence of GSK3 $\beta$  on both tumor and normal stem cell phenotypes. This knowledge can be applied for the development of novel cancer treatments that target GSK3 $\beta$ .

One of the concerns about targeting GSK3 $\beta$  for cancer treatment is that its inhibition may promote the progression of existing tumors [reviewed in Takahashi-Yanaga<sup>(22)</sup>, Jope *et al.*,<sup>(25)</sup> Luo<sup>(26)</sup> and McCubrey *et al.*<sup>(27)</sup>]. A number of



studies have focused on the putative tumor suppressive role of GSK3 $\beta$  (Table S1).<sup>(SR30–58)</sup> Many of these report inactivation or activation of GSK3 $\beta$  as a mediating event in pathways leading to tumor progression or suppression, respectively. An inverse association between tumor expression of GSK3 $\beta$  (either total or active form pGSK3 $\beta$ <sup>Y216</sup>) and the survival of cancer patients has been reported.<sup>(SR33,36,37)</sup> Other studies found causal links between pharmacological GSK3 $\beta$  inhibition and/or depletion of GSK3 $\beta$  expression (e.g. gene knockout and RNA interference) or activity (e.g. recombinant kinase-dead or constitutively active mutant form) and tumor cell survival, proliferation<sup>(SR40,52)</sup> and susceptibility to cancer therapies.<sup>(SR32,34,58)</sup> GSK3 $\beta$  inhibition strategies and the development of GSK3 $\beta$ -targeted agents therefore require careful evaluation to determine whether the tumor promoting function of GSK3 $\beta$  is counteracted by its putative tumor suppressor function in different cancer types.

The development and administration of GSK3 $\beta$  inhibitors for the treatment of chronic diseases, including diabetes



mellitus, neurodegenerative disorders and cancer, requires serious awareness of the safety issues. A major concern is whether and how GSK3 $\beta$  can be targeted to treat disease, because its systemic inhibition may lead to unwanted effects by disrupting multiple biological pathways (Fig. 1c). Most notably, long-term inhibition of GSK3 $\beta$  may initiate tumorigenesis due to its role in suppressing proto-oncogenic pathways, in particular that are mediated by  $\beta$ -catenin [reviewed in Takahashi-Yanaga<sup>(22)</sup>, Jope *et al.*,<sup>(25)</sup> Luo<sup>(26)</sup> and McCubrey *et al.*<sup>(27)</sup>]. A previous study showed that genetic deletion of GSK3 $\beta$  in mammary epithelial cells resulted in  $\beta$ -catenin activation and induced intraepithelial neoplasia that progressed to the development of adenosquamous carcinoma.<sup>(SR51)</sup> Moreover, overexpression of wild-type and constitutively active mutant GSK3 $\beta$  in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-mediated, transformation-resistant mouse epidermal cells suppressed EGF-mediated and TPA-mediated anchorage-independent growth in soft agar and tumorigenicity in rodents (Table S1).<sup>(SR56)</sup> However, there is currently no direct evidence to support tumor development *in vivo* following treatment with a GSK3 $\beta$  inhibitor [reviewed in Miyashita *et al.*<sup>(11)</sup> and SR27]. As discussed in previous studies that report cancer therapeutic effects of GSK3 $\beta$  inhibition (Table 1), none of the available experimental GSK3 $\beta$  inhibitors induces neoplastic transformation of non-neoplastic (normal) cells or tumor development in experimental animal models [reviewed in Miyashita *et al.*<sup>(11)</sup> and SR27]. Long-term prescription of lithium, the only GSK3 $\beta$  inhibitor approved for the treatment of bipolar disorder since the 1950s, has not been associated with an increased risk of cancer or death from cancer.<sup>(SR59)</sup> Post-translational regulation of GSK3 $\beta$  activity via the phosphorylation of S9 and Y216 (pGSK3 $\beta$ <sup>S9</sup> versus pGSK3 $\beta$ <sup>Y216</sup>) (Fig. 1b) in response to various stimuli could partly underlie a mechanism that protects normal cells from the detrimental effects of GSK3 $\beta$  inhibition.

Despite the concerns outlined above, clinical trials for neurodegenerative diseases and cancer have tested some seed pharmacological GSK3( $\beta$ ) inhibitor compounds and also approved medicines with the ability to inhibit GSK3 $\beta$  activity (Table S2). The former trials include AZD-1080 (AstraZeneca) for the treatment of Alzheimer's disease (phase I), and NP031112 (tideglusive; Noscira SA) for the treatment of progressive supranuclear palsy (NCT01049399: phase IIb)<sup>(SR60,61)</sup> and of Alzheimer's disease (NCT01350362: phase II).<sup>(SR62,63)</sup> Clinical trials for cancer treatment have used LY2090314 (Eli Lilly) alone for acute leukemia (NCT01214603: phase II), and the same compound in combination: (i) with gemcitabine, the combined folate, 5-FU and oxaliplatin (FOLFOX) regimen or the combined gemcitabine and nab-paclitaxel regimen for metastatic pancreatic cancer (NCT01632306: phase I/II); and

(ii) with pemetrexed and carboplatin for advanced or metastatic solid cancer (NCT01287520: phase I).<sup>(SR64,65)</sup> Our group is undertaking the phase I/II clinical trials by repurposing approved GSK3 $\beta$ -inhibiting medicines (combined cimetidine, lithium, olanzapine and valproate regimen) in combination with gemcitabine for advanced pancreatic cancer (UMIN000005095) and with temozolomide for recurrent glioblastoma (UMIN000005111) (T. Furuta, H. Sabit, D. Yu, K. Miyashita, M. Kinoshita, M. Kinoshita, Y. Hayashi, Y. Hayashi, T. Minamoto, M. Nakada, unpublished data, 2016).

Currently, information regarding the side effects of GSK3 $\beta$  inhibitors is limited because the clinical trials have evaluated only a small number of seed compounds and also because lithium chloride is the only currently approved inhibitor for clinical use. It is, therefore, difficult to predict what serious adverse events, if any, will occur in patients treated with GSK3 $\beta$  inhibitors. The extent of GSK3 $\beta$  inhibition and the differential effects between normal and diseased cells/tissues (therapeutic window) are critical determining factors for therapeutic efficacy and undesirable side effects. It is clear that GSK3 $\beta$  activity is deregulated in various pathological conditions, including many, but not all, cancer types (Table 1).<sup>(10–12,22,25–27)</sup> Identifying the range of GSK3 $\beta$  inhibition that is efficient for treatment of disease but which results in minimal detrimental effects to normal tissues and organs will be important for the clinical application of GSK3 $\beta$  inhibitors. Numerous studies have shown that, in addition to differential phosphorylation at S9 and Y216 residues (Fig. 1a,b), the subcellular localization of GSK3 $\beta$  together with various upstream pathways can regulate the expression and activity of this kinase [reviewed in Beurel *et al.*<sup>(10)</sup> and Takahashi-Yanaga<sup>(22)</sup>]. New classes of GSK3 $\beta$  inhibitors that spatially and temporally control the expression and activity of GSK3 $\beta$  may therefore be required to reduce the risk of adverse events such as carcinogenesis that may be associated with long-term GSK3 $\beta$  inhibition.

## Acknowledgments

We thank Dr Barry Iacopetta (University of Western Australia) for critical review of the manuscript. This study was supported by Grant-in-Aids for Scientific Research (TD, IVP, TS, MN and TM) from the Japanese Ministry of Education, Science, Sports, Technology and Culture; from the Ministry of Health, Labour and Welfare, from the Japan Society for the Promotion of Science, and the Extramural Collaborative Research Grant of Cancer Research Institute, Kanazawa University.

## Disclosure Statement

The authors have no conflict of interest to declare.

## References

- Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Rev Cancer* 2013; **19**: 1423–37.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–74.
- Friedl P, Wolf K. Tumor-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003; **3**: 362–74.
- Wan L, Pantel K, Kang Y. Tumor metastasis: moving new biological insights into the clinic. *Nat Med* 2013; **19**: 1450–64.
- Holohan C, Van Schaeybroek S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer* 2013; **13**: 714–26.
- Ramos P, Bentires-Alj M. Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. *Oncogene* 2015; **34**: 3617–26.
- Al-Lazikani B, Banerji U, Workman P. Combinatorial drug therapy for cancer in the post-genomic era. *Nat Biotechnol* 2012; **30**: 679–92.
- Papadatos-Pastos D, De Miguel Luken MJ, Yap TA. Combining targeted therapeutics in the era of precision medicine. *Br J Cancer* 2015; **112**: 1–3.
- Alexander S, Friedl P. Cancer invasion and resistance: interconnected processes of disease progression and therapy failure. *Trends Mol Med* 2012; **18**: 13–26.
- Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther* 2015; **148**: 114–31.
- Miyashita K, Nakada M, Shakoori A *et al.* An emerging strategy for cancer treatment targeting aberrant glycogen synthase kinase 3 $\beta$ . *Anti-Cancer Agents Med Chem* 2009; **9**: 1114–22.
- McCubrey JA, Steelman LS, Bertrand FE *et al.* GSK-3 as potential target for therapeutic intervention in cancer. *Oncotarget* 2014; **5**: 2881–911.

- 13 Beck B, Blanpain C. Unravelling cancer stem cell potential. *Nat Rev Cancer* 2013; **13**: 727–38.
- 14 Maugeri-Saccà M, Vigneri P, De Maria R. Cancer stem cells and chemosensitivity. *Clin Cancer Res* 2011; **17**: 4942–7.
- 15 Borah A, Raveendran S, Rochani A, Maekawa T, Kumar DS. Targeting self-renewal pathways in cancer stem cells: clinical implications for cancer therapy. *Oncogenesis* 2015; **4**: e177.
- 16 Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871–90.
- 17 Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev* 2009; **28**: 15–33.
- 18 Pribluda A, de la Cruz CC, Jackson EL. Intratumoral heterogeneity: from diversity comes resistance. *Clin Cancer Res* 2015; **21**: 2916–23.
- 19 Tabassum DP, Polyak K. Tumorigenesis: it takes a village. *Nat Rev Cancer* 2015; **15**: 473–83.
- 20 Ebos JML. Prodding the beast: assessing the impact of treatment-induced metastasis. *Cancer Res* 2015; **75**: 3427–35.
- 21 Beurel E, Michalek SM, Jope RS. Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3). *Trends Immunol* 2010; **31**: 24–31.
- 22 Takahashi-Yanaga F. Activator or inhibitor? GSK-3 as a new drug target. *Biochem Pharmacol* 2013; **86**: 191–9.
- 23 Doble BW, Patel S, Wood GA, Kockeritz LK, Woodgett JR. Functional redundancy of GSK-3 $\alpha$  and GSK-3 $\beta$  in Wnt/ $\beta$ -catenin signaling shown by using an allelic series of embryonic stem cell lines. *Dev Cell* 2007; **12**: 957–71.
- 24 Clevers H, Nusse R. Wnt/ $\beta$ -catenin signaling and diseases. *Cell* 2012; **149**: 1192–205.
- 25 Jope RS, Yukaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem Res* 2007; **32**: 577–95.
- 26 Luo J. Glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) in tumorigenesis and cancer chemotherapy. *Cancer Lett* 2009; **273**: 194–200.
- 27 McCubrey JA, Davis NM, Abrams SL et al. Diverse roles of GSK-3: tumor promoter-tumor suppressor, target in cancer therapy. *Adv Biol Regul* 2014; **54**: 176–96.
- 28 Shakoori A, Ougolkov A, Yu ZW et al. Deregulated GSK3 $\beta$  activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem Biophys Res Commun* 2005; **334**: 1365–73.
- 29 Mai W, Miyashita K, Shakoori A et al. Detection of active fraction of glycogen synthase kinase 3 $\beta$  in cancer cells by nonradioisotopic in vitro kinase assay. *Oncology* 2006; **71**: 297–305.
- 30 Mai W, Kawakami K, Shakoori A et al. Deregulated glycogen synthase kinase 3 $\beta$  sustains gastrointestinal cancer cells survival by modulating human telomerase reverse transcriptase and telomerase. *Clin Cancer Res* 2009; **15**: 6810–9.
- 31 Miyashita K, Kawakami K, Mai W et al. Potential therapeutic effect of glycogen synthase kinase 3 $\beta$  inhibition against human glioblastoma. *Clin Cancer Res* 2009; **15**: 887–979.
- 32 Tan J, Zhuang L, Leong HS, Iyer NG, Liu ET, Yu Q. Pharmacologic modulation of glycogen synthase kinase-3 $\beta$  promotes p53-dependent apoptosis through a direct Bax-mediated mitochondrial pathway in colorectal cancer cells. *Cancer Res* 2005; **65**: 9012–20.
- 33 Shakoori A, Mai W, Miyashita K et al. Inhibition of GSK-3 $\beta$  activity attenuates proliferation of human colon cancer cells in rodents. *Cancer Sci* 2007; **98**: 1388–93.
- 34 Ougolkov AV, Fernandez-Zapico ME, Savoy DN, Urrutia RA, Billadeau DD. Glycogen synthase kinase-3 $\beta$  participates in nuclear factor  $\kappa$ B-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res* 2005; **65**: 2076–81.
- 35 Wilson W 3rd, Baldwin AS. Maintenance of constitutive I $\kappa$ B kinase activity by glycogen synthase kinase-3 $\alpha/\beta$  in pancreatic cancer. *Cancer Res* 2008; **68**: 8156–63.
- 36 Kotliarova S, Pastorino S, Kovell LC et al. Glycogen synthase kinase-3 inhibition induces glioma cell death through c-MYC, nuclear factor- $\kappa$ B, and glucose regulation. *Cancer Res* 2008; **68**: 6643–51.
- 37 Korur S, Huber RM, Sivasankaran B et al. GSK3 $\beta$  regulates differentiation and growth arrest in glioblastoma. *PLoS ONE* 2009; **4**: e7443.
- 38 Tang QL, Xie XB, Wang J et al. Glycogen synthase kinase-3 $\beta$ , NF- $\kappa$ B signaling, and tumorigenesis of human osteosarcoma. *J Natl Cancer Inst* 2012; **104**: 749–63.
- 39 Cao Q, Lu X, Feng YJ. Glycogen synthase kinase-3 $\beta$  positively regulates the proliferation of human ovarian cancer cells. *Cell Res* 2006; **16**: 671–7.
- 40 Yin Y, Kizer NT, Thaker PH et al. Glycogen synthase kinase 3 $\beta$  inhibition as a therapeutic approach in the treatment of endometrial cancer. *Int J Mol Sci* 2013; **14**: 16617–37.
- 41 Liao X, Zhang L, Thrasher JB, Du J, Li B. Glycogen synthase kinase-3 $\beta$  suppression eliminates tumor necrosis factor-related apoptosis-inducing ligand resistance in prostate cancer. *Mol Cancer Ther* 2003; **2**: 1215–22.
- 42 Mazor M, Kawano Y, Zhu H, Waxman J, Kypta RM. Inhibition of glycogen synthase kinase-3 represses androgen receptor activity and prostate cancer cell growth. *Oncogene* 2004; **23**: 7882–92.
- 43 Zhu Q, Yang J, Han S et al. Suppression of glycogen synthase kinase 3 activity reduces tumor growth of prostate cancer in vivo. *Prostate* 2011; **71**: 835–45.
- 44 Darrington RS, Campa VM, Walker MM et al. Distinct expression and activity of GSK-3 $\alpha$  and GSK-3 $\beta$  in prostate cancer. *Int J Cancer* 2012; **131**: E872–83.
- 45 Naito S, Bilim V, Yuuki K et al. Glycogen synthase kinase-3 $\beta$ : a prognostic marker and a potential therapeutic target in human bladder cancer. *Clin Cancer Res* 2010; **16**: 5124–32.
- 46 Bilim V, Ougolkov A, Yuuki K et al. Glycogen synthase kinase-3: a new therapeutic target in renal cell carcinoma. *Br J Cancer* 2009; **101**: 2005–14.
- 47 Pal K, Cao Y, Gaisina IN et al. Inhibition of GSK-3 induces differentiation and impaired glucose metabolism in renal cancer. *Mol Cancer Ther* 2014; **13**: 285–96.
- 48 Zeng FY, Dong H, Cui J, Liu L, Chen T. Glycogen synthase kinase 3 regulates PAX3-FKHR-mediated cell proliferation in human alveolar rhabdomyosarcoma cells. *Biochem Biophys Res Commun* 2010; **391**: 1049–55.
- 49 Chen EY, DeRan MT, Ignatius MS et al. Glycogen synthase kinase 3 inhibitors induce the canonical WNT/ $\beta$ -catenin pathway to suppress growth and self-renewal in embryonic rhabdomyosarcoma. *Proc Natl Acad Sci USA* 2014; **111**: 5349–54.
- 50 Wang Z, Smith KS, Murphy M, Piloto O, Somervaille TCP, Cleary ML. Glycogen synthase kinase-3 in MLL leukaemia maintenance and targeted therapy. *Nature* 2008; **455**: 1205–9.
- 51 Mirlashari MR, Randen I, Kjeldsen-Kragh J. Glycogen synthase kinase-3 (GSK-3) inhibition induces apoptosis in leukemic cells through mitochondria-dependent pathway. *Leuk Res* 2012; **36**: 499–508.
- 52 Zeng J, Liu D, Qiu Z et al. GSK3 $\beta$  overexpression indicates poor prognosis and its inhibition reduces cell proliferation and survival of non-small cell lung cancer cells. *PLoS ONE* 2014; **9**: e91231.
- 53 Vincent EE, Elder DJ, O'Flaherty L et al. Glycogen synthase kinase 3 protein kinase activity is frequently elevated in human non-small cell lung carcinoma and supports tumour cell proliferation. *PLoS ONE* 2014; **9**: e114725.
- 54 Yoshino Y, Ishioka C. Inhibition of glycogen synthase kinase-3 $\beta$  induces apoptosis and mitotic catastrophe by disrupting centrosome regulation in cancer cells. *Sci Rep* 2015; **5**: 13249.
- 55 Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378**: 607–20.
- 56 Wen PY, Kasari S. Malignant gliomas in adults. *N Engl J Med* 2009; **359**: 492–507.
- 57 Sun T, Rodriguez M, Kim L. Glycogen synthase kinase 3 in the world of cell migration. *Dev Growth Differ* 2009; **51**: 735–42.
- 58 Nowicki MO, Dmitrieva N, Stein AM et al. Lithium inhibits invasion of glioma cells: possible involvement of glycogen synthase kinase-3. *Neuro Oncol* 2008; **10**: 690–9.
- 59 Williams SP, Nowicki MO, Liu F et al. Indirubins decrease glioma invasion by blocking migratory phenotypes in both the tumor and stromal endothelial cell compartments. *Cancer Res* 2011; **71**: 5374–80.
- 60 Kitano A, Shimazaki T, Chikano Y et al. Aberrant glycogen synthase kinase 3 $\beta$  is involved in pancreatic cancer cell invasion and resistance to therapy. *PLoS ONE* 2013; **8**: e55289.
- 61 Chikano Y, Domoto T, Furuta T et al. Glycogen synthase kinase 3 $\beta$  sustains invasion of glioblastoma via the focal adhesion kinase, Rac1 and c-Jun N-terminal kinase-mediated pathway. *Mol Cancer Ther* 2015; **14**: 564–74.
- 62 Machesky LM. Lamellipodia and filopodia in metastasis and invasion. *FEBS Lett* 2008; **582**: 2102–11.
- 63 Paz H, Pathak N, Yang J. Invading one step at a time: the role of invadopodia in tumor metastasis. *Oncogene* 2014; **33**: 4193–202.
- 64 Bid HK, Roberts RD, Manchanda PK, Houghton PJ. RAC1: an emerging therapeutic option for targeting cancer angiogenesis and metastasis. *Mol Cancer Ther* 2013; **12**: 1925–34.
- 65 Sulzmaier FJ, Jean C, Schlaepfer DD. FAK in cancer: mechanistic findings and clinical applications. *Nat Rev Cancer* 2014; **14**: 598–610.
- 66 Cook DR, Rossman KL, Der CJ. Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease. *Oncogene* 2014; **33**: 4021–35.
- 67 Ying X, Jing L, Ma S et al. GSK3 $\beta$  mediates pancreatic cancer cell invasion in vitro via the CXCR4/MMP-2 pathway. *Cancer Cell Int* 2015; **15**: 70.
- 68 Yoshino Y, Suzuki M, Takahashi H, Ishioka C. Inhibition of invasion by glycogen synthase kinase-3 $\beta$  inhibitors through dysregulation of actin reorganization via down-regulation of WAVE2. *Biochem Biophys Res Commun* 2015; **464**: 275–80.
- 69 Pyko IV, Nakada M, Sabit H et al. Glycogen synthase kinase 3 $\beta$  inhibition sensitizes human glioblastoma cells to temozolomide by affecting O<sup>6</sup>-methylguanine DNA methyltransferase promoter methylation via c-Myc signaling. *Carcinogenesis* 2013; **34**: 2206–17.

- 70 Shimasaki T, Ishigaki Y, Nakamura Y *et al.* Glycogen synthase kinase 3 $\beta$  inhibition sensitizes pancreatic cancer cells to gemcitabine. *J Gastroenterol* 2012; **47**: 321–33.
- 71 Knudsen ES, Wang JYJ. Targeting the RB-pathway in cancer therapy. *Clin Cancer Res* 2010; **16**: 1094–9.
- 72 David Engelmann D, Pützer BM. The dark side of E2F1: in transit beyond apoptosis. *Cancer Res* 2012; **72**: 571–5.
- 73 Grassilli E, Narloch R, Federzoni E *et al.* Inhibition of GSK3 $\beta$  bypass drug resistance of p53-null colon carcinomas by enabling necroptosis in response to chemotherapy. *Clin Cancer Res* 2013; **19**: 3820–31.
- 74 Kawazoe H, Bilim VN, Ugolkov AV *et al.* GSK-3 inhibition in vitro and in vivo enhances antitumor effect of sorafenib in renal cell carcinoma (RCC). *Biochem Biophys Res Commun* 2012; **423**: 490–5.
- 75 Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* 2009; **9**: 265–73.
- 76 Kuhlmann JD, Hein L, Kurth I, Wimberger P, Dubrovskaya A. Targeting cancer stem cells: promises and challenges. *Anticancer Agents Med Chem* 2015; **16**: 38–58.
- 77 Li Y, Lu H, Huang Y *et al.* Glycogen synthase kinases-3 $\beta$  controls differentiation of malignant glioma cells. *Int J Cancer* 2010; **127**: 1271–82.
- 78 Kroon J, in't Veld LS, Buijs JT, Cheung H, van der Horst G, van der Pluijm G. Glycogen synthase kinase-3 $\beta$  inhibition depletes the population of prostate cancer stem/progenitor-like cells and attenuates metastatic growth. *Oncotarget* 2014; **5**: 8986–94.
- 79 Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med* 2004; **10**: 55–63.
- 80 Trowbridge JJ, Xenocostas A, Moon RT, Bhatia M. Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. *Nat Med* 2006; **12**: 89–98.

## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Table S1.** Previous studies reporting the putative tumor suppressor roles of GSK3 $\beta$ .

**Table S2.** Clinical trials of GSK3 $\beta$  inhibitors for treatment of diseases.

**Data S1.** Supplementary references.