Review

FoxM1 and Wnt/β-Catenin Signaling in Glioma Stem Cells

Aihua Gong¹ and Suyun Huang¹,²

Abstract

Cancer stem cells may be responsible for tumor initiation and maintenance. The molecular mechanisms that control cancer stem cells are related to alterations in various signaling pathways, including the Wnt/β-catenin signaling pathway. The canonical Wnt/β-catenin signaling pathway is one of the major signaling systems in stem and progenitor cells, and aberrant activation of the Wnt/β-catenin signaling pathway is common in human cancers. As with β-catenin, FoxM1 has been found to play important roles in a number of cancers. In this review, we discuss the evidence that FoxM1 affects the expression and function of a variety of genes that are critical to the survival, proliferation, invasion, angiogenesis, and self-renewal of cancer stem cells. We highlight the pivotal roles of the Wnt/β-catenin and FoxM1 signaling pathways in neural stem and progenitor cells and glioma stem cells. We also discuss the evidence for cross-talk between the β-catenin and FoxM1 signaling pathways in the regulation of the stemness and tumorigenicity of glioma stem cells.

Cancer Res; 72(22); 5658–62. ©2012 AACR.

Introduction

FoxM1 is a member of the Forkhead box (Fox) transcription factor family. FoxM1 is ubiquitously expressed in embryonic tissues, but is extinguished in differentiated cells (1, 2). FoxM1 is involved in the self-renewal and proliferation of stem cells, including neural stem cells (NSC) and epithelial progenitor cells (3–5), and is a key cell-cycle regulator of both the transition from G1 to S-phase and progression to mitosis (1). FoxM1 is also essential for chromosome segregation, and the loss of FoxM1 causes centrosome amplification and mitotic catastrophe (1). FoxM1 is substantially elevated in most human tumors and contributes to oncogenesis in many tissue types. In melanoma cells, for example, FoxM1 is stabilized and activated by CDK4/6 and protects cancer cells from senescence by inducing expression of G1 to S-phase genes and suppressing the levels of reactive oxygen species (6). In malignant glioma, FoxM1 is a downstream component of the Wnt/β-catenin signaling pathway and plays critical roles in tumorigenicity (4).

Malignant gliomas are associated with high rates of morbidity and mortality. Most human gliomas seem to have a set of pathways that are disrupted (pRb, p53, and PTEN) and a set that are abnormally active (telomerase, EGFR, and Akt; ref. 7). However, the molecular mechanisms underlying the development and progression of gliomas remain poorly understood. A recently proposed hypothesis about the role of cancer stem cells in glioma may bring a new perspective to our understanding of glioma biology and therapy. Cancer stem cells are the subpopulation of tumor cells that have self-renewal, multipotent, and tumor-initiating abilities. Several recent studies have indicated that cancer stem cells are present in glioblastoma multiforme (GBM); such cells are known as glioma stem cells (GSC), GBM stem cells, or GBM-initiating cells (7).

GSCs share many properties with neural stem and progenitor cells, and the deregulation and/or abnormal operation of key signaling pathways in GSCs potentially leads to the development of brain tumors. The Wnt signaling pathway is critical to regulating the self-renewal, proliferation, and differentiation of NSCs and progenitor cells in the brain. Emerging evidence has suggested that deregulation of the Wnt pathway is associated with brain tumors, including gliomas. The canonical, or β-catenin–dependent, Wnt signaling pathway is initiated by the binding of Wnt proteins to receptors of the frizzled and low density lipoprotein receptor-related protein/β2-macroglobulin receptor (LRP) families on the cell surface. The signal is transduced through several cytoplasmic relay components to β-catenin, which then enters the nucleus and forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) to activate the transcription of Wnt target genes (8). Interestingly, in the search for a β-catenin–binding partner, FoxM1 was found to be a novel downstream component of the Wnt/β-catenin signaling pathway.

A deeper understanding of the mechanisms that govern normal brain development would likely provide insight into the molecular basis of brain tumors. In this review, we focus on recent insights into our understanding of the Wnt/β-catenin signaling pathway and of FoxM1 in the development of normal brain and the formation and progression of glioma. We also define the interlocking roles of Wnt, FoxM1, and β-catenin in the regulation of cell proliferation, invasion, and...
tumorigenicity in glioma and identify new targets for therapeutic intervention.

**Wnt/β-catenin is a major signaling pathway in brain development**

Neural precursor or progenitor cells, which are located in the ventricular and subventricular zones, give rise to the neurons, astrocytes, and oligodendrocytes that make up the functioning brain. Findings from several studies have suggested that Wnt signaling is required at several stages of central nervous system (CNS) development. Wnt signaling controls the initial formation of the neural plate as well as many subsequent patterning decisions in the embryonic nervous system, including those governing the formation of the neural crest. Wnt signaling also has critical roles in dorsal forebrain specification and NSCs and precursor cell formation in the ventricular zone (9). Overexpression of Wnt3 is sufficient to increase the rate of adult hippocampal progenitor and stem cell neurogenesis in vitro and in vivo. In contrast, blockade of Wnt signaling reduces adult hippocampal progenitor neurogenesis in vitro and in vivo (10).

Mechanistically, Wnt signaling regulates both the self-renewal and differentiation of neural stem and progenitor cells. Wnt signaling directly mediates the self-renewal of neural stem/progenitor cells in vitro and in vivo (11). Also, studies of loss-of-function and gain-of-function mutations of CTNNB1 in the CNS have shown that Wnt signals direct cell differentiation; specifically, when constitutively active β-catenin was expressed in vivo, cell-cycle exit was reduced in the ventricular zone, leading to an enlarged brain, whereas deletion of CTNNB1 in cortical progenitors decreased the proliferation and migration defects and markedly reduced brain tissue mass (12). Taken together, these results highlight the importance of Wnt signaling in the normal development of the CNS.

**FoxM1 plays a critical role in brain development**

FoxM1 is expressed in all embryonic tissues, particularly in proliferating cells of epithelial and mesenchymal origin. The role of FoxM1 in normal development was first noted in the myocardium, liver, and lungs (1). Recently, mounting evidence indicates that FoxM1 is required for both the proliferation and differentiation of neuronal precursors. High-throughput screening in situ hybridization studies revealed that FoxM1 is highly expressed in NSCs of the germinal zone (ventricular and subventricular zones) throughout early CNS development (E14, E17, P0; ref. 13). This specific germinal zone expression pattern of FoxM1 suggests that the protein plays a specific role in neural stem cell biogenesis.

Moreover, a study using a FoxM1 knockout mouse model confirmed that FoxM1 regulates mitotic entry in cerebellar granule neuron precursors (1). FoxM1-deficient cerebellar granule neuron precursors showed a significant delay in the G1 to M transition and lower expression levels of the FoxM1 target genes cyclin B1 and Cdc25b (1, 2). Another study revealed that FoxM1 is required for cell proliferation in the *Xenopus laevis* neuroectoderm (1). Furthermore, we recently found that FoxM1 is a novel component of the Wnt signaling pathway and that FoxM1–β-catenin interaction is required for the activation of the canonical Wnt signaling pathway in NSCs (4).

Findings from these studies suggest that the components of the Wnt signaling pathway, including FoxM1, are involved in NSC self-renewal and brain development.

**Emerging role of the Wnt/β-catenin signaling pathway in glioma**

Wnt signaling is divided into 2 different pathways: the canonical, or Wnt/β-catenin, pathway is involved in the determination of cell fate, whereas the noncanonical pathway is involved in the control of cell movement and tissue polarity. The activation of both Wnt pathways is associated with the development and progression of brain tumors. Genetic alterations in the Wnt/β-catenin signaling pathway are commonly found in human tumors, including medulloblastoma but are not found in glioma. Glioma formation and progression are associated with many Wnt signaling pathway components, including positive regulators (Wnt ligands, β-catenin, PLAG2, FoxM1, and receptors FZL and DVL) and negative inhibitors [secreted frizzled-related protein (sFRP), dickkopf (Dkk), paternally expressed gene 3 (PEG3/Pwl), and ß2-macroglobulin (ß2M)]. Moreover, functional studies have revealed that these factors are involved in the regulation of the migration, invasion, and proliferation of malignant glioma cells and the self-renewal of GSCs (4, 14–18). For example, siRNA knockdown of Wnt2 and β-catenin inhibits cell proliferation and invasion and induces apoptosis in human U251 glioma cells (16).

On the other hand, epigenetic silencing of the Wnt pathway inhibitor gene frequently occurs in glioma, including promoter hypermethylation of SFRP1, SFRP2, SFRP4, SFRP5, dickkopf (Dkk1, Dkk3), naked (Nkd1, Nkd2), and PEG3/Pwl1. Promoter hypermethylation of the imprinted gene PEG3/Pwl1 and sFRPs is a significant event in primary de novo GBM, whereas promoter hypermethylation of the Wnt receptor LRP antagonist Dkks is associated with secondary GBM (17).

Other studies have further highlighted the critical roles of Wnt signaling in gliomagenesis. For example, integrated genomic and biologic analyses have identified PLAGL2, which inhibits cell differentiation to promote the self-renewal of NSC/progenitor cells and glioma-initiating cells as a potent proto-oncogene in malignant gliomas (18). The differentiation suppressive activities of PLAGL2 are due, in part, to the activation of Wnt/β-catenin signaling. Furthermore, PLAGL2 amplification correlates with increased β-catenin levels in GBM samples (18).

The nuclear localization of β-catenin is the hallmark of an active Wnt pathway. However, the frequency of mutations in the APC or CTNNB1 (β-catenin) gene seems to be substantially lower than the frequency of nuclear accumulation of β-catenin in gliomas, suggesting that mutations are not the major molecular event that leads to the nuclear accumulation of β-catenin in gliomas. Given that both FoxM1 and β-catenin are activated in glioblastoma, we investigated whether FoxM1 regulates the nuclear localization of β-catenin and the self-
FoxM1 is a critical regulator of gliomagenesis and progression

With use of transcriptome microarray analysis, massively parallel signature sequencing, and bioinformatics analysis, several studies showed that the expression of FoxM1 in high-grade anaplastic astrocytomas and glioblastomas is significantly higher than that in low-grade astrocytomas (19). Moreover, the Cancer Genome Atlas data of 201 GBM specimens confirmed the overexpression of FoxM1 in GBM specimens.

In previous studies, investigating the functional significance of FoxM1, we found that FoxM1B was the predominant FoxM1 isoform in human gliomas (20). The expression level of FoxM1 protein in human glioma tissue was directly correlated with tumor grade in human glioma tissue, and the expression level of FoxM1 protein in human GBM tissue was inversely correlated with patient survival. With use of GBM animal models, we showed that increased FoxM1 expression in glioma cells enhanced their tumorigenicity, invasiveness, and angiogenesis (21–23). Moreover, FoxM1 promoted the uncontrolled proliferation, invasion, and angiogenesis of GBM cells, presumably by decreasing the protein expression of its target gene p27kip1 and increasing the transcription of its target genes Skp2, cyclin D1, MMP-2, and VEGF (21–23).

In another study, because the expression of FoxM1 was detected in low-grade glioma and FoxM1 was identified as a novel target of human papillomavirus type 16 E7 protein, which might be important for transformation of normal cells, we sought to determine whether FoxM1 functions as an oncogene and plays a role in the transformation of astrocytes. A previous study showed that FoxM1 transgenic mice did not spontaneously develop brain tumors, indicating that overexpression of FoxM1 itself is insufficient to induce gliomas. Because both the inactivation of the pRb pathway and overexpression of FoxM1 occur in more than 80% of gliomas, and inactivation of p53 occurs in 30% of gliomas (7), we also sought to determine whether overexpression of FoxM1 and loss of Rb and p53 cooperate to induce glioma formation. We found that FoxM1 overexpression cooperated with loss of p53 and Rb to initiate cellular transformation and gliomagenesis in normal human astrocytes (21). FoxM1-mediated astrocyte transformation and GBM formation through multiple mechanisms, including the increased activation of Akt and expression of survivin, cyclin E, and cyclin D1.

Because FoxM1 induces the promoter activity of β-catenin target genes c-Myc and cyclin D1, and the level of FoxM1 correlates with the activity of Wnt/β-catenin signaling in GBM-initiating cells, we investigated a possible link between FoxM1 and β-catenin activation. First, we identified β-catenin as a potential interacting partner of FoxM1 by liquid chromatography/tandem mass spectrometry analysis (4). Then, we carried out various in vitro and in vivo experiments that revealed a direct interaction between FoxM1 and β-catenin proteins. FoxM1 and β-catenin were colocalized in both the cytoplasm and nucleus, and Wnt3a stabilized both proteins and increased their nuclear accumulation in the cells, indicating that FoxM1 is a downstream component of the Wnt signaling pathway. FoxM1 promoted β-catenin nuclear translocation in both NSCs and GSCs, was recruited by β-catenin to the β-catenin–TCF/LEF transcription activation complex, and promoted the assembly of the transcription activation complex.

We established the biologic relevance of this interaction by showing that knockdown of FoxM1 or β-catenin in GSCs abolished their self-renewal and tumor initiation abilities. Moreover, constitutive activated β-catenin (β-catenin–nuclear localization signal) rescued the inhibitory effect of knockdown of FoxM1.
of FoxM1 on the tumorigenicity of GSCs. Furthermore, knockdown of β-catenin in FoxM1-dependent tumorigenic glioma cells abolished the cells' tumorigenicity indicating that β-catenin mediates the tumorigenic effect of FoxM1. The significance of the FoxM1 and β-catenin relationship and its clinical relevance were determined by using 40 human GBM specimens. In human GBM, FoxM1 expression directly correlated with β-catenin activation (nuclear β-catenin level) as well as with the expression of the stem cell marker nestin. Taken together, these findings suggest that FoxM1 is a major component of the Wnt/β-catenin signaling pathway and plays a critical role in the maintenance of stemness and tumorigenesis in GSCs (Fig. 1). However, FoxM1 may not be directly involved in the malignant transformation of NSCs, as FoxM1 transgenic mice do not undergo spontaneous production of brain tumors.

Recently, integrated genomic analysis has identified clinically relevant subtypes of glioblastoma, including proneural, neural, classical, and mesenchymal subtypes (24). We have analyzed FoxM1 mRNA expression in a TCGA data set and found that all 4 subtypes have a wide range of FoxM1 expression, among which the neural group has a relatively low level of FoxM1 (unpublished data). Thus, it is unclear whether FoxM1 is a subtype-specific factor. Future studies to analyze the levels of FoxM1 mRNA, protein, and phosphorylation will be needed to determine the intricate relationship between GBM subtypes and FoxM1 status.

**FoxM1 and Wnt/β-catenin signaling in other tumors**

In addition of glioma, many other tumors have overactivated signaling of both FoxM1 and Wnt/β-catenin, including medulloblastoma, colon cancer, and hepatocellular carcinoma (HCC; ref. 2). In medulloblastoma, the most frequent brain tumor in children, 15% of cases have an activated Wnt/β-catenin signaling pathway because of mutations in components of the pathway, including β-catenin (25). FoxM1 is essential for the proliferation of medulloblastoma cells, and the expression levels of FoxM1 protein significantly correlated with worse patient survival (26). Colon cancer has a well-established association with overactivated Wnt/β-catenin signaling as well as with FoxM1 overexpression. Moreover, Wnt/β-catenin signaling may depend on FoxM1 in colon cancer, as FoxM1 plays an important role in colon cancer development, which was revealed in a study that used conditional FoxM1 knockout mice to show that β-catenin–TCF-4 signaling is reduced in Foxm1−/− colon tumors (27). Aberrant activation of Wnt/β-catenin signaling has been implicated as a major mechanism of HCC tumorigenesis. FoxM1 promotes HCC development and constitutes a therapeutic target for HCC treatment (28). However, future studies are needed to determine whether FoxM1 has similar functions in these tumors as in glioma, such as interacting with β-catenin and regulating the self-renewal of cancer stem cells.

**Future studies**

The deregulation of Wnt signaling and FoxM1 may play important cooperative roles in glioma initiation and progression; however, many mechanisms governing the development and progression of gliomas need to be elucidated.

First, the mechanisms causing FoxM1 overexpression in tumors, including GBM, are unclear. FoxM1 overexpression is not due to gene amplification in GBM (19). Our previous study findings indicated that Wnt may control FoxM1 protein expression in GBM cells; thus, dysregulated Wnt signaling may induce FoxM1 overexpression in GBM.

Second, FoxM1 regulates sFRP1 expression in endothelial cells (29); therefore, it is worthwhile to determine whether FoxM1 regulates Wnt signaling via other Wnt components in addition to direct interaction with β-catenin.

Third, recent studies have identified various miRNAs involved in regulating Wnt/β-catenin signaling, including miR-9, miR-200a, and miR-135; however, no miRNAs have yet been identified that regulate FoxM1. Also, recent research has indicated that miRNAs have an important role in regulating normal stem cell and cancer stem cell self-renewal by overriding cell-cycle checkpoints to sustain continual division (30). FoxM1 is a key protein controlling the G1 to S and G2 to M checkpoints; thus, it will be interesting to determine the role of miRNA in the regulation of FoxM1 during the maturation of cancer stem cells. Furthermore, it will be interesting to determine whether the same miRNAs modulate the function of FoxM1 and Wnt/β-catenin signaling, hence overseeing the tumorigenicity of cancer stem cells.

Finally, the noncanonical Wnt pathway may be involved in gliomagenesis and progression. Future studies of the roles of FoxM1 in the noncanonical Wnt pathway, as well as studies of the connection between the 2 Wnt pathways, should provide valuable insight into the mechanisms governing glioma development and progression.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: A. Gong, S. Huang

Writing, review, and/or revision of the manuscript: A. Gong, S. Huang

Study supervision: A. Gong, S. Huang

**Acknowledgments**

The authors thank Joe Munch and Tamara Locke in MD Anderson’s Department of Scientific Publications for editing the manuscript.

**Grant Support**

S. Huang is supported by grants from the National Cancer Institute (R01CA157933, R01CA152309, and R21CA152623).

Received March 9, 2012; revised June 25, 2012; accepted July 6, 2012; published OnlineFirst November 8, 2012.

**References**


FoxM1 and Wnt/β-Catenin Signaling in Glioma Stem Cells

Aihua Gong and Suyun Huang


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-0953

Cited articles
This article cites 30 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/72/22/5658.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/72/22/5658.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.