FoxM1 Drives a Feed-Forward STAT3-Activation Signaling Loop That Promotes the Self-Renewal and Tumorigenicity of Glioblastoma Stem-like Cells

Ai-hua Gong1,2, Ping Wei1, Sicong Zhang1,3, Jun Yao4, Ying Yuan5, Ai-dong Zhou1, Frederick F. Lang1, Amy B. Heimberger1, Ganesh Rao1, and Suyun Huang1,3

Abstract

The growth factor PDGF controls the development of glioblastoma (GBM), but its contribution to the function of GBM stem-like cells (GSC) has been little studied. Here, we report that the transcription factor FoxM1 promotes PDGF-A-STAT3 signaling to drive GSC self-renewal and tumorigenicity. In GBM, we found a positive correlation between expression of FoxM1 and PDGF-A. In GSC and mouse neural stem cells, FoxM1 bound to the PDGF-A promoter to upregulate PDGF-A expression, acting to maintain the stem-like qualities of GSC in part through this mechanism. Analysis of the human cancer genomic database The Cancer Genome Atlas revealed that GBM expresses higher levels of STAT3, a PDGF-A effector signaling molecule, as compared with normal brain. FoxM1 regulated STAT3 transcription through interactions with the β-catenin/TCF4 complex. FoxM1 deficiency inhibited PDGF-A and STAT3 expression in neural stem cells and GSC, abolishing their stem-like and tumorigenic properties. Further mechanistic investigations defined a FoxM1-PDGF-A-STAT3 feed-forward pathway that was sufficient to confer stem-like properties to glioma cells. Collectively, our findings showed how FoxM1 activates expression of PDGF-A and STAT3 in a pathway required to maintain the self-renewal and tumorigenicity of glioma stem-like cells. Cancer Res; 75(11); 2337–48. ©2015 AACR.

Introduction

Glioblastoma is the most lethal primary malignant brain tumor in adults. Recent evidence from human and animal studies suggests that cancer stem cells are the cellular origin of glioma. GBM stem-like cells (GSC) can drive brain tumorigenesis through differentiation into highly proliferative tumor cells and transdifferentiation into endothelial cells and vascular pericytes in animal models (1–3). GSCs are resistant to chemotherapy and radiotherapy, and therefore they give rise to tumor recurrence by sustaining long-term tumor growth (4, 5).

Several studies have demonstrated that gliomagenesis involves aberrant signaling through PDGF pathway (6–14). Activation of the PDGF/PDGFR pathway may be triggered by binding between PDGFR (PDGFRα and PDGFRβ) with PDGF ligands (PDGF-A, B, C, and D). However, high expression of PDGF-A was found in 70% of gliomas, and PDGF-A expression directly correlates with grade with higher expression in glioblastoma multiforme (GBM; refs. 8, 15). Moreover, inhibition of PDGF-A was able to inhibit growth of GBM cell lines in vivo, indicating an important role of PDGF-A in mediating the growth of glioma cells (16, 17). Furthermore, Jackson and colleagues (18) showed that the PDGF-A pathway may link adult neural stem cells (NSC) to glioma, because infusion of PDGF-AA (PDGF-A homodimer) into the lateral ventricles led to formation of atypical hyperplasias, whereas withdrawal of PDGF-AA resulted in regression of hyperplasias and increased differentiation of NSCs. Thus, the study provides compelling evidence that PDGF-A signaling regulates NSC self-renewal, and increased PDGF-A signaling may be important for glioma initiation (18). However, whether GSCs express PDGF-A is unknown. Also, the mechanism driving PDGF-A expression in glioma has not been elucidated.

The STAT3 is a critical signaling node in tumor that can be activated by a panel of cytokines and growth factors, such as EGF, PDGF, and IL6, as well as by oncogenic proteins, such as Src and Ras (19–21). STAT3 is activated at high frequency in human tumors, including GBM (22, 23). Recently, STAT3 activity was found to be required for maintenance of the stem-like characteristics of GSCs (24). Moreover, IL6, bone marrow X-linked nonreceptor tyrosine kinase, and EZH2 activate STAT3 signaling to maintain self-renewal and tumorigenic potential of GSCs (25–27), confirming the importance of STAT3 in tumor stem cells.

PDGF controls the development of glioblastoma (GBM), but its contribution to the function of GBM stem-like cells (GSC) has been little studied. Here, we report that the transcription factor FoxM1 promotes PDGF-A-STAT3 signaling to drive GSC self-renewal and tumorigenicity. In GBM, we found a positive correlation between expression of FoxM1 and PDGF-A. In GSC and mouse neural stem cells, FoxM1 bound to the PDGF-A promoter to upregulate PDGF-A expression, acting to maintain the stem-like qualities of GSC in part through this mechanism. Analysis of the human cancer genomic database The Cancer Genome Atlas revealed that GBM expresses higher levels of STAT3, a PDGF-A effector signaling molecule, as compared with normal brain. FoxM1 regulated STAT3 transcription through interactions with the β-catenin/TCF4 complex. FoxM1 deficiency inhibited PDGF-A and STAT3 expression in neural stem cells and GSC, abolishing their stem-like and tumorigenic properties. Further mechanistic investigations defined a FoxM1-PDGF-A-STAT3 feed-forward pathway that was sufficient to confer stem-like properties to glioma cells. Collectively, our findings showed how FoxM1 activates expression of PDGF-A and STAT3 in a pathway required to maintain the self-renewal and tumorigenicity of glioma stem-like cells. Cancer Res; 75(11); 2337–48. ©2015 AACR.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

A.-h. Gong, P. Wei, and S. Zhang contributed equally to this article.

Corresponding Author: Suyun Huang, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-834-6252; Fax: 713-834-6257; E-mail: suhuang@mdanderson.org

doi: 10.1158/0008-5472.CAN-14-2800

©2015 American Association for Cancer Research.
of STAT3 signaling in GSCs. Inhibition of STAT3 expression in GBM cells and GSCs results in downregulated STAT3 phosphorylation (24, 28), indicating that the level of STAT3 is important to its phosphorylation. Moreover, STAT3 may drive downstream targets gene expression in the absence of phosphorylation (29, 30). However, the molecular mechanisms underlying STAT3’s own gene expression in glioma are poorly defined.

The transcription factor FoxM1 is also substantially elevated in a majority of human tumors and contributes to oncogenesis in different organs, including brain (31, 32). Independent profiling studies showed that the expression of FoxM1 in high-grade anaplastic astrocytomas and glioblastomas is significantly higher than that in low-grade astrocytomas (33, 34). Overexpression of FoxM1 in GBM specimens was further confirmed by a study that analyzed a set of data from The Cancer Genome Atlas (TCGA; ref. 35). Our previous studies showed that FoxM1 promotes the uncontrolled proliferation, invasion, and angiogenesis of GBM cells (36, 37).

In the present study, we examined the mechanisms underlying PDGF-A expression in glioma cells and GSCs. We show that FoxM1 plays critical roles in both expression and activation of PDGF-A. We also show a unique role of FoxM1 in STAT3 expression and activation. Collectively, our findings demonstrate that FoxM1 is required for the self-renewal and tumorigenicity of GSCs and that FoxM1-induced expression of PDGF-A and STAT3 and activation of STAT3 is a key mechanism for FoxM1-induced GSC self-renewal and tumorigenicity.

Materials and Methods

Cell lines and culture conditions

Human glioma Hs683 and SW1783 cells and 293T cells were obtained from the ATCC and cultured in DMEM with 10% FBS. The cell lines were tested and authenticated by DNA typing at the MD Anderson Cancer Center Characterized Cell Line Core. GBM stem cell lines were isolated from fresh surgical specimens of GBM patients at the MD Anderson Cancer Center Characterized Cell Line Core. The cell lines were tested and authenticated by DNA typing at the ATCC and cultured in DMEM with 10% FBS.

Immunohistochemical staining

Human tissue sections of GBM were de-paraffinized and then stained with a primary antibody against human FoxM1, pSTAT3, or PDGF-A. To quantify FoxM1 and PDGF-A expression, staining of each protein was scored as 0–8 according to the percentage of positive cells and staining intensity, as we previously described (39). Two separate individuals who were blinded to the slides examined and scored each sample. An average value of the two scores was reported.

Intracranial tumor assay

GSC and glioma cells were injected intracranially into nude mice as described previously (31). Animals showing general or local symptoms were euthanized; the remaining animals were euthanized 90 days after tumor cell injection. Tumor formation was determined by histologic analysis of hematoxylin–eosin (H&E)-stained brain sections. Tumor volumes were measured by using length (a) and width (b) in H&E-stained sections and were calculated using the equation: \( V = \frac{a \times b^2}{2} \) (40). All mouse experiments were approved by the Institutional Animal Care and Use Committee of MD Anderson Cancer Center.

Western blot analysis

Standard Western blotting was done with antibody against FoxM1, β-catenin, or β-actin (Santa Cruz Biotechnology, PDGF-A (OriBio), SSEA-1, or Nestin (BD Transduction Laboratories), SOX2 or CD133 (Abcam), GFAP (DAKO), STAT3, pSTAT3 (Y705), Tuj-1, or OCT4 (Cell Signaling).

Neurosphere formation assays

The sphere formation assay was performed by plating dissociated single cells at a density of 1 cell/μL in 96-well plates, and counting the number of spheres that formed after 7 days of culture in neurosphere medium (38).

Promoter reporters and dual luciferase assays

Cells were transfected with PDGF-A promoter reporter plasmids (41) or TOPFlash (38) or STAT3 promoter reporter. Transfection efficiency was normalized by cotransfection with a β-actin-RL reporter containing a Renilla luciferase gene under the control of a human β-actin promoter. The activities of firefly luciferase and Renilla luciferase were quantified using the dual-luciferase reporter assay system (Promega).

Statistical analysis

Correlations between positive staining for FoxM1 and positive staining for PDGF-A or pSTAT3 in the GBM specimens were assessed using Pearson correlation. The significance of the in vitro results was determined with the Student t test (two-tailed). The significance of in vivo survival study was determined by the log-rank test. \( P < 0.05 \) was considered to be significant.

Results

FoxM1 regulates PDGF-A in GSCs and NSCs

To examine the role of PDGF-A in GSCs, we first detected PDGF-A expression in SW1783 glioma cells and in GSC11, GSC20s, GSC17, GSC6-27, and GSC267 GBM stem cells. PDGF-A expression was much higher in GSCs than in SW1783 cells (Fig. 1A), suggesting that higher PDGF-A expression may be associated with GSC phenotype. Next, we examined the expression levels of FoxM1, a transcriptional factor associated with GSCs, in SW1783 cells and GSCs. The levels of FoxM1 might be associated with the levels of PDGF-A in the cells (Fig. 1A). To determine whether the finding has clinical relevance, we evaluated the expression of PDGF-A and FoxM1 in the 154 GBM tumors with RNA sequencing data available from TCGA (42). When the GBM tumors were characterized into FoxM1high and FoxM1low subpopulations, higher PDGF-A RNA expression was enriched in the FoxM1high subgroup (Fig. 1B, \( P < 0.001 \)). Moreover, we examined the protein levels of FoxM1 and PDGF-A in serial sections of 50 human primary GBM samples by immunohistochemical analyses. The levels of PDGF-A expression significantly correlated with the levels of FoxM1 expression in these samples (Fig. 1C).
To determine whether FoxM1 is an upstream regulator of PDGF-A, we examined PDGF-A expression in stable FoxM1-knockdown GSCs. Knocking down FoxM1 significantly downregulated PDGF-A protein and mRNA levels as compared with sh-control (Fig. 1D–F and Supplementary Fig. S1A and S1B). To ascertain the effect of FoxM1 on PDGF-A expression, we examined PDGF-A expression in FoxM1-null NSCs. Deletion of FoxM1 in FoxM1<sup>−/−</sup> NSCs resulted in diminished sphere formation ability (Fig. 1G) and diminished expression of PDGF-A (Fig. 1H). Taken together, these results indicated that FoxM1 plays an important role in regulating PDGF-A expression.

To test whether FoxM1 directly regulates PDGF-A expression, we first analyzed the sequence of the PDGF-A promoter by using the FoxM1 consensus binding sequences and identified two putative FoxM1-binding sites (Fig. 2A). Moreover, knocking down FoxM1 in GSC11 and GSC20s cells resulted in decreased PDGF-A promoter activity in the cells (Fig. 2B and C), whereas overexpression of FoxM1 in 293T cells increased PDGF-A promoter activity (Fig. 2D). Next, we generated various mutants of PDGF-A promoter (Fig. 2A). The mutants containing mutations in binding site 1, binding site 2, or both sites and the deletion mutant A200 exhibited lower promoter activity than wild-type PDGF-A promoter in 293T cells (Fig. 2E). Also, FoxM1 directly interacts with the PDGF-A promoter and regulates its expression.
disruption of one or both of the FoxM1-binding sites significantly attenuated PDGF-A promoter activity in GSC11 and GSC20s cells (Supplementary Fig. S2A and S2B), indicating that the FoxM1-binding sites were critical for PDGF-A promoter activation.

To provide direct evidence that FoxM1 binds to the endogenous PDGF-A promoter during transcription in vivo, we performed chromatin immunoprecipitation (ChIP) assays using GSC11 cells. Both of the FoxM1-binding regions of the PDGF-A promoter bound specifically to endogenous FoxM1 protein.
FoxM1 knockdown also reduced the size and number of spheres (Fig. 3B and D and Supplementary Fig. S3A and S3B) and suppressed the expression of stem cell markers (Supplementary Fig. S3C) but upregulated the expression of GFAP (Supplementary Fig. S3C). However, FoxM1 knockdown exhibited much stronger inhibitory effects on GSC self-renewal than did PDGF-A knockdown, as determined by the size and number of spheres in each group (Fig. 3B and D).

To determine the role of PDGF-A in FoxM1-mediated stemness of GSCs, we tested whether exogenous PDGF-A rescued the inhibitory effects of FoxM1 knockdown on the stemness of GSCs. Exogenous PDGF-AA (50 ng/mL) only partially rescued the effect of downregulation of FoxM1 on the self-renewal of GSC11 and GSC20s cells (Fig. 3F and G; Supplementary Fig. S3D and S3E) or the effect of FoxM1 depletion on the self-renewal of NSCs (Supplementary Fig. S3F).

**Figure 3.**
FoxM1 maintains the stemness of GSCs partially via PDGF-A. A, Western blotting of PDGFRA phosphorylation levels in GSC11 and GSC20s cells expressing sh-control or sh-PDGF-A. B, photographs of neurosphere of GSC11 and GSC20s cells expressing control, FoxM1, or PDGF-A shRNA. Bar, 20 μm. C and D, neurosphere formation efficiency of the cells in B. Values are mean ± SD for triplicate samples. E, Western blotting of stem cell and differentiation markers in GSC11 and GSC20s cells expressing sh-control or sh-PDGF-A. F, photographs of neurosphere formation of GSC11-sh-control and GSC11-sh-FoxM1 cells treated with or without PDGF-AA (50 ng/mL) for 10 days. Bar, 10 μm. G, SOX2 expression detected by Western blotting in GSC11-sh-FoxM1 and GSC20s-sh-FoxM1 cells treated with or without PDGF-AA (50 ng/mL) for 72 hours. H, relative cell proliferation of GSC11 and GSC20s cells expressing control, FoxM1, or PDGF-A shRNA in 72 hours was determined by in vitro cell proliferation assay. ***, P < 0.01.
Inhibition of FoxM1 decreased cell proliferation and increased chemosensitivity of GSCs to temozolomide

Because cell proliferation is ultimately required, although not sufficient, for the self-renewal of GSCs, we examined the effects of FoxM1 or PDGF-A on GSC proliferation. FoxM1 or PDGF-A knockdown significantly decreased cell proliferation of GSC11 and GSC20s (Fig. 3H). Also, a small fraction of apoptotic cells was observed in FoxM1 knockdown cells and to a less extent in PDGF-A knockdown cells (Supplementary Fig. S4A). Furthermore, GSCs have been postulated to have intrinsic resistance to chemotherapy including temozolomide, a standard chemotherapy for newly diagnosed GBM patients. Because the above finding indicated that FoxM1 is important to the stemness of GSC, thus, we determined whether FoxM1 inhibition leads to an increase in sensitivity to temozolomide. FoxM1 knockdown significantly decreased cell viability of GSC11, GSC20s, and GSC267 cells after temozolomide treatment as compared with sh-control (Supplementary Fig. S4B and S4D). PDGF-A knockdown also significantly decreased cell viability after temozolomide treatment, but to a less extent than FoxM1 knockdown (Supplementary Fig. S4B and S4D). Together, these results indicate that FoxM1 inhibition decreased cell proliferation and increases the chemosensitivity of GSCs to temozolomide.

FoxM1 regulates both STAT3 expression and STAT3 activation

We next examined the mechanism by which the FoxM1–PDGF-A axis regulates self-renewal of GSCs. Because STAT3 is a critical node in PDGF signaling, we investigated whether STAT3 is a downstream component of FoxM1 signaling in GSCs. Knocking down PDGF-A or FoxM1 reduced STAT3 phosphorylation in both GSC11 and GSC20s cells (Fig. 4A and B). However, surprisingly, knocking down FoxM1 resulted in not only decreased STAT3 phosphorylation but also decreased STAT3 expression (Fig. 4B). In contrast with FoxM1 knockdown, PDGF-A knockdown decreased STAT3 phosphorylation but did not change STAT3 expression (Fig. 4A). Moreover, deletion of FoxM1 in FoxM1fl/fl NSCs resulted in a strikingly lowered STAT3 expression compared with the level in FoxM1 wild-type NSCs (Fig. 4C), indicating that STAT3 is a downstream target of FoxM1. These results suggest that FoxM1 plays dual roles in STAT3 signaling, i.e., FoxM1 regulates both STAT3 expression and STAT3 activation.

STAT3 expression is elevated in GBM specimen and correlated with FoxM1 expression

To determine the importance of STAT3 in GBM, we have assessed the gene expression of STAT3 from TCGA on 594 cases of GBM tumors and 10 cases of normal brain tissues, and found that most GBM tumors expressed higher STAT3 than normal tissue (Fig. 4D). Moreover, STAT3 knockdown inhibited the self-renewal of both GSC11 and GSC20s cells (Fig. 4E) and downregulated the levels of pSTAT3 and SOX2 (Fig. 4F), indicating that STAT3 is required for maintaining the stemness of GSCs.

The above results provide a compelling rationale for studying the mechanisms for the regulation of STAT3 expression in tumor cells. We evaluated the association of STAT3 and FoxM1 expression in the GBM tumors from TCGA dataset, which were characterized into FoxM1high and FoxM1low subpopulations as described in Fig. 1B, and found that higher STAT3 expression was enriched in the FoxM1high subgroup (Fig. 4G). Consistently, the level of activated STAT3 detected by nuclear staining of phosphorylated STAT3 protein also correlated with the level of FoxM1 expression in the 50 GBM specimens described in Fig. 1C (Fig. 4H).

FoxM1 increases STAT3 expression by enhancing β-catenin/TCF4 binding to the STAT3 gene promoter

We showed that FoxM1 can bind to β-catenin to enhance expression of its target genes (38), and a previous study reported that β-catenin may affect STAT3 transcription (43). Thus, we tested whether FoxM1 regulates STAT3 expression via β-catenin. Knocking down β-catenin or inhibiting TCF4, the key transcriptional factor of β-catenin pathway, by TCF4-DN (dominate-negative TCF4) significantly decreased the transcriptional activity of β-catenin in GSCs, as indicated by TOP-flash activity (Fig. 5A and B). Knocking down FoxM1 also significantly decreased the transcriptional activity of β-catenin in GSCs (Fig. 5A and B). Consistent with this finding, knocking down FoxM1 or β-catenin or inhibiting TCF4 in GSCs significantly decreased STAT3 mRNA expression (Fig. 5C and D), suggesting that FoxM1 regulates STAT3 expression via the β-catenin/TCF4 transcriptional complex. Moreover, by using primers flanking the TCF4-binding site (−571 to −565 bp) in the STAT3 promoter, we found that both β-catenin and FoxM1 bound to the STAT3 promoter (Fig. 5E). Furthermore, β-catenin knockdown inhibited FoxM1 binding to the STAT3 promoter, and FoxM1 knockdown inhibited β-catenin binding to the STAT3 promoter (Fig. 5E), indicating that β-catenin and FoxM1 are dependent on each other for binding to the promoter. These results demonstrated that FoxM1 enhances STAT3 expression by promoting β-catenin/TCF4 binding to the STAT3 promoter.

FoxM1 regulates growth factor– and cytokine-induced STAT3 activation

To determine the mechanism by which FoxM1 maintains the stemness of GSCs, we investigated whether FoxM1 affects STAT3 activation by growth factors and cytokines in GSCs. Exogenous PDGF-AA induced phosphorylation of STAT3 in both sh-control and sh-FoxM1 cells (Fig. 6A). However, the pSTAT3 level increased less in sh-FoxM1 than in sh-control cells, indicating that FoxM1 knockdown inhibited STAT3 activation induced by PDGF-AA because of lower expression of total STAT3. Next, we investigated whether FoxM1 affects STAT3 activation by EGF or IL6 in GSCs, because STAT3 is a common node in multiple stem cell maintenance pathways, including EGF and IL6, both of which promote self-renewal and tumorigenic potential of GSCs by activating STAT3 (27). Both EGF and IL6 immediately activated STAT3, but the GSCs with FoxM1 knockdown were less responsive to EGF or IL6 treatment than were the normal control cells (Fig. 6A). These
results indicated that FoxM1 regulates growth factor– and cytokine-induced STAT3 activation.

Constitutively active STAT3 rescues the inhibitory effects of FoxM1 knockdown on the stemness and tumorigenicity of GSCs

To ascertain FoxM1 regulates the stemness of GSCs by affecting STAT3, we tested whether adding back activated STAT3 could reverse the effects of FoxM1 inhibition by using GSC cell lines that stably expressed STAT3C (constitutively active STAT3). In sphere formation assays, STAT3C largely rescued the consequences of FoxM1 knockdown: the sphere formation efficiency in sh-FoxM1-STAT3C GSCs was up to 90% than that in sh-control-vector GSCs (Fig. 6B; Supplementary Fig. S5A). Moreover, SOX2 expression levels were restored in sh-FoxM1-STAT3C GSCs compared with sh-control-vector GSCs (Fig. 6C). These results indicated that activated STAT3 is important for FoxM1’s impact on the stemness of GSCs.

Next, we examined whether ectopic expression of STAT3C rescued tumor growth inhibition caused by FoxM1 knockdown. All mice injected with GSC11 and GSC20s cells expressing sh-control developed GBM, but the mice injected with sh-FoxM1 GSCs did not, as indicated by tumor volumes (Fig. 6D). Forced expression of STAT3C in sh-FoxM1 cells largely abolished the tumor growth inhibition (Fig. 6D) and the decreased survival of mice by FoxM1 downregulation (Fig. 6E). Furthermore, Sox2 and Ki67, a cellular marker for proliferation, were highly expressed in the tumor tissues of sh-control group and sh-FoxM1 + STAT3C group (Supplementary Fig. S5B). These data demonstrated that STAT3 is critical for FoxM1’s promotion of the stem cell phenotype and tumorigenic potential of GSCs.
non-GSC glioma cells overexpressing FoxM1 but deficient in STAT3. SW1783 and Hs683 glioma cells have low levels of the FoxM1 protein (31). Overexpression of FoxM1 in SW1783 and Hs683 cells caused them to exhibit GSC characteristics: SW1783-FoxM1 and Hs683-FoxM1 cells were able to form neurospheres and expressed stem cell markers SSEA-1, SOX2, and Nestin (Fig. 7A–D; Supplementary Fig. S6). Furthermore, overexpression of FoxM1 in SW1783 and Hs683 cells significantly increased PDGF-A and STAT3 expression levels and activity of STAT3 (Fig. 7A; Supplementary Fig. S7A). In contrast, knockdown of STAT3 in SW1783-FoxM1 and Hs683-FoxM1 cells abolished elevated expression of stem cell markers SSEA-1, SOX2, CD133, OCT4, and Nestin (Fig. 7E). Taken together, these findings indicated that FoxM1 overexpression is responsible for reprogramming non-stem glioma cells to GSC-like cells and that FoxM1’s impact on stemness of glioma cells depends on STAT3.

Furthermore, SW1783 and Hs683 cells did not form brain tumors in nude mice, but SW1783-FoxM1 and Hs683-FoxM1 cells did form brain tumors, which possessed features of human GBM, including necrosis and invasion (Fig. 7F; Supplementary Fig. S7B). The tumors also expressed SSEA-1 and Nestin (Supplementary Fig. S7C and S7D). These results indicate that FoxM1 promotes glioma cells reprogramming to GSC-like cells and initiates tumor formation via PDGF-A and STAT3.

Discussion

Here, we demonstrate that FoxM1 is required for the expression of PDGF-A and STAT3 in GSCs. Constitutively active STAT3 rescued the effects of FoxM1 knockdown on the stemness of GSCs, indicating that FoxM1 signals through STAT3 in GSCs. Overexpression of FoxM1 reprogrammed glioma cells to
GSC-like cells and enhanced their PDGF-A expression and STAT3 activation, whereas inhibition of STAT3 in GSCs diminished the FoxM1-induced stemness. Therefore, FoxM1-induced expression of PDGF-A and STAT3 represents a novel and critical mechanism for controlling GSC self-renewal and tumorigenesis (Fig. 7G).

**FoxM1 plays dual roles in PDGF-A signaling**

In this study, we found that FoxM1 plays dual roles in PDGF-A signaling to maintain GSC growth. FoxM1 regulates both PDGF-A expression and activation of STAT3. Overexpression of PDGF-A signaling components has been observed in GBMs, possibly associated with tumor initiation and malignant progression. For example, exogenous PDGF-AA or PDGF-A long isoform (PDGF-A_{L}) can, to different extents, induce a glioma-like mass (18, 44). These findings imply that autocrine or paracrine PDGF is a key signal for glioma initiation and progression. We found that PDGF-A signaling driven by FoxM1 was required for self-renewal of GSCs and tumorigenesis, which further confirms the role of PDGF-A in glioma initiation and progression.

PDGF-A promoter structure and function have previously been investigated (45). Several transcription factors, such as SP-1, Egr-1, and Pura, regulate PDGF-A expression in vitro via promoter binding pattern (45). The current study is the first, however, to show that FoxM1 regulates the transcription of PDGF-A. Our study also elucidated a mechanism for overexpression of PDGF-A in glioma for the first time.

**FoxM1 establishes a link between PDGF signaling and Wnt signaling associated with GSC biology**

A growing body of evidence suggests that PDGF signaling is required for NSC differentiation and oligodendrocyte precursor...
cell proliferation (46–48). Hyperactivated PDGF signaling is a common event in gliomagenesis and has been implicated in tumor initiation. On the other hand, our recent studies suggested that the Wnt-FoxM1/β-catenin pathway is critical for GSC maintenance and tumorigenicity (38). Thus, our results from the current study not only support the importance of PDGF signaling in GSC biology but also uncover a key node in FoxM1-mediated crosstalk between the two key forms of stem cell signaling: PDGF signaling and Wnt/β-catenin signaling.

FoxM1 and STAT3 are part of the key signaling node in GSC self-renewal

Recent accumulating evidence indicates that FoxM1 is required for maintaining stemness and self-renewal of stem cells, including neuronal precursors and GSCs (38, 49, 50). For example, FoxM1 stimulates Bmi1 expression to promote self-renewal of neural stem/progenitor cells (50). Also, FoxM1 signaling is essential for proliferation, self-renewal, and tumorigenesis of GSCs by regulating β-catenin activation (38).

STAT3 regulates diverse cellular processes, including cell growth, differentiation, and apoptosis, and is frequently activated during tumorigenesis. Moreover, STAT3 is required for maintenance of the stem-like characteristics of GSCs (24). Whereas the above findings suggest that both FoxM1 and STAT3 are critical for NSC and GSC growth, our results in the current study establish a direct link between FoxM1 and STAT3 and reveal an example for transcription regulation network in maintaining renewal of GSCs. This finding further suggests that the FoxM1 and STAT3 transcription regulation network is a potential target for novel therapeutic strategies against malignant glioma.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: A.-h. Gong, P. Wei, S. Zhang, Y. Yuan, S. Huang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.-h. Gong, P. Wei, S. Zhang, S. Huang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.-h. Gong, P. Wei, S. Zhang, J. Yao, Y. Yuan, S. Huang
Writing, review, and/or revision of the manuscript: A.-h. Gong, S. Zhang, Y. Yuan, A.-d. Zhou, A.B. Heimberger, G. Rao, S. Huang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.F. Lang, A.B. Heimberger, S. Huang
Study supervision: S. Huang

References


Acknowledgments

The authors thank Drs. Tucker Collins and Byoung Nagai for PDGF-A pro-motor [wild-type and mutants] and Stephanie Deming in MD Anderson’s Department of Scientific Publications for editing the manuscript.

Grant Support

This work was supported in part by NCI grants R01 CA157933, R01CA182684, R21CA152623, and P50CA127001, and the National Science Foundation of China (no. 81337218 to A.-h. Gong).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 20, 2014; revised March 25, 2015; accepted March 26, 2015; published OnlineFirst April 1, 2015.
FoxM1 Drives a Feed-Forward STAT3-Activation Signaling Loop That Promotes the Self-Renewal and Tumorigenicity of Glioblastoma Stem-like Cells

Ai-hua Gong, Ping Wei, Sicong Zhang, et al.

Cancer Res 2015;75:2337-2348. Published OnlineFirst April 1, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-2800

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2015/04/01/0008-5472.CAN-14-2800.DC1

Cited articles
This article cites 49 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/11/2337.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/75/11/2337.full.html#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.