

## TGF- $\beta$ /SMAD/GLI2 Signaling Axis in Cancer Progression and Metastasis

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### Abstract

The Hedgehog (HH) and TGF- $\beta$  signaling pathways represent essential regulators of cell proliferation and differentiation during embryogenesis. Pathway deregulation is a characteristic of various cancers. Recently, evidence for a convergence of these pathways at the level of the GLI2 transcription factor in the context of tumor initiation and progression to metastasis has emerged. This short review summarizes recent knowledge about GLI2 function and mechanisms of action downstream of TGF- $\beta$  in cancer. *Cancer Res*; 71(17); 5606–10. ©2011 AACR.

### Introduction

GLI2 is a Kruppel-like transcription factor that was initially thought to function essentially as an effector of the Hedgehog (HH) pathway. Several recent reports indicate that GLI2 is ubiquitously induced by the TGF- $\beta$  pathway. We herein review the most relevant literature associated with the role of GLI2 as an important mediator of some of the oncogenic and prometastatic activities associated with both HH and TGF- $\beta$  signaling.

### Hedgehog Signaling in Cancer

HH signaling components [HH ligands (Sonic, Indian, and Desert HH)] and the cell surface receptors Patched-1 (PTCH1) and Smoothed (SMO) play a major role in embryonic patterning and tumor development. In the absence of HH ligands, PTCH1 maintains SMO in an inactive state. Upon binding of HH to PTCH1, SMO repression by PTCH1 is alleviated, SMO translocates to the primary cilium, and signaling is transduced, leading to activation and nuclear translocation of GLI transcription factors (Fig. 1; ref. 1). The latter contribute to cancer progression via regulation of cell-cycle progression and apoptosis.

The direct role of the HH signaling pathway in tumorigenesis was initially established through the identification of loss-of-function mutations in the *PTCH* gene in patients with familial and sporadic basal cell carcinomas (BCC) of the skin.

Inappropriate HH pathway activation, estimated as elevated *GLI1* expression, has since been described in an ever-growing number of tumors, including esophageal squamous cell sarcomas, transitional cell carcinomas, small cell lung carcinomas, and bladder, ovarian, gastrointestinal, and pancreatic carcinomas, as well as cutaneous melanoma (reviewed in ref. 2). HH signaling/GLI factors provide a survival advantage to tumor cells and have also been implicated in cancer stem cell renewal and survival.

Targeting the HH pathway for cancer treatment by means of SMO antagonists has shown remarkable efficacy in both preclinical and clinical settings against tumors with identified mutations in the upstream components of the pathway (e.g., pancreatic carcinoma and BCC of the skin). On the other hand, a number of tumors were found to be oblivious to HH signaling inhibition even though they exhibited high expression of *GLI1*, suggesting the existence of alternate pathways that lead to expression of downstream HH mediators.

### TGF- $\beta$ Signaling in Cancer

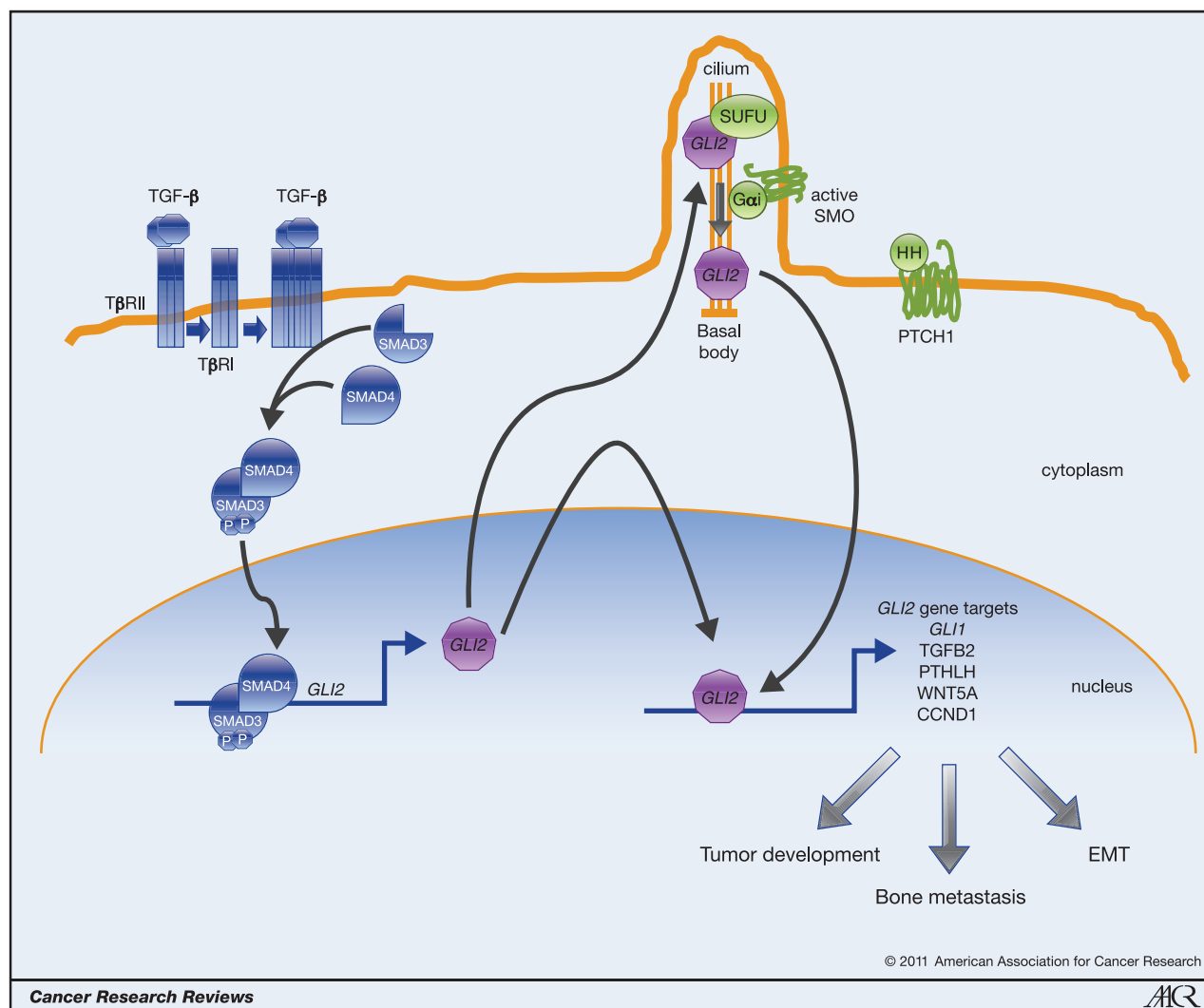
TGF- $\beta$  family members signal via membrane-bound, heteromeric, serine–threonine kinase receptor complexes, whose activation by TGF- $\beta$  ligands leads to phosphorylation of proteins of the SMAD family. The latter, in turn, accumulate in the nucleus and act as transcription factors to regulate target-gene expression, acting either directly on SMAD-specific *cis*-elements on DNA or via physical interaction with other transcription factors acting on their cognate DNA recognition sites (Fig. 1; ref. 3). Negative control of the cell cycle drives the tumor suppressor functions of TGF- $\beta$  in normal and premalignant tissues. On the other hand, TGF- $\beta$ , which is secreted abundantly by tumor cells as well as by the local microenvironment, promotes invasion and metastases of various neoplasms through autocrine and paracrine mechanisms (4, 5). Of note, TGF- $\beta$  induces epithelial–mesenchymal transition (EMT), whereby epithelial tumor cells acquire an invasive, mesenchymal-like

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**Figure 1.** Schematic representation of TGF- $\beta$  and HH signaling in cancer. TGF- $\beta$  ligand activation of specific cell surface serine-threonine kinase receptors activates the SMAD cascade, resulting in transcriptional activation of the *GLI2* gene. GLI2 protein may either regulate target gene expression and exert prooncogenic activities downstream of TGF- $\beta$  signaling or contribute to HH-driven signaling, which occurs via SMO activation in the primary cilium following HH binding to the pathway inhibitory receptor PTCH1.

phenotype accompanied by changes in the expression of cell-cell adhesion molecules and secretion of metalloproteinases, leading to metastasis (6). TGF- $\beta$  is a critical mediator of bone metastasis, whereby complex bidirectional interactions between tumor cells and the bone microenvironment increase bone destruction and establishment of metastases in the bone (7).

### TGF- $\beta$ Pathway Targeting in Melanoma and Breast Cancer Cells

TGF- $\beta$  signaling blockade has proved to be efficient at preventing the development of a variety of tumor types. For example, the small-molecule T $\beta$ RI kinase inhibitor SD-208 increased survival following orthotopic implantation of glioma cells (8). Most recently, we showed that

systemic administration of SD-208 to mice bearing human melanoma bone metastases significantly reduced the development and progression of the osteolytic lesion area. This was associated with decreased tumor burden as well as increased survival in a dose-dependent manner (9). SD-208 was similarly effective in preventing the development and progression of MDA-MB-231 breast cancer bone metastases (10). Another T $\beta$ RI kinase inhibitor, SM16, has shown remarkable efficacy to inhibit the growth of TGF- $\beta$ -producing primary 4T1 murine mammary carcinoma, as well as metastasis to lung, via immune-mediated mechanisms (11).

TGF- $\beta$  signaling blockade by other modalities [i.e., overexpression of either a dominant-negative form of TGF- $\beta$  receptor type II (12) or SMAD7 (13) in breast cancer cells and melanoma cells (14, 15)] was also shown to be effective in

reducing bone metastases. Of note, a TGF- $\beta$ /SMAD-dependent gene bone metastasis signature initially identified by Kang and colleagues (16) in breast cancer cells was found in highly metastatic melanoma cell lines (15) and was inhibited by both SMAD7 overexpression (15) and the T $\beta$ RI kinase inhibitor SD-208 (9). Thus, similar mechanisms are likely to be involved in TGF- $\beta$ -driven bone metastases from melanoma and breast cancer cells.

Anti-TGF- $\beta$  therapies that have shown promising results in both preclinical and clinical settings are diverse and include systemic administration of small-molecule T $\beta$ RI/RII kinase inhibitors (see above examples), neutralizing antibodies and soluble receptors that act as ligand traps and inhibit the activity of all 3 TGF- $\beta$  isoforms by preventing their binding to cell surface TGF- $\beta$  receptors, and tumor delivery of anti-sense oligonucleotides targeting TGF- $\beta$  expression for the treatment of metastatic tumors overexpressing active TGF- $\beta$  (reviewed in ref. 17).

### GLI2 Is a Target of Multiple Signaling Pathways: Direct Induction by the TGF- $\beta$ /SMAD Pathway

Although GLI activation may result from HH ligand- or HH receptor-induced signaling, there is mounting evidence that noncanonical signaling events lead to the expression of HH mediators of the GLI family, including TGF- $\beta$ , fibroblast growth factor (FGF), epidermal growth factor (EGF), and mitogen-activated protein kinase (MAPK) signaling (reviewed in refs. 18 and 19). Evidence that GLI-dependent transcription may occur even in the absence of upstream HH signals is suggested, for example, by the fact that *Gli2* and *Gli3* are widely expressed in the developing embryo, including in regions that are far from SHH production and may be expressed downstream of FGF signaling (20).

We identified GLI2 as a direct TGF- $\beta$ /SMAD target that is independent of HH signaling in a variety of cell types, including primary and immortalized skin and lung fibroblasts, keratinocytes, MDA-MB-231 breast adenocarcinoma cells, and other human cancer cell lines derived from pancreatic carcinoma, glioblastoma, and cutaneous melanoma (21). Using several experimental approaches, including siRNA knockdown, we demonstrated that GLI2 induction by TGF- $\beta$  is directly under the control of SMAD3. Subsequent GLI1 induction by TGF- $\beta$  was shown to require GLI2 and to occur independently of SMO activity. Cloning of the 5' regulatory region of the human *GLI2* gene identified both SMAD and  $\beta$ -catenin recruitment to the TGF- $\beta$ -responsive region of the promoter (22), hinting that the WNT/ $\beta$ -catenin pathway may also be able to regulate GLI2 expression.

In a study comparing the proapoptotic and cytostatic effects of HH pathway inhibition in a series of human pancreatic carcinoma cell lines, Thayer and colleagues (23) identified a subset of cancer cells that express high *GLI* levels yet are resistant to cyclopamine, a compound that interferes with SMO activity and prevents HH-induced *GLI1* expression. This suggested to us that high *GLI* expression is not due to constitutive HH activation, and we hypothesized that constitutive TGF- $\beta$  pathway activation may actually be responsible

for *GLI* expression in these cyclopamine-resistant cell lines. We then demonstrated that pharmacologic inhibition of autocrine TGF- $\beta$  signaling efficiently slowed the growth of these cell lines and reduced *GLI2* expression (21), and that siRNA knockdown of *GLI2* in these cyclopamine-resistant cell lines also caused growth inhibition. These experiments were the first to identify TGF- $\beta$  signaling as a relevant target for therapeutic intervention in a cellular context characterized by high *GLI* expression and lack of responsiveness to HH targeting (21).

Induction of GLI2 by TGF- $\beta$  has been confirmed by several independent groups. For example, in an experimental model of breast cancer, Hu and colleagues (24) recently found that TGF- $\beta$  signaling with increased *GLI2* expression is implicated in the progression from ductal carcinoma *in situ* to invasive carcinoma. Consistent with our initial observations (21), the authors found that TGF- $\beta$  increases *GLI2* expression and GLI-dependent transcription, influencing myoepithelial cell differentiation and progression to invasion. Edson and colleagues (25) reported that ovarian-specific *Bmpr1a Bmpr1b* double-mutant mice develop granulosa cell tumors that exhibit dysregulated TGF- $\beta$  signaling associated with increased *GLI2* (and *GLI1*) expression. The authors also showed that *GLI2* is a TGF- $\beta$ -regulated gene in normal granulosa cells, and suggested that GLI factors may contribute to cancer progression in the ovary.

There is ample evidence that GLI2 plays a direct functional role in the development of solid tumors. Overexpression of Gli2 in mouse skin by use of a *Keratin 5* promoter is sufficient to produce BCCs (26). Inversely, *GLI2* knockdown in prostate cancer cells reduces anchorage-independent colony formation, delays tumor xenograft growth *in vivo*, and enhances paclitaxel chemosensitivity (27, 28). Likewise, in hepatocellular carcinoma cell lines, *GLI2* knockdown inhibits cell proliferation through the regulation of genes implicated in the cell cycle and apoptosis (29). *GLI2* knockdown also reduces the invasiveness of melanoma cells through downregulation of E-cadherin and decreases their capacity to form bone metastases (30). Of note, pharmacologic interference with GLI function or *GLI1* knockdown led to robust cytotoxicity in human colon carcinoma cells, whereas a SMO inhibitor had little effect (31).

### GLI2 in Melanoma Progression

We recently established that GLI2 modulates critical events associated with melanoma progression (30). We showed that basal *GLI2* expression in melanoma cells largely depends on autocrine TGF- $\beta$  signaling and that high *GLI2* expression is associated with a mesenchymal transition and loss of E-cadherin expression. In epithelial cancers, loss of E-cadherin is a hallmark of the EMT, a complex phenotypic conversion that involves changes in morphology, differentiation, and cell-cell adhesion, and acquisition of a motile behavior that is functionally associated with poor prognosis in various cancers (32). Likewise, a mesenchymal transition is characteristic of melanoma switching from an early radial growth phase

to a vertical growth phase of primary melanoma, a critical event that leads to metastatic spreading (33).

Of interest, GLI1, a direct GLI2 target, was shown to induce an EMT in rat kidney epithelial cells via induction of the E-cadherin repressor SNAIL (34). In melanoma, we found that high *GLI2* expression was associated with increased cell invasiveness *in vitro* and the capacity to form bone metastases in mice (30). Within human melanoma primary tumors, *GLI2* expression was heterogeneous: tumor areas expressing high levels of *GLI2* exhibited little E-cadherin expression and were often at the invasive tumor front, whereas regions with low *GLI2* expression showed strong pericellular E-cadherin staining. We also found that *GLI2* expression increased with disease progression. A direct link between *GLI2* expression and aggressiveness of melanoma cells was established, as *GLI2* knockdown in highly invasive melanoma cells (i.e., strongly expressing *GLI2*) dramatically reduced their capacity to invade Matrigel and form bone metastases in nude mice, thus providing direct evidence for the relevance of GLI2 targeting to treat melanoma skeletal metastases. Retrospectively, we found that SMAD7 overexpression and pharmacologic inhibition of T $\beta$ RI activity, both of which are efficient at reducing melanoma bone metastases (see below and refs. 9 and 15), reduced both basal and TGF- $\beta$ -induced *GLI2* expression in melanoma cells, an event that likely contributes to its anti-metastatic activity.

### TGF- $\beta$ and Bone Metastasis

The link between TGF- $\beta$  signaling and the osteoclastogenic factor PTHrP has long been established and implicates both SMAD- and non-SMAD-dependent mechanisms (reviewed in ref. 7). Targeted inhibition of the TGF- $\beta$  pathway is effective at inhibiting breast cancer bone metastasis in nude mice, partly due to reduced PTHrP expression (12). Recently, Johnson and colleagues (35) confirmed our initial identification of *GLI2* as a TGF- $\beta$ -regulated gene in the MDA-MB-231 breast carcinoma cell line (21), and determined that stable overexpression of a repressor form of GLI2 in these cells inhibited the formation of osteolytic bone metastases in mice, as well as PTHrP expression. Additional TGF- $\beta$ -dependent events are likely critical for MDA-MB-231 metastasis to bone, because in a thorough analysis of genes overexpressed in osteolytic bone metastases generated by MDA-MB-231 cells in nude mice, *IL11* and *CTGF* were identified as critical TGF- $\beta$ -regulated genes that cooperate with *OPN* and *CXCR4* to act as metastasis-enhancing genes (16). In that setting, PTHrP itself was not expressed in the highly metastatic cell populations. In our own studies of melanoma metastasis to bone, we identified *PTHrP*, *IL11*, *CXCR4*, and *OPN* as genes regulated by TGF- $\beta$  whose expression was greatly reduced by both SMAD7 overexpression (15) and systemic administration of the T $\beta$ RI inhibitor SD-208 (9). These 2 approaches severely reduced melanoma bone metastasis formation and/or diminished established metastases. These reports highlight a remarkable similarity between the mechanisms involved in TGF- $\beta$ -driven bone metastases from melanoma and breast cancer.

### An HH/TGF- $\beta$ Vicious Cycle?

As shown above, TGF- $\beta$  is a potent transcriptional regulator of GLI2, which results in GLI1 activation independently from the HH signaling cascade (21). In addition to facilitating direct GLI2-dependent oncogenic events, it is also plausible that TGF- $\beta$  may prime or potentiate HH responsiveness by elevating the available pool of GLI2, a critical substrate that is necessary for HH response (Fig. 1).

Investigators have identified functional cross-talk between the TGF- $\beta$  and HH pathways, whereby HH signaling leads to the expression of TGF- $\beta$  family members that may be necessary for SMO-dependent tumorigenesis. For example, it has been shown that SHH promotes motility and invasiveness of gastric cancer cells through TGF- $\beta$ -mediated activation of the ALK5-SMAD3 pathway (36). Similarly, in a mouse model of Smo-mediated BCC development, activation of the TGF- $\beta$  signaling pathway was observed and appeared to be critical for Smo-mediated cancer development, possibly via immunosuppressive mechanisms (37). Inversely, transcriptional upregulation of SHH was recently shown to contribute to TGF- $\beta$ -induced EMT in non-small cell lung cancer cells (38).

Taken together, these reports indicate that TGF- $\beta$  and HH signaling may form a vicious cycle of promoting and amplifying the metastatic process, whereby GLI2 and its downstream target GLI1 play a major role in allowing tumor cell invasion and resistance to apoptosis.

A number of reports indicate the potential therapeutic benefit of targeting either the TGF- $\beta$ /SMAD or SHH/GLI signaling pathway to counter the neoplastic process, consistent with their respective prooncogenic capacities. Major drawbacks include widespread toxicity due to blockade of numerous vital functions associated with growth factor signaling together with possible off-target effects. Suppression of *GLI2* expression or suppression of GLI2 function independently of its upstream activators may therefore represent a valuable therapeutic option for the treatment of several cancers (39) because it would also circumvent the difficulty of identifying the relevant upstream signals leading to *GLI2* expression.

In conclusion, the critical role played by GLI2 downstream of TGF- $\beta$  signaling in driving cancer progression toward metastasis in an HH-independent manner has been documented in distinct cancer types. GLI2 promotes a mesenchymal transition of tumor cells characterized by loss of E-cadherin expression, as well as secretion of soluble factors such as matrix metalloproteinases and osteoclastic molecules, including PTHrP. All of these events contribute to the acquisition of a more aggressive phenotype and metastasis.

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No potential conflicts of interest were disclosed.

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