# Therapeutic Inhibition of Hedgehog-GLI Signaling in Cancer: Epithelial, Stromal, or Stem Cell Targets?

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**Hedgehog (HH)-GLI signaling is a developmental patterning pathway used by many tumors for bulk proliferation that has been shown also to regulate cancer stem cell self-renewal and survival. Surprisingly, a recent study by Yauch et al. (2008) proposes that HH-GLI signaling acts only on the tumor stroma. The mode of action of HH-GLI signaling in cancer may shape the development of therapeutic antagonists.**

Intercellular patterning signaling pathways involved in organizing the body plan in the embryo, including Hedgehog (HH), Wnt, and Notch, have been identified as key players in human cancers. The mechanisms of their actions in normal and pathological conditions are under intensive investigation, as this knowledge may lead to not only an understanding of tumor ontogeny (and therefore of developmental plasticity) but also the design of new therapies. This is of great significance, as these pathways participate in incurable metastatic cancers.

A milestone in understanding cancer as a developmental problem is the identification of cancer stem cells that self-renew, give rise to the tumor bulk, and reinitiate tumor development. Standard chemo- and radiotherapies can greatly reduce tumor bulk but may be less effective on cancer stem cells, which may induce cancer recurrence. The key challenge has been in identifying the molecular mechanisms that maintain and support cancer stem cell self-renewal and survival.

HH-GLI signaling (Figure 1A), one of the most critical pathways in animal patterning, has been implicated in several unrelated sporadic human cancers, including cancers of the skin, brain, prostate, stomach, pancreas, and lung. As these organs are unrelated in developmental origin, site, or function, a common dependence of cancer stem cells on HH-GLI signaling for survival and self-renewal, paralleling its roles in normal development and homeostasis, could underlie its widespread involvement in human cancers.

Direct evidence that HH-GLI signaling has a critical role in cancer stem cells derives from a number of recent studies in different tumor types (Figure 1B). In glioblastoma multiforme (GBM), self-renewal and survival of clonal gliomasphere-forming cells depend on SMOOTHENED (SMOH) and GLI1 activities, as tested by inhibition with the natural small molecule cyclopamine, a specific SMOH inhibitor, and interference with siRNAs (Clement et al., 2007; Bar et al., 2007). GBM stem cell cultures respond to cyclopamine and exogenous Sonic hedgehog (SHH) ligand by downregulating or upregulating *GLI1* transcription (which marks a cell's response to HH pathway activity), respectively. The growth of gliomasphere-derived intracranial GBMs is greatly reduced by systemic treatment with cyclopamine or the conditional activation of SMOH by lentiviral-mediated RNA interference (Clement et al., 2007). These effects are specific, as the inhibitory activity of shRNAs against SMOH and of cyclopamine are rescued by GLI1, consistent with their epistatic relationship in the HH pathway (Figure 1A). $CD133^+$ GBM cancer stem cells express *GLI1* and display a HH-GLI-responsive stemness signature that includes *NANOG*, *SOX2*, *OCT4*, and *BMI1* (Clement et al., 2007).

HH-GLI signaling also appears to be active in $CD44^+CD24^{-/low}Lin^-$ putative breast cancer stem cells, as these express *GLI1* (Liu et al., 2006), and treatments with cyclopamine or anti-HH blocking antibody reduce the clonogenicity of $CD19^+CD37^+$ multiple myeloma cancer stem cells (Peacock et al., 2007).

Sources of HH ligands include $CD133^+$ cancer stem cells and tumor-induced vasculature in GBMs (Clement et al., 2007) and the stroma in lymphomas and multiple myelomas (Dierks et al., 2007).

Given the results summarized above, the proposed effect of tumor-derived HH only on surrounding stroma reported by Yauch et al. (2008) is intriguing (Figure 1C). Yauch et al. show that tumor cells expressing HH can induce a response in adjacent stromal cells and report that treatment of human xenografts of such cancers, including those of the prostate, pancreas, and ovary, with cyclopamine or another blocker of SMOH (HhAntag) does not lead to significant tumor regression at doses that affect GLI-dependent luciferase reporter activity. They then document a response to SMOH blockers in the mouse stromal compartment, but not in human epithelial cells, and show that SMOH-dependent functions in stroma are required for xenograft growth. Thus, unlike the reported inhibition of epithelial tumor growth by blocking HH-GLI signaling in cancers with detectable cell-autonomous pathway-activating mutations (e.g., basal cell carcinoma), Yauch et al. do not detect inhibition of epithelial cells by HH-GLI pathway blockade in HH ligand-dependent tumors and conclude that this pathway acts only on the stroma.

These are interesting results that raise three important issues:

(1) The reported lack of response by epithelial tumor cells to HH-GLI modulation contrasts with extensive data from several laboratories with cell lines and patient-derived primary cultures of melanomas

and brain, prostate, pancreas, and lung cancers, among others, using recombinant SHH, anti-HH blocking antibodies, cyclopamine, and siRNAs (e.g., Dahmane et al., 2001; Thayer et al., 2003; Sanchez et al., 2004; Karhadkar et al., 2004; Stecca et al., 2007). While Yauch et al. (2008) did not observe a correlation of cyclopamine action with *GLI1* or *PTCH1* downregulation, their assays may have been performed too late (24 hr after treatment) for detection of such a response, in contrast to previous studies where cyclopamine clearly downregulated *GLI1* and *PTCH1* ~4 hr posttreatment followed by recovery (Clement et al., 2007; Stecca et al., 2007; B. Stecca, F. Varnat, and A.R.A., unpublished data). Their xenograft results also differ from prior studies that included rescue by expression of GLI1 and conditional interference of *SMOH* in epithelial tumor cells (e.g., Karhadkar et al., 2004; Clement et al., 2007; Dierks et al., 2007). The reasons for these discrepancies are unknown. Different response kinetics of stromal versus epithelial cells could partly explain the results. Nevertheless, whereas pharmacological treatments can affect multiple cell types, lentiviral-mediated silencing of SMOH only in epithelial cells is efficient in inhibiting the growth of intracranial and subcutaneous GBMs and subcutaneous melanoma xenografts (Clement et al., 2007; Stecca et al., 2007), demonstrating the requirement of HH-GLI in epithelial tumor cells.

(2) Yauch et al. demonstrate a paracrine action from HH ligand-expressing epithelial cells on surrounding mouse stroma. This is interesting and reinforces the critical role of the stromal microenvironment on epithelial tumor growth. Caution is required, however, regarding the extension of such data to the human stroma, as this is quickly replaced by its mouse counterpart in patient-derived tumor xenografts (Yauch et al., 2008). Moreover, detailed in situ hybridization analysis of human tissue suggests autocrine signaling, as the prostate epithelium is the site of robust *SHH* and *GLI1* expression, and PSA$^+$ prostate cancer epithelial cells respond to exogenous SHH and anti-HH blocking antibodies and cease proliferation in response to cyclopamine treatment (Sanchez et al., 2004). Human metastatic melanomas also strongly coexpress *SHH*, *GLI1*, and *PTCH1* in epithelial tumor cells and not in the surrounding stroma (Stecca et al., 2007). In contrast, rodents display paracrine Hh signaling from the prostate epithelium to the mesenchyme (Pu et al., 2004). Whether HH-GLI affects the stroma of human tumors in addition to the epithelium and its cancer stem cells (Figure 1D) requires investigation.

(3) The new SMOH blocker used by Yauch et al. appears limited in its efficacy. Administration of their proprietary SMOH blocker HhAntag reduced tumor volume by 20%–50% after ~20 days, which contrasts with the eradication of engrafted human melanomas and prevention of their recurrence by local cyclopamine treatment for 20 days, and with the elimination of their metastatic growth in the lungs by systemic cyclopamine treatment (Stecca et al., 2007). Methodological differences could explain the varying results, as systemic (intraperitoneal) cyclopamine treatment also has limited effects on subcutaneous xenograft growth (reducing it by ~30%), likely due to inefficient drug delivery (Stecca et al., 2007). However, if blocking HH-GLI signaling were to affect only the stroma and have limited effects, the development of antagonists for many kinds of devastating cancers could lack support. On the contrary, the action of HH-GLI on cancer stem cells provides a solid basis for the development of better antagonists, independent of whether they also act on the stroma.

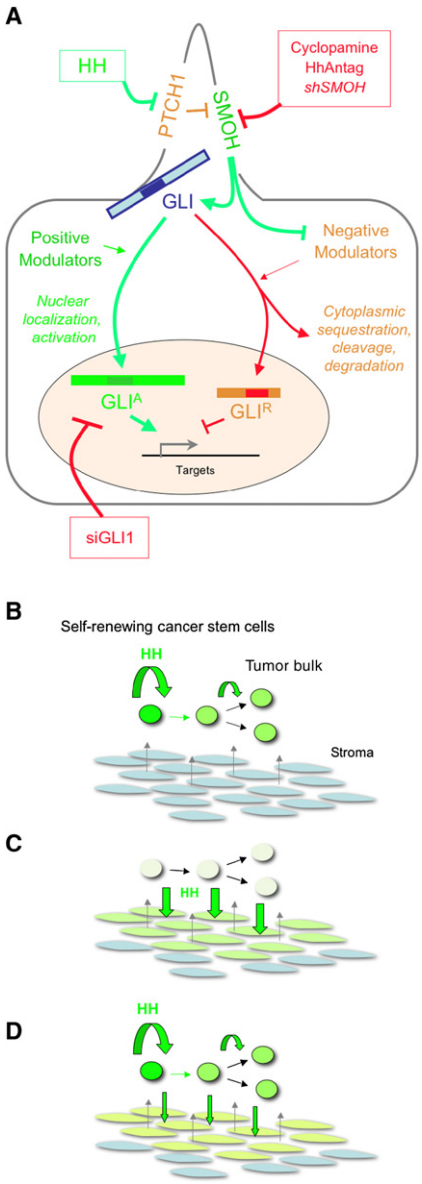


**Figure 1. Modulations and Modes of Action of HH-GLI Signaling in Cancer**

(A) Schematic diagram of HH-GLI signaling, with the action of inhibitors noted. Regulation of the GLI transcription factors occurs in the primary cilium before they enter the nucleus and regulate gene expression.

(B–D) Diagrams of the different possible modes of action of HH signaling in cancer stem cells and on the tumor bulk (B), on stromal cells only (C), or on both epithelium and stroma (D).

Cancers harbor many mutations, but they appear to be fully dependent on the inappropriate activity of a few developmental pathways. Elucidating the mechanisms by which HH-GLI signaling regulates cancer stem cell self-renewal and survival, and extending the current findings to additional cancer types, should pave the way for new rational and specific drugs to treat presently incurable

cancers. The evidence is promising, and the possibilities wide open.

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# Leaving Home Early: Reexamination of the Canonical Models of Tumor Progression

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**A recent report in *Science* from the Varmus laboratory (Podsypanina et al., 2008) puts an interesting twist on the origins of metastatic cells, suggesting that metastases can arise in ways that are very different from those widely believed.**

The accepted canon of tumor progression involves the initial development of tumorigenicity by cells within the site of primary tumor formation. These cells remain in that site unless they become invasive, with an associated tendency to intravasate (enter blood vessels) and disseminate via the circulation to distant sites in the body. Subsequently, disseminated cells may escape blood vessels (extravasate), form micrometastases, and, with very low efficiency, succeed in forming macroscopic metastases (colonization) (Fidler, 2002, 2003; Fidler et al., 2007; Thiery, 2002).

The factors that enable the neoplastic cells within primary tumors to invade and metastasize are surely complex. Some of these determinants may already be implanted in the precursors of primary tumor cells relatively early in the course of tumor progression. For example, the differentiation program of a normal cell of origin as well as the spectrum of somatic alterations (mutations and promoter methylation events) sustained by its lineal descendants within a primary tumor are both likely to affect the probability of evolving highly malignant cell traits (Bernards and Weinberg, 2002; Ince et al., 2007). In addition, the stromal microenvironment of a primary carcinoma is also likely to contribute heterotypic signals that influence the eventual development of invasive traits (Bhowmick et al., 2004).

Once these various factors converge on individual tumor cells and impart to them an invasive phenotype, these cells may gain ready access to the systemic circulation, providing them with channels that carry them to distant sites in the body. More often than not, the destination sites are likely to be dictated by the accidental trapping of relatively large cancer cells in the small-diameter microvessels present in most organs. The lung is a favored site of initial dissemination, as its capillary bed is the first encountered by circulating tumor cells after they have entered into the venous circulation and made an initial pass through the heart.

Most cells (>95%) are cleared from sites of initial trapping in the lungs (for example) within a day or two. Moreover, the fate of the survivors that succeed in extravasating is hardly clear. Some may survive as indolent micrometastases for extended periods of time without losing their viability, while most eventually disappear. Only on very rare occasions do the cells in micrometastases succeed in proliferating vigorously and forming a macroscopic metastasis—the colonization process (Fidler, 2003).