Therapeutic Targeting of the Hedgehog-GLI Pathway in Prostate Cancer

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Abstract

The Hedgehog-GLI signaling pathway is important in animal development and tumorigenesis. Recent findings indicate that the growth and survival of human prostate cancer cells rely upon sustained signaling from the Hedgehog-GLI pathway. These findings have prompted a novel rational strategy for therapeutic treatment of prostate tumors, including metastatic tumors. (Cancer Res 2005; 65(8): 2990-2)

Hedgehog signaling has been implicated in the control of many aspects of vertebrate development. The three Hedgehog proteins of humans and mice—Sonic, Indian, and Desert—are expressed in specific, largely nonoverlapping regions and cell types. All three Hedgehog proteins act through the transmembrane proteins Patched1 (PTCH1) and Smoothened (SMOH), which regulate the transcriptional activity of three GLI zinc-finger transcription factors (Fig. 1*A*; refs. 1, 2). In the male reproductive tract, Sonic Hedgehog (SHH) signaling is necessary for the formation of the external genitalia and for the development of the prostate (3, 4). Although prostate organogenesis in the mouse is SHH independent, SHH is expressed in the developing prostatic epithelium and inhibition of SHH signaling causes defects in ductal patterning (5) and a reduction of epithelial cell proliferation (6).

In adult humans, we and others have shown that deregulation of the SHH-GLI pathway is involved in a number of tumors, such as basal skin cell carcinomas, medulloblastomas, and pancreatic and lung cancers (1, 7, 8). Based on our initial finding that sporadic human prostate tumors express *GLI1* (9), a very reliable marker of SHH signaling, we hypothesized that SHH-GLI function is involved in prostate cancer.

To test this hypothesis, we first analyzed the expression of SHH pathway components in prostate cancer and normal adult tissue. *In situ* hybridization showed that *GLI1*, *PTCH1*, and *SHH* are normally coexpressed in adult prostate epithelial cells as well as in prostate carcinomas (10). This is consistent with the idea that tumor cells derive from the expansion of normal SHH-expressing prostatic epithelium. In our study, we did not detect *GLI1* expression in the surrounding normal or tumor stroma, raising the possibility that SHH acts in an autocrine fashion in adult prostate epithelial cells. The levels of expression of *SHH, PTCH1, GLI1, GLI2*, and *GLI3* are variable but were often up-regulated in tumor versus matched control samples of the same patients. Similarly, SHH protein levels are elevated in tumors where it is present as a secreted protein in the glandular lumen. A significant correlation between high SHH expression, tumor presence, and high cell proliferation was detected, but no correlation was found with Gleason score or other clinical parameters.

To test the relevance of sustained SHH-GLI signaling for prostate tumor cell growth, we first used the plant alkaloid cyclopamine, a selective inhibitor of SMOH (Fig. 1*A*; ref. 11), on three established prostate cancer cell lines derived from metastatic lesions that are commonly used to study prostate cancer (LNCaP from a prostate cancer lymph node metastasis and PC3 and DU145 from bone metastases). Cyclopamine treatment for 48 hours greatly inhibited the proliferation of LNCaP cells (>80% reduction in BrdUrd incorporation), reduced the proliferation of PC3 cells by 30%, but did not affect the proliferation of DU145 cells (10). In addition, we cultured primary prostate carcinomas that express prostate-specific antigen *PSA*, *SHH*, *PTCH1*, and *GLI1*. The growth of all primary cultures was dependent on SHH pathway activity as cyclopamine treatment led to a major (>70%) decrease in BrdUrd incorporation (10).

Expression of ligand (SHH) and final mediator (GLI1) in the prostatic epithelia suggested autocrine signaling as in the case of lung and digestive tract tumors (12, 13). We observed that addition of blocking anti-SHH antibody inhibited the proliferation of three of four tumors. Moreover, addition of recombinant SHH protein increased the proliferation in two of four tumors (10). Treatment of LNCaP, PC3, or DU145 cells with either SHH protein or blocking antibody did not produce any effects. Thus, whereas autocrine signaling may take place in prostate tumors *in situ*, these metastatic cell lines seem to have lost responsiveness to ligand and may have activation of the pathway downstream of ligand-receptor binding.

Activation of the SHH pathway leads to GLI protein function (Fig. 1*A*). However, mouse *Gli1* is apparently not necessary for development or tumorigenesis (14). In contrast, *GLI1* is the only *GLI* gene consistently expressed in the three human cell lines tested, raising the possibility that it plays a central role in SHH signaling in the men's prostate. To test this idea, we used specific 21-nucleotide-long small interfering RNAs (siRNA). Although siRNAs could not be used on primary cells due to low levels of lipofection, *GLI1* siRNA treatment produced a 60% reduction in BrdUrd incorporation in transfected LNCaP cells (10). This is the first demonstration of the requirement of *GLI1* in human cells and suggests that the point of activation in LNCaP occurs at the level of SMOH or upstream (because cyclopamine inhibited proliferation), but downstream of SHH (as the blocking antibody produced no effect). More interestingly, *GLI1* siRNA led to a 30% reduction in cell proliferation of DU145 cells. Because these cells are not sensitive to cyclopamine, the siRNA results suggest activation of the pathway downstream of SMOH and upstream or at the level of GLI1 function. *GLI1* siRNA produced a smaller effect on PC3 cells, likely due to the low efficiency of lipofection, which is always incomplete, or to the compensation mechanisms

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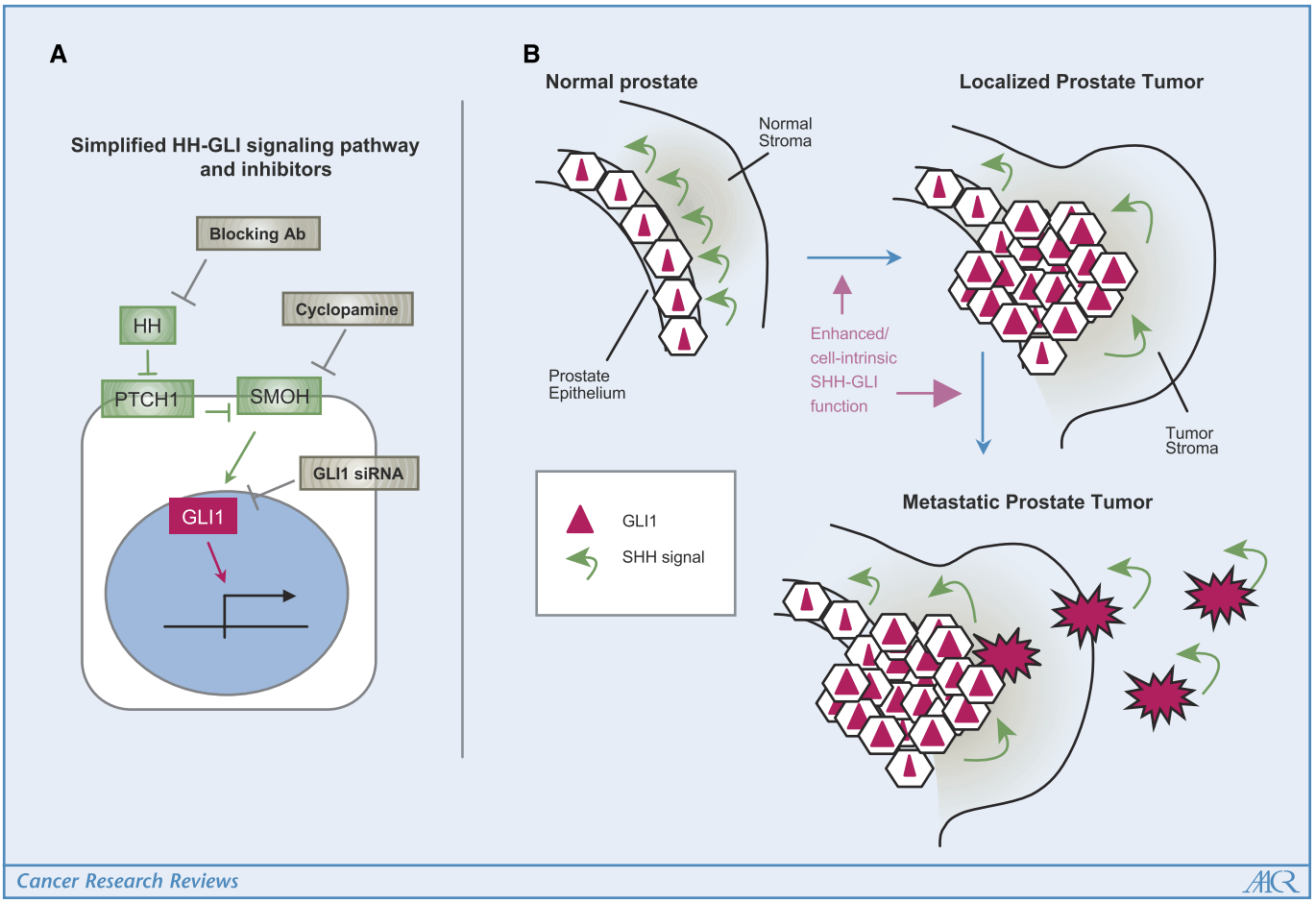

Figure 1. *A,* schematic representation of key components of the Hedgehog-GLI pathway. The ligand Hedgehog (*HH*) binds to and blocks the activity of the transmembrane protein Patched1 (*PTCH1*), thereby releasing the inhibition of Smoothened (*SMOH*) and activating GLI1 function. The diagram also represents the site of action of different types of inhibitors used on prostate cancer cells (10): a blocking antibody (*Ab*) against SHH, the SMOH inhibitor cyclopamine, and siRNA targeting *GLI1*. *B,* proposed model of prostate cancer development from normal epithelia to metastasis. Inappropriate maintenance/elevation of SHH-GLI pathway activity leads to enhanced/sustained GLI1 function in epithelial cells, triggering an increase in cell proliferation and tumor formation. Elevated levels of GLI1 function are proposed to underlie and be required for metastasis.

mediated by other GLI proteins (as they express *GLI2* and this gene can share some functions with *GLI1*; refs. 15, 16). Taken together, our results show the dependence of prostate cancer cell proliferation on SHH-GLI pathway activity and suggest activation of the SHH-GLI pathway at different levels in different tumors (10). Moreover, they show the requirement of *GLI1* in human cancer. Considering the role of this pathway in normal prostate development and its potential involvement in adult prostate homeostasis, we suggest that the normal patterning role of SHH-GLI signaling is deregulated in prostate tumors, perhaps acting on epithelial stem cells.

In parallel with our research, a second study proposed that SHH induces *GLI1* in the stroma of prostate tumors (17), suggesting that SHH-GLI signaling might play a role in critical tumor-stromal interactions, although these finding have not been reproduced (10). SHH was also shown to increase LNCaP cell growth in mouse xenografts (17); in a third study, SHH was shown to induce angiogenesis and facilitate the growth of PC3 cell xenografts (18).

A fourth study also showed the existence of autocrine signaling by SHH in the adult prostate and in prostate cancer (19). This study showed that cyclopamine is an effective inhibitor of cell proliferation for prostate tumor cultures and s.c. xenografts, that GLI1 overexpression can bypass the inhibitory effect of cyclopamine as expected, and that it can enhance the transformation and tumorigenesis of prostate cells. These data, together with the effect of *GLI1* siRNA described above (10), confirm that GLI1 is necessary and sufficient for prostate cancer and underline its potential for diagnosis as well as a therapeutic target, as first shown for basal cell carcinomas (20). Moreover, an analysis of fresh metastastic lesions as well as localized tumors and adjacent normal tissue showed that normal and tumorigenic tissue express *SHH* and *IHH* (Indian Hedgehog) with no correlation with tumor aggressiveness, although a clear up-regulation of *PTCH1* and *GLI1* in metastatic samples was observed when compared with benign prostate tumors or normal tissue (19). Two discrepancies with our data need to be resolved as the DU145 and PC3 cell subcultures used (19) were sensitive to cyclopamine and blocking anti-SHH antibodies, respectively.

A fifth study shows that *PTCH1* and *HIP1* (Hedgehog interacting protein 1) are highly expressed in a number of metastatic and high-grade prostate tumors (21). It is also shown that tumors with low Gleason scores express SUFUH (a negative regulator of the pathway), whereas most tumors with high Gleason scores do not, or instead express high levels of SHH. These data suggest that prostate cancer progression correlates with activation of the SHH-GLI pathway

either by loss of a repressor component like SUFUH (which acts downstream of SMOH) or by overexpression of the ligand. Finally, this work confirmed that prostate cancer cell lines are sensitive to cyclopamine and, interestingly, that cyclopamine-treated PC3 cells have reduced invasiveness, suggesting that SHH signaling regulates not only proliferation and progression but also tumor invasiveness.

In summary, the findings from our work and those from other studies taken together suggest a simple conceptual model for prostate cancer initiation, progression, and metastasis (Fig. 1*B*), which may be applicable to many, if not all, Hedgehog-GLI–dependent tumors (1). Under normal conditions, activity of the SHH-GLI pathway is involved in the homeostasis of the prostatic epithelium, perhaps acting on prostate stem cells and in regeneration when required. Epigenetic events, hormonally-related changes, direct or indirect alterations in pathway components, or other causes leading to enhanced GLI1 function result in tumor initiation and cancer development. Other factors may then alter the character of the tumor. Metastases are proposed to arise due to the acquisition of elevated cell-intrinsic signaling or to an overall ligand-dependent increase in signaling (10, 18), all cases leading to enhanced GLI1 function (10). More experiments are clearly needed to understand the complexity and variability of prostate cancer, to define the role of the SHH-GLI signal in the cross-talk between prostate tumor cells and their surrounding stroma, and to determine the interaction with other signaling pathways previously implicated in prostate cancer. Nevertheless, the new data confirm our hypothesis (9), but they are otherwise surprising and unexpected as no other role for SHH signaling in prostate cancer had been suggested previously.

Practically, we suggest that monitoring SHH-GLI pathway activity would be a good routine diagnostic for a number of cancers, including those of the prostate, and that pathway inhibitors, with emphasis on interfering with GLI1 function, will provide new therapeutics for prostate cancers and other lethal cancers.

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