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# Unraveling the therapeutic potential of the LncRNA-dependent noncanonical Hedgehog pathway in cancer

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Acquired resistance to Hedgehog pathway inhibitors has been reported in the clinical setting and upregulation of noncanonical Hedgehog signaling is one of the major underlying mechanisms behind this resistance. As demonstrated in our recent study, greater clinical efficacy might be achieved by focusing on downstream targets of the chemokine-activated noncanonical Hedgehog signaling pathway such as *BCAR4* and phospho-GLI2 (Ser149).

Accumulating evidence demonstrates that long noncoding RNAs (lncRNAs) regulate gene expression by interacting with major pathways related to cell growth, proliferation, differentiation, and survival.[1] Alterations in the function of these lncRNAs promote tumor formation, progression, and metastasis in many cancer types.[2,3] However, the precise molecular mechanisms underlying the functions of lncRNAs in cancer are not fully understood; this hinders the development of reliable clinical strategies based on lncRNA biomarkers that might ultimately result in therapeutic treatments for various cancers. A broader understanding of the functional mechanisms of lncRNAs and the regulatory networks in which they are involved would serve to greatly deepen our understanding of their function in many cancers and help to uncover more effective therapeutic options through the modulation of their function. LncRNAs are an emerging concept that enhances our understanding of cancer development and metastasis while also representing a novel regulatory layer to consider when attacking this disease. Despite the involvement of lncRNAs in cancer, cancer studies that only examine classical pathways largely neglect to study lncRNAs. The role of lncRNAs should be examined with regard to cancer progression because classical means have been insufficient to explain why many types of cancer return even after targeted therapeutic regimens that target canonical pathways (e.g., the Hedgehog pathway). A perspective that incorporates lncRNAs into the study of cancer biology could be the conceptual advance that draws us closer to the next generation of breakthroughs in cancer research and treatment.

In our laboratory, we recently discovered a lncRNA called *BCAR4* that is upregulated in breast cancer and contributes to metastasis by directing cooperative epigenetic regulation downstream of chemokine signals and activating the noncanonical Hedgehog/GLI2 transcriptional program.[4] Mechanistically, the binding of *BCAR4* to SNIP1 and PNUTS in response to CCL21 releases inhibition by SNIP1 of p300-dependent histone acetylation that in turn enables the *BCAR4*-recruited PNUTS to bind H3K18ac and relieve inhibition of RNA Pol II via activation of the phosphatase PP1. This mechanism activates a noncanonical Hedgehog/GLI2 transcriptional program that promotes cell migration. *BCAR4* expression correlates with advanced stages of breast cancer, and the therapeutic delivery of locked nucleic acids (LNAs) targeting *BCAR4* strongly suppresses breast cancer metastasis in mouse models.[4] Interestingly, the lncRNA *BCAR4* is also overexpressed in many other cancer types and its expression is strongly correlated with cancer metastasis.[4] Notably, the phosphorylation of GLI2 (Ser149), which is a signature event of the chemokine-activated noncanonical Hedgehog pathway, is dramatically upregulated in multiple cancer tissues.[4] These findings certainly warrant further investigation to establish *BCAR4* and phospho-GLI2 (Ser149) as new prognostic and therapeutic factors of cancer progression.

Although a preponderance of data support the idea that Hedgehog signaling correlates with the potential for cancer


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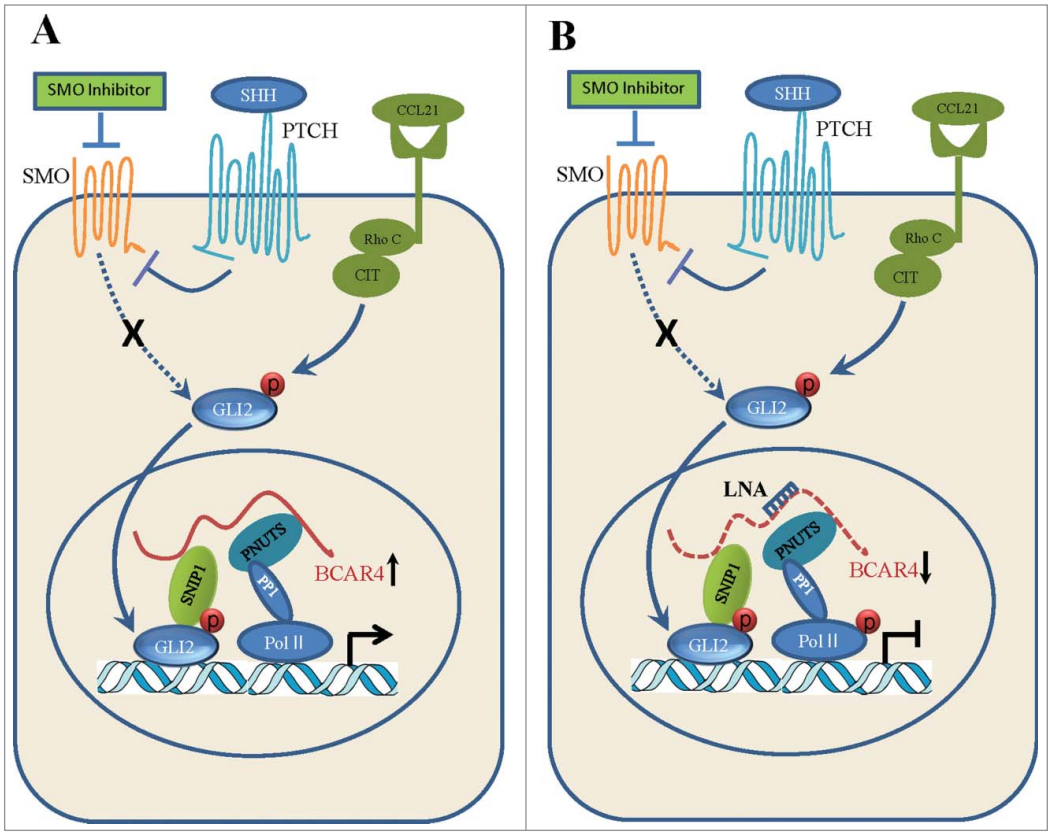


**Figure 1.** Targeting the lncRNA-dependent noncanonical Hedgehog pathway by LNA in combination with classical Hedgehog pathway inhibitors. (**A**) Canonical activation of the GLI2 pathway occurs through Hedgehog (HH) ligands, such as binding of SHH to the PTCH receptor, followed by SMO activation, which induces GLI2 phosphorylation and nuclear translocation for binding to the downstream target genes. SMO inhibitors have been developed that target the canonical Hedgehog signaling pathway to treat some types of cancers; however, acquired resistance has occurred. In our studies we demonstrated that binding of the lncRNA *BCAR4* to SNIP1 and PNUTS in the presence of CCL21 treatment releases the inhibitory activity of SNIP on p300-dependent histone acetylation. The resulting H3K18ac binds to PNUTS, which relieves inhibition of RNA Pol II via activation of the PP1 phosphatase. This mechanism activates a noncanonical hedgehog/GLI2 signaling pathway and may contribute to drug resistance in some types of cancers. (**B**) Depletion of *BCAR4* by LNA significantly impairs breast cancer metastasis *in vivo*. We propose that a rational combination of SMO inhibitors and LNA targeting *BCAR4* would be more efficacious for the treatment of some types of human cancer.

metastasis as well as therapy-resistant behavior, the efficacy of current Hedgehog pathway inhibitors for cancer treatment has been disappointing because of the eventual emergence of acquired resistance to such inhibitors and upregulation of the noncanonical GLI signaling pathway, which is independent of the upstream Hedgehog pathway.[5] Hedgehog pathway inhibitors represent new opportunities for targeted therapies of cancer, as shown for advanced basal cell carcinoma and medulloblastoma;[6] however, clinical trials in solid tumors, including breast cancer, have not yet been performed because of the critical role that Hedgehog signaling plays in embryonic development and homeostasis (e.g., bone turnover).[7] The newly identified lncRNA-dependent noncanonical Hedgehog signaling pathway in breast cancer sheds light on appropriate patient populations who would respond optimally to treatment; acquired resistance to Hedgehog pathway inhibitors has been reported in both preclinical and clinical settings and upregulation of the noncanonical GLI signaling pathway is one of the major underlying mechanisms behind this resistance. Therefore, combination treatments with Hedgehog pathway inhibitors in conjunction with a LNA-based lncRNA targeting strategy could improve the effectiveness of therapies for cancer. Accumulating evidence has demonstrated that the frequency of GLI activation that bypasses upstream Hedgehog signaling argues for the development of GLI inhibitors to diminish downstream effects, yet very few specific GLI inhibitors show promise.[5] Given the evidence available, it appears that targeting *BCAR4* and phospho-GLI2 (Ser149) downstream of chemokine-activated noncanonical Hedgehog signaling in advanced cancers would be more efficacious and may alleviate the need to stratify upstream GLI activation signals (**Fig. 1**).

In summary, the results of our study reveal the prognostic value of *BCAR4* and phospho-GLI2 (Ser149) in predicting metastasis and specific mortality in multiple cancer types. Given that clinical trials for Hedgehog inhibitors in patients with solid tumors have been hindered by upregulation of the noncanonical Hedgehog signaling pathway, the results of our study suggest the effectiveness of targeting the lncRNA-dependent noncanonical Hedgehog pathway either independently or in conjunction with traditional Hedgehog pathway inhibitors to improve the treatment of metastatic cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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