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

Zhen Xing¹, Chunru Lin¹,²,*, and Liuqing Yang¹,²,³,*

¹Department of Molecular and Cellular Oncology; The University of Texas MD Anderson Cancer Center; Houston, TX USA; ²Cancer Biology Program; The University of Texas Graduate School of Biomedical Sciences; Houston, TX USA; ³The Center for RNA Interference and Non-Coding RNAs; The University of Texas MD Anderson Cancer Center; Houston, TX USA

Keywords: Long non-coding RNA, BCAR4, signaling pathway, Hedgehog GLI2, breast cancer, lung metastasis, cancer treatment, locked nucleic acid

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
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. Hedgehog pathway inhibitors represent new opportunities for targeted therapies of cancer, as shown for advanced basal cell carcinoma and medulloblastoma; 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). The newly identified IncRNA-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 IncRNA 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. 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 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 IncRNA-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.
References

1. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 2013; 14:699-712; PMID:24105322; http://dx.doi.org/10.1038/nrm3679.

2. Trimarchi T, Bilal E, Ntziachristos P, Fabbrri G, Dalla-Favera R, Tsirigos A, Aifantis I. Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. Cell 2014; 158:593-606; PMID:25083870; http://dx.doi.org/10.1016/j.cell.2014.03.005.

3. Walsh AL, Tuzova AV, Bolton EM, Lynch TH, Perry AS. Long noncoding RNAs and prostate carcinogenesis: the missing linc? Trends Mol Med 2014; 20:428-436; PMID:24836411; http://dx.doi.org/10.1016/j.molmed.2014.03.005.

4. Xing, Z, Lin A, Li C, Liang K, Wang S, Liu Y, Park PK, Qin L, Wei Y, Hawke DH, et al. lncRNA directs cooperative epigenetic regulation downstream of chemokine signals. Cell 2014; 159:1110-1125; PMID:25416949; http://dx.doi.org/10.1016/j.cell.2014.10.013.

5. Amakye D, Jagani Z, Dorsch M. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. Nat Med 2013; 19:1410-1422; PMID:24202594; http://dx.doi.org/10.1038/nm.3389.

6. Von Hoff DD, LoRusso PM, Rudin CM, Reddy JC, Yauch RL, Tibes R, Weiss GJ, Borad MJ, Hano CL, Brahmer JR, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med 2009; 361:1164-1172; PMID:19726763; http://dx.doi.org/10.1056/NEJMoa0905360.

7. Low JA, de Sauvage FJ. Clinical experience with Hedgehog pathway inhibitors. J Clin Oncol 2010; 28:5321-5326; PMID:21041712; http://dx.doi.org/10.1200/JCO.2010.27.9943.