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LncRNA BCAR4 wires up signaling transduction in breast cancer

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Long noncoding RNAs (lncRNAs) are dysregulated in many cancer types and are believed to play crucial roles in regulating several hallmarks of cancer biology. Currently, most studies support the concept that lncRNAs are involved in either transcriptional or post-transcriptional processes via binding/targeting epigenetic modifiers or hRNP complexes. The discovery of new biological functions of lncRNA and novel RNA binding proteins suggests that lncRNAs may be implicated in a broad spectrum of biological processes such as signal transduction, allosteric regulation of cytoplasmic enzymatic activities, among other potential processes. In a recent report that we have made, based on open-ended lncRNA pulldown technology and a series of systematic analyses, we suggest that lncRNAs also play critical roles in the regulation of noncanonical Hedgehog/GLI 2 signal transduction pathways in cancer cells, which further broadens the scope of known lncRNA functions and aids in the discovery and design of more effective and evidence-based therapeutic targets for the treatment of human cancers and other diseases.

Keywords: lncrna, bcar4, breast cancer, metastasis, signal transduction, Hedgehog/GLI, locked nucleic acids (LNA)

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Classification of Distinct LncRNAs Based on Their Genomic Context

It is quite surprising that the human genome produces such a vast number of long non coding RNAs (lncRNAs), the study of which has benefitted greatly from many powerful technologies and approaches to characterize the essence of these non-protein coding transcripts, which include the following: high-throughput RNA-sequencing (RNA-seq) technologies, large-scale whole genome sequencing, tiling arrays (ChIP-chip, transcriptome mapping), serial analysis of gene expression (SAGE), as well as profiling of specific histone marks (such as H3K4-H3K36 domains) in human cells.[1,2] In addition, considerable efforts have been made to combine the analysis of large-scale sequencing data and experimental validation approaches in order to annotate new RNA species. Benefiting from the progress made by the RefSeq, Ensembl, and GENCODE Consortium within the framework of the ENCODE Project, many lncRNAs have been comprehensively standardized and annotated, resulting in an integrated and curated lncRNA database that represents an invaluable resource for future studies of lncRNAs.[3,4]

LncRNAs can be classified into distinct groups based on their different features such as genomic location, molecular function/effects, mechanisms/modes of action.[5] Currently, one of the most broadly used and relatively convenient ways of classification relies on the corresponding genomic context, *i.e.*, the position in the chromosome where the lncRNA is transcribed. Additionally, it is becoming clear that the residing genomic localization also helps predict the functional roles of a category of lncRNAs. With this perspective, lncRNAs can be characterized as: 1) lincRNAs (large intergenic noncoding RNAs), including well studied and cancer associated *Xist*,[6,7] H19,[8] HOTAIR,[9,10] NEAT2/MALAT1,[11-13] lincRNA-RoR,[14] lincRNA-P21.[15] Many lincRNAs are initially discovered by analyzing intergenic (non-protein-coding) chromatin-state maps that mark actively transcribed regions that are initiated by RNA Pol II (K4-K36 domain: H3K4me3 at the

promoter and H3K36me3 along the whole transcripts), which is also the typical histone modification pattern for actively transcribed protein-coding genes. LincRNAs can be transcribed from thousands of genomic loci and it is estimated that the total number of human lincRNAs is around 3,300 with high evolutionary conservation.[16,17] Furthermore, it was revealed that nearly 20% of lincRNAs associate with the chromatin-modifying complex PRC2 and affect gene expression programs.[18] Another study found that nearly 30% of lincRNAs expressed in mice embryonic stem cells are associated with at least one of 11 chromatin modifying factors,[19] suggesting potentially similar functional mechanisms for many lincRNAs.[16,18] Two) Natural antisense transcripts. They are transcribed with the opposite orientation to the sense DNA strand of their mRNA/lncRNA counterparts at the same or separate genomic loci and form perfect/imperfect pairs.[20,21] They are prevalent in eukaryotes, including humans, mice, yeast, *Drosophila*, and *Aragidopsis*.[22] Functionally, they have been proposed to control multiple layers of gene regulation including transcription, mRNA splicing, and translation,[22] particularly regulating neighboring conjugated sense gene expression.[21,22] For example, *Kcnq1ot1* antisense silences its flanking genes via deposition of inactive chromatin-specific histone modifications.[23] Currently, the highest estimated number of human NATs is around 6,000.[24] Three) Pseudogenes. By definition, pseudogenes are dysfunctional counterparts of genes that have lost protein-coding potential due to accumulation of mutations during genome evolution.[25] They are generally identified by computational analysis of genomic sequences using complex algorithms and are characterized by homology to a known gene and nonfunctionality.[26] It is estimated that the human genome contains more than 18,000 pseudogenes.[27] More and more evidence suggests that pseudogenes may have physiological significance by their direct interactions with DNA or transcripts of the parental protein-coding genes.[25] Recently, it was revealed that the mRNA levels of the tumor suppressor PTEN and oncogenic KRAS are regulated by their pseudogenes *PTENP1* and *KRASP1*, attributing a novel biological role to expressed pseudogenes in cancer progression.[28] Four) Long intronic ncRNAs. They are transcribed from intronic regions of protein-coding genes and it has been revealed that about 81% and 70% of all spliced human and mouse protein-coding genes, respectively, have transcriptionally active introns.[29] LncRNAs transcribed from introns are generally produced through the post-splicing process and are indicative of gene transcription events, which affects many other genes and regulates their expression.[30] For example, it was reported that a number of intronic RNA sequences are capable of binding to the core component EZH2, and in another case, overexpression of the intronic RNA for the gene *SMYD3* was sufficient to reduce endogenous SMYD3 mRNA and protein levels in human cancer cells.[31] Five) Other uncharacterized and divergent transcripts. It was reported that the human genome also produces many diverse and heterogeneous RNA species from transcription start sites and even the regulatory enhancer regions. Usually, these classes of lncRNAs have extremely low abundance in cells and their biological function remain largely elusive. Although lncRNAs can be classified into different groups based on the above criteria, it is still difficult to know the exact total number of distinct human lncRNAs. The combination of several well-established high-confidence lncRNA databases estimated that the total number of lncRNAs (lincRNAs+NATs+intronic lncRNAs+ pseudogenes+others) is at around 111,000 transcripts (integrated statistics from LNCipedia, Feb. 2015).

Functional Significance of LncRNAs and Their Underlying Molecular Mechanisms

It is now clear that the human genome encodes numerous lncRNAs and are now recognized as another crucial layer of the functional outputs of the mammalian genome with bona fide, widespread biological functions across diverse biological processes;[32,33] however, compared to protein-coding genes, there is still little knowledge regarding the biological roles of lncRNAs due to technical limitations and the intrinsic properties of lncRNAs, such as short half-life and extremely low levels. Several well-investigated cases have reported on the implicated roles of lncRNAs in X-chromosome inactivation,[6,7] imprinting,[34,35] control of pluripotency in lineage differentiation,[14,19] as well as some diseases such as cancer.[32,36,37] 1). X-chromosome inactivation (XCI) is an early developmental process by which one X chromosome is transcriptionally silenced in female mammals. It is now well known that the lncRNA *Xist* acts as a major effector during the XCI process. The inactive X chromosome is coated with *Xist*, which is essential for the initiation and maintenance of XCI.[6] Another lncRNA *Tsix* is a gene antisense to *Xist* located at the X-inactivation center and has a role in regulating the early steps of X-inactivation but not the silencing step.[38,39] Recently, one study has suggested that the RNA *Xist* silences X-chromosome transcription by directly interacting with SHARP, recruiting SMRT, activating HDAC3, and deacetylating histones to exclude Pol II across the X chromosome.[7] 2) Genomic imprinting affects 1% of genes in mammals and results in a monoallelic, parental-specific expression pattern, which is achieved by putting epigenetic marks, such as DNA methylation, at specific gene loci in gametes.[40] The majority of imprinted clusters contain a lncRNA, which is crucial for maintaining imprinted gene signatures. For example, the lncRNAs *Kcnq1/Kcnq1ot1* and *Airn* are involved in the imprinting of related genomic loci by occupying the chromatin and recruiting the chromatin remodeling complex to achieve the imprinting effects.[41-43] 3) Previous studies have identified the governing transcription factors required for maintaining pluripotency, namely, Oct4, Nanog, Sox2, Klf4, and c-Myc.[44] By performing a shRNA-mediated loss-of-function screening, one recent study showed that 26 lincRNAs are required for the maintenance of pluripotency of mES cells. The authors found that knockdown of dozens of lincRNAs resulted in a departure from the pluripotent state and upregulation of differentiation programs.[19]

Mechanistically, a handful of studies have implicated lncRNAs in recruiting/directing the chromatin modifying complexes at specific genomic loci to modify chromatin structures and further regulate the gene expression program. Indeed, RNA has been speculated to be an integral component of chromatin in addition to proteins and DNA since long ago. The discovery of lncRNAs and further understanding of their biology greatly helps us to appreciate how RNA species are involved in pathways of chromatin modification. Benefitting from the development and standardization of new techniques such as lncRNA-pulldown, RNA immunoprecipitation (RIP), Cross-linking and RNA Immunoprecipitation (CLIP), and Chromatin Isolation by RNA Purification (ChIRP),[36,45,46] the list of chromatin modification complexes associated with lncRNAs is growing steadily.[47] For example, *HOTAIR* associates with 2 different chromatin modification complexes PRC2 and LSD1/CoREST/REST and functions as a scaffold/guider to assemble these factors to genomic DNA to repress gene expression in the HOXD locus.[9] *HOTTIP* binds the adaptor protein WDR5 directly and targets WDR5/MLL complexes across HOXA to maintain active chromatin.[48] The lateral mesoderm-specific lncRNA *Fendrr* is essential for proper heart and body wall development in the mouse. *Fendrr* associates with the PRC2 and TrxG/MLL complexes to regulate H3K27 trimethylation and H3K4 trimethylation at the promoter regions of several transcription factors.[49] Beyond the aforementioned major theme regarding lncRNAs in chromatin states regulation, which has been extensively studied, lncRNAs can also directly regulate transcription by interference with RNA Pol II[50,51] by acting as decoys for transcription factors[52] or by affecting the localization of transcription factors to achieve fine-tuned gene expression programs.[53] Intriguingly, at the transcriptional level, a set of lncRNAs can function same with defined chromatin enhancers to promote the expression of neighboring protein-coding genes.[54] In addition, it has been reported that the lncRNAs *TUG1* and *NEAT2* are involved in gene activation or repression through organization of distinct nuclear substructures, such as Polycomb bodies and interchromatin granules in response to growth signals, with lncRNAs as the key functional players that bind either methylated or unmethylated Pc2.[13] LncRNAs also regulate mRNA processing including splicing[55] and editing,[56] as well as post-transcriptional events such as controlling the initiation of translation and mRNA stability via direct base pairing.[57] Recently, there is evidence supporting mutual regulation between lncRNAs and miRNAs. For example, the lncRNAs *PTENP1* and *KRASP1* have been found to serve as "sponges" to bind miRNA, thereby sequestering miRNAs away from their mRNA targets.[28] The lncRNA H19 has been found to be a developmental reservoir of miR-675 that suppresses growth and Igf1r.[58,59]

Although lncRNAs function through a variety of interesting mechanisms, it is obvious that the current emphasis is still on how lncRNAs regulate transcription or post-transcriptional processes.[32] Most assuredly, lncRNAs should be involved in a wide array of tasks in cells given their biochemical versatility, various cellular localizations, as well as large amounts of uncharacterized candidates. For example, a substantial proportion of lncRNAs resides within, or is dynamically shuttled, to the cytoplasm where they may regulate protein localization, modification, and even intrinsic enzymatic activity. A recent study found that lnc-DC bound directly to STAT3 in the cytoplasm, which promoted STAT3 phosphorylation by preventing STAT3 binding with SHP1 as well as subsequent dephosphorylation by SHP1 in the regulation of dendritic cell differentiation.[60]

The Implication of LncRNAs in Cancer

Compared to mRNA levels in cells, most types of lncRNA are present at relatively low levels. However, many of the lncRNAs show tissue specific expression patterns. Further lncRNA profiling in multiple cancer cell lines and clinical tissues has made it increasingly clear that many lncRNAs are expressed in a disease-, or developmental stage-specific manner, suggesting that they have specific biological significance, human disease relevance, and diagnostic applicability.[32,61]. Specifically, it has been reported that the expression levels of dozens of lncRNAs are correlated with cancer development and progression (**Table 1**); furthermore, gain-/loss-of-function analyses in various models indicate the importance of these lncRNAs in many cancer types.[32,62] For example, the lncRNAs *PCGEM1* and *PRNCR1* are highly expressed in prostate cancers and regulate androgen dependent or independent cancer cell growth, revealing their potential as therapeutic targets by targeting lncRNA-dependent regulatory networks in human prostate cancers.[36] *HOTAIR* is a highly expressed lncRNA in metastatic breast, liver, colorectal, gastrointestinal, and pancreatic cancer cells/tissues and its expression level in primary tumors is a powerful predictor of eventual metastasis and death.[10,63] Other lncRNAs have been found to be tumor suppressors by operating as a transcriptional repressor. For example, *Linc-p21* is induced by p53 and mediates p53-dependent gene repression in mice cells[15] and acts in a reciprocal way with HIF-1α to modulate the Warburg effects in human cells.[64] In addition, lncRNAs have been shown to have prognostic and diagnostic value in a number of cancers.[65] In spite of these advances, the contributions of most lncRNAs to the hallmarks of cancer biology continues to be poorly characterized.[66] In addition, mechanism studies of lncRNAs are still heavily focused on the interaction between lncRNAs and epigenetic modifiers or other chromatin associated factors. However, more exciting and even surprising findings should be anticipated after the development and utilization of new open-ended techniques and systematic assays in the fields of lncRNA and cancer research. This will also help us understand the molecular details about the interaction between lncRNAs and their protein partners, which are required for the design of effective therapeutic targets for the treatment of human cancers. In the next section, we will introduce how we have combined many open-ended and systematic methods to investigate the *in vitro* and *in vivo* functions of

Table 1. The involvement of lncRNAs in various cancers

lncRNA	Function/mechanisms	Cancer type	Refs
7SK	Targeting/associated with HEXIM	Gastric	91
ANRIL	Epigenetic regulation of CDKN2A/B	Prostate	92
BANCR	N/A	Non-small lung cancer	93
BCAR4	Required for GLI2-dependent transcription	Breast/Prostate	83
BCYRN1	N/A	Breast/Esophagus/Ovarian	94
CCAT1	N/A	Gastric/colorectal	95,96
H19	Gene regulation/miRNA sponges	Breast/liver/prostate	8,97–99
HOTAIR	Epigenetic regulation/chromatin targeting	Breast/colorectal	10,100
LncRNA-LALR1	Activating Wnt/β-catenin	Liver cancer	101
MALAT1	Sequester/gene expression	Lung/colorectal	102,103
MEG3	N/A	Cervical	104
MIR31HC	Regulation of HSP90	Hepatocellular/colorectal	105
PCAT1	Regulating BRCA2 and homologous recombination	Prostate	106
PVT1	N/A	Breast/ovarian	107
PCGEM1	Regulating AR receptor	Prostate	36,108
PRNCR1	Regulating AR receptor	Prostate	36
TUG1	Epigenetic regulation	Non-small lung cancer	109
UCA1	Gene regulation	Breast	110
XIST	Imprinting	Hematologic cancer	111

the lncRNA BCAR4 in regulating a noncanonical Hedgehog pathway. We will further discuss the potential of targeting the lncRNA BCAR4 for the treatment of human cancers.

Potential Involvement of LncRNAs in Hedgehog Pathways in Cancer

The evolutionarily conserved canonical Hedgehog (Hh) system plays an important role in organogenesis and the pattering phase of normal early development from *Drosophila* to humans but has also been linked to tumorigenesis.[67] In the canonical Hh pathway, 3 secreted ligands have been identified, namely Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH). These ligands bind to the negative regulatory receptor Patched (PTCH), a 12-transmembrane protein receptor, which inhibits the activation of Smoothened (SMO), a 7-transmembrane effector, by preventing its surface translocation into the cilium in the absence of Hh ligands. The binding between the ligands and PTCH receptors results in abolishment of the repression effect of PTCH on SMO and the activation of SMO orchestrates a signaling cascade, eventually leading to the final activation of GLI zinc finger transcriptional factors containing GLI1/2/3[68]. In addition, protein kinase A, glycogen synthase kinase 3β, and casein kinase 1α also form a complex with other signaling components to regulate the activation of GLI transcription factors.[69]

In adults, the canonical Hh pathway is either largely inactive or essentially undetectable in most cells but is implicated and reactivated in the development of cancers including brain, lung, breast, prostate, and skin cancers.[70] This reactivation is due either to ligand-dependent (autocrine or paracrine mechanisms to Hh ligands from tumor cells or stromal cells) or ligand-independent (a variety of mutations in the downstream components) mechanisms.[67] GLI gene amplification was first reported in malignant glioma.[71] Subsequently, inactivating mutations in PTCH or activating mutations in SMO were identified in nearly 90% of sporadic basal cell carcinoma.[72,73] In addition, genetic mice models further shed light on the functional importance of the Hh pathway in tumorigenesis; for example, heterozygous loss of Ptch1 results in an increased tendency to develop basaloid tumors while transgenic expression of GLI1 in the epidermis results in skin tumors in mice.[74,75] Aside from the aberrant reactivation of the canonical Hh pathway in cancers, the effector of GLI also directly interacts with other distinct cancer-associated signaling pathways, acting independently of the HH-PTCH1-SMO-GLI paradigm. For example, in esophageal adenocarcinomas, tumor necrosis factor-α (TNF-α) induced activation of the mammalian target of rapamycin (mTOR)-S6 kinase (S6K) via direct phosphorylation, results in GLI1 activation in a Hh ligand independent manner.[76] Several studies have also demonstrated that TGF-β-SMAD3, RAF-MEK-MAPK, and PI3K-AKT cascades also lead to stabilization or increased expression of GLI in distinct cancer types, inducing the expression of Hh ligand-independent and GLI-dependent genes.[77-79] This kind of crosstalk constitutes noncanonical Hh/GLI pathway activation.[67] Genomic analysis has identified hundreds GLI target genes, many of which show tissue-/cell-specific patterns. However, a portion of them are common targets in distinct cell lines. These include genes involved in cell proliferation and survival (*CCND1/2, MYCN, IGFBP6, BCL2*) and genes involved in angiogenesis and metastasis (*VEGF, TGF-β, SNAIL, MUC5AC*).[68,70] In breast cancer, the potential roles of Hh signaling have not been well defined. It has been reported that the *PTCH2* mutation exists in both the primary tumor and brain metastasis of a patient with aggressive basal-like breast cancer (BLBC).[80] There is also evidence that SMO and Hh are ectopically expressed in BLCB or invasive ductal carcinoma (IDC).[81,82] Also, there have been reports of increased GLI activity in breast

cancers; however, this activity occurs in the absence of Hh ligands, which raises the possibility that there might be other regulators, such as lncRNAs, that might contribute to GLI activation, especially since lncRNAs are known to directly bind transcription factors.

The LncRNA BCAR4 Regulates Non-canonical Hh/GLI2 Pathway in Breast Cancer Cells

Recent work from our lab demonstrated how a lncRNA, BCAR4, functions to orchestrate a noncanonical Hh cascade to activate GLI2-dependent gene transcription and to promote cancer cell metastasis[83] (**Fig. 1**). Initially, we sought to identify human breast cancer relevant lncRNAs by analyzing clinical breast cancer tissue samples with a lncRNA array. BCAR4 ranked first in the candidate list and showed the most dramatic upregulation in breast cancer tissues. RNAScope analysis further supported the conclusion that higher BCAR4 expression is correlated with advanced lymph node metastasis stage and shorter survival time for breast cancer patients. Additionally, Oncomine data-mining showed that elevated BCAR4 expression is not only correlated to invasive breast cancer but also to ERBB2/ER/PR negative breast cancer status. The assortment of unbiased and compelling evidence strongly supports BCAR4 as a driving force in the process of breast cancer progression and metastasis.

Since most lncRNAs act in-trans to functional in cells, understanding the interacting protein partners of a lncRNA will by necessity reveal pertinent information to help determine the roles of a specific lncRNA. Interestingly, the putative protein complex, which associates with BCAR4, contains 2 RNA-binding proteins (SNIP1 and PNUTS), one kinase (CIT), and one transcription factor (GLI2). Furthermore, we performed a series of robust and thorough tests including EMSA, to study *in vitro* RNA-protein binding, as well as an RNA pull-down-Dot Blot assay to characterize the protein binding domains of the lncRNA. Our data support the notion that the primary RNA sequence (50–100 nt) may play a critical role in determining the specific RNA-protein interaction. This also suggests the potential explanation for the low sequence conservation of the lncRNA across different species as only a 50–100 nt fragment in the whole lncRNA gene appears to be essential for its interaction with proteins. Indeed, as it has been suggested that the secondary structure of lncRNAs mediates their functional interactions with protein factors,[84] we speculated that this short fragment might possess highly structured regions critical for its function.

GLI2 is one of the 3 effectors (GLI1/2/3) that are downstream of the Hh pathway and its post-translational modifications, such as phosphorylation, have been reported to be critical for its activity. Our data show that CIT kinase can directly phosphorylate GLI2 at S149, which induces its nuclear translocation, binding to promoters of downstream genes, and activating transcription. The importance of GLI2 (S149) phosphorylation is supported by its correlation with invasive breast cancer status and its widespread existence in other tested cancer types (including lung, liver, colorectal, and ovarian cancers) as revealed by immunohistochemistry in a large number of clinical tissue samples. In addition, treatment cells with several cancer metastasis-related chemokines or growth factors including CCL21, CXCL21, IGF-1, or TGF-β induced GLI2 phosphorylation at S149 to differing degrees. To examine the genomic occupancy of BCAR4, we performed a Chromatin Isolation by RNA Purification (ChIRP) assay and found that after nuclear translocation and activation of phosphor-GLI2 (S149), BCAR4 is recruited to the promoters of GLI2-dependent downstream genes. Consistently, knockdown of BCAR4 dramatically suppresses CCL21-induced expression of GLI downstream target genes. Recent findings suggest that the Hh/GLI pathway is critical for cancer metastasis.[68,70] Our loss-of-function studies in cells showed that knockdown of BCAR4 significantly inhibits the metastatic ability of multiple breast cancer cell lines; consistently, overexpression of full-length BCAR4, but not the deletion mutants which abolished SNIP1 and PNUTS binding, respectively, dramatically increased cell invasion and GLI2 target gene expression in a non-metastatic breast cancer cell line. These data strongly suggest the importance of BCAR4 in the phospho-GLI2-mediated transcriptional activation of a subset of genes, which contribute to breast cancer cell migration and invasion.

Mechanistically, we found that BCAR4 acts as a "double-unlock-switch" which is required for GLI2-dependent gene activation. Our research, and that of others, has found that the DUF domain of SNIP1 binds to the catalytic domains of p300 to inhibit its HAT activity;[83,85] however, CCL21-induced binding of BCAR4 to

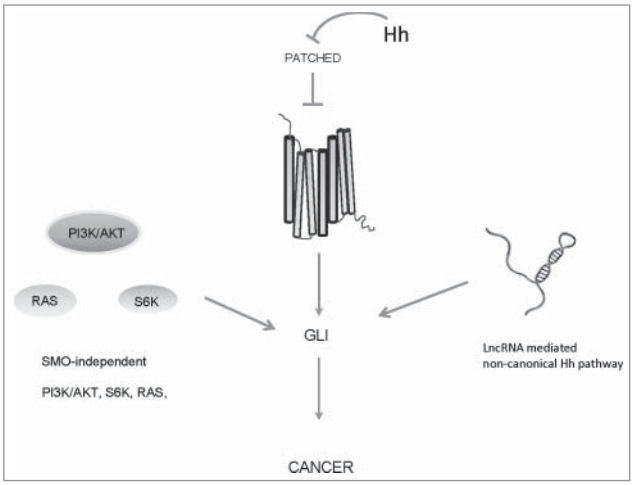

Figure 1. LncRNA mediated non-canonical Hh pathway.

the DUF domain of SNIP1 releases its interaction with the catalytic domain of p300, leading to the activation of p300. Subsequently, the activated p300 enhances the acetylation of H3K18 on the promoters of GLI2 target transcription units, which further releases the inhibitory roles of PNUTS on PP1 phosphatase enzymatic activity; this consequently modulates the phosphorylation of Pol II Ser5 at GLI2 target gene promoter regions and activates transcription. Therefore, on a molecular level, these findings demonstrate how a lncRNA, BCAR4, through its direct interactions with RNA binding proteins SNIP1 and PNUTS, acts as a key to bridge signal-induced epigenetic regulation of general transcription machinery during the activation of GLI2 target genes in breast cancer cells.

While targeted therapies against selected proteins in breast cancer are promising, they are limited due to the development of resistance. Our goal was to uncover clues for a more efficacious breast cancer treatment from a lncRNA point-of-view. There has been a long-standing interest in targeting noncoding RNAs by using a Locked Nucleic Acids (LNA)-based antisense oligonucleotides strategy, as there have been several successful applications that have targeted miRNAs in cancer.[86] To evaluate the therapeutic potential of BCAR4, we used in vitro synthesized LNAs to knockdown endogenous BCAR4 expression. Significantly, 2 individual LNA treatments substantially reduced lung metastases, providing the first demonstration of the pharmacologic value of lncRNAs in human cancers.

Concluding Remarks and Future Perspectives

In summary, our findings are the first to show how a lncRNA directs cooperative epigenetic regulation downstream of specific chemokine signals, thereby contributing to breast cancer metastasis.[83] Cell signaling, mediated mostly by membrane receptors, intracellular kinases, and nuclear transcriptional factors, is part of a complex system of communication that governs and coordinates basic cellular activities. The active involvement of lncRNA in this process makes it more likely that lncRNAs are innate components of the classical protein-mediated signal transduction pathways in cells. Intriguingly, we observed that BCAR4 is also highly expressed in other organ malignancies including lung squamous cell carcinoma, skin malignant melanoma, kidney clear cell carcinoma, colon adenocarcinoma, and rectum adenocarcinoma. In addition, knockdown of BCAR4 in HCT116, H1299, HepG2, and Hey8 cells significantly impaired the migration and invasion of these cells;[83] this suggests that the lncRNA BCAR4 may also contribute to the metastatic potential of these cancers by regulating GLI2-dependent gene activation. Indeed, the TCGA database showed that elevated levels of the BCAR4 transcript are correlated with prostate cancer metastasis. It is necessary to investigate, in cancer cell lines from other cancer types, whether or not BCAR4 directly binds SNIP1 and PNUTS. In addition, it is important to examine the potential extracellular signals which induce GLI2 (S149) phosphorylation to comprehend the general human cancer relevance of this BCAR4-dependent GLI2 transcriptional program. Here we posit that a large proportion of cancer susceptibility may be the result of dysregulated lncRNAs. Examination of these lncRNA genes and their functional mechanisms will broaden our understanding of their biology, human cancer relevance, as well as their contributions to the hallmarks of cancers.[66] Most importantly, these studies will accelerate their integration into clinical applications for diagnosis, prevention, and therapeutic treatment of human cancers. We are still in the early stages of the expanding field of lncRNA research and there is no doubt that development of new technologies and methods for characterizing the functions of lncRNAs will continue to accelerate the pace of exciting discoveries. For example, a new approach has been developed that allows for the direct evaluation of RNA structure in living cells and the assessment of dynamic changes in RNA structure in different cell states.[87] In addition, RNA aptamers have been developed that bind fluorophores resembling the fluorophore in GFP, named Spinach, and is markedly resistant to photobleaching, which can be used to examine the localization of lncRNAs in cells.[88]

The development of acquired resistance to the targeting of specific cancer signals is clearly a complex phenomenon involving multiple pathways and has frequently been a challenge in cancer therapeutics; the Hh pathway is no exception to this phenomenon. Currently, the major antagonists against the Hh pathway target SMO, such as vismodegib, sonidegib, BMS-833923, PF04449913, and LY2940680, which have been studied in clinical trials for many cancers types.[67] For example, the strategy of blocking SMO with vismodegib alone has been approved clinically for the treatment of advanced BCC,[89] and responses have been observed for BCC treatment with either sonidegib or BMS-833923 alone.[90] However, acquired resistance has been observed in both the preclinical and clinical settings due to the activation of noncanonical Hh pathway, amplification of downstream Hh target genes, or resistance mutations of SMO.[67] One of the biggest challenges in treating drug-resistant tumors is the underlying complexity of the networking pathways; a detailed understanding of the mechanisms leading to GLI activation (canonical, noncanonical, and crosstalk) would allow for the development of the appropriate targeted therapies and improved outcomes. While consideration of lncRNAs in this pathway does add additional complexity, it provides the rational basis for the targeting of either lncRNA alone or as part of a combination therapy with established inhibitors to overcome drug resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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