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A New Lnc in Metastasis: Long Non-Coding RNA Mediates the Pro-metastatic Functions of TGF-β

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Abstract

TGF-β signaling promotes metastasis by controlling the expression of downstream target genes. In this issue of *Cancer Cell*, Yuan et al. discover a novel TGF-β-induced lncRNA, lncRNA-ATB, that stimulates EMT through sequestering miR-200s and facilitates colonization by stabilizing *IL-11* mRNA, thus promoting both early and late steps of cancer metastasis.

The transforming growth factor-β (TGF-β) pathway plays crucial roles during development and homeostasis and exerts strong anti-proliferative effects on normal and premalignant cells. However, advanced stage cancers often become insensitive to the tumor-suppressive actions of TGF-β. Instead, advanced cancers benefit from TGF-β's profound metastasis-promoting effects, such as epithelial-to-mesenchymal transition (EMT) induction, angiogenesis promotion, altered extracellular matrix deposition, immune suppression, and increased metastatic colonization (Ikushima and Miyazono, 2010; Massague, 2008). These pro-metastatic responses to TGF-β are mediated by a variety of downstream effector proteins, including transcription factors (e.g. AP-1, ID1, SNAIL, SLUG, TWIST, and ZEB1/2), cytokines, growth factors and other ligands (e.g. ANGPTL4, PTHrP, IL-11, JAGGED1, PDGF-B, CTGF, and VEGF), matrix proteins and proteases (e.g. TNC, MMPs) (Ikushima and Miyazono, 2010; Massague, 2008), and a growing number of microRNAs (miRNAs) (Butz et al., 2012).

In recent years, long non-coding RNAs (lncRNAs), a new class of non-coding RNAs longer than 200 nucleotides, have been recognized to regulate a wide variety of physiological and pathological processes through diverse mechanisms. For example, lncRNAs ANRIL and HOTAIR promote tumor growth or metastasis by recruiting chromatin-remodeling complexes to alter gene transcription, while tumor-suppressing lncRNA GAS-5 and tumor-promoting lncRNA HULC act as decoys for glucocorticoid receptor and miR-372, respectively (Wapinski and Chang, 2011). Although the diversity and abundance of lncRNAs seem to rival that of mRNAs in any given cell type, there is little understanding of

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crucial lncRNAs functioning downstream of the TGF-β pathway. In this issue of *Cancer Cell*, Yuan et al. report a novel TGF-β induced lncRNA that amplifies the pro-metastatic effect of TGF-β via two independent mechanisms (Yuan et al., 2014).

Aiming to identify TGF-β regulated lncRNAs involved in EMT, Yuan et al. profiled lncRNA expression in human hepatocellular carcinoma (HCC) cells after TGF-β treatment. The authors focused their attention on lncRNAs that might function as potential competing endogenous RNAs (ceRNAs) against the miR-200 family miRNAs, which are suppressed by TGF-β signaling and are potent inhibitors of EMT through targeting of two master EMT transcription factors ZEB1 and ZEB2 (Figure 1). One such lncRNA, aptly named lncRNA-Activated by TGF-β (lncRNA-ATB), stood out by virtue of containing three predicted miR-200 binding sites. Binding of lncRNA-ATB by miR-200s was confirmed by RNA immunoprecipitation (RIP) of lncRNA-ATB, luciferase assays, and anti-AGO2 RIP. LncRNA-ATB is non-polyadenylated, localizes primarily in the cytoplasm, and has three close homologs in the human genome. Notably, lncRNA-ATB is also up-regulated by TGF-β in MCF7 breast cancer cell line and SMAD4-deficient SW480 colorectal cancer cell line, implying that lncRNA-ATB may be activated through the SMAD-independent, non-canonical TGF-β pathway. LncRNA-ATB increases ZEB1 and ZEB2 mRNA and protein levels through competitively binding and sequestering miR-200s, thereby inducing EMT (Figure 1). Remarkably, depletion of lncRNA-ATB is sufficient to abolish TGF-β-induced EMT in HCC cells, even though TGF-β is known to strongly induce many other EMT drivers, such as SNAIL, SLUG, and TWIST. These findings suggest that lncRNA-ATB may represent an essential node in the EMT regulatory network.

While mutations in miR-200 binding sites or miR-200 overexpression abolished lncRNA-ATB's function in stimulating EMT, they only partially eliminated the pro-metastatic effect of lncRNA-ATB, suggesting that other mechanisms are at play. The authors first tested the role of lncRNA-ATB in different steps of the metastatic cascade. While lncRNA-ATB overexpression increases tumor dissemination in a miR-200-dependent manner, lncRNA-ATB promotes liver and lung colonization of HCC cells independent of miR-200s. Genome-wide RIP revealed *IL-11* mRNA as one of the top transcripts bound by lncRNA-ATB. *IL-11* is a TGF-β target gene that has been shown to promote bone metastasis of breast cancer (Kang et al., 2003) and liver colonization of colorectal cancer by activating the pro-survival GP130/STAT3 signaling pathway (Calon et al., 2012). Yuan et al. showed that lncRNA-ATB binds to and stabilizes *IL-11* mRNAs, stimulates autocrine IL-11 production, thus triggering STAT3 signaling in tumor cells to promote colonization (Figure 1).

LncRNA-ATB is up-regulated in HCC samples compared to paired noncancerous hepatic tissues and significantly correlates with liver cirrhosis, vascular invasion, and reduced recurrence-free and overall survival of HCC patients. *LncRNA-ATB* levels are also significantly higher in portal vein tumor thrombus (PVTT), the main route for intrahepatic metastasis of HCC cells, compared to primary tumor tissues. Furthermore, increased *lncRNA-ATB* levels significantly correlate with increased *ZEB1/2* and *IL-11* mRNA levels and decreased *CDH1* (encoding for E-cadherin). Collectively, these data highlight strong clinical relevance and prognostic value for lncRNA-ATB and suggest its potential as a promising biomarker and therapeutic target.

This exciting study revealed a novel TGF-β-induced lncRNA that promotes both early and late steps of HCC metastasis by enhancing the pro-metastatic effects of TGF-β signaling in EMT and colonization. These findings also raised important questions that warrant future explorations. First, as lncRNA-ATB is responsive to TGF-β induction even in SMAD4-deficient cells, this indicates that non-canonical SMAD-independent pathways downstream of TGF-β (Moustakas and Heldin, 2005) are involved and that lncRNA-ATB might be able to mediate the pro-metastatic function of TGF-β in the context of Smad deficiency. Further studies are needed to connect TGF-β signaling to lncRNA-ATB activation and investigate the potential regulation of lncRNA-ATB by other oncogenic signaling pathways that are active in the tumor microenvironment. On the other hand, among >20,000 lncRNAs in the human genome, other lncRNAs (including those identified but not explored further in the current study) are also likely to be involved in mediating tumor-suppressive or tumor-promoting effects of TGF-β. The field remains wide open to identify these TGF-β-responsive lncRNAs and elucidate their mechanisms of action.

It is also worth noting that the pro-metastatic effects of lncRNA-ATB rely on miR-200s and IL-11, whose expressions are previously known to be regulated by TGF-β through transcriptional mechanisms independent of lncRNA-ATB. Thus, lncRNA-ATB functions by enhancing the existing network of pro-metastatic TGF-β signaling (Figure 1). It will be of great interest to put this new link into the tempo-spatial context of TGF-β signaling dynamics during tumor progression. How are the levels of lncRNA-ATB maintained in disseminated tumor cells during colonization after the cells depart from the TGF-β-rich primary tumor microenvironment? Is lncRNA-ATB expression maintained by a bistable control mechanism just like the miR-200/ZEB double-negative feedback loop, or perhaps lncRNA-ATB has a long half-life? Is lncRNA-ATB also involved in mediating the paracrine signaling effect of TGF-β in stromal cells during metastasis, as was previously described for the production of IL-11 from cancer-associated fibroblasts (Calon et al., 2012)?

Despite these questions, the strong clinical significance of lncRNA-ATB in HCC suggests its potential utility as a therapeutic target. Soluble antisense oligonucleotides against lncRNA-ATB or other agents that block lncRNA-ATB's interactions with target miRNAs and mRNAs may be developed to specifically block the pro-metastatic branch of TGF-β signaling (Wahlestedt, 2013). To avoid potential detrimental side effects, it will be essential to understand the normal physiological function of lncRNA-ATB as well as its three closely related homologs. In this regard, it will also be important to characterize the role of other miRNAs and mRNAs that bind to lncRNA-ATB as they may also mediate lncRNA-ATB's function during development, homeostatsis, and cancer progression. Despite these challenges, the discovery of lncRNA-ATB represents an exciting step forward toward harnessing lncRNAs, formerly among the "dark matters" of the genome, for therapeutic intervention against cancer.

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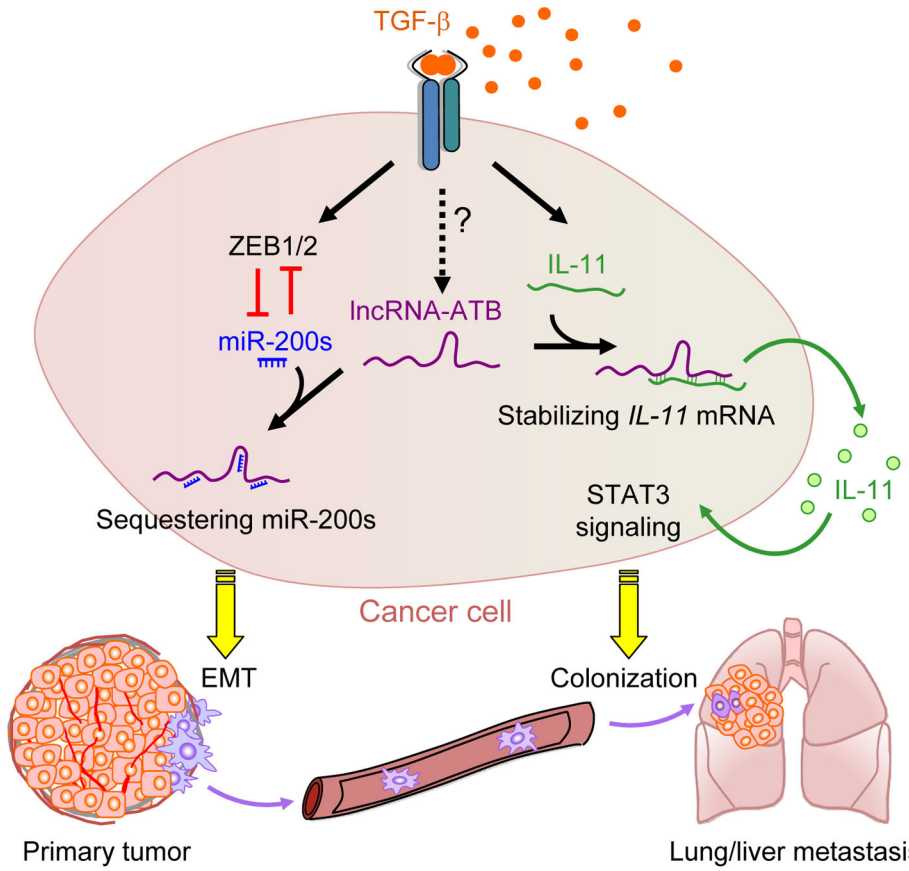

Figure 1. LncRNA-ATB Acts Downstream of TGF-β to Promote Different Steps of Cancer Metastasis

TGF-β signaling promotes metastasis by altering the expression of a variety of downstream genes, including many protein-coding mRNAs, miRNAs, and in the current study, a long non-coding RNA lncRNA-ATB. TGF-β signaling induces lncRNA-ATB, which reinforces the pro-metastatic TGF-β response via two distinct mechanisms. LncRNA-ATB competitively binds to miR-200s and sequesters them away from their mRNA targets *ZEB1* and *ZEB2*, which encode two key EMT promoting transcription factors that repress the expression of E-cadherin and the miR-200s themselves, thus promoting EMT. LncRNA-ATB also binds to and stabilizes *IL-11* mRNA, thereby increasing autocrine IL-11-STAT3 signaling to enhance the survival and metastatic colonization of disseminated tumor cells in the lung and liver. While ZEB1/2 and IL-11 are known to be activated by Smad-dependent pathways downstream of TGF-β receptor activation, lncRNA-ATB is activated by a Smad-independent non-canonical pathway that remains to be identified.