IncRNA promotes protein translation to metastasis

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Long noncoding RNAs (lncRNAs) are a novel class of regulators that play crucial roles in development and disease. Here we highlight the findings by Huang and colleagues that a regulatory lncRNA (treRNA) acts as a scaffold for a new ribonucleic protein complex that inhibits translation of E-cadherin and promotes cell invasion. This work underscores the potential importance of lncRNAs in cancer metastasis.

Genome-wide transcriptome sequencing efforts have led to the discovery of thousands of long noncoding RNAs (lncRNAs). lncRNAs are polyadenylated transcripts, typically more than 200 base pairs in length, that show polymerase II transcription initiation and elongation marks (Ulitsky and Bartel, 2013). Little is known about the biological roles of lncRNAs, but several potential mechanisms for the action of nuclear and cytoplasmic lncRNAs have been proposed. Nuclear lncRNAs mainly regulate the transcription of target genes by recruiting chromatin-modifying complexes to specific regions (Rinn and Chang, 2012), while cytoplasmic lncRNAs may interact with other RNAs, serve as molecular decoys for microRNAs and RNA-binding proteins, or function as cytoplasmic scaffolds of RNA–protein complexes. Interestingly, cytoplasmic lncRNAs may also interact with target mRNAs and protein complexes

to regulate mRNA stability and translation (Carrieri *et al*, 2012; Yoon *et al*, 2012; Kretz *et al*, 2013). Recently, lncRNAs have been found to play important roles as drivers of tumour progression and as oncogenic suppressors in various cancer types. A report by Gumireddy *et al* (2013) in this issue now adds another lncRNA to this list by identifying a role for translational regulatory lncRNA (treRNA) in cellular invasion and metastasis.

Gumireddy *et al* (2013) examined the expression of treRNA in matched primary and lymph node metastatic tumours and found higher treRNA levels in all metastatic tumours, suggesting that increased treRNA expression could be associated with metastasis. To investigate treRNA function, the authors ectopically expressed the lncRNA in the non-invasive breast cancer cell line MCF7 and observed increased cell migration and invasion capacity. Conversely, knockdown of treRNA in A549 cells (a metastatic cell line with high endogenous expression of treRNA) strongly suppressed cell migration and invasion. Using an *in vivo* bioluminescence system, the authors furthermore found that treRNA-expressing MCF7 cells promoted lung metastasis, while depletion of treRNA in A549 cells reduced the formation of lung tumours relative to controls, substantiating a potential role for treRNA in metastasis.

Figure 1 TreRNA promotes the formation of a protein complex (treRNA–RNP) consisting of hnRNP K, FXR1, FXR2, PUF60, and SF3B3. treRNP interacts with the 3'UTR of E-cadherin and other transcripts, and inhibits translation.

Loss of E-cadherin is known to allow epithelial cells to undergo changes in cell morphology and motility in order to adopt mesenchymal characteristics, a process known as epithelial-to-mesenchymal transition (EMT; Valastyan and Weinberg, 2011). Close examination of MCF7 cells with overexpression of treRNA revealed loss of the epithelial protein markers E-cadherin, zonula occludens (ZO-1), and β -catenin, while the mesenchymal markers fibronectin and vimentin were upregulated, indicating that the cells underwent partial EMT. The mRNA levels for the epithelial markers remained constant, indicating that regulation by treRNA occurs at the protein synthesis level. Interestingly, treRNA expression was predominant in the cytoplasm, and polysome analysis confirmed that treRNA affects the translational efficiency of E-cadherin mRNA.

But how exactly does treRNA affect E-cadherin protein expression? Gumireddy *et al* (2013) further show that treRNA associates with the RNA-binding proteins hnRNP K, FXR1, FXR2 and PUF60, as well as with SF3B3, a subunit of splicing factor 3b. Among those proteins, hnRNP K, FXR1, FXR2, and PUF60 were necessary for treRNA-mediated suppression of E-cadherin translation. Moreover, hnRNP K, FXR1, and FXR2 were also required for treRNA-mediated metastasis formation *in vivo*. RNAi-mediated depletion of the three RNA-binding proteins diminished the number of lung metastases, and combinations of double and triple knockdowns completely abolished the ability of treRNA to induce metastases in the lung.

Mechanistically, pull-down experiments indicated that treRNA expression is necessary for binding of PUF60-SF3B3 to hnRNP K, FXR1, and FXR2 to form a ribonucleic complex (RNP) that associates with the translation initiation factor eIF4G1. Furthermore, the authors found that the 3'UTR of

E-cadherin mRNA is required for translation suppression by the treRNA-RNP complex. The E-cadherin 3'UTR contains multiple potential binding sites for hnRNP K, FXR1, and FXR2 and mutating these binding sites in reporter constructs served to rescue the suppressive effect of treRNA, confirming these sites as docking platforms for treRNA-RNP complexes.

Collectively, the findings presented by Huang, Gumireddy and colleagues provide evidence that lncRNAs can function as cytoplasmatic scaffolds for the formation of RNP complexes (Figure 1). Such RNP complexes interact with target mRNAs to control the rate of protein synthesis for key regulators of the EMT process. However, the study of Gumireddy *et al* (2013) also raises several intriguing questions. First, how does treRNA specifically interact with regulatory RNA-binding proteins? Second, how many targets does treRNA-RNP have? Third, it remains to be determined how the expression of treRNA is generally controlled, and more specifically whether transcription factors that induce EMT (e.g., TWIST, SNAI1, ZEB1, and others) have a role here. Last but not least, the *in vivo* role of treRNA should be examined using mouse models for EMT and cancer metastasis. Nevertheless, in the past few years several studies have indicated that lncRNAs are associated with and promote metastasis (Ji *et al*, 2003; Gupta *et al*, 2010). Although the mechanism of action of these lncRNAs seems to be very different in nature, the intricate interaction between lncRNAs and tumour metastasis may have important implications for cancer diagnosis and therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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