Editorial

# *PNUTS* at the crossroads of tumorigenesis and metastasis formation

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*Provenance:* This is an invited Editorial commissioned by the Section Editor Dr. Chunlin Ou (Cancer Research Institute of Central South University, Changsha, China).

*Comment on:* Grelet S, Link LA, Howley B, *et al*. A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression. Nat Cell Biol 2017;19:1105-15.

Submitted Nov 24, 2017. Accepted for publication Dec 25, 2017.

doi: 10.21037/jtd.2017.12.135

**View this article at:** http://dx.doi.org/10.21037/jtd.2017.12.135

Around 75% of the human genome is transcribed (1,2). Of these genomic transcripts, at most 2% encode functional proteins within their open reading frames. The remaining 98% are non-coding RNAs that lack open reading frames. This majority of RNAs consists to 20% of short non-coding RNAs. These are 20–200 nt in length and include the subgroup of micro RNAs (miRNAs). The remaining 80% of the non-coding RNAs are long non-coding RNAs (lncRNAs), which are 200 nt to 10 kb in length (*Figure 1*).

While initially believed to be "junk" RNA, increasing evidence illustrates that non-coding RNAs can be critical regulators of gene and protein expression and hence key regulators of cell fate decisions (1-4). Since these decisions include all steps of cellular transformation and tumorigenesis (2), analyses to comprehensively understand pro- and anti-oncogenic functions of these regulatory RNA molecules are warranted.

Grelet and colleagues have elucidated that a splice switch in the *PNUTS/PPP1R10* RNA is relevant for metastasis-associated gene expression programs and tumor growth (5). The *PNUTS* pre-RNA can give rise to the *PNUTS* mRNA or an alternatively spliced isoform that represents a tumor-relevant lncRNA of *PNUTS* (lncRNA *PNUTS*) (*Figure 2*). This lncRNA is generated through a removal of 61 bases in the 5'-region of exon 12, leading to a frameshift in the transcript's open reading frame. As the lncRNA *PNUTS* is not translated into a protein and only occurs in the monosomal fraction of ribosomes, it is truly non-coding (5). This is of importance in light of the fact that the PNUTS protein, which is also known as serine/threonine phosphatase-1 regulatory subunit-10, contributes to cancer cell migration in conjunction with the lncRNA *BCAR4* (6).

How did Grelet and colleagues identify lncRNA *PNUTS* as a regulator of the metastatic process? Based on their previous finding that the heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) controls oncologically important RNA processing (7,8), the authors knocked down hnRNP E1 in tumorigenic epithelial murine NMuMG breast cells and performed a RNA microarray analysis. Data collected with this approach suggested that the knockdown of hnRNP E1 led to an increase in lncRNA *PNUTS*. A knockdown of hnRNP E1 in epithelial human A549 lung carcinoma cells verified the enhanced processing of *PNUTS* pre-RNA into lncRNA *PNUTS* instead of *PNUTS* mRNA in the absence of hnRNP E1 (5). This finding is consistent with the ability of hnRNP E1 to control alternative splicing (9,10).

Grelet *et al*. provide several lines of evidence that suggest a tumorigenic role for lncRNA *PNUTS*. First, the expression levels of lncRNA *PNUTS* are higher in human breast tumor tissue compared to normal breast tissue. Furthermore, the mesenchymal human MDA-231 breast adenocarcinoma cell line and two lung and bone metastases thereof express higher amounts of lncRNA *PNUTS* than epithelial breast tumor cell lines. This difference in lncRNA *PNUTS* expression correlates with a decrease in the levels

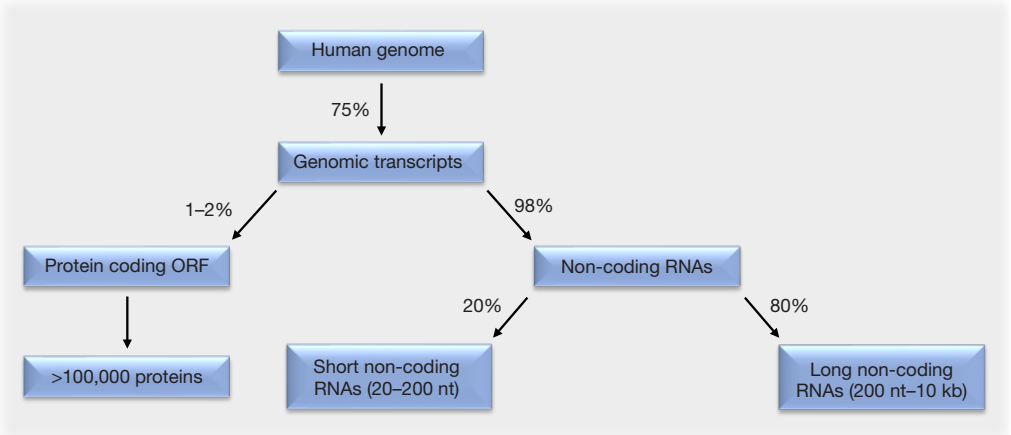


**Figure 1** Estimated ratio of RNA species in mammalian cells. See text for details. ORF, open reading frame.

of the epithelial marker E-cadherin, an increase in the mesenchymal marker vimentin, and higher levels of the transcription factors ZEB1 and ZEB2 (5). Together with other transcription factors, ZEB1 and ZEB2 promote the epithelial-to-mesenchymal transition (EMT). This essential developmental process is hijacked by cancer cells during tumorigenesis to develop tumor metastases at distant sites (11-13). Overexpression of the lncRNA *PNUTS* in A549 and NMuMG cells verified that this lncRNA promotes the accumulation of mesenchymal marker proteins together with an increased migratory and invasive potential (5).

At the molecular level, hnRNP E1 binds to exon 12 of the *PNUTS* pre-RNA to control its splicing (5). Stimulation with the EMT-promoting cytokine TGFβ activates the kinase AKT2, which phosphorylates hnRNP E1 and thereby disrupts its interaction with the *PNUTS* pre-RNA (*Figure 2*). As this effect occurs transiently, it may rather contribute to the initiation of TGFβ-induced EMT than to the progression of the metastatic cascade. On the other hand, the constantly elevated levels of lncRNA *PNUTS* in tumors and mesenchymal cell lines may indicate additional functions of this molecule. It will be interesting to see in this context whether the frequently observed overexpression and hyperactivation of AKT2, which correlates positively with the aggressiveness of breast and other cancers (14), is linked to the ratio of *PNUTS* RNA species.

How does the lncRNA *PNUTS* regulate EMT? The lncRNA *PNUTS* acts as a competing endogenous RNA sponge for the miRNA miR-205 (5), which has a significant impact on EMT (15). Although the lncRNA *PNUTS* can bind and inactivate six miR-205 molecules, the *PNUTS* mRNA does not sequester this miRNA. Structural features and ribosomal hindrance are discussed as potential mechanisms underlying this finding (5).

The formation of metastases *in vivo* is a complex process that involves a plethora of factors. Grelet and colleagues analyzed the impact of lncRNA *PNUTS* in a mammary fat pad transplantation model using NOD/SCID mice and epithelial MDA-468 cells or mesenchymal MDA-231 lung metastasis cells. They could show that an overexpression of lncRNA *PNUTS* in MDA-468 cells increased and that an attenuation of lncRNA *PNUTS* expression in MDA-231 cells decreased tumor growth, respectively. The authors also show that lncRNA *PNUTS* levels determine the primary growth of MDA-231 cells in fat pads (5). Therefore, it will be interesting to see the relative contributions of altered EMT signaling and hitherto unknown effects of the lncRNA *PNUTS* on tumor cell growth. It is equally thought-provoking why MDA-231 breast cancer cells and metastases thereof have equal levels of the lncRNA *PNUTS* but express divergent levels of epithelial and mesenchymal marker proteins. Answers to these questions might contribute to a better understanding of the complex regulatory mechanisms involved in the process of metastatic cancer cell growth.

The identification of lncRNA *PNUTS* as critical modulator of EMT supports the view that EMT is a main driver of the metastatic spread of breast cancer cells (16). At present, it is also possible that lncRNA *PNUTS* expression is causally associated with a decreased chemosensitivity of mesenchymal cancer cells (17,18). Another crucial question is whether there are further upstream regulators of the *PNUTS* lncRNA/mRNA splice switch and if these might represent feasible drug targets. There could be additional

 

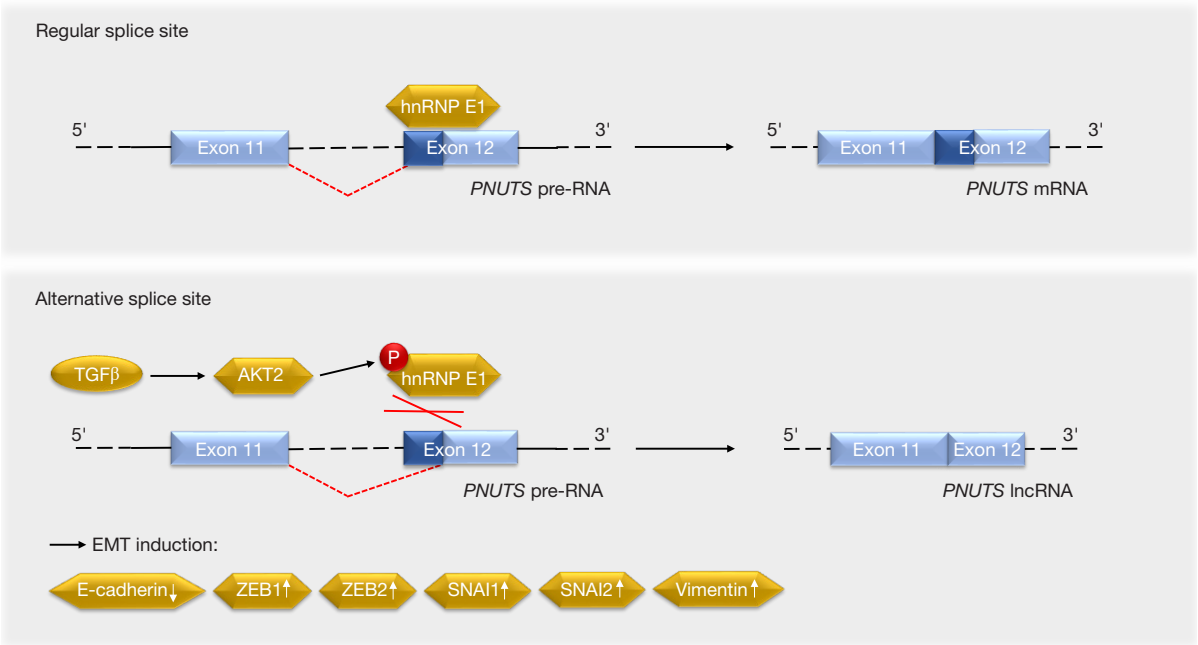


**Figure 2** Regulation of *PNUTS* pre-RNA splicing and impact on EMT-associated processes. hnRNP, heterogeneous nuclear ribonucleoprotein; lncRNAs, long non-coding RNAs; EMT, epithelial-to-mesenchymal transition.

mechanisms beyond TGFβ and AKT signaling; for example, a possible interplay between epigenetic modifiers and RNA-dependent tumor-relevant mechanisms (3,19). One may also consider that further miRNAs might be competed out by the lncRNA *PNUTS* (5). Since the miR-205 itself can exert pro- and anti-oncogenic functions in an ambivalent manner (15), additional work is necessary to generate safe and effective RNA-based therapeutics. Such drugs may pave the way for innovative and effective treatment strategies against diverse cancer types. Furthermore, as elevated lncRNA *PNUTS* expression is an early event in TGFβ-mediated EMT (5), it could be an early prognostic marker for EMT-mediated drug resistance (17,18).

Taken together, in their work Grelet *et al.* (5), the team around Philip H. Howe and colleagues has revealed for the first time that bifunctional RNA isoforms are able to dynamically regulate biologically important cellular signaling pathways. Moreover, as these authors demonstrate that TGFβ and AKT signaling trigger the alternative splicing of the *PNUTS* pre-RNA, the expression levels of the lncRNA *PNUTS* might serve as a biomarker to define patient subgroups, whose primary tumors are prone to metastasis formation. These patients might particularly benefit from targeted therapies that abrogate TGFβ signaling and AKT activation (14,20).

## Acknowledgements

*Funding:* This work was supported by the Wilhelm Sander-Stiftung (grant number, #2010.078).

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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**Cite this article as:** Kiweler N, Krämer OH. *PNUTS* at the crossroads of tumorigenesis and metastasis formation. J Thorac Dis 2018;10(2):560-563. doi: 10.21037/jtd.2017.12.135

 