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EDITORIAL

Thymosin beta-4x LINCs SPAAR to its non-coding function

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This editorial refers to 'The LINC00961 transcript and its encoded micropeptide SPAAR regulate endothelial cell function' by H.L. Spencer *et al.*, pp. 1981–1994.

Gene expression control and RNA biology is exceedingly complex. During evolution, genetic information expanded progressively and became increasingly more complex as more 'non-coding' RNA appeared. Evolution is particularly rapid for non-coding RNAs longer than 200 nucleotides (lncRNAs), which often exhibit highly specialized modes of action as well as low, yet dynamic and specific, tissue expression.[1] Owing to these characteristics, it is attractive to speculate that many biological functions and traits arose in part from the action of lncRNAs.[1,2]

The term 'non-coding RNA' strongly implies that an RNA does not code for a peptide/protein.[1] This definition, however, can be somewhat ambiguous considering that all RNA is essentially 'non-coding' until a transcript is identified. With the refinement of mass spectrometry and ribosome foot-printing techniques, as well as the improved prediction and classification of open reading frames, it is becoming clear that a significant number of long 'non-coding' RNAs are in fact translated into peptides.[3] The identification of 'coding potential' of a lncRNA does not provide any useful information on the biological relevance of the peptide. However, it does lead to the RNA being labelled as 'mis-annotated'.[3] Consequently, attempting to convince the community of a non-coding function of this now coding RNA will become significantly more difficult. This is for good reason, as a number of newly identified peptides were found to be of great biological importance.[3,4]

Translational research on lncRNAs must overcome several challenges: The large size and complex genetic structure of lncRNAs makes it difficult to modulate their expression. In addition, the low species conservation of most human lncRNAs precludes the use of rodent model systems.[5,6] It is conceivable therefore that translational research into lncRNAs that display species conservation would be more appealing since their biological importance is ultimately easier to demonstrate. This approach, however, has a higher probability of uncovering protein-coding potential. As evolution can be more rapid if no amino acid sequence has to be conserved, high conservation of an RNA bears greater probability of unidentified peptide-coding potential.

Spencer *et al.*[7] made an observation along this line: the authors searched for RNAs that emerged during the course of differentiation of human embryonic stem cells into endothelial cells. With the sequencing depth and selection criteria applied, they succeeded in identifying six long non-coding RNAs that were enriched and specific to endothelial cells. Of these, only one (linc00961) was found to be conserved between mouse and man. However, linc00961 was recently shown to code for an important peptide[8] and was thus re-annotated as SPAAR—'small regulatory polypeptide of amino acid response'.

Whereas many researchers would probably not have considered linc00961 any further, Spencer *et al.* wondered whether it also functions

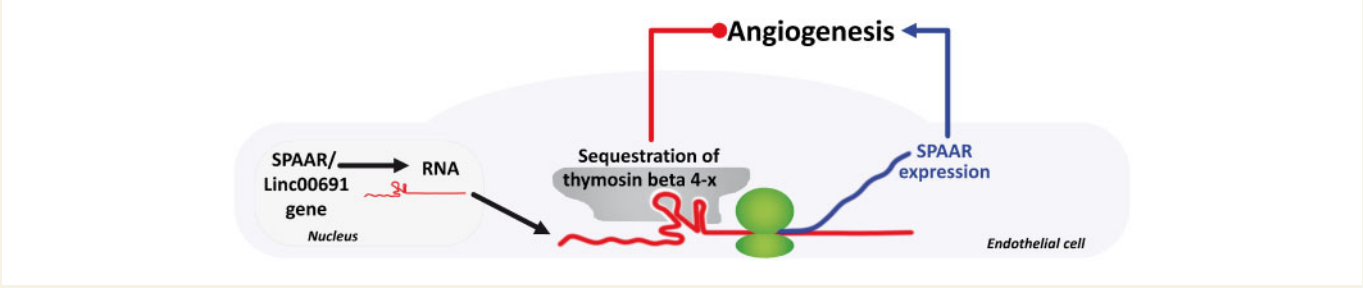

Figure 1 Model for the bi-functional RNA SPAAR/linc00961. From its coding region, the peptide SPAAR is transcribed, which promotes angiogenesis. A non-coding region of the RNA sequesters thymosin beta-4x and thereby reduces the angiogenic function.

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as a 'non-coding' RNA. To study this, they not only performed loss of function experiments on the whole gene but also selectively overexpressed the SPAAR coding sequence and a linc00961 mutant lacking the SPAAR start codon (LV-Δ ΔATG961). Collectively, these experiments demonstrated that the linc00961 locus at large promotes an endothelial angiogenic phenotype and that this effect is mediated by the SPAAR peptide. The remaining section of linc00961 (LV-Δ ΔATG961), in contrast, has an anti-angiogenic function. In subsequent mechanistic experiments, the authors identified that LV-Δ ΔATG961 interacts with the pro-angiogenic protein thymosin beta-4x [9] and that depletion of thymosin beta-4x had a similar anti-angiogenic effect as LV-Δ ΔATG961 overexpression (*Figure 1*).

The study by Spencer *et al*. is important for several reasons. It reports an interesting, conserved fine-tuning mechanism of endothelial angiogenic function, which could be exploited therapeutically. It also adds a new layer of complexity to RNA biology: within a single mRNA molecule, the non-coding portion can limit the functional importance of the gene product through an indirect mechanism. Finally, it provides an important reference to lncRNA researchers by demonstrating that coding potential does not necessarily prohibit an important non-coding function of an lncRNA.

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