Temporal and spatial post-transcriptional regulation of zebrafish tie1 mRNA by long non-coding RNA

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1 BACKGROUND AND OVERVIEW
Tyrosine kinase containing Immunoglobulin and Epidermal growth factor homology-1(Tie1), an endothelial and hematopoietic cell specific receptor tyrosine kinase1, is an important regulator of angiogenesis and critical for the maintenance of vascular integrity2. We have previously reported the identification of non-coding natural antisense transcripts (NATs) from the tie1 locus in zebrafish, mice and humans3. We have also demonstrated that two of these NATs – one in zebrafish and another in humans – are capable of binding tie1 mRNA in the cytoplasm, and down regulating tie1 mRNA levels4. However, this mechanism of down regulation is not well known. Here, we analyzed the mechanism involved in the tie1 antisense (tie1AS) RNA mediated regulation of tie1 mRNA in zebrafish embryos.

The zebrafish tie1AS long non-coding RNA that regulates tie1 mRNA is 804 bases long. The first 148 bases of the tie1AS locus in zebrafish, mice and humans are homology

Figure 2A: Schematic representation of the tie1 mRNA and the antisense IncRNA. Probes shown above (purple, green and red) were used in assays in Fig 2 & 4.

RESULTS

3 tie1AS binds Elav1 in vitro

4 tie1AS binds Elav1 in vivo

5 Elav1 binds tie1AS through AU element

6 tie1 mRNA, tie1AS IncRNA and Elav1 protein expression overlap in the head but not in trunk or tail

7 Three distinct approaches were used to characterize the functional relevance of the interaction between tie1AS and Elav1

1. Constitutively active MO approach

2. Photoactivatable MO approach

3. CRISPRi: KD of tie1AS approach

8 Merged

9 Control MO

10 SUMMARY OF RESULTS

1. tie1AS IncRNA binds Elav1 in vivo and in vitro through a 10 nt AU rich element.

2. Elav1 – tie1AS interaction takes place in zebrafish embryo head between 28 - 31 hpf.

3. Disturbing Elav1 – tie1AS interaction through 3 independent methods led to 2-fold increase (p<0.05) in tie1 mRNA levels and a decrease in tie1AS RNA levels only in the head between 28 - 31 hpf (data not shown).

4. The changing levels of RNA correlate with reduced ventricular space, smaller eye size, dilated primordial midbrain channels (PMBCs) and an increase in endothelial cells within the PMBCs.

11 Hypothesis of mechanism

tie1AS uses a 10 bases long AU rich element at the 5’end to interact with Elav1. The interaction of Elav1 with tie1AS, places Elav1 close to the poly(A) tail of tie1 mRNA where it interacts with the poly(A) tail of tie1 mRNA and recruits proteins to destabilize tie1 mRNA. A similar scenario has been reported in Elav1 orthologue, HuR, mediated destabilization and degradation of nucleophosmin mRNA.

Elav-family of proteins have been shown to interact with poly(A) sequences and AU rich element simultaneously in vitro.

References:

T.A.C. is a recipient of the AHA post-doctoral fellowship. This research has been supported in part by NIH grant HL123308 to P.P.