A novel class of small tRNA-like noncoding transcripts arising from the human NEAT1-MALAT1 region critically influences innate immunity and angiogenesis

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Background: The evolutionary conserved NEAT1-MALAT1 gene cluster encounters high interest in cardiovascular medicine and oncology. The cluster generates large primary transcripts which remain nuclear, whereas novel tRNA-like transcripts (mascRNA, menRNA) enzymatically generated from these precursors translocate to the cytosol. We previously found that NEAT1 and MALAT1 deficient mice display accelerated atherosclerosis and vascular inflammation due to immune dysfunctions.

Methods: While the previously investigated mice were deficient in the entire NEAT1 or MALAT1 locus, here we aimed to selectively disrupt only tRNA-like transcripts "menRNA" arising from NEAT1, or "mascRNA" arising from MALAT1. To none of these a biological function has been assigned so far. Both lncRNAs give rise to transcripts of vastly different size (NEAT1: 23kb MENb, 3.7kb MENe, 59nt "menRNA"; MALAT1: 8.3 kb primary, 59nt "mascRNA"), and traditional knockout methods are unable to selectively inactivate one of the small transcripts only. Through CRISPR/Cas9 editing we therefore developed human monocyte-macrophage cell lines with short deletions in the respective tRNA-encoding sequences to disrupt normal menRNA or mascRNA formation, respectively. These editing procedures do not affect transcription of the respective lncRNA parent transcripts, and also not disturb regular formation of the triple-helix structures at their 3'-ends which support stabilization of the respective lncRNAs (Fig. 1).

Results: We found the tRNA-like transcripts menRNA and mascRNA critically influence innate immunity and angiogenesis. In addition to common anomalies resulting from their selective CRISPR-Cas9 mediated deletion (Fig. 1), there are specific disturbances associated with either ∆masc or ∆men cells (Fig. 2).

Both ∆mascRNA and ∆menRNA human monocytes show profoundly altered ribosomal RNA/protein and tRNA-modifying enzyme expression, display anomalous growth/ angiogenetic factor expression, fundamentally change angiogenetic patterns in co-cultures with human endothelial cells, and have gravely disturbed innate immune responses (LPS, DNA and RNA viruses) (Fig. 1).

CRISPR-engineered ∆menRNA cells share remakable similarities with human post-MI PBMCs, suggesting the NEAT1-menRNA system may significantly contribute to post-MI residual inflammatory risk despite optimal standard therapy (Fig. 2).

Conclusions: Beyond prior work in knockout mice documenting immune function of the NEAT1-MALAT1 cluster, the current study identifies menRNA and mascRNA as important novel components of human innate immunity with relevance for angiogenetic processes. These data provide a second mechanistic link for the apparent relevance of the NEAT1-MALAT1 gene cluster in cardiovascular and malignant diseases. As prototypes of a novel class of small noncoding RNAs (distinct from miRNAs and siRNAs) they may constitute cytosolic therapeutic targets.

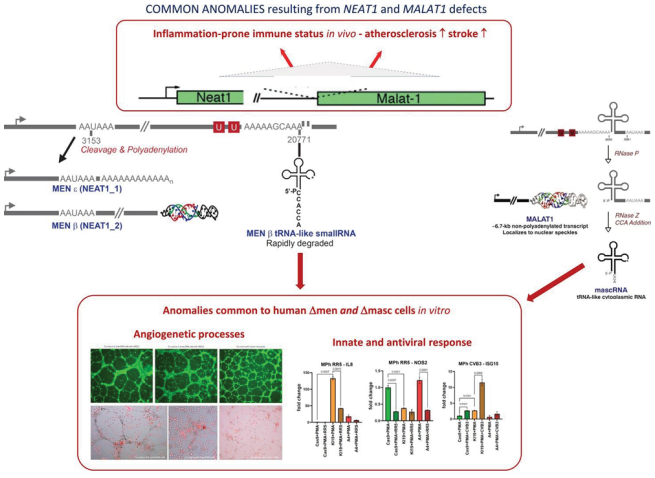

Figure 1. Common anomalies

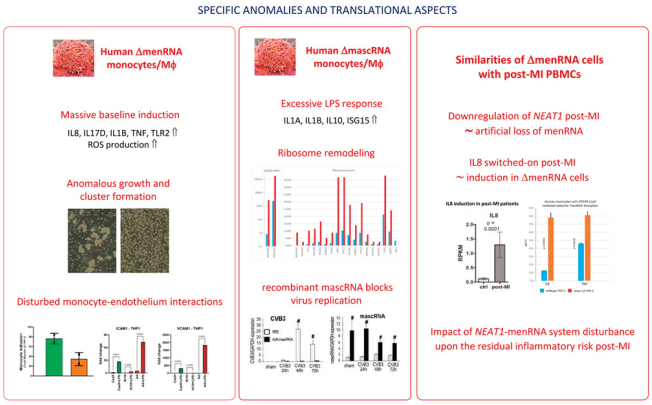

Figure 2. Specific anomalies