Experimental and population-genetic evidence for inflammation control functions of long noncoding RNAs and a novel tRNA-like transcript arising from the human NEAT1-MALAT1 genomic region

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Background: Uncontrolled inflammation is a key driver of atherosclerosis, myocardial infarction (MI), and multiple other diseases. Beyond proteins and microRNAs, long noncoding RNAs (lncRNAs) are implicated in inflammation control. We previously reported suppression of lncRNA NEAT1 in circulating immune cells of post-MI patients. In mice lacking lncRNAs NEAT1 or MALAT1 we observed major immune disturbances affecting monocyte-macrophage and T cell differentiation and rendering the immune system unstable and highly vulnerable to immune stress. Here, we report functions of a novel tRNA-type transcript arising from the NEAT1-MALAT1 gene cluster, and on genetic heterogeneity of this region in the human population.

Methods and results: While previously investigated mice were deficient in the entire NEAT1 or MALAT1 locus, we here aimed to selectively disrupted only the novel 59-nt tRNA-like transcript "menRNA" with hitherto unknown functions. Through CRISPR/Cas9 editing we developed 4 human THP-1 monocyte-macrophage cell line clones with deletions of different extension all of which prevented, however, normal transcript folding and formation of "menRNA". Transcriptome mapping of all clones by RNA-sequencing identified dysregulation of innate immunity-related genes (IFI16, IFITM3, IRAK3, IRF2BP2, IRF3), chemokine and interleukin receptors (CCR10, IL11RA, IL12RB2, IL23A), cell surface receptors (CD37, CD40LG, CD72, FOCAD, ITGA6, MAEA, THY1), macrophage function-associated genes (ELANE, GRN, MIF, MMP25, MST1P2, PRTN3), tRNA-processing transcripts (GARS, QRSL1P3, QTRT1P1, THG1L, VARS), and small nucleolar RNAs (SNORA26.62.64, SNORD65.112). These data and functional assays indicate functions of NEAT1-derived "menRNA" distinct from those previously described for MALAT1-derived mascRNA.

As multiple data suggest inflammation control functions of the NEAT1-MALAT1 region, we investigated the extent of genetic variability of this region in humans. In cohorts from the SHIP study coordinated by the Institute for Community Medicine Greifswald, screening of this region for sequence variants and possible phenotype associations was conducted the results of which are given in Figure 1. Consistent with prior findings, a MALAT1 SNP with very low minor allele frequency (MAF=0.01) was associated (p=0.0062) with systemic low level inflammation (CRP >3.0 mg/L). Unexpected was the association (p<0.01) of eight SNPs (low MAF=0.09 for all) with BMI >35 kg/m^2 and LDL >164 mg/dl.

Conclusions: First, selective disruption of menRNA formation in human monocyte-macrophages provides evidence that this novel type of noncoding RNA has immunoregulatory functions. Second, the phenotype associations of SNPs within the NEAT1-MALAT1 gene cluster warrant further in-depth investigation of the molecular basis of these associations, and of their allele frequencies in cardiovascular disease patient cohorts.

The first three and the last authors contributed equally to this work.

Genetic heterogeneity of the human *NEAT1-MALAT1* genomic region

SNP associated with systemic low level inflammation CRP >3.0 mg/L

rsID	CHR	POS_GRCh38	REF	ALT	MAF	AA/AB/BB	N	beta	se	p-value	gene
rs113637620	11	65.495.816	T	C	0,0113	7323/168/1	7494	0,5087	0,1859	0,0062	*MALAT1*

SNPs associated with class II obesity BMI >35 kg/m2 and LDL >164 mg/dl

rsID	CHR	POS_GRCh38	REF	ALT	MAF	AA/AB/BB	N	beta	se	p-value	gene
11_65185874_IN_DEL	11	65.418.403	GA	G	0,0903	6519/1302/61	7883	-0,179	0,0638	0,0050	*NEAT1*
rs508286	11	65.440.647	A	G	0,0941	6463/1355/65	7883	-0,172	0,0615	0,0052	*NEAT1*
rs580933	11	65.429.413	C	G	0,0912	62/1315/6506	7883	-0,1743	0,0625	0,0053	*NEAT1*
rs550015	11	65.418.877	A	G	0,0922	63/1329/6491	7883	-0,1757	0,0634	0,0056	*NEAT1*
rs481335	11	65.439.998	T	C	0,0931	6479/1340/64	7883	-0,169	0,0617	0,0061	*NEAT1*
rs673753	11	65.438.546	C	T	0,0931	6479/1340/64	7883	-0,1686	0,0616	0,0062	*NEAT1*
rs475967	11	65.439.446	A	G	0,0947	6454/1362/66	7883	-0,1661	0,0616	0,0070	*NEAT1*
rs550894	11	65.444.469	C	A	0,0910	6510/1313/61	7883	-0,1681	0,0629	0,0075	*NEAT1*

Figure 1