Dr. Muredach Reilly

Most human complex trait genetic signals lie in intergenic regions, implicating regulatory elements, including long intergenic non-coding RNAs (lincRNAs). These are typically not conserved in mouse and often cell-specific, raising challenges to mechanistic study but also providing a major opportunity for advances in human health and disease. Here, we embrace this challenge in interrogation of three non-conserved lincRNAs that we prioritized from deep RNAseq and preliminary functional studies in human macrophages. Macrophage activation to diverse functional states plays a central role in cardiometabolic diseases (CMD). We hypothesize that human lincRNAs modulate macrophage inflammatory and metabolic functions that impact complex CMD. To date regulatory effects of a few lincRNAs on macrophage biology have been reported, but the vast majority of human macrophage lincRNAs has yet to be studied. Through RNAseq of primary human monocyte-derived macrophages (HMDM), we identified 2,776 multi-exon lincRNAs of which >80% are not annotated in mouse. Based on (i) modulation during macrophage activation, (ii) overlap with genetic signals for CMD, (iii) macrophage enrichment and abundance, (iv) ChIP-seq binding of CEBPa and PU.1 proteins, and (v) similar expression pattern in human induced pluripotent stem cells (hiPSC) derived macrophages (IPSDM), we selected a set of 22 lincRNAs for validation and preliminary translation. From these, we focus here on three lincRNAs, not expressed in mouse macrophages, that have preliminary evidence of human macrophage functions. RP11-10J5.1 is markedly induced during inflammatory M1 activation, has increased expression in human atherosclerosis and attenuates iNOS expression and apoptosis (Aim 1). In contrast, RP11-184M15.1 is highly induced during M2 macrophage activation and modulates M2 phenotype (Aim 2). Finally, RP11-472N13.3 associates with central human obesity and attenuates macrophage IFNg signaling (Aim 3). Because large-scale genetic manipulation in primary human macrophages is limiting, we propose CRISPR/Cas9 gene-editing in IPSDM to pursue functional genomic interrogations of these CMD-relevant lincRNAs. Key findings will be corroborated by knockdown (KD) and overexpression (OE) of lincRNAs in HMDM. Localization, miR binding and protein interactions will be defined by RNA fluorescence in situ hybridization, MS2-tagged RNA affinity purification, confirmed with QPCR, and pull-down with biotinylated lincRNA probes coupled to mass-spectrometry and with RNA immunoprecipitation. KD and OE of interacting partners will test lincRNAs functional roles via specific miR or proteins target(s). Human disease relevance will be determined by localizing lincRNAs to macrophages in coronary atherosclerosis and visceral adipose and through interrogation of lincRNA cis-regulatory variants, identified via macrophage allele specific expression, in CMD genetic datasets.