Quaking Post-Transcriptionally Promotes Differentiation of Monocytes Into Pro-Atherogenic Macrophages by Controling Pre-mRNA Splicing and Gene Expression

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Aim: Atherosclerosis is accelerated by excessive monocyte recruitment, influx and differentiation into pro-inflammatory macrophages. While the genome-wide mRNA expression profiles of human monocytes and pro-inflammatory macrophages are well-established, their transcriptomes are ultimately defined by factors, such as RNA-binding proteins, that modulate pre-mRNA splicing patterns and mRNA transcript abundance. This prompted us to investigate the role of the RNA-binding protein Quaking in regulating global changes in pre-mRNA splicing and mature mRNA expression as human monocytes acquire the pro-inflammatory macrophage identity.
Methods: We employed RNA-sequencing and splicing-sensitive microarrays to determine genome-wide changes in pre-mRNA splicing and mRNA expression upon conversion of human monocytes into pro-inflammatory macrophages, including those derived from a unique Quaking haploinsufficient patient.
Results: Using laser-capture micro-dissection and immunohistochemistry, we discovered that expression levels of Quaking mRNA and protein are low in monocytes of early human atherosclerotic lesions, but abundant in macrophages of advanced plaques. Depletion of Quaking protein using both siRNA and GapmeR technology significantly impaired monocyte adhesion and migration; delayed differentiation into pro-inflammatory macrophages while maintaining the capacity to adopt the anti-inflammatory phenotype; and diminished foam cell formation in vitro and in vivo. RNA-sequencing and microarray analysis of human monocyte and macrophage transcriptomes revealed striking changes in Quaking-dependent pre-mRNA splicing and mRNA transcript levels, with gene ontology analyses identifying an enrichment in transcripts involved in cellular migration and lipid metabolism. Furthermore, these studies uncovered common as well as novel alternatively spliced transcripts with unknown biological functions in monocytes and macrophages.
Conclusions: Our studies illustrate a central role for Quaking in post-transcriptionally guiding pro-inflammatory macrophage identity and function.

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