Abstract: The RNA editor ADAR2 links inflammation to functional recovery from ischemic diseases

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Topic(s): Basic Science - Vascular Biology and Physiology: Genetics, Epigenetics, ncRNA

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Background/Hypothesis: The RNA editor ADAR2 catalyzes the deamination of adenosine to inosine, a process that is called RNA editing, however, its role in endothelial cell (EC) biology and particularly in cardiovascular disease remains unknown.

Methods/Results: RNAi-based silencing of ADAR2 (siADAR2; 85±5%) in primary ECs followed by RNA sequencing and a GO analysis revealed that ADAR2 may regulate the EC pro-inflammatory response through the control of gp130 mRNA, a receptor subunit of IL-6. WB and qRT-PCR validated that siADAR2 decreased gp130 expression by >50% in human and murine ECs, while overexpression of ADAR2 conferred the opposite. Accordingly, siADAR2 reduced IL-6 trans-signalling, evidenced by 75% decreased STAT3 phosphorylation, expression of the downstream adhesion genes E-selectin and vascular cell adhesion molecule-1, and leukocyte adhesion onto ECs in vitro. Intravital microscopy showed that after administration of IL-6, both rolling and adhesion of leukocytes over the inflamed vascular wall were impaired in Adar2-/-tg by >50%. Likewise, leukocyte transmigration towards IL-6 inflamed tissues (sterile peritonitis) was suppressed in Adar2-/-tg mice by >50%. Inducible endothelial restricted ADAR2 deficient mice displayed a similarly reduced transmigration of leukocytes in inflamed tissues. Moreover, ADAR2 controlled IL-6-triggered EC pro-angiogenic effects in an ex vivo aortic ring assay, while no effect was observed, in the absence of IL-6 stimulus, in postnatal retinal angiogenesis in Adar2-/-tg mice. Mechanically, small RNA high-throughput sequencing revealed a profound upregulation of two specific miRNAs, miR-199a-5p and miR-335-3p, after siADAR2 which both target gp130 mRNA in human and murine ECs. Simultaneous treatment of ECs with siADAR2 and miRNA-inhibitors restored gp130 levels. RNA editing studies of primary miR-199a1/2 and miR-335 identified several edited sites close to Drosha cleavage sites. RNA-immunoprecipitation studies showed increased binding of Drosha to these pri-miRs upon siADAR2 by >5-fold, while co-silencing of ADAR2 and Drosha rescued the gp130 levels. ADAR2 is 2-fold induced by hypoxia in ECs and ischemic tissues. Accordingly, Adar2-/-tg mice displayed a 40% decreased blood-flow recovery, 3 weeks after hindlimb ischemia. Histology revealed 53% reduced neovascularization and 80% decreased leukocyte infiltration within the ischemic regions in Adar2-/-tg. Moreover, cardiac recovery after myocardial infarction was impaired in Adar2-/-tg mice (34±3% vs 43±3% control) 4 weeks after myocardial infarction, assessed by MRI. Importantly, ADAR2-gp130 axis was associated with cardiac recovery after mechanical-assisted unloading in patients with end-stage heart failure.

Conclusion: Vascular endothelial cell ADAR2 edits the stem-loops of specific primary miRNAs that target the IL-6 receptor subunit gp130 mRNA, thus, controlling gp130-dependent IL-6 signalling and functional recovery from ischemic diseases.
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