The RNA-binding Protein ADAR2 Controls Interleukin-6-induced Endothelial Cell Pro-inflammatory Response by Regulating MicroRNA Biogenesis

Aikaterini Gatsiou, Federica F Lunella, Carolin Amrhein, J.W. Goethe Univ Frankfurt, Frankfurt am Main, Germany; Stefan Guenther, Claudia Garcia Gonzalez, Andre Schneider, Thomas Braun, Max-Planck Inst, Bad Nauheim, Germany; Andreas M Zeiher, Stefanie Dimmeler, **Konstantinos Stellos**, J.W. Goethe Univ Frankfurt, Frankfurt am Main, Germany

Background: RNA binding proteins (RBPs) are key players in posttranscriptional regulation of mRNAs. Adenosine deaminase acting on RNA-2 (ADAR2) enzyme binds to double-stranded RNAs and controls brain development. The role of ADAR2 in endothelial cell function has not been described so far.

Methods and Results: ADAR2 is expressed in human and murine endothelial cells and is 2-fold induced by hypoxia *or* hind limb ischemia in mice ($P<0.05$ for all). ADAR2 deficiency resulted in 73±12% impairment of leukocyte infiltration, in 53±4% reduced neovascularization, and a 40±6% decreased blood-flow recovery of ischemic muscle tissues in a hindlimb ischemia mouse model ($P<0.001$ for all). Mechanistically, ADAR2 knockdown causes a 2-fold downregulation of 522 transcripts, whereas 113 transcripts are found to be upregulated in RNA-seq experiments. Among the endothelial cell enriched, highly ADAR2-regulated transcripts was interleukin-6 signal transducer (IL6ST also known as glycoprotein-130, gp130), the receptor of interleukin-6 (IL-6). Silencing of ADAR2 resulted in a downregulation of gp130 mRNA and protein expression in endothelial cells by 65±5% and 50±5%, respectively ($P<0.001$ for both). Similarly, the expression of gp130 mRNA was decreased by 50±25% ($P<0.05$) in murine lung endothelial cells, which derived from transgenic ADAR2-null mice. Silencing of ADAR2 reduced by at least 2-fold the IL-6-mediated STAT3 phosphorylation, the mRNA expression of the IL-6-induced downstream genes MCP-1, VCAM-1 and E-selectin, and the IL-6-induced platelet and leukocyte adhesion on endothelial cells and endothelial cell network formation ($P<0.05$ for all). Of interest, ADAR2 regulated gp130 mRNA stability. Co-silencing of ADAR2 and Drosha completely restored the ADAR2-regulated gp130 mRNA expression indicating that ADAR2 regulates the maturation of specific microRNAs (miRs). Indeed, ADAR2 regulates by at least 2-fold the expression of 20 microRNAs, including of miR-579 and miR-320e, which are predicted to bind to the 3'-untranslated region of IL6ST mRNA.

Conclusions: This study shows for the first time that ADAR2 controls miR biogenesis and thus IL-6/STAT3 signalling in endothelial cells.

Disclosures:
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