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A-to-I editing of microRNA-487b alters target gene selection and promotes neovascularization after ischemia

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Introduction: microRNA miR487b has been shown to play an important role in cardiovascular pathology, despite having relatively few putative targets. Adenosine-to-inosine (A-to-I) editing of microRNAs by ADARs can cause a shift in target-site selection.

Purpose: To investigate whether miR487b is subject to A-to-I editing during ischemia and how this affects the pool of target genes of miR487b.

Methods and Results: cDNA was prepared from muscle tissue of C57BL/6 mice subjected to hind limb ischemia. Using Sanger sequencing and sequence specific endonuclease digestion, we identified and confirmed A-to-I editing in the seed sequence of miR487b. Subsequent quantification revealed post-ischemic regulation of miR487b editing. In the ischemic gastrocnemius, primary-miR487b editing increased from 6.7±0.4% before to 11.7±1.6% (P=0.02) one day after ischemia. Edited primary-miR487b is processed into a mature microRNA, miR487b-ED, which is also upregulated following ischemia.

Primary human vascular cells were used to show that miR487b editing is conserved in human miR487b and that its precursor associates with both ADAR1 and -2. With specific in vitro siRNA knockdowns, we found that both ADAR1 and -2 can edit miR487b.

The 3’UTR of IRS1 contains a binding site for both miR487b and miR487b-ED, whereas BMP1 contains six separate binding sites for miR487b-ED. Luciferase reporter gene assays using both endogenous and mutated IRS1 and BMP1 binding sites demonstrated that editing of miR487b’s seed sequence does result in a complete shift in target site selection. In an identical context, binding of miR487b-ED induced stronger luciferase silencing than miR487b binding. Furthermore, a subset of putative target genes were validated to confirm specific regulation by either miR487b or miR487b-ED in gain-of-function experiments using primary human vascular cells.

In contrast to wildtype miR487b’s target pool, miR487b-ED’s novel targetome is enriched for angiogenesis-associated pathways. Indeed, microRNA overexpression experiments showed that miR487b-ED causes a 2-fold increase in neovascularization compared to miR487b in an ex vivo aortic ring sprouting assay and also increases wound healing in scratch assays on both human vascular endothelial cells and fibroblasts. Finally, whole exome microarray was performed to measure average targetome expression after hind limb ischemia. We found that only miR487b-ED’s targetome is actively repressed during active neovascularization after ischemia.

Conclusions: MiR487b is edited in its seed-sequence in both mice and humans. Editing of miR487b is upregulated after ischemia and causes a shift in target gene selection, resulting in a novel, pro-angiogenic microRNA, miR487b-ED, with a unique targetome. Our results demonstrate that microRNA-editing plays an...
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active role in neovascularization.