Abstract

Blood vessel formation and maturation is induced by the activation of perivascular cells (PVC) and endothelial cells. Factors triggering this activation are not fully known, but recent studies indicate that microRNAs (miRNAs) can regulate the activation of endothelial cells and their interaction with PVC to form new blood vessels. However, the relevance of miRNAs in PVC remains unclear and important miRNAs involved in PVC activation are not yet defined. Therefore, the aim of this thesis was to identify PVC-specific miRNAs, to clarify their role in PVC-induced angiogenic processes and to elucidate the underlying molecular mechanisms of miRNA action in PVC. Using an in vitro angiogenesis coculture system and miRNA transcriptome technology miR-126-3p, miR-146a and miR-155-5p could be identified among 700 tested miRNAs to be uniquely upregulated in activated PVC. The miR-126-3p showed the strongest, hundred fold increase in expression and represented the most likely miRNA to modulate the activation of PVC. The increase of miR-126-3p in PVC promotes the formation of long directional protrusions and cell-cell contacts on a complex basement membrane-like substrate, whereas no changes could be observed on individual vascular basement membrane components, like laminin 511, collagen IV and nidogen 1. Moreover, intercellular interactions of PVC with HUVEC were increased in a two-dimensional coculture system on a complex basement membrane-like substrate. Subsequent luciferase binding assays and immunoblot analyses identified Spred1, Plk2 and Irs1 as direct target genes of miR-126-3p in PVC. The siRNA-mediated knockdown of the miR-126-3p target genes Spred1 or Plk2 in PVC caused increased cell-cell and cell- matrix interactions, mimicking the cellular phenotype of increased miR-126-3p levels in PVC. Furthermore, immunoblot analyses indicated a direct link between reduced Spred1 and Plk2 levels and activated ERK1/2 and AKT signaling. In order to test the relevance of miR-126-3p for in vivo angiogenesis the expression of miR-126-3p and its in vivo function were studied. In expression analyses increased levels of miR-126-3p could be detected in isolated primary perivascular cells at embryonic stage 11.5 and 13.5, pointing to a role of miR-126-3p in PVC during angiogenesis in vivo. Gain of expression experiments in chicken embryos demonstrated that miR-126-3p modulates the interaction of perivascular progenitor cells of the neural crest with cells of the cranial vasculature in ovo, most likely to stabilize the developing vascular structures. Taken together, the results in this thesis demonstrated for the first time a relevance of miR-126-3p for PVC activation. miR-126-3p is upregulated in PVC upon interaction with HUVEC to inhibit the expression of Spred1 and Plk2 target genes and thereby activates ERK1/2 and AKT signaling pathways in PVC. This activation promotes cell-cell contacts and their interaction on complex basement membrane substrates. Hence, the increase of miR-126-3p in PVC seems to be important to stabilize newly formed vascular structures during angiogenesis, whereas reduced levels of miR-126- 3p promote the loss of intercellular interactions and thereby vessel destabilization.