

Bart Krist - The role of microRNA-378a in skeletal muscle differentiation, angiogenesis and hind limb ischemia in mice

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Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels including peripheral arterial disease (PAD) affecting arms and legs. PAD is a common type of CVD, especially in elderly patients and is the third leading cause of CVD-related morbidity after coronary artery disease and stroke. The symptoms of PAD include intermittent claudication, skin ulcers and tissue necrosis and in the most severe cases may manifest as critical limb ischemia (CLI). Since the effectiveness of traditional treatment, particularly for CLI, is poor, there is still a need for more efficient therapeutic approaches including the enhancement of angiogenesis in the ischemic tissue.

MicroRNAs are non-coding RNAs that play important roles in the regulation of gene expression. Previous studies, including research from our department found that miR-378a effects tumor angiogenesis. Previous studies from other research groups also indicated that miR-378a, which is highly expressed in skeletal muscle, may influence the differentiation of myoblasts. Therefore, we hypothesize that miR-378a might be of significance for ischemic muscle regeneration and the development of new therapeutic strategies.

The objective of this study was to investigate the role of miR-378a in differentiation of muscle progenitor cells, angiogenic potential of myoblasts and differentiated myotubes and in regeneration after hind limb ischemia in mice.

We showed that the expression of both miR-378a strands, miR-378a-3p and miR-378a-5p, was high *in vitro* in murine myoblast cell line, C2C12, and *in vivo*, in murine skeletal muscles. We did not observe any influence of hypoxia on miR-378a expression in myoblasts. On the other hand, the level of miR-378a increased during myoblast differentiation, both under normoxia and hypoxia. Silencing of miR-378a neither under normoxia nor under hypoxia affected the differentiation of C2C12 since we did not observe changes in the level of myogenic markers such as myosin, myogenin and skeletal myosin. Thus, although miR-378a is upregulated during muscle differentiation, it seems to be dispensable for this process.

On the other hand, we showed that myoblasts lacking miR-378a show impaired angiogenic potential towards aortic endothelial cells on Matrigel and in fibrin gel. Such proangiogenic effect of miR-378a was also visible in case of myotubes. Accordingly, we observed a tendency to decreased sprouting from cross sections of aortas isolated from miR-378a-deficient mice and placed into Matrigel (vs. wild type counterparts). Nonetheless, we found no effect of miR-378a on the sprouting potential of choroid endothelium *ex vivo*. Those results suggest that the proangiogenic effect of miR-378a may be specific for aortic endothelial cells.

Taking into consideration proangiogenic action of miR-378a in tumors and shown in this study proangiogenic role in muscle cells, we aimed to assess the effect of miR-378a on the reperfusion and

subsequent regeneration of the ischemic muscles in a murine model of hind limb ischemia (HLI). Surprisingly, the expression of miR-378a as well as its host gene, *ppargc1b*, decreased upon induction of ischemia in wild type mice. However, neither local nor systemic miR-378a overexpression using AAV vectors affect the reperfusion of the ischemic limb. Also no difference in the blood flow recovery was observed between wild type mice injected intramuscularly with either anti-miR-378a-3p, anti-miR-378a-5p or scrambled control. In order to verify that miR-378a is dispensable for skeletal muscle regeneration, we examined the response of miR-378a-knockout mice to HLI. However, at day 7 and day 21 after the surgery such animals revealed similar reperfusion rate as wild type counterparts. Moreover, no significant effect of the lack of miR-378a was observed in case of the number of arterioles or mRNA level of VEGF and CD31 in the ischemic muscles. Likewise, miR-378a did not affect the percentage of regenerative muscle fibers.

Interestingly, we revealed that miR-378a affect inflammatory cells subpopulations in the ischemic muscles. We observed slightly decreased percentage of overall CD45-positive cells, macrophages and T cells in the ischemic muscles of wild type mice injected either intramuscularly or intravenously with AAV-vectors encoding miR-378a (vs. GFP group). The number of dendritic cells was lower only when miR-378a was enhanced systemically. Importantly, we detected a significant increase in CD45-positive cells and macrophages in ischemic muscles of miR-378a-knockout mice at day 7 after HLI in comparison to wild type mice. This observation correlates with our findings obtained using miR-378a overexpressing mice. miR-378a-dependent differences did not significantly contribute to the restoration of blood flow in the ischemic muscles, however, they may suggest a role for miR-378a in the regulation of inflammation and leukocyte infiltration to the ischemic tissue.

Overall, we revealed a proangiogenic role of miR-378a in muscle cells, both myoblasts and myotubes, towards endothelial cells, whereas undermined its role in muscle differentiation. Moreover, we showed that miR-378a is dispensable for skeletal muscle regeneration in response to HLI, since it did not affect neither the percentage of regenerative muscle fibers nor reperfusion rate. On the other hand, an anti-inflammatory role for miR-378a in the ischemic muscle was suggested.