Inhibition and Promotion of Angio- and Arteriogenesis

Regulatory function of miRNA-100 via mTOR

Technology

RNA-interference (RNAi) is a RNA dependent gene silencing process. MicroRNAs (miRNAs) are short, non-coding RNAs which regulate protein expression by imperfect mRNA base-pairing, hence inhibition of the target gene. These miRNA are found in all eukaryotic cells and it has become clear that changes in expression levels of several miRNAs are linked to various cancer types, cardiovascular diseases and inflammatory processes. For the first time we recently showed that miRNA-100 has a regulatory function in angiogenesis and arteriogenesis, also we showed that this anti-proliferative function of miRNA-100 is based on direct inhibition of the kinase mTOR (FRAP1) that is important for cellular growth, proliferation and survival.

Innovation

- Overexpression of miRNA-100 leads to decreased levels of mTOR and reduced blood vessel growth
- Antagonistic inhibition of miRNA-100 causes stimulated endothelial proliferation, increased angiogenesis and increased levels of mTOR
- Deletion of of miRNA-100 binding site for mTOR reversed inhibition of proliferation

Application

- Medical application of miRNA-100 mimicking compounds against tumor progression, as endogenous miRNA-100 is decreased in ovarial and endometrial cancer, metastatic prostate cancer and hepatoblastoma.
- Application of miRNA-100 inhibiting compounds (“Antagomirs”) to stimulate collateral artery growth in cardiovascular disease.
- New approach to regulate angiogenesis and arteriogenesis in mTOR related diseases
- Direct impact on cellular growth and proliferation
- As miRNA-based therapeutics imitates the natural microRNA, they have the potential of fewer side effects compared to classical pharmaceuticals (e.g. rapamycin).

Market Potential

- Cardiovascular disease and Cancer are the first and second most frequent causes of death in industrial nations and angiogenesis plays a crucial role in both pathologies.
- Increasing relevance for miRNAs in therapy and diagnosis
- Presumably relevant for treatment of mTOR-related diseases, like transplant rejection, renal failure or in-stent restenosis.

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MicroRNA-100 regulates neovascularization by suppression of mammalian target of rapamycin in endothelial and vascular smooth muscle cells

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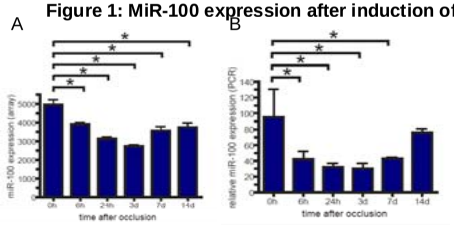
Introduction

The adaptive growth of blood vessels is an important protective mechanism in cardiovascular disease. However, the underlying regulatory mechanisms of this process are only partly understood. Recently, small endogenous RNAs (microRNAs) were found to play an important role in embryonic and postnatal vascular development. Here, we used microRNA-transcriptome analysis following induction of hindlimb ischemia in mice to screen for microRNAs involved in adaptive blood vessel growth following arterial occlusion.

Methods and Results

Using microRNA-arrays, we explored the microRNA-expression profile during adaptive neovascularization. We show that the microRNA-100 is significantly downregulated after induction of hindlimb ischemia in mice. It is expressed in endothelial and vascular smooth muscle cells and has inhibitory effects on neovascularization in vitro and in vivo involving the regulation of its target gene mTOR.

Figure 1: MiR-100 expression after induction of hindlimb ischemia



A: MiR-100 expression following femoral artery ligation as quantified by microarray analysis

B: MiR-100 expression as quantified by stemloop quantitative PCR.

C: In situ hybridization for miR-100 expression combined with immuno-fluorescent staining for endothelial cells and nuclei shows miR-100 expression in CD31 positive blood vessels from the mouse hindlimb.

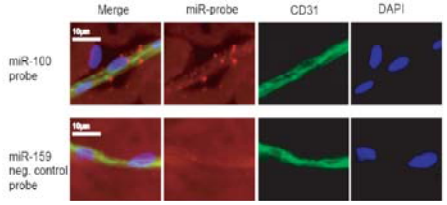
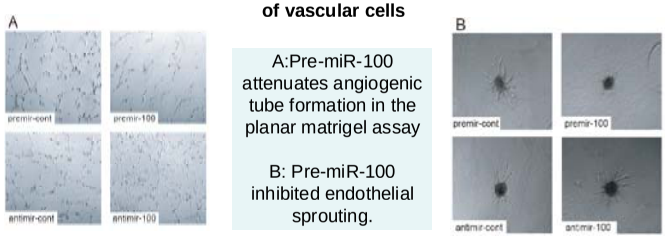


Figure 2: MiR-100 regulates angiogenic sprouting and proliferation of vascular cells



A:Pre-miR-100 attenuates angiogenic tube formation in the planar matrigel assay

B: Pre-miR-100 inhibited endothelial sprouting.

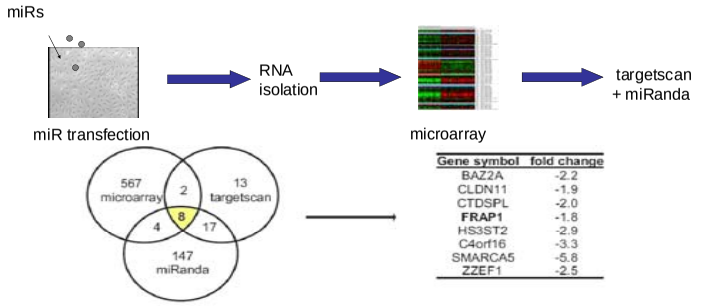
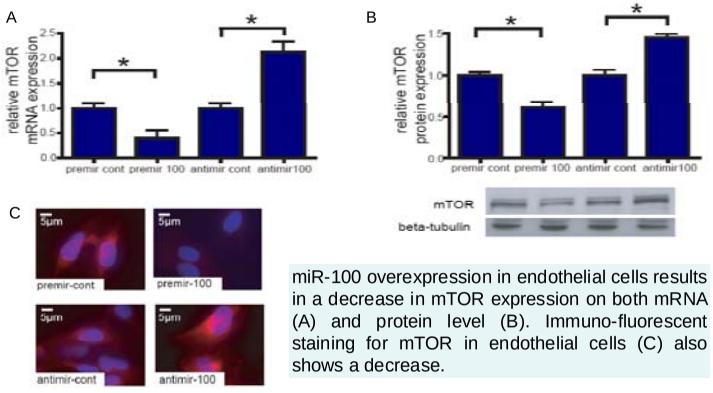


Figure 3: Identificiation of miR-100 target genes Among the 581 significantly downregulated genes with a more than 1.5 fold decrease in expression following pre-miR-100 overexpression were 8 mRNAs with a miR-100 target site predicted. We chose mammalian target of rapamycin because of its known pro-angiogenic properties.

Figure 4: mTOR is a miR-100 target in endothelial cells



miR-100 overexpression in endothelial cells results in a decrease in mTOR expression on both mRNA (A) and protein level (B). Immuno-fluorescent staining for mTOR in endothelial cells (C) also shows a decrease.

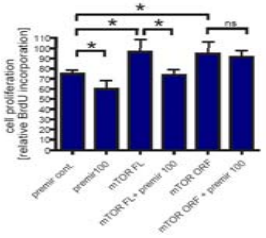
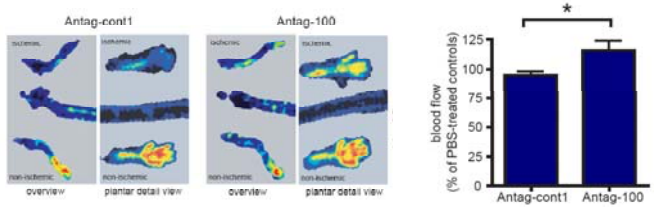


Figure 5: mTOR, being a miR-100 target, confers its effect on cell proliferation
Expression of ORF-mTOR, lacking the 3'-UTR miR-100 binding site, but not FL-mTOR, including the miR-100 binding site, rescues the miR-100 effect on proliferation completely.

Data are mean ± SEM and represent mean values from at least three experiments. *= p<0.05 vs. the corresponding control. ORF=open reading frame, FL=full length.

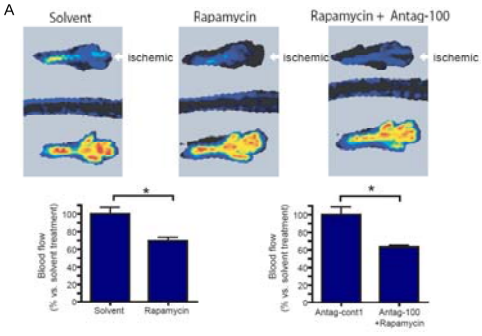
MiR-100 inhibition by specific antagomirs *in vivo* stimulated angiogenesis and resulted in functional improvement of perfusion after femoral artery occlusion in mice. In contrast, treatment with the mTOR-inhibitor rapamycin had the opposite effect.

Figure 6: miR-100 inhibition by antagomir treatment stimulates perfusion restoration following hindlimb ischemia in vivo.



Laser Doppler perfusion measurements revealed a significant stimulation of blood flow recovery at day 7 following femoral artery ligation by antagomir-100 treatment compared to a control antagomir.

Figure 7: mTOR inhibition attenuates perfusion restoration after femoral artery ligation and abrogates the stimulatory effect of miR-100 inhibition.



A: Rapamycin treatment resulted in a significant reduction of laser Doppler flow. Also, it abrogated the stimulatory effect of antagomir-100 injection, showing that the therapeutic effect of miR-100 inhibition is dependent on intact signalling of its target molecule mTOR.

B: Real-time PCR-based analysis of isolated endothelial cells from ischemic hindlimbs revealed that mTOR expression is inversely correlated with miR-100 expression levels, upregulated in response to hindlimb ischemia and further increased after miR-100 inhibition in ischemic hindlimbs.

Conclusion

Our data demonstrates that miR-100 has an anti-angiogenic function and represses mTOR-signalling in endothelial and vascular smooth muscle cells. Inhibition of miR-100 could be a novel approach for the modulation of blood vessel growth and other mTOR-dependent processes.