AngiomiR-126 expression and secretion from circulating CD34\(^+\) and CD14\(^+\) PBMCs: role for proangiogenic effects and alterations in type 2 diabetics

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Angiogenesis

- AngiomiR-126 expression in PBMCs of type 2 diabetic patients and healthy controls

- Differences between CD34+/CD14 +, CD34+/CD14 -, CD34-/CD14 +, and CD34-/CD14 - PBMC subsets

- miR-126 levels in supernatant, microparticles and exosomes

- PBMCs are suggested to raise angiogenesis in ischemia.

- Diabetic patients have a reduced cardiovascular repair function and an impaired neovascularization.
• CD34⁺ - hematopoietic progenitor cells, tumor cells
• CD14⁺-monocytes, macrophages

• microRNAs (miRs) are non-coding RNA molecules, which regulate gene expression

• AngiomiRs are microRNAs regulating angiogenesis
Methods in vitro

- Positive and negative selection of CD34/CD14 cells
- MicroRNA RT PCR array
- Anti-miR-126 and miR-mimic-126 transfection
- In vitro tube formation assay (matrigel plugs, co-culture with Human aortic endothelial cells)
Methods in vivo

- Matrigel basement membrane matrix with
  - miR-mimic-126,
  - anti-miR-126,
  - scrambled RNA,
  - supernatant,
  - microvesicles and
  - exosomes
  was injected subcutaneousley in mice along the abdominal midline

- Male NRMI nu/v mice were used for transplantation of human PBMC subpopulation

- Diabetes was induced in C57BL/6 mice with streptozotocin
Positive selection

1. Steps 1–4: Mix excess primary antibody with magnetic secondary antibody.

2. Steps 5–9: Incubate and remove excess primary antibody.


Negative selection

4. Steps 11–14: Incubate and wash to remove contamination.

5. Step 15: Target is enriched.

6. Steps 16 and 17: Perform amplification directly on the bead substrate, resulting in target genomic DNA ready for high-throughput sequencing.

Generating whole bacterial genome sequences of low-abundance species from complex samples with IMS-MDA; Nature Protocols 8, 2404–2412 (2013) doi:10.1038/nprot.2013.147 Published online 07 November 2013
Results

MicroRNA expression

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Antiangiogenic miR

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Transfection

**Before Pulse**
- Cell membrane

**During E-field**
- Introduce genes/drugs
- Electric field induces a voltage across cell membrane

**After Pulse**
- Cell "heals" with gene/drug inside
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Microvesicles and Exosomes

• Supernatant:
  – Centrifugation of PBMCs in cell culture for 1000g for 10min

• Microvesicles:
  – Pellet of centrifugated supernatant (16 000g for 60min)

• Exosomes:
  – Pellet of centrifugated microvesicle-supernatant (120 000g for 60min)

• 220 000g-pellet:
  – Pellet of centrifugated exosome-supernatant (220 000g for 60min)
Effects of High Glucose/Diabetes

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Discussion

• Higher AngiomiR-126 expression and secretion in microvesicles and exosomes in CD34+ cells

• Modulation of AngiomiR-126 expression has a critical effect on proangiogenic capacity

• Reduced AngiomiR-126 expression in patients with Diabetes
• After myocardial infarction in AngiomiR-126 knock out mice, decreased vascular growth in the border zone

• Proangiogenic AngiomiR-126 is enriched in microvesicles and exosomes, which leads to increased tube formation capacity

• MiR-100 expression inhibits proliferation for endothelial cells and tube formation

• MiR-10b promotes endothelial cell migration and tube formation
Thank you for your attention!