Endothelial progenitor cells and vasculogenesis in diabetes

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Endothelial Progenitor Cells

- Re-endothelialization and Neovascularization

Endothelial Progenitor Cells

Endothelial progenitor cells are a bone marrow–derived cell population that circulate and participate in both vasculogenesis and vascular homeostasis.

Circulating Endothelial Progenitor Cells, Vascular Function, and Cardiovascular Risk


CONCLUSIONS

In healthy men, levels of endothelial progenitor cells may be a surrogate biologic marker for vascular function and cumulative cardiovascular risk. These findings suggest that endothelial injury in the absence of sufficient circulating progenitor cells may affect the progression of cardiovascular disease.
Circulating Endothelial Progenitor Cells and Cardiovascular Outcomes

Nikos Werner, M.D., Sonja Kosiol, M.D., Tobias Schiegl, M.D., Patrick Ahlers, M.D., Katrin Walenta, M.D., Andreas Link, M.D., Michael Böhm, M.D., and Georg Nickenig, M.D.

ABSTRACT

CONCLUSIONS
The level of circulating CD34+KDR+ endothelial progenitor cells predicts the occurrence of cardiovascular events and death from cardiovascular causes and may help to identify patients at increased cardiovascular risk.
Healthy EPCs are resistant to oxidative stress

- High intrinsic levels of
  - MnSOD

- Protect EPCs during inflammation and vascular repair

\[ \text{O}_2^- \xrightarrow{\text{MnSOD}} \text{H}_2\text{O}_2 \xrightarrow{\text{Catalase, Glutathione Peroxidase}} \text{H}_2\text{O} \]

Dernbach E, et al, Blood. 2004 Dec 1;104(12):3591-7
Stress
- pH ↓
- ROS
- Hypoxia

Loss of function
- Senescence
- Apoptosis

Protection
- Anti-apoptotic
- Anti-oxidant
  - e.g. MnSOD

- „Stemness“
- Proliferation
- Angiogenic function
Diabetic Wound Healing
Prevalence of Diabetes in US

Diabetic Wound Healing and Amputation

Hospitalizations for Nontraumatic Lower Extremity Amputation

http://www.cdc.gov/diabetes/statistics/lea/fig1.htm
Angiogenesis is the rate-limiting step of wound healing.
A. Angiogenesis by mobilization of EPCs from the bone marrow

EPCs → Capillaryplexus → Mature network

Angiogenesis by second intention

EPCs → Wound contraction

Kumar: Robbins and Cotran: Pathologic Basis of Disease, 7th ed
Hypothesis

Diminished antioxidant protection contributes to EPC dysfunction in diabetic wound healing.
Type II Diabetic Mice (db/db) are hyperglycemic and obese.

Mouse Weight

*P<0.001
n=4-5

Peripheral Blood Glucose

*P<0.001
n=4-5
EPC Marker
Marrow Day 7

Hoechst
FITC-Isolectin
Dil-acLDL-
Combined
EPC therapy improved the rate of wound healing in type 2 diabetic mice.

Day of Wound Closure
0 2 4 6 8 10 12 14 16
% Wound Closure
-20
0
20
40
60
80
100
db/+ (n=10)
db/db (n=10)
db/db with db/+EPC (n=10)
db/db with db/db-EPC (n=10)
MnSOD Gene Therapy of Diabetic EPC Accelerates Wound Healing
Integration of transplanted EPCs on day 6 of wound healing
MicroRNAs

- Single-stranded RNA
- 21-23 nucleotides
- Post-transcriptional regulation
- Modulate or fine-tune cellular phenotypes
Angiogenesis-related miRNAs

- **Pro-angiogenic**
  - mir-130a
  - mir17-92 cluster
  - *let-7f & mir-27b*
  - mir-378, mir-210

- **Anti-angiogenic**
  - mir-221 & mir222
  - mir-15, mir-16, mir-20a & mir20b

Hypothesis

Pro-angiogenic miRNA let-7f and mir-27 restore decreased MnSOD in EPCs, resulting in reduced oxidative stress and improved angiogenesis in diabetic wound repair.
Diabetic EPCs possess deficient expression of microRNA let-7f and mir27b
Ex vivo angiogenesis in diabetic EPCs is impaired, which can be rescued by let-7f and mir-27b
MicroRNA let-7f and mir-27b rescue decreased MnSOD expression in diabetic EPCs
AMPK and Angiogenesis

AMPK activation

- Promoted endothelial cells angiogenesis\(^1\)
- Increased EPC differentiation and angiogenesis\(^2\)

Let-7f promotes AMPK activation

**Thr172**

p-AMPK

**AMPK**

**Ser79**

p-ACC

**β-actin**

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<th>Control</th>
<th>STZ</th>
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<tr>
<td>Scramble</td>
<td>let-7f inhi</td>
<td>Scramble</td>
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*\( p < 0.05 \) vs. Scrambled control

*\( p < 0.05 \) vs. Scrambled control and STZ+let-7f mim, \( n=5 \)
Let-7f improves EPC functions via AMPK activation

*\(p<0.05\) vs. Control and STZ+let-7f mim, \(n=5\)

Comp C: Compound C, selective AMPK inhibitor
Let-7f promotes MnSOD expression via AMPK activation in EPCs

* Thr172 p-AMPK
* MnSOD
* β-actin

![Graph showing MnSOD/actin levels](image)

Control  | STZ    | STZ+AICAR
---       | ---    | ---
MnSOD/actin | * | |

* p<0.05 vs. Control and STZ+AICAR, n=5

AICAR: selective AMPK agonist

![Graph showing MnSOD/actin levels](image)

Control  | Scramble | let-7f mim | let-7f mim + Comp C
---       | ---      | ---        | ---
MnSOD/actin | * | | |

* p<0.05 vs. Control and STZ+let-7f mim, n=5
Let-7f elevates MnSOD activity via AMPK activation

*p < 0.05 vs. Control and STZ+let-7f mim, n=4
Let-7f reduces mitochondrial superoxide via AMPK activation

MitoSOX Red: an indicator of mitochondrial superoxide production

* $p<0.05$ vs. Control and STZ+let-7f mim, n=5
Let-7f and Protein Phosphatase 2A

- Let-7f plays its role by suppressing target mRNA
- Let-7f has binding sites for protein phosphatase 2A (PP2A)
- PP2A was identified to inactivate AMPK

The level of PP2A, an endogenous AMPK inhibitor, is elevated in EPCs of type 1 diabetic mice.
Let-7f inhibits PP2A mRNA expression

* $p<0.05$ vs. Control and STZ+let-7f mim, n=5
Let-7f suppresses PP2A protein

* $p<0.05$ vs. Control and STZ+let-7f mim, n=4
MicroRNA let-7f improves EPC-mediated angiogenesis via AMPK activation and MnSOD induction in diabetes
The level of p66$^{\text{shc}}$, a ShcA protein promoting oxidative stress, is increased in diabetic EPCs.
Increased TSP-2 secretion in diabetic EPCs
Excisional wounds have delayed healing with diminished local blood flow in diabetic mice.
Normal EPC therapy is superior than diabetic EPCs in improving wound closure and blood flow.
Summary Two

In type 2 diabetes:

1. MicroRNA mir-27b augments EPC angiogenesis through TPS-1 inhibition.
Conclusion

MicroRNA let-7f and mir-27b improve EPC angiogenesis through different pathways in diabetes

Perspective

Augmentation of EPC functions by targeting miRNAs may provide a novel strategy to enhance angiogenesis in patients with diabetes.
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