Exosomes derived from ischemic cerebral endothelial cells and neural stem cells enhance the coupling of neurogenesis and angiogenesis by transfer of microRNAs

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Disclosure

- None
Introduction

➢ Stroke induces angiogenesis and neurogenesis.
➢ Exosomes mediate the coupling of angiogenesis and neurogenesis after stroke.

Ischemic cerebral endothelial cell (CEC)-exosomes enhance neural progenitor cell (NPC) proliferation

**EM**

<table>
<thead>
<tr>
<th>protein</th>
<th>130kDa</th>
<th>95kDa</th>
<th>63kDa</th>
<th>39kDa</th>
<th>26kDa</th>
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</thead>
<tbody>
<tr>
<td>CD31</td>
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<td></td>
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<tr>
<td>Alix</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CD63</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* p<0.05, n=3

Brdu+DAPI

- control
- non-ischemic exosomes
- ischemic exosomes

% of Brdu+ cells (mean±SE)

- control
- non-ischemic exosomes
- ischemic exosomes
Ischemic CEC-exosomes enhance neuronal and oligodendrocyte differentiation of NPCs

**Tuj1 + DAPI**
- Control
- Non-ischemic exosomes
- Ischemic exosomes

**NG2 + DAPI**
- Control
- Non-ischemic exosomes
- Ischemic exosomes

**Graphs:**
- % of Tuj1+ cells (mean±SE)
  - Control
  - Non-ischemic CEC-exosomes
  - Ischemic CEC-exosomes
  - *p<0.05, n=3

- % of NG2+ cells (mean±SE)
  - Control
  - Non-ischemic CEC-exosomes
  - Ischemic CEC-exosomes
  - *p<0.05, n=3
miRNAs within CEC-exosomes are transported to NPCs

miRs in CEC-exosomes

- ECs
  - miRNA: 221
- miRNA: 91
  - ECs exos
  - miRNA: 164

miR transfer from CECs to NPCs

- Fold change (mean±SE)
  - rno-miR-494
  - rno-miR-195
  - rno-miR-411
  - rno-miR-32
  - rno-miR-125a-5p
  - rno-miR-146a

- Recipient NPCs
  - con-ECs exos
  - ip-ECs exos

- Fold change (mean±SE)
  - miR-146a
  - miR-125a-5p
  - miR-411

- * p<0.05, n=3
Inhibition of exosome release impairs miRNA transfer from CEC to NPCs

CECs → CEC exosome release

Rab27 siRNA

CEC-exosomes

Recipient NPCs

Fold change (mean±SE)

miR-146a  miR-125a

con-ECs non-target  con-ECs exos  con-ECs exos
Rab27a si  Rab27b si

dip-ECs non-target  dip-ECs exos  dip-ECs exos
Rab27a si  Rab27b si

*  #
CEC-derived exosomes transfer miRNAs to NPCs and promote their proliferation

miRNA tailored exosomes

miRNA oligos

Transfection

Cells

miRNA tailored exosomes

Recipient cells

Brdu + DAPI

Mimic con  miR-146a mimic  miR-125a mimic

The percentage of positive cells (mean±SE)

Brdu/DAPI

* vs non-target p<0.05
CEC-derived exosomes transfer miRNAs to NPCs and promote neuronal differentiation.

Tailored Exosomes

<table>
<thead>
<tr>
<th>Mimic con</th>
<th>miR-146a mimic</th>
<th>miR-125a mimic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuj1</td>
<td>Tuj1</td>
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</tr>
<tr>
<td>Merge</td>
<td>Merge</td>
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<tbody>
<tr>
<td>NG2</td>
<td>NG2</td>
<td>NG2</td>
</tr>
<tr>
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<td>Merge</td>
<td>Merge</td>
</tr>
</tbody>
</table>

The percentage of positive cells (mean ± SE)

- Tuj1
- NG2

* vs non-target exos p<0.05
miRNA transmission reduces target genes in recipient NPCs

miR-146
- IRAK1
- TRAF6

miR-125a
- BAK1
- BMF
- KLF13

![Bar charts showing fold change in target genes](chart.png)

* vs naïve, p<0.05, n=3
NPC-exosomes increase capillary tube formation and migration of CECs.
Altered miRNAs in ischemic NPC-exosomes are transported to CECs

**miRs in NPC-exosomes**

Exosome release

NPCs → NPC exosome release

Rab27 siRNA

**miR transfer**

![Graph showing miR-106b-3p and miR-125b-3p expression levels in different conditions.](image)

* p<0.05, n=3
NPC-exosomes transfer miRNAs to CECs and induces tube formation
Summary

➢ Exosomes mediate the coupling of angiogenesis and neurogenesis.
➢ Ischemic CEC-exosomes enhance neurogenesis and oligodendrogeneration.
➢ Exosomes derived from either non-ischemic or ischemic NPCs promote in vitro angiogenesis.
➢ Exosome-transmitted miRNAs regulate stroke-induced neurogenesis and angiogenesis.
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