Exosome and arrhythmia

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Burden of Arrhythmia

01 Increasing Incidence
02 Economic & Medical Problem
03 Unpredictable
04 Curable method

We need new tool for Arrhythmia treatment
Gene and Stem cell Tx

- No good prevention & prediction
- Sudden cardiac death (CV death : 3rd cause of death)

- Limitation of current Tx

- Current modalities to manage arrhythmia are not sufficient to reverse and to cure the disease.
# Limitation of stem cell therapy

## Long-term effect of stem cell therapy

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Number of patients</th>
<th>Cell type</th>
<th>Dose</th>
<th>Route of delivery</th>
<th>Timing of delivery</th>
<th>Primary end point</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute myocardial infarction</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>BOOST</td>
<td>60</td>
<td>nBMC</td>
<td>128 ml</td>
<td>i.c.</td>
<td>Day 6±1</td>
<td>LVEF ↑</td>
<td>Effect diminished after 18 and 61 months</td>
</tr>
<tr>
<td>REPAIR-AMI</td>
<td>187</td>
<td>mnBMC</td>
<td>50 ml</td>
<td>i.c.</td>
<td>Day 3-6</td>
<td>LVEF ↑</td>
<td>NA</td>
</tr>
<tr>
<td>Leuven-AMI</td>
<td>66</td>
<td>mnBMC</td>
<td>130 ml</td>
<td>i.c.</td>
<td>Day 1</td>
<td>LVEF ↔</td>
<td>Regional contractility ↑ Infarct size ↓</td>
</tr>
<tr>
<td>ASTAMI</td>
<td>97</td>
<td>mnBMC</td>
<td>50 ml</td>
<td>i.c.</td>
<td>Day 6±1</td>
<td>LVEF ↔</td>
<td>NA</td>
</tr>
<tr>
<td>FINCELL</td>
<td>77</td>
<td>mnBMC</td>
<td>80 ml</td>
<td>i.c.</td>
<td>Day 3</td>
<td>LVEF ↑</td>
<td>NA</td>
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<tr>
<td><strong>Ischemic heart failure</strong></td>
<td></td>
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<tr>
<td>REGENERATE</td>
<td>117</td>
<td>mnBMC (unselected vs CD34+/ CXCR4+)</td>
<td>50–70 ml (unselected) 100–120 ml (selected)</td>
<td>i.c.</td>
<td>Day 3–12</td>
<td>LVEF ↑ with both cell types</td>
<td>NA</td>
</tr>
<tr>
<td>HEBE</td>
<td>189</td>
<td>mnBMC vs mnPBC</td>
<td>60 ml (mnBMC) 150 ml (mnPBC)</td>
<td>i.c.</td>
<td>Day 3–8</td>
<td>Regional contractility ↔</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Table 1</strong></td>
<td>Randomized trials in patients with acute myocardial infarction or ischemic heart failure</td>
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</tbody>
</table>

Strategies to improve delivery and selective targeting of cardiovascular RNA therapeutics
Extracellular vesicles

- Exosome, microvesicle, apoptotic body

Research for exosome

1. Exosome Production
- Plasma membrane proteins
  - Multivesicular body (MVB)
  - Endocytosis
  - Backfusion

Exosomes

2. Ex vivo Exosome Modification
- Introduction of exogenous nucleic acids, e.g., via electroporation
- Small molecule loading

3. Exosome Delivery
- Receptor mediated endocytosis
- Macropinocytosis
- Lipid raft mediated endocytosis
- Multivesicular body (MVB)
  - Cytosolic cargo release
  - Endosomal membrane backfusion

Native components:
- lactadherin
- lamp2b
- CD63
- cytosolic protein e.g., hsp90
- native RNA

Engineered components:
- lactadherin C1C2 domain with synthetic tag
- lamp2b with synthetic tag
- exogenous RNA
- electroporated exogenous RNA
- small molecule drug

YONSEI UNIVERSITY COLLEGE OF MEDICINE
SEVERANCE CARDIOVASCULAR HOSPITAL
Potentiated Exosome: Hypoxic stress
Antiarrhythmic Potential of Mesenchymal Stem Cell Is Modulated by Hypoxic Environment

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Seoul, Incheon, and Gwangju Republic of Korea; and Providence, Rhode Island

Potentiated Microvesicle (MV): hypoxia-treated human MSC

A. Cell culture media → Supernatant
   - 300 x g, 10 min → Dead cell
   - 750 x g, 5 min → Cell debris
   - 1500 x g, 5 min → Apoptotic body
   - 14000 x g, 45 min, 3 times → Extracellular vesicles

B. 200 nm

C. Concentration (particles/ml)
   - Evs Concentration
     Mode: 172 nm
     Mean: 200 nm

D. | Cell | EVs |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>HSP70</td>
<td></td>
</tr>
<tr>
<td>Annexin</td>
<td></td>
</tr>
<tr>
<td>Integrin</td>
<td></td>
</tr>
<tr>
<td>CD63</td>
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</tr>
</tbody>
</table>

E. PBS
   - PKH26 α-tubulin DAPI

F. cTNT PKH26
   - PBS
   - EVs

EV(HM) significantly reduced infarct size (24±2% vs. 8±1%, p<0.001), and diminished arrhythmias by recovering electrical conduction, I(Na) current, and Cx43 expression.
EV(HM) also reversed reductions in Wnt1 and β-catenin levels and increases in GSK3β induced after IR injury.

miRNA-26a was significantly increased in EV(HM), compared with EV(NM), in real-time PCR.
Hypoxia MV protect IR injury by inhibiting GSK-3β via miR-26a

**P<0.01

AHA 2016

Potentiated MV & AF: hypoxia-treated human MSC

Recovery of Calcium regulation with MV

MV prevent the disarrangement of microtubules

MV attenuated reductions of $I_{CaL}$ current

Control                          TP                             MV+TP

Tubulin  Acetylated tubulin  Merge

Control (NP)  

TP  

TP+MV

Kim YR, APHRS 2016
Genetically modified exosome
Exosomes expressing cardiac-targeting peptide (CTP)-Lamp2b on the exosomal membrane (CTP-Exo) were generated by introducing vectors encoding CTP-Lamp2b into HEK 293 cells.

The expression of CTP-Lamp2b peptide on exosomes was stabilized by attaching glycosylation sequences.
Isolation, characterization, and delivery of exosomes

A

CTL BF TR

CTP

B

Cell CTL CTP CTL CTP

Exosome

Lamp2

CD81

FLAG

Alix

GAPDH

C

Con-Exo

Concentration (×10^6/mL)

0 1 2 3

Size (nm)

0 200 400 600

CTP-Exo

Concentration (×10^6/mL)

0 1 2 3

Size (nm)

0 200 400 600
Delivery of exosomes containing CD81-mCherry in HEK and H9C2 cells

The delivery of the exosome was not different between CTP-Exo and CTL-Exo in HEK 293 cells, whereas the delivery of CTP-Exo was 16% greater than that of CTL-Exo in H9C2 cells (P = 0.047).
The delivery of exosomes to target organs of mouse

- Compared with CTL-Exo, the in vivo delivery of exosomes to the hearts of mice was increased by 15% with CTP-Exo (P = 0.035).
- The delivery to livers and spleens was not different between the two exosomes.

Kim H, Joung B, et al. BBRC 2018;499:303-8
Human peripheral blood-derived exosomes for miRNA delivery
Delivery of gene or protein to exosome

Candidate gene and protein

RAGE siRNA

Heat shock protein, vimentin
VEGF 벡터
Wnt pathway
miRNA

Electroporation을 이용한 전달

ADV fiber
Lamp-2b
+ -

ADV fiber
Lamp-2b
+ -
Delivery of miR-21 mimic or inhibitor loaded exosomes to cardiac cells

In H9C2 and HL-1 cells, miR-21 expression was successfully regulated by treatment with human peripheral blood derived-exosomes loaded with an miR-21 mimic or inhibitor compared with untreated cells.
Effect of miR-21 mimic or inhibitor loaded exosomes on modulation of target expression

- The mRNA and protein expression levels of Smad7, PTEN and MMP2, which are involved in cardiac fibrosis, were associated with the uptake of miR-21 mimic- or inhibitor-loaded exosomes.
The Effects of miR-21 mimic or inhibitor loaded exosomes on cardiac fibrosis in mice

- The in vivo mRNA and protein expression of Smad7, PTEN and MMP2 were altered following treatment with miR-21 mimic- or inhibitor-loaded exosomes.

Exosome as a diagnostic tool
AF progression

- Hypertension
- Heart failure
- Start atrial remodelling

Years

SR

paroxysmal

persistent

permanent

remodelling

ECV

ECV
Characterization of exosomes

For the discovery phase, exosomes were isolated from the serum of patients with supraventricular tachycardia (SVT) as the controls (n = 5) and with paroxysmal AF (n = 4) and persistent AF (n = 5) for microarray analysis of miRNAs.
44 miRNAs were expressed significantly higher (>1.5-fold) in patients with PeAF, but not in patients with PaAF, relative to the levels in patients with SVT control. The expression of 5 miRNAs (miRNA-103a, -107, -320d, -486, and let-7b) was elevated by more than 4.5-fold in patients with persistent AF.
qRT-PCR quantitation of expressions of five miRNAs in the SVT-controls and PeAF groups

For the validation phase, miRNAs were analyzed using quantitative RT-PCR analysis in exosomes from the serum of patients with SVT control (n = 20) and patients with persistent AF (n = 40).

Functional pathways of the five higher expressed miRNAs

- These miRNAs and their target genes were involved in atrial function and structure, oxidative stress, and fibrosis pathways.
Potentiated Exosome:
Exosome from AF patients

Control (PSVT) → AF

Serum
300 x g, 10min
Dead cell
Supernatant
375 x g, 5min
Cell debris
Supernatant
1500 x g, 5min
Apoptotic body
Supernatant
14000 x g, 45min
3 times
Exosome

miR-1
Let-7a-2-3p
Let-7d-3p
Let-7c-3p
Let-7e-5p
Let-7f-2-3p
Let-7g-3p
Let-7b-3p
Let-7i-3p
Let-7i-5p
Let-7e-5p
miR-30a-3p
miR-30d-5p
miR-30c-1-3p
miR-30b-3p
miR-30e-3p
miR-29a-3p
miR-29b-3p
miR-29b-1-5p
miR-29b-2-5p
miR-29c-5p
miR-26b-5p
miR-26a-1-3p
miR-26a-2-3p
miR-26b-3p

Unpublished data

F/F0
Non-Pacing no Tubacin Co-Exo AF-Exo
Pacing

Ac-tubulin
Tubulin
HDAC6
GAPDH

Control
AF

Min
Max
Conclusion

1. The current modalities to manage arrhythmia are not sufficient to reverse and to cure the disease.

2. Gene and potentiated exosome might be a new tool to treat arrhythmia in the future.

3. Serum exosomal miRNAs might be used as novel biomarkers to reflect the progression of AF.
Thank you for your attention!

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