Potential of microRNA-223 for polarization of macrophages to M2 phenotype
Characterization of miR-223/HAPEI NPs

Z-Average (d.nm): 200.6
PdI: 0.266
Intercept: 0.931

Result quality: Good

Size Distribution by Intensity

Record 2: HAPEI and miRNA 7-15-15.1
Expression level of miR-223 in M1 (LPS+IFN-γ treated) and M2 (IL4 treated) macrophages (J774A.1)

![miR-223 expression graph]

- Untreated cells
- M1 (LPS+IFN-γ treated)
- M2 (IL4 treated)
Polarization of M1 macrophages using miR-223-lipofectamin for 24 h in J774A.1

iNOS2-M1 marker  

Arg1-M2 marker

Normalized iNOS-2 expression level

Normalized Arg-1 expression level

Medium  miR-223  Scrambled
Transfection of miR-223 and plasmid-miR223 in J774A.1 macrophages-Taqman assay of miR-223

miR-223 expression level

miR-223 duplex was better than pDNA-miR-223 to induce high expression of miR-223 after transfection
Transfection study of miR-223 duplex at different doses in J774A.1

- No correlation of transfected dose and miR-223 expression
- Transfection efficiency of lipofectamine was better than that of HA-PEI
- Transfection with miR-223 induced much higher miRNA expression than with pDNA-miRNA
Transfection study of pDNA-miR-223 duplex at different doses in J774A.1 macrophages

miR-223 expression

Fold change compared to control

24 h
48 h

different pDNA-miR-223 doses
In vitro polarization of miR-223 and plasmid-miR223 (24h) in J774A.1 macrophages
Polarization of J774 with HA-PEI/plasmid-miR-223

Plasmid miR-223 significantly decreased level of iNOS, other than increase Arginase level
Anti-inflammatory effect of pDNA miR-223 in J774 cells

Pre-transfected J774 with pDNA miR-223 for 24h, then treated with LPS and IFN-gamma for 6h

Fold change compared to untreated cells

- HAPEI-pDNA miR-223
- Naked pDNA miR-223
Studies in peritoneal macrophages—a better model of macrophages
Uptake of Cy5-HAPEI/miR-223 in peritoneal macrophages

<table>
<thead>
<tr>
<th>Control</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI-nuclei</td>
<td>Cy5-HAPEI</td>
<td>Merged</td>
<td>DAPI-nuclei</td>
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</tbody>
</table>

10 um
Transfection study in peritoneal macrophages

Taqman assay

miR-223 expression (Fold change compared to untreated)

- Untreated
- Blank HAPEI
- HA-PEI/miR-223
- Lipofectamine/miR-223

24h

48h
Polarization study of miR-223 (100 nM) in peritoneal macrophages

### iNOS

- Control
- M1
- LF-miR
- HA-PEI/miR

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2</td>
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<tr>
<td>M1</td>
<td>1.2</td>
</tr>
<tr>
<td>LF-miR</td>
<td>0.8</td>
</tr>
<tr>
<td>HA-PEI/miR</td>
<td>0.6</td>
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</tbody>
</table>

### Arg

- Control
- M1
- LF-miR
- HA-PEI/miR

<table>
<thead>
<tr>
<th>Condition</th>
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<tr>
<td>Control</td>
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<tr>
<td>M1</td>
<td>1.6</td>
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<tr>
<td>LF-miR</td>
<td>1.0</td>
</tr>
<tr>
<td>HA-PEI/miR</td>
<td>1.8</td>
</tr>
</tbody>
</table>
In vitro anti-inflammatory effect in peritoneal macrophages at 48h post-transfection

TNF-α at 48h post-transfection

IL1-b expression
In vitro anti-inflammatory effect in peritoneal macrophages at 48h post-transfection

IL6 expression

![IL-6 mRNA expression graph]
*In vivo* polarization study in C57BL/6 mice

peritoneal macrophages