Role of MicroRNA-1 (miR-1) in chordoma and its therapeutic potentials

Zhenfeng Duan
Biological principles of miRNA (miR)

[Diagram showing the process of miRNA biogenesis and function]
Differential expression of microRNA (miRNA) in chordoma reveals a role for miRNA-1 in Met expression

Zhenfeng Duan¹, Edwin Choy¹, G. Petur Nielsen², Andrew Rosenberg², John Iafrate ², Cao Yang¹, Joe Schwab¹, Henry Mankin¹, Ramnik Xavier³, Francis J. Hornicek¹

Article first published online: 29 DEC 2009
DOI: 10.1002/jor.21055
Copyright © 2009 Orthopaedic Research Society

Journal of Orthopaedic Research
Volume 28, Issue 6, pages 746–752, June 2010
### Top decreased expression of miRNAs in both chordoma tissues and chordoma cell lines

<table>
<thead>
<tr>
<th>Name of miRNA</th>
<th>Normal (mean value)</th>
<th>Tumor tissues and cell lines (mean value)</th>
<th>Fold</th>
<th>p-value (&lt;0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-1</td>
<td>27,231</td>
<td>31</td>
<td>878</td>
<td>6.75E-04</td>
</tr>
<tr>
<td>miRNA-206</td>
<td>16,049</td>
<td>29</td>
<td>553</td>
<td>8.89E-04</td>
</tr>
<tr>
<td>miRNA-133b</td>
<td>15,709</td>
<td>68</td>
<td>231</td>
<td>4.41E-03</td>
</tr>
<tr>
<td>miRNA-95</td>
<td>4,243</td>
<td>19</td>
<td>223</td>
<td>2.85E-03</td>
</tr>
<tr>
<td>miRNA-133a</td>
<td>16,095</td>
<td>75</td>
<td>215</td>
<td>4.49E-03</td>
</tr>
<tr>
<td>miRNA-1268</td>
<td>2,043</td>
<td>134</td>
<td>145</td>
<td>4.90E-03</td>
</tr>
<tr>
<td>miRNA-101</td>
<td>875</td>
<td>21</td>
<td>42</td>
<td>4.19E-03</td>
</tr>
<tr>
<td>miRNA-139-5p</td>
<td>705</td>
<td>17</td>
<td>41</td>
<td>5.90E-03</td>
</tr>
</tbody>
</table>
Real-time RT-PCR and Northern blot detection of mature miR-1 in chordoma tissues

### A

**Amplification Plot**

- N1
- N2
- T1
- T2

### B

**Amplification Plot**

- N1
- N2
- T1
- T2

### C

Relative Expression

---

### D

- miRNA-1
- 28S
- 18S
Expression of miR-1 target gene HDAC4 and Met proteins in chordoma cell lines and chordoma tissues
miR-1 expression in other type of tumors

- Notochord
- Normal Muscle
- Liposarcoma
- Chordoma
- TC-71
- U-2OS
- KHOS
- MCF-7

Relative Expression
Transfection of miRNA-1 into chordoma cells UCH1 suppresses Met expression and inhibits cell growth.

**A**
Absorbance (cell growth) over hours after pre-miRNA transfection.

**B**
Absorbance (cell growth) with varying concentrations of pre-miRNA-1 (nmol).

**C**
Transfection of miRNA-1 into chordoma cells UCH1 suppresses Met expression and inhibits cell growth.

**D**
Western blot analysis showing suppression of Met expression by miRNA-1 transfection.
Met expression was most frequently detected in chordoma (94.4%), followed by chondrosarcoma (54.2%) and osteosarcoma (23.3%).

Met oncoprotein plays a leading role in the metastatic process in chordoma, and that a c-Met-HGF pair is involved in chordoma malignancy.
FISH analysis demonstrated that MET gene amplification is not the cause of MET overexpression in chordomas.
Two different precursors (hsa-miR-1-1 and hsa-miR-1-2) of miR-1

Primary miRNA

hsa-mir-1-2

hsa-mir-1-1

5’-UGGAAUGUAAAGAAGUAUGUAU-3’
mature miR-1
Similar to previous findings, large copy number losses (green), involving chromosomes 18 was more common than copy number gains.
Downregulation of microRNAs miR-1, -206 and -29 stabilizes PAX3 and CCND2 expression in rhabdomyosarcoma

Lihua Li, Aaron L Sarver, Setara Alamgir and Subbaya Subramanian


MicroRNA regulation of the paired-box transcription factor Pax3 confers robustness to developmental timing of myogenesis.

Goljanek-Whysall K, Sweetman D, Abu-Elmagd M, Chapnik E, Dalmay T, Hornstein E, Münsterberg A.
miR-1 regulates PAX3 expression through sequence-specific binding to its 3’ UTR

<table>
<thead>
<tr>
<th>Gene/miRNAs position</th>
<th>Predicted pairing</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX3 3’ UTR 3133-3139</td>
<td>5’...GAGGUAAGGACAAGAAAAUCUCCU...</td>
</tr>
<tr>
<td>miR-206 3’</td>
<td>GGUGUUGUGAAGGGAAUGUAGGU</td>
</tr>
<tr>
<td>miR-1 3’</td>
<td>UAUGUAUAAGAAAGUAGGAGGU</td>
</tr>
<tr>
<td>PAX3 3’ UTR 2158-2164</td>
<td>5’...CAUCGAAGGCUAAAGAAACAUCCA...</td>
</tr>
<tr>
<td>miR-206 3’</td>
<td>GGUGUUGUGAAGGGAAUGUAGGU</td>
</tr>
<tr>
<td>miR-1 3’</td>
<td>UAUGUAUAAGAAAGUAGGAGGU</td>
</tr>
</tbody>
</table>

![Graph showing Firefly/Renilla expression](image)

- PAX3-3’ UTR-sGG
- PAX3-3’UTR1-m-sGG
- PAX3-3’UTR2-m-sGG
- PAX3-3’UTRDm-sGG

![Graph showing miR-1, miR-206, and miR-1 and -206 effects](image)

- Firefly/Renilla comparison

![Western blot analysis](image)

- PAX3-FKHR
- PAX3
- GAPDH

Wild type PAX3 with 3’ UTR

PAX3-FOXO1 fusion transcript

![Diagram showing miR-1 and -206 effects](image)
## Altered expression of miR-1 in different cancers

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>miR-1 down regulation</th>
<th>miR-1 target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>Primary lung cancer tissue and serum</td>
<td>MET;FoxP1;HDAC4;Slug</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Different colon cancer tissues</td>
<td>MET</td>
</tr>
<tr>
<td>Genitourinary cancer</td>
<td>Cancer cell lines and tissues</td>
<td>LASP1;TAGLN2;SRSF9;PTMA;PNP1</td>
</tr>
<tr>
<td>Head &amp; Neck cancer</td>
<td>laryngeal carcinoma and MSSCC</td>
<td>TAGLN2;PTMA;FN1;PNP</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Thyroid adenomas and carcinomas</td>
<td>CCND2;CXCR4;CXCL12</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>HCC cell lines and tumor tissues</td>
<td>MET;FoxP1;HDAC4;ET-1</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Sarcoma cell lines and sarcoma tissues</td>
<td>MET;CCND2;HDAC4</td>
</tr>
</tbody>
</table>
Oncogenes and oncogenic pathway targets of miR-1 in cancers

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Genbank Accession #</th>
<th>Chromosome location</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>NM_000245.2</td>
<td>7q31</td>
<td>Proto-oncogenic receptor tyrosine kinase</td>
</tr>
<tr>
<td>HDAC4</td>
<td>NM_006037.3</td>
<td>2q37.3</td>
<td>Histone deacetylase activity and represses transcription</td>
</tr>
<tr>
<td>Pim-1</td>
<td>NM_002648.3</td>
<td>6p21.2</td>
<td>Proto-oncogene</td>
</tr>
<tr>
<td>FOXP1</td>
<td>NM_01244810.1</td>
<td>3p14.1</td>
<td>Regulate gene transcription; tumor suppressor</td>
</tr>
<tr>
<td>TAGLN2</td>
<td>NM_003564.1</td>
<td>1q21-q25</td>
<td>Earliest marker of differentiated smooth muscle</td>
</tr>
<tr>
<td>PNP</td>
<td>NM_000270.3</td>
<td>14q13.1</td>
<td>Purine metabolism</td>
</tr>
<tr>
<td>PTMA</td>
<td>NM_002823.4</td>
<td>2q37.1</td>
<td>Enhance cell-mediated immunity</td>
</tr>
<tr>
<td>CXCR4</td>
<td>NM_001008540.1</td>
<td>2q21</td>
<td>Chemokine receptor</td>
</tr>
<tr>
<td>CCND2</td>
<td>NM_001759.3</td>
<td>12p13</td>
<td>Regulator of cyclin-dependent kinase</td>
</tr>
<tr>
<td>SRSF9</td>
<td>NM_003769.2</td>
<td>12q24.31</td>
<td></td>
</tr>
<tr>
<td>FN1</td>
<td>NM_212482.1</td>
<td>2q34</td>
<td>Involved in cell adhesion, growth, migration</td>
</tr>
<tr>
<td>ETS1</td>
<td>NM_001143820.1</td>
<td>11q23.3</td>
<td>Proto-oncogene</td>
</tr>
<tr>
<td>endothelin 1</td>
<td>NM_001955.4</td>
<td>6p24.1</td>
<td>Involve in vascular disorders</td>
</tr>
<tr>
<td>Slug</td>
<td>NM_003068.4</td>
<td>8q11</td>
<td>Transcriptional repressor and has antiapoptotic activity</td>
</tr>
<tr>
<td>CXCL12</td>
<td>NM_199168.3</td>
<td>10q11.1</td>
<td>Chemotactic for lymphocytes</td>
</tr>
</tbody>
</table>
Therapeutic Inhibitors of the c-MET Signaling Pathway
A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells

Rajib Ghosh a,1, Lalithya C. Singh a,1, Jason M. Shohet b, Preethi H. Gunaratne a,*

a Department of Biology & Biochemistry, University of Houston, 4800 Calhoun Road, Houston, TX 77204, USA
b Texas Children's Cancer Center Baylor College of Medicine, Houston, TX 77030, USA

miR-stemloop pathway

miR-duplex pathway

miR-stemloop (miR-sl)

S-PEG

cAuNPs

miRNA/miRNA* duplexes (miR-dp)

S-PEG

cAuNPs

Endosome

Endosome

Dicer

TRBP

mRNA/miRNA* duplexes

Passenger strand degradation

mRISC loading on target mRNA

m7G

Translational inhibition or

m7G

mRNA cleavage

a

b

c

d
Dextran-nanoparticle delivers miR-199a-3p into cancer cells.
Conclusion

• Chordoma has a distinct miRNA (miR) expression profile.

• miR-1 expression is significantly decreased in both chordoma tissues and cell lines. miR-1 is downregulated in 93.7% of the tumors and its decrease significantly correlated with Met overexpression.

• Downregulation of miR-1 is associated with poor prognosis in chordoma.

• Overexpression of miR-1 in chordoma cells inhibit cell growth and reduced replication potential, miR-1 have a tumor suppressor function in chordoma by directly regulating Met oncogene.

• miR-1 would be a viable small molecule candidate in clinical trials testing Met Inhibitors or directly deliver miR-1 to treat chordoma.
Acknowledgements

- The Chordoma Foundation
- The Stephan L. Harris Fund
- Gattegno and Wechsler funds
- Sarcoma Foundation of America (SFA)
- Jennifer Hunter Yates Foundation
- Stanton Fund
- InteRNA Technologies - miRNA therapeutics for cancer
- National Cancer Institute (NCI), UO1 grant
- Academic Enrichment Fund of MGH Orthopaedics