MicroRNA-29a Reveals Oncogenic Role on Myeloid Malignancies by Regulating DNMT3A

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Introduction

• MicroRNAs (miRNAs) are a small non-coding RNAs
• Have been implicated in regulating gene expression
• Involved in an increasing number of biological processes and..
• Play role in regulating normal hematopoiesis.
miRNA Mechanism

1. **Transcription**
   - microRNA gene
   - Hairpin RNA

2. **Processing**
   - Duplex RNA
   - microRNA

3. **microRNA-protein complex**
   - Perfectly matching mRNA
   - No translation
   - Partially matching mRNA
   - Chopped up!
MicroRNA Activity in Cancer: Tumor Suppressive or Oncogenic

**Oncogenic miRNAs**
- Called “oncomirs” promote tumor development by negatively inhibiting tumor suppressor genes and/or genes that control cell differentiation or apoptosis
- Some of them: miR10-b, miR-17, miR-21, miR-155

**Tumor suppressor miRNAs**
- Target of them is oncogenes in cell differentiation, invasion, apoptosis, proliferation, metastasis
- Some of them: Let 7 family, miR 34b, miR145, miR205, miR200
### miRNA Oncogenes or Tumor Suppressor Genes in human Cancer

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Dysregulation</th>
<th>Function</th>
<th>Validated targets</th>
<th>Oncogene (ONC) or tumour suppressor (TS)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15a and miR-16-1</td>
<td>Loss in CLL, prostate cancer and multiple myeloma</td>
<td>Induces apoptosis and inhibits tumorigenesis</td>
<td>BCL2, WT1, RAB9B and MAGE83</td>
<td>TS</td>
<td>15,20,23, 30,52,69</td>
</tr>
<tr>
<td>let-7 (a, b, c, d, e, f, g and h)</td>
<td>Loss in lung and breast cancer and in various solid and haematopoietic malignancies</td>
<td>Induces apoptosis and inhibits tumorigenesis</td>
<td>RAS, MYC and HMGA2</td>
<td>TS</td>
<td>22,26, 42,70</td>
</tr>
<tr>
<td>miR-29 (a, b and c)</td>
<td>Loss in aggressive CLL, AML (11q23), MDS lung and breast cancers and cholangiocarcinoma</td>
<td>Induces apoptosis and inhibits tumorigenicity. Reactivates silenced tumour suppressor genes</td>
<td>TCL1, MCL1 and DNMTs</td>
<td>TS</td>
<td>30,64, 71,72</td>
</tr>
<tr>
<td>miR-34</td>
<td>Loss in pancreatic, colon, breast and liver cancers</td>
<td>Induces apoptosis</td>
<td>CDK4, CDK6, cyclin E2, EZF3 and MET</td>
<td>TS</td>
<td>56–58</td>
</tr>
<tr>
<td>miR-145</td>
<td>Loss in breast cancer</td>
<td>Inhibits proliferation and induces apoptosis of breast cancer cells</td>
<td>ERG</td>
<td>TS</td>
<td>31</td>
</tr>
<tr>
<td>miR-221 and miR-222</td>
<td>Loss in erythroblastic leukaemia</td>
<td>Inhibits proliferation in erythroblasts</td>
<td>KIT</td>
<td>TS</td>
<td>30</td>
</tr>
<tr>
<td>miR-221 and miR-222</td>
<td>Overexpression in aggressive CLL, thyroid carcinoma and hepatocellular carcinoma</td>
<td>Promotes cell proliferation and inhibits apoptosis in various solid malignancies</td>
<td>p27, p57, PTEN and TIMP3</td>
<td>ONC</td>
<td>43,51,73</td>
</tr>
<tr>
<td>miR-155</td>
<td>Upregulated in aggressive CLL, Burkitt’s lymphoma and lung, breast and colon cancers</td>
<td>Induces cell proliferation and leukaemia or lymphoma in mice</td>
<td>MAF and SHIP1</td>
<td>ONC</td>
<td>32–34, 36,37</td>
</tr>
<tr>
<td>miR-17–92 cluster</td>
<td>Upregulated in lymphomas and in breast, lung, colon, stomach and pancreatic cancers</td>
<td>Induces proliferation</td>
<td>E2F1, BIM and PTEN</td>
<td>ONC</td>
<td>19,34,35, 40,41</td>
</tr>
<tr>
<td>miR-21</td>
<td>Upregulated in glioblastomas, AML (11q23), aggressive CLL and breast, colon, pancreatic, lung, prostate, liver and stomach cancers</td>
<td>Induces apoptosis and increases tumorigenicity</td>
<td>PTEN, PDCD4, TPM1 and TIMP3</td>
<td>ONC</td>
<td>31,37–39, 44–50</td>
</tr>
<tr>
<td>miR-372 and miR-373</td>
<td>Upregulated in testicular tumours</td>
<td>Promotes tumorigenicity in cooperation with RAS</td>
<td>LATS2</td>
<td>ONC</td>
<td>74</td>
</tr>
</tbody>
</table>

AMl, acute myeloid leukaemia; BCL2, B cell leukaemia/lymphoma 2; BIM, Bcl2-interacting mediator of cell death; CLL, chronic lymphocytic leukaemia; DNMT, DNA methyltransferase; HMGA2, high mobility group AT-hook 2; LATS2, large tumour suppressor homologue 2; MCL1, myeloid cell leukaemia sequence 1; MDS, myelodysplastic syndrome; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; SHIP1, SH2 domain-containing inositol-5’-phosphatase 1; TCL1, T cell lymphoma breakpoint 1; TIMP3, tissue inhibitor of metalloproteinases 3; TPM1, tropomyosin 1; WT1, Wilms tumour 1.
Dysregulation of miRNA expression in human leukemia, inspiring numerous explorations of the functional role of miRNAs in leukemogenesis.

<table>
<thead>
<tr>
<th>Poor prognosis</th>
<th>Favorable prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>miR-15a/16-1</strong>, miR-17/92, miR-21, miR-148, miR-155, miR-222</td>
<td><strong>miR-29, miR-34, miR-132, miR-150, miR-181b, miR-212, miR-223, miR-650, miR-708</strong></td>
</tr>
<tr>
<td>miR-26a, miR-28c, miR-130b, miR-146a, miR-148, miR-362-5p</td>
<td><strong>miR-10a, miR-23a, miR-17-92, miR-30e, miR-130a, miR-150, miR-199b, miR-203, miR-217, miR-318, miR-320, miR-328, miR-451</strong></td>
</tr>
<tr>
<td>let-7a, let-7i, miR-7, miR-8, miR-16, miR-33, miR-92a, miR-100, miR-142-3p, miR-146a, miR-181a/c, miR-193a, miR-198, miR-210, miR-215, miR-216, miR-335, miR-369-5p, miR-496, miR-518d, miR-599, miR-633, miR-1290</td>
<td><strong>miR-10a, miR-18a, miR-27a, miR-124a,miR-126, miR-128b, miR-134, miR-150, miR-151-5p, miR-191, miR-214, miR-221, miR-222, miR-223, miR-342, miR-345,miR-451, miR-454, miR-484, miR-486, miR-487, miR-561a,miR-572, miR-580, miR-624, miR-627, miR-708, miR-709</strong></td>
</tr>
<tr>
<td>let-7a-3, miR-9-5p, miR-26a,miR-29b,miR-29c, miR-34a (TP53 unaltered), miR-124, miR-124-1, miR-126, miR-128-1, miR-146a, miR-155, miR-155-5p, miR-181b,miR-181b-5p, miR-188-5p, miR-191, miR-194, miR-196b, miR-199a, miR-210, miR-219-5p, miR-220a, miR-320, miR-331, miR-335, miR-375, miR-378, miR-644, miR-3151</td>
<td><strong>let-7a-2-3p, miR-10a, miR-20a, miR-25, miR-29a, miR-29b, miR-34a (TP53 biallelic altered), miR-96, miR-135a, miR-142, miR-150, miR-181, miR-203, miR-204, miR-212, miR-409-3p</strong></td>
</tr>
<tr>
<td>miR-130b, miR-155</td>
<td><strong>miR-126, miR-145, miR-223</strong></td>
</tr>
</tbody>
</table>

The figure represents a summary of miRNAs associated with a poor or a favorable prognosis in CLL, CML, ALL, AML and ATL. Highlighted in red and green are the miRNAs that are found most frequently associated with an unfavorable or favorable outcome, respectively, across different human leukemias.
MicroRNA investigation methods

**A**

- Expression data for differentially expressed mRNA (Genes)
- Expression data for differentially expressed miRNAs

Identifying miRNA-target gene pairs and calculating correlation index between the miRNA-gene pair by MAGIA tool.

miRNA-gene pairs with a R value of ≤ -0.4 has been filtered

Generating the network in Cytoscape with enriched 152 genes and 17 miRNA

**B**

Graphical representation of miRNA-gene interaction network with Log FC values.
MiR-29 family

(Hwang et al., 2007; Garzon et al., 2009a).
miR-29 family members target multiple genes to mediate their biologic effects.
Epigenetic and DNA methyltransferase

• Epigenetic is defined as “The changes in gene expression that are not due to any alteration in the DNA sequence”.

• In humans, DNA is methylated by three enzymes, DNA methyltransferase 1, 3a, and 3b (DNMT1, DNMT3a, DNMT3b).
  • **DNMT3a and 3b**: are the *de novo* methyltransferases that set up DNA methylation patterns early in development.
  • **DNMT1**: is the maintenance methyltransferase that is responsible for copying DNA methylation patterns to the daughter strands during DNA replication.

• DNA methylation is important in:
  • Transcriptional gene silencing
  • Maintain genome stability
  • Embryonic development
  • Genomic imprinting
  • X chromosome inactivation (females)
DNMT3A mutation in hematological malignancies

• In 2010, *DNMT3A* mutation was first recognized in association with three groups in acute myeloid leukemia (AML), revealing changes in mutation frequencies for up to 22%.

• *DNMT3A* mutations have been reported in several hematological disorders. These mutations are recurrent in AML patients and related to poor event-free and overall survival.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3 (ITD, TKD)</td>
<td>37 (30, 7)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>24</td>
</tr>
<tr>
<td>NPM1</td>
<td>24</td>
</tr>
<tr>
<td>KIT</td>
<td>14</td>
</tr>
<tr>
<td>TET2</td>
<td>10</td>
</tr>
<tr>
<td>WT1</td>
<td>10</td>
</tr>
<tr>
<td>CEBPA</td>
<td>10</td>
</tr>
<tr>
<td>NRAS</td>
<td>10</td>
</tr>
<tr>
<td>IDH2</td>
<td>8</td>
</tr>
<tr>
<td>IDH1</td>
<td>6</td>
</tr>
<tr>
<td>ASXL1</td>
<td>4</td>
</tr>
<tr>
<td>KRAS</td>
<td>2.5</td>
</tr>
<tr>
<td>PHF6</td>
<td>2.5</td>
</tr>
<tr>
<td>RUNX1</td>
<td>5</td>
</tr>
<tr>
<td>PTEN</td>
<td>1.5</td>
</tr>
<tr>
<td>TP53</td>
<td>2</td>
</tr>
<tr>
<td>MLL</td>
<td>10</td>
</tr>
</tbody>
</table>

Patel et al. NEJM 2012
miR-29A and Epigenetic modulation through DNMT3A

• Deregulation of microRNA-29 (miR-29a/b) family and epigenetic modulating genes as DNMT3a have been associated with the pathogenesis of myeloid leukemia.
• The down-regulation of *miR-29b* is thought to promote DNA hypermethylation in AML since *miR-29b* can directly target *DNMT3A, DNMT3B*, and DNMT1 via *Sp1*

(Langemeijer et al., 2009; Tefferi et al., 2009a,b,c; Patel et al., 2012)
Research Aims..

• Study the expression of *miR-29a* and *DNMT3A* as a signature for chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) patients.

• Address the correlation of miRN-29a and DNMT3A in CML patients with BCR/ABL in CML patients.

• The implications of this investigation on the development of miRNA-directed therapies in leukemia.

• The role of *miR-29* in regulating epigenetic modifiers, cellular proliferation, apoptosis, and hematopoietic differentiation, and the role of these functional changes in myeloid pathogenesis.
Work plan

Patients diagnosed with myeloid leukemia ➔ RNA extraction

miR-29a expression ➔ DNMT3A expression

Examine the expression of miRNA and target genes in patient samples
Samples were collected from King AbdulAziz University Hospital
Age: The study includes pediatric and adult patients
Patients were selected based on the final diagnosis after all laboratory investigation completed.
Result: The Correlation between miR-29a and the target gene DNMT3A in AML and CML patients

![Bar graph showing the correlation between miR-29a and DNMT3A in Control, AML, and CML patients.](image-url)
## MiR-29a/ DNMT3A expression

### miR-29a expression

<table>
<thead>
<tr>
<th></th>
<th>Upregulated</th>
<th>Down regulated</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>27.27%</td>
<td>54.5%</td>
<td>18.18%</td>
</tr>
<tr>
<td>CML</td>
<td>27.27%</td>
<td>45.4%</td>
<td>27.27%</td>
</tr>
</tbody>
</table>

### DNMT3A expression

<table>
<thead>
<tr>
<th></th>
<th>Upregulated</th>
<th>Down regulated</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>0</td>
<td>81.8%</td>
<td>18.18%</td>
</tr>
<tr>
<td>CML</td>
<td>0</td>
<td>72.7%</td>
<td>27.27%</td>
</tr>
</tbody>
</table>
miR-29A and DNMT3A expression in AML patients

The chart shows the relative quantification (RQ) of miR-29a, DNMT3A, and control in AML patients. The y-axis represents RQ values, and the x-axis indicates different patient samples. The bars indicate the expression levels with error bars, and the significance levels are marked with asterisks (***). The control group is represented by blue bars, miR-29a by cyan bars, and DNMT3A by gray bars.
miR-29A and DNMT3A expression in CML patients

![Bar graph showing expression levels of miR-29A and DNMT3A in CML patients.](image)

**Legend:**
- miR-29a
- DNMT3A
- Control

**Sample Groups:**
- Ph- Ph- Ph+ Ph+ Ph+ Ph+ Ph+ Ph+ Ph+ Ph+ Ph+ Ph+

*Presenter’s work*
The correlation between miR-29a expression and CML patients with BCR/ABL fusion

Molecular Cancer

Short communication

MicroRNA expression profiling in Imatinib-resistant Chronic Myeloid Leukemia patients without clinically significant ABL1-mutations

Edurne San José-Enériz1, José Román-Gómez2, Antonio Jiménez-Velasco3, Leire Garate1, Vanesa Martin2, Lucia Cordeu1, Amaia Vilas-Zornoza1, Paula Rodríguez-Otero1, María José Calasanz4, Felipe Prósper1 and Xabier Agirre*1

regulated in cluding miR-29a in resistant CML patients.
Why the Expression is Low in the Majority of the Cases?

- Mutation in miR-29A
- Mutation in DNMT3A
- Global hypomethylation
- Other
- Treatment
DNMT3A sequencing for R882H/S on exons 23

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0026906
NGS Sequencing for DNMT3A

• 48 selected exons in 20 genes found to be commonly mutated in AML.

• These genes to be associated with myelodysplastic syndromes, myeloproliferative neoplasms.

• Sequencing using HiSeq/MiSeq.

• Rapid analysis of raw sequence data for known/predicted pathogenic variants.
Epigenetic Inhibitors as Cancer Therapies

Current States of Epigenetic Targets for Inhibitors

<table>
<thead>
<tr>
<th>In development</th>
<th>Preclinical</th>
<th>Clinical trials</th>
<th>FDA approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAT; KMT; RMT; KDM; Chromatin readers</td>
<td>CBP/EP300; DOT1L1; EZH2; KDM1A</td>
<td>DNMT; HDAC; JAK2; Aurora, PARP; BET (BRD2/3/4)</td>
<td>DNMT (Azacitidine and Decitabine); HDAC (Vorinostat and Romidepsin); JAK2 (Ruxolitinib)</td>
</tr>
</tbody>
</table>
miR-29a as a therapeutic target

• A role for microRNA expression as a prognostic factor in various tumors has only been recently described.
• While no enough information is available regarding their involvement in the response to chemotherapy.
• Recent studies suggest that miR-15b and miR-16, and miR-27a and miR-451 modulate multidrug resistance by target-ing BCL2 and MDR1, respectively.
• Research on mice, miR-29a oligonucleotide mimics recapitulate the effects of hypomethylating agents, 5-azacytidine and decitabine, by demethylating the promoters of tumor suppressors estrogen.
Transplanting mice with HSPCs overexpressing miR-29a results in increased myeloid and reduced lymphoid chimerism 8–12 weeks post-transplant, and is accompanied by splenomegaly as well as megakaryocytic and granulocytic hyperplasia in the bone marrow and spleen, consistent with a myeloproliferative phenotype.

Combination regimens including miR-29 with cytarabine and/or daunorubicin could also help elucidate its therapeutic efficacy as a stimulant of myeloid differentiation in myeloid leukemias.
Summary

Finally, data demonstrates that...

*miR-29a* is critical for targeting BCR/ABL1 through its regulation of *DNMT3a*.

Thus, the use of the epigenetic effect via micro RNA or methylation regulated genes may yield clinically useful biomarkers in hematopoietic malignancies.
Acknowledgment

Dr. Aisha Elaimi
Prof. Mohammed Al Qahtani
Prof. Adel Abuzenadah
Prof. Adeel chudary
Prof. Mamdouh Qari

Ms. Manal Shabaad
Ms. Zinab Allal
Ms. Abrar Alqahtani
Ms. Hanadi Qudaih
Molecular genetic staff
Cytogenetic staff

Prof. Faten Alsayes
Dr. Abeer Hussein
Dr. Maha badawi

MS.c students
Ms. Reham Sultan
Mr. Anas Oun