The Role of microRNA in Treatment of Chemo-resistant Breast Cancer

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Breast cancer is the 2nd most common cause of cancer deaths.

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Mortality</th>
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<tr>
<td><strong>Women 774,370</strong></td>
<td><strong>Women 271,520</strong></td>
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<tr>
<td>30% Breast</td>
<td>26% Lung &amp; bronchus (40,728)</td>
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<td>14% Lung &amp; bronchus</td>
<td>9% Colon &amp; rectum</td>
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<td>9% Colon &amp; rectum</td>
<td>7% Pancreas</td>
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<td>6% Uterine corpus</td>
<td>6% Ovary</td>
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<td>5% Thyroid</td>
<td>4% Non-Hodgkin lymphoma</td>
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<td>4% Non-Hodgkin lymphoma</td>
<td>3% Leukemia</td>
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<td>4% Melanoma of skin</td>
<td>3% Uterine corpus</td>
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<td>3% Kidney &amp; renal pelvis</td>
<td>2% Liver &amp; intrahepatic bile duct</td>
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<td>3% Ovary</td>
<td>2% Brain/Other nervous system</td>
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<td>3% Pancreas</td>
<td>23% All other sites</td>
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<td>16% All Other Sites</td>
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</table>

Source: American Cancer Society, 2011.
Drug resistance accounts for treatment failure in more than 90% of patients with metastatic breast cancer.

**Preferred Agents**
- Anthracyclines
  - Doxorubicin
  - Epirubicin
- Taxanes
  - Paclitaxel
  - Docetaxel
- Anti-metabolites
  - Gemcitabine
  - Vinorelbine
  - Cyclophosphamide
  - Etoposide
  - Fluorouracil Cl

**Other Regimens: endocrine therapy**
- Tamoxifen
- Aromatase inhibitors

**First-line Agents for Her2+ Disease**
Trastuzumab with
- Paclitaxel
- Docetaxel
- Vinorelbine
- lapatinib

**Bottom line**
Overcoming mechanisms of resistance is crucial for the effective management of breast cancer, particularly once the disease has metastasized.
Drug Resistance in Breast Cancers

- Probable causes of cancer-specific drug resistance:
  - Random drug-induced mutational events
  - Epigenetic alteration of gene function
  - Karyotypic changes

- Convincing evidence for association of epigenetic events with increased resistance of cancer cells to chemotherapeutic agents

- miRNA and drug resistance: increased sensitivity of breast cancer patients to Anthracycline-based chemotherapy may be related to deletion of 11q, a region containing miR-125b
Why study microRNA?
microRNA biogenesis and function

MicroRNAs: small but mighty
MicroRNA & Cancer

- 50% of human miRNAs are associated with fragile sites on different chromosome that are associated with cancer

- miR-125-1 on 11q24: deleted in breast, lung, ovarian, and cervical cancer
- miR-15a and 16-1 on 13q14: deleted in 65% of B cell-CLL, 20-40% multiple myelomas

- Strong correlation between abrogated expression of miRNA and oncogenesis

- miR-143 and 145 significantly reduced in colorectal tumors

- miR-19 amplified on lymphoma: if inactivating it, it impaires the growth of lymphoma

- HMGA2 and miRNA 763 regulation of oncogene as tumor suppressor gene. Let 7 reduced in HMGA2 amplified (regulator)

- **miRs could be both oncogenic and tumor-suppressing**
What is so special about microRNA?

- Manipulation of miRNA levels represents the next generation of therapeutic strategies. Current cancer therapies face major hurdles, including insufficient disruption of oncogenic pathways, drug-induced toxicity and acquired drug resistance.

- MicroRNAs function through subtle regulation of a large number of factors. This is in contrast to current therapies that target individual genes and gene products.

- MicroRNA: critical role in cancer + unique mechanism of action = new class of targeted therapeutics
What is so special about microRNA?

- Two distinct therapeutic modalities:
  - miRNAs that acquire a gain of function can be inhibited with miRNA inhibitor
  - miRNAs that exhibit a loss of function can be restored with miRNA mimics.

- miRNA mimics have the same sequence as naturally occurring miRNAs and target the same genes as the endogenous miRNAs, making off-target effects unlikely.

- * miRNAs are normal constituents of healthy cells, the introduction of miRNA into healthy cells is not likely to result in toxicity and tumor-specific targeting is less likely to be necessary.
What is so special about microRNA?

- miRNA levels can be altered by systemic delivery: target-binding affinity facilitates the development of much shorter miRNA mimic/inhibitor-based drugs, thereby circumventing the need for complex delivery vehicles.

- Feasibility of successful delivery to target tissue without degradation by biofluids.
  - Original Article

- Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA.

**Bottom Line**

Stable and easy to deliver
Presence or absence of miRNA

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miRNA Expression or not
BC cell lines show a wide range of drug sensitivity

- paclitaxel
- trastuzumab
- gemcitabine
- etoposide
- docetaxel
- tamoxifen
- doxorubicin

Log\(_{10}(ED_{50})\)

- Sensitive
- Intermediate
- Resistant

ED\(_{50}\)
High throughput approach to identify drug sensitizers (de) miRNAs.

Transfection

Drug Exposure

End Point Assay

Candidate Selection

Validation

Similar drugs [nM]

Drugs with different mechanisms [nM]

Measure viability ratio: mean_{paclitaxel}/mean_{carrier}

FDR ≤ 0.05, tail of ratio distribution
miRNA/s Regulate/s Drug Response in Breast Cancers

miRNA inhibitor library

- Tam v.r. < 0.85: 43
- Tmab v.r. < 0.85: 28
- Pact v.r. < 0.85: 47

$P < 0.05$
- 10
- 12
- 19

974

Cell viability

- miR-9x1: 0.012
- miR-2x4: 0.013

Tam
- carrier: 0.0
- drug: 0.2

Pac
- carrier: 0.016
- drug: 0.016

Tmab
- carrier: 0.013
- drug: 0.013
Significantly lower expression of miR-2x4 in relapsed metastatic breast cancers
miR-2x4 acts as a potent tumor suppressor
Systemic delivery approaches

1. Lipid-based formulation

2. PLGA Nanoparticles
   - miRNA/Drugs
   - Targeting Ligand
Systemic delivery of miR-2x4 eliminates tumor growth

Dose: 1 mg/kg B.W
Every 6-days for 30 days

60 days after the last dose

Neg. Control
miR-Rx4
Neg. Control
miR-2x4

N= 20
N= 20
MicroRNAs: safe and viable therapeutic agent

Bottom Line

No liver toxicity
miR-2x4 putative gene targets

P-glycoprotein binds to ezrin at amino acid residues 149 through 242 in the ferm domain and plays a key role in the multi-drug resistance of human osteosarcoma.

A Novel Role for BDNF-TrkB in the Regulation of Chemotherapy Resistance in Head and Neck Squamous Cell Carcinoma.
miR-2x4 putative gene targets

**Ezrin**
- is a member of ezrin/radixin/moesin (ERM) family
- promotes cytoskeletal reorganization by coupling functions of the plasma membrane and actin cytoskeleton of the cell
- has been implicated in the tumor growth and metastasis of several adult and pediatric tumors
- strong multifocal expression has been associated with poor prognosis of several tumors

**BDNF**
- plays a critical role in the development of nervous system by binding and subsequently activating the tyrosine kinase receptor TrkB
- BDNF/TrkB pathway promotes proliferation, angiogenesis and tumor invasiveness
- overexpression of BDNF/TrkB has been implicated in poor prognosis of several solid tumors

What determine effective miRNA targeting

You may target, but not down regulated. How we can see effective targeting?
Inverse expression correlation between miR-2x4 and its target genes
Bona fide targets of miR-2x4

**BDNF 3'UTR**

*Control*  *2x4 Inhibitor*  *2x4 mimic*

**BDNF**

*Actin*

**mut BDNF 3'UTR**

**mut-Ezrin 3'UTR**

*Control*  *2x4 mimic*  *2x4 Inhibitor*

**Ezrin**

*Tubulin*
miR-2x4 mechanism of action

Control  miR-2x4

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>miR-2x4</th>
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<td>pAKT</td>
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<td>p4E-BP1</td>
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<td>β-Actin</td>
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mechanisms of resistance to trastuzumab: presence of upregulation of HER2 downstream signaling pathways.

miR-2x4 mechanisms of action: Paclitaxel resistance

Paclitaxel resistance

- Altered tubulin isotype expression
- Tubulin mutation
- Alteration to tubulin through post-translational modification
- Altered expression of MAPs

Stathmin (STMN1)

- microtubule destabilizer that can bind to tubulin dimers and stimulate microtubule destabilization
- STMN1 mRNA levels are upregulated in breast cancer patients with more aggressive disease
What’s Next

Treatment

* Detailed pharmacology and toxicity studies:

* Phase I clinical trial:

Diagnostic

Screening of circulating miR-2x4 in blood samples of triple negative metastatic breast cancer patients

More

Screening of small compound library to find hits for miR-2x4 targets
Why study microRNA?
Summary

1. miRNAs are important players in regulating drug sensitivity/resistance in cancers

2. Identification of miRNAs involvement in drug sensitivity/resistance may be exploited for future therapeutic interventions

3. Insight into the mechanism of breast cancer growth and progression

4. Prognostic marker to identify patients who might benefit most from specific drug treatment

5. Taken together, the identification of miRNAs that mediate chemoresistance could lead to more efficient selection of treatments at the patient level and an improvement in response rates at the population level.
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