Negative Regulation of c-MYC Oncogenic Activity Through the Tumor Suppressor PP2A-B56α

Mahnaz Janghorban, PhD
Dr. Rosalie Sears lab

2/8/2015
Zanjan University

Content

1. Background (keywords: c-Myc, PP2A, SET, CIP2A)
2. Methods and results
3. Summary
Translational research: from bench to bedside

Cancer is loss of normal cell growth control

Adopted from Anke Sparmann et al., 2006
Central Dogma

Where does it go wrong?
Onco-proteins increase cell growth

Tumor suppressors stall cell growth
Kinases and phosphatases are deregulated in cancer

Breast cancer is a second leading cause of death in female

Leading New Cancer Cases and Deaths – 2013 Estimates

<table>
<thead>
<tr>
<th>Estimated New Cases*</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>238,599 (28%)</td>
<td>321,830 (26%)</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>118,080 (14%)</td>
<td>131,600 (11%)</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>73,680 (9%)</td>
<td>73,680 (9%)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>54,610 (6%)</td>
<td>68,000 (6%)</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>45,060 (5%)</td>
<td>50,000 (5%)</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,433 (5%)</td>
<td>40,433 (5%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>37,600 (4%)</td>
<td>37,600 (4%)</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>29,620 (3%)</td>
<td>29,620 (3%)</td>
</tr>
<tr>
<td>leukemia</td>
<td>27,880 (3%)</td>
<td>27,880 (3%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,740 (3%)</td>
<td>22,740 (3%)</td>
</tr>
<tr>
<td>All sites</td>
<td>854,790 (100%)</td>
<td>854,790 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Estimated Deaths</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>110,110 (14%)</td>
<td>110,110 (14%)</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>69,140 (9%)</td>
<td>69,140 (9%)</td>
</tr>
<tr>
<td>Urinary corpus</td>
<td>49,560 (6%)</td>
<td>49,560 (6%)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>45,310 (6%)</td>
<td>45,310 (6%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>33,140 (4%)</td>
<td>33,140 (4%)</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>24,720 (3%)</td>
<td>24,720 (3%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,480 (3%)</td>
<td>22,480 (3%)</td>
</tr>
<tr>
<td>Stomach</td>
<td>12,220 (4%)</td>
<td>12,220 (4%)</td>
</tr>
<tr>
<td>Bladder</td>
<td>10,820 (4%)</td>
<td>10,820 (4%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,590 (3%)</td>
<td>10,590 (3%)</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,780 (3%)</td>
<td>8,780 (3%)</td>
</tr>
<tr>
<td>All sites</td>
<td>306,920 (100%)</td>
<td>306,920 (100%)</td>
</tr>
</tbody>
</table>

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

©2013, American Cancer Society, Inc., Surveillance Research
Breast cancer is classified into various subtypes based on differential immunohistochemical staining for ER, PR, HER2.

Overview of project:
**Negative Regulation of c-MYC Oncogenic Activity through the Tumor Suppressor PP2A-B56α**

- MYC is an onco-protein
- PP2A-B56α is a tumor suppressor
- SET and CIP2A are onco-protein

How can we target this pathway?
Can we “Activate PP2A and Inhibit MYC”? 

William B. Coleman, 2010
MYC is downstream of many growth factor signals

MYC is a transcription factor that controls cell functions
MYC overexpression results in tumor formation

- Low levels of MYC: normal
- High levels of MYC: cancer

MYC activation regulates pathways required for tumor formation

- Immune Activation
- Cellular Senescence
- Angiogenesis
- Metabolic Reprogramming
- Autonomous Proliferation

Yulin Li et al., 2014
MYC and breast cancer

MYC is overexpressed in 45% of breast cancers:

- gene amplification
- transcriptional regulation and mRNA stabilization
- protein overexpression and stabilization

MYC protein is regulated by phosphorylation at 2 sites

- Serine 62 phosphorylation (pS62) stabilizes MYC in response to mitogens or Ras signaling
- Threonine 58 phosphorylation (pT58) destabilizes MYC and requires prior Serine 62 phosphorylation.
- Mutations at or around T58 occur in Burkitt's lymphomas >>>> stable MYC

Sears et al., 2000
Papas and Lautenberger, 1985
Bhatia et al., 1993
MYC protein is stabilized in cancer

Increased PS62 and decreased PT58 of Myc in breast cancer cell lines

A. Increased PS62 and decreased PT58 of Myc in breast cancer cell lines

Xiaoli Zhang, 2009
MYC is stabilized by increased pS62-MYC in human breast tumors

Zhang et al., PNAS 2012

Reversing pS62-MYC by PP2A

growth stimulatory signals
(Receptor Tyrosine Kinase activation)

MYC
CDK, ERK
Ras
PI3K

MYC
GSK3β

MYC

growth stimulatory signals
(Receptor Tyrosine Kinase activation)

MYC
CDK, ERK
Ras
PI3K

PP2A-B56α
PP2A is a heterotrimeric serine/theronine phosphatase in cell

Protein Phosphatase 2A=PP2A

PP2A regulates 30-50% of cellular phosphatase activity
PP2A inhibition is an important step in cell transformation

- Simian virus 40 (SV40)
- SV40 LT inhibits RB and P53

SV40 ST displaces multiple regulatory B subunits from the AC core

- siRNA targeting different B subunits showed that:
  - B56α, B56γ, and PR72/PR130 are involved in human cell transformation (Hahn, 2010)
Cellular inhibitor of PP2A

- CIP2A (Cancerous Inhibitor of PP2A)
- SET (I2PP2A-Inhibitor 2 of PP2A)

PP2A in inhibited by cellular inhibitors

C. Hahn, 2008
Targeting MYC through enhancing its degradation pathway in breast cancers

Overall Hypothesis

1. PP2A-B56α reduces MYC oncogenic activity
2. SET and CIP2A increase MYC oncogenic activity
3. Activating PP2A by targeting SET (or CIP2A) can serve as a good therapeutic approach for breast cancers
Targeting MYC by antagonizing PP2A inhibitors in breast cancer

1- Are SET and CIP2A upregulated in breast cancer?

2- How can we activate PP2A in tumors?

SET and CIP2A are frequently overexpressed in human breast cancer

Janghorban et al., PNAS 2014
Increased SET, CIP2A, and pS62-MYC protein levels occur in patient samples

Increased SET, CIP2A, and pS62-MYC protein levels occur in breast cancer cell lines

MCF10A:
Immortalized
Nontransformed
"normal"

Janghorban et al., PNAS 2014
SET or CIP2A knockdown decreases growth of breast cancer cell lines in vitro

Soft agar assay testing anchorage-independent cell growth

SET or CIP2A knockdown decreases oncogenic potential on breast cancer cells

Janghorban et al., PNAS 2014
Targeting MYC by antagonizing PP2A inhibitors in breast cancer

1- Are SET and CIP2A upregulated in breast cancer?

- SET and CIP2A are overexpressed in breast cancer
- Knocking down SET or CIP2A decreases tumor cell survival

2- How can we activate PP2A in tumors?

**OP449 Peptides**

- OP449 developed by Oncotide
- Binds to SET
- Reactivates PP2A

MP Vitek et al., 2011
OP449 decreases the growth of breast cancer cells and induces apoptosis

Janghorban et al., PNAS 2014

OP449 decreases the growth of cells from patient samples

Janghorban et al., PNAS 2014
OP449 decreases the oncogenic potential of cells in vitro

Janghorban et al., PNAS 2014

SET or CIP2A knockdown decreases S62-phosphorylated MYC

Janghorban et al., PNAS 2014
**OP449 decreases pS62-MYC**

![Graph showing the effect of OP449 on pS62-MYC levels in different cell lines (MDA-MB-231, HCC38, MDA-MB-436, MDA-MB-468).](image)

**OP449 decreases MYC transcriptional activity**

![Flowchart showing the process of Chromatin Immunoprecipitation (ChIP) and the quantification of MYC transcriptional activity.](image)

Janghorban et al., PNAS 2014
OP449 suppresses breast tumor growth

![Graphs showing tumor growth inhibition](image)

Janghorban et al., PNAS 2014

OP449 increases PP2A activity in tumors

![Graphs showing PP2A activity](image)

Janghorban et al., PNAS 2014
Summary

- SET and CIP2A are overexpressed in breast cancers and breast cancer cell lines
- SET or CIP2A knockdown decreases tumorigenic potential of breast cancer cell line
- SET or CIP2A knockdown decreases in phosphoS62-MYC and MYC transcriptional activity
- OP449 treatment inhibits growth of BCC in vitro and in vivo and induces apoptosis

PP2A activator kills cancer cells
Thank You!