Novel role of NMI (N-myc interactor) in breast cancer with mitochondrial dysfunction and as a potential target for cancer therapy

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I have no conflict of interest to declare
NMI (N-myc interactor)

Binding to TFs functions are still unknown.
**NMI (N-myc interactor) in cancer**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>NMI expression</th>
<th>Findings</th>
<th>Study type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Lost with increasing stage</td>
<td>Inhibits EMT, lung metastasis, and tumor growth</td>
<td>Cell lines, xenograft, Patient samples</td>
<td>Fillmore et al., 2009, Devine et al., 2014</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>Increased expression with grade</td>
<td>Promotes growth by regulating G1/S transition</td>
<td>Patient samples, xenograft, cell lines (U251,U87)</td>
<td>Meng et al., 2014</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Unknown</td>
<td>Binds N-myc</td>
<td>Cell line (kelly neuroblastoma)</td>
<td>Bannasch et al., 1999</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Unknown</td>
<td>Binds IFP35, induced by IFNα</td>
<td>Cell line (HT-29)</td>
<td>Zhou et al., 2000</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Unknown</td>
<td>Induced by IFN γ</td>
<td>Cell line (Hela)</td>
<td>Li et al., 2012</td>
</tr>
<tr>
<td>Acute T cell leukemia</td>
<td>Unknown</td>
<td>Induced by IFN γ binds with IFP35</td>
<td>Cell line (Jurkat)</td>
<td>Chen et al., 2000, Nagel et al., 2011</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>SNPs detected</td>
<td>SNPs are associated with reduced risk of ovarian cancer</td>
<td>Patient samples</td>
<td>Quaye et al., 2009</td>
</tr>
</tbody>
</table>

*Hawley et al., J. Cancer (2016)*

**The biological roles are all diverse depending on tumors**
Previous results

NMI overexpression is related to poor response to chemotherapy in breast cancer

Suppression of NMI increases sensitivity to chemotherapy

Cell Viability Assay (CellTiter-Glo Luminiscenct)
NMI knock-down model

<table>
<thead>
<tr>
<th></th>
<th>MDA-MB-231</th>
<th>BT20</th>
<th>MDA-MB-468</th>
</tr>
</thead>
<tbody>
<tr>
<td>siNMI</td>
<td>0.75</td>
<td>0.85</td>
<td>0.90</td>
</tr>
<tr>
<td>siControl</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>


1.275

P = 0.031

nCR (n = 78) CR (n = 78)

Number of spheroids

Oncogene
Study design

Mechanism analysis

Step 1. In vitro and in vivo tests
- siRNA gene (NMI)
- 3D cell proliferation assay
- Cell-titer Glo assay
- Seahorse mitochondrial function test
- Immunofluorescence
- Electronic microscope
- Xenograft mouse model

Proteomics

Step 2. Sample preparation and MS analysis
- Sample preparation
  - Xylene, Acetone PPT, modified FASP, Desalting
  - Ostsiewicz et al. 2010
- LC-MS/MS (Orbitrap Q Exactive-plus, Thermo Fisher)
- MaxQuant & Perseus

Step 3. Data analysis
- Pathway enrichment
  - Gene ontology (David, ToppGene)
- Network analysis
  - String, Cytoscape
NMI enhances 3-D cell proliferation

3D morphogenesis (on Matrigel)

<table>
<thead>
<tr>
<th>MCF10A (Normal-like)</th>
<th>Knock-down model</th>
<th>Overexpression model</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLOC</td>
<td>2D proliferation</td>
<td>2D proliferation</td>
</tr>
<tr>
<td>NMI (overexpression)</td>
<td>3D proliferation</td>
<td>3D proliferation</td>
</tr>
</tbody>
</table>

Knock-down model:
- MDA-MB-231
- MDA-MB-468
- BT20

Overexpression model:
- pLOC
- NMI

**Figure A**
- A: Schematic diagram of cell growth phases:
  - Proliferative
  - Growth-arrested

**Figure B**
- Tabel showing proliferation and matrigel conditions for each cell line:
  - MDA-MB-231
  - MDA-MB-468
  - BT20
  - Matrigel
  - Matrigel + collagen mix

**Image**
- Representative images of cell proliferation and morphogenesis under different conditions.
NMI induces tumor growth and metastasis

**Tumor-initiating capacity**

- **MDA-MB-231**
  - shnon
  - shNMI

- **BT20**
  - CD24
  - CD44

**in vivo xenograft**

- **MDA-MB-231** (shnon vs. shNMI)
- **Liver** (shnon vs. shNMI)

**Graphs**

- Tumor volume (mm$^3$) vs. Days
- Tumor weight (g)

**Statistical Significance**

- p < 0.0001

**Images**

- MDA-MB-231 xenografts
- Liver with tumors
- CD44 and CD24 expression levels

**Legend**

- shnon
- shNMI
Pathways related to NMI1?

NMI gene modulation
- siRNA
- Expression vector transfection
- MDA-MB-468
- MDA-MB-231
- BT20

LC-MS/MS, proteomics
- High-pH Reversed-Phase Fractionation
- Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer

Protein identification
- PPI network analysis

Bioinformatics
- GENEONTOLOGY
- STRING
- Cytoscape
Up proteins: 197
Down proteins: 144

oxidation-reduction process
NADH metabolic process
glucose catabolic process
acyl-CoA metabolic process
ATP generation from ADP
ATP metabolic process
fatty-acyl-CoA biosynthetic process
response to oxygen levels
cellular respiration
NMI Knock down_GOCC (Cellular Component)

1. mitochondrion
2. mitochondrial matrix
3. mitochondrial part
4. cytoplasmic vesicle part
5. mitochondrial envelope
6. whole membrane
7. mitochondrial membrane
8. sperm fibrous sheath
9. tricarboxylic acid cycle enzyme complex
10. myelin sheath
11. endosome membrane
12. organelle envelope

Hit Count in Query List

Log10(p-value)

PPI network analysis
NMI drives oxidative phosphorylation (OXPHOS)

Seahorse -MMP

knock-down model

Mitochondria!

Cell metabolism 2016

GoBP) oxidative phosphorylation process

EM

Cytoescape

Mitochondria

ATP energy

GOBP) oxidative phosphorylation process

EM

Cytoescape

Mitochondria

ATP energy

Nearest neighbor

Cell metabolism 2016

Seahorse -MMP

knock-down model

Mitochondria!

Cytoescape

Mitochondria

ATP energy

Nearest neighbor

Cell metabolism 2016
Loss of NMI increases hydrogen peroxide ($H_2O_2$) production

Biochem. J. 2009
NMI regulates enzymes (SOD and GPX) of redox homeostasis

**SOD activity**

**GPX activity**

*J of Cell Bio. 2018*
NMI involves anti-ROS process by nuclear translocation of NRF2

Nrf2 pathway activation by reactive oxygen species (ROS)

NMI promotes nuclear translocation of NRF2

BBA (2018)

Redox enzyme production (x)
Conclusions

NMI involves anti-ROS process by nuclear translocation of NRF2

ROS

NMI

GSK3B

NFR2

AKT

nucleus

Translation

Transcription

Mitochondria

SOD

GPX

Redox homeostasis

Tumor cell survival

<Physiologic condition in Tumor cells>
Conclusions

NMI involves anti-ROS process by nuclear translocation of NRF2
Conclusions

NMI involves anti-ROS process by nuclear translocation of NRF2

- ROS
  - NMI
    - GSK3B
      - AKT
        - Transcription
        - ROS
          - NRF2
          - AKT
            - Transcription
            - nucleus
  - Mitochondria
    - SOD
    - GPX
      - Redox homeostasis
    - Tumor cell survival
    - ROS damage and tumor cell death
  - Pfizer

<Physiologic condition in Tumor cells>
<NMI loss in Tumor cells>
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