DEAD-BOX RNA HELICASE DDX20 ENHANCES YAP SUMOYLATION FOR YAP-TEAD DEPENDENCE AND STATIN SENSITIVITY IN TRIPLE NEGATIVE BREAST CANCER

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This work is supported by a grant from the National Medical Research Council, Singapore to APK: Interplay between Mevalonate and Hippo Pathways Regulates DDX20 Transcription via YAP-TEAD Complex in Triple Negative Breast Cancer. NMRC OF-IRG (2017) S$991,000.
Triple Negative Breast Cancers (TNBC)

- Estrogen Receptor -
- Progesterone Receptor -
- HER2 -

10-20%

Poor prognosis

Early age of onset

Chemotherapy ✓

Hormone Therapy ✗

Targeted Therapy ✗

The anti-cancer effects of statins

Simvastatin induces derepression of PTEN expression via NFκB to inhibit breast cancer cell growth.

Kanuqula et al., FEBS J, 2011

Statin-induced inhibition of breast cancer proliferation and invasion involves attenuation of iron transport: intermediacy of nitric oxide and antioxidant defence mechanisms.

Kanuqula et al., FEBS J, 2011

Statin induces inhibition of triple negative breast cancer via a PI3K pathway.

Yeon Hee Park et al., BBRC, 2013
**DDX20/DP103**

**Q-E-A-D** box family RNA helicase.

Motif 1

Motif 1a

Motif 1b

Motif II

Motif III

Motif IV

Motif V

Motif VI

Unwinding cancers from the D-E-A-D(H)...

**Solid tumors**

Prostate: DDX5, DHX9

Lung: DDX2, DDX3, DDX5, DHX9

Breast: DDX2, DDX3, DDX5/17, DDX10, DDX18, DDX20, DDX21, DDX24, DDX43, DHX9

Colon: DDX3, DDX6, DDX5/17, DHX32

Testicular: DHX9

CML: DDX10, DDX43

CLL: DDX10, DDX3X

ALL: DDX3, DDX5, DDX53

AML: DDX6, DDX10, DDX43


Cai W et al., J Natl Cancer Inst. 2017
Positive correlation between DDX20 expression and mevalonate pathway genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Low expression</th>
<th>High expression</th>
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<tbody>
<tr>
<td>DDX20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHCR7</td>
<td></td>
<td>0.067</td>
</tr>
<tr>
<td>SC4MOL</td>
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<tr>
<td>CYP51A1</td>
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<td>SQLE</td>
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<td>FDPS</td>
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<td>NSDHL</td>
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<td>ACAT2</td>
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<td>HMGCS1</td>
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<td>0.218</td>
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<tr>
<td>HMGCR</td>
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<td>0.222</td>
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</table>

(n=1325)
Question 1: Can DDX20 be used as a biomarker for statin sensitivity?
DDX20 expression positively correlates with statin sensitivity

Rho = -0.57
p = 0.0185

Ref:
Mori S et al., PLoS One, 2009
DDX20 is highly expressed in triple negative breast cancer cells

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
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<tbody>
<tr>
<td>MCF-10A</td>
<td></td>
<td></td>
<td>Normal Epithelial</td>
</tr>
<tr>
<td>BT474</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T47D</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BT549</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>-</td>
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</tbody>
</table>

![Graph showing relative expression of DDX20 mRNA](image)

![Image showing Western blot analysis of DDX20 and β-actin expression](image)
TNBCs are more sensitive to simvastatin than ER+.
Question 2: How does statin treatment affect DDX20 expression?
TNBCs are more sensitive to simvastatin than ER+.

**BT474**

- DDX20
- β-actin

**MDA-MB-231**

- DDX20
- β-actin
Simvastatin decreases DDX20 expression in mice
Simvastatin decreases DDX20 in Breast Cancer Patients

Pre-treatment

Post-treatment (with simvastatin)

200 X

* denotes p<0.05
Statins inhibit YAP via GGPP-RhoA.

Metabolic control of YAP and TAZ by the mevalonate pathway.

Sorrentino G et al., Nat Cell Biol, 2014
Question 4: Does YAP/TEAD regulate DDX20 transcription?
TEAD4 binds to DDX20 promoter
Simvastatin disrupts TEAD4 binding to DDX20 promoter
TEAD4/YAP regulates DDX20

![Graph showing relative expression of DDX20 mRNA](image)

- control
- siYAP

![Western blots showing DDX20, YAP, and GAPDH](image)

- GFP-YAP
  - Control
  - +dox

- DDX20
- YAP
- GAPDH

![Graph showing relative expression of DDX20 mRNA](image)

- control
- siTEAD4

- DDX20
- TEAD4
- GAPDH

![Western blots showing DDX20, YAP-5SA, YAP-5SA/S94A, and GAPDH](image)

- EV
- YAP-5SA
- YAP-5SA/S94A

- DDX20
- GAPDH
DDX17 interacts with YAP

Colocalization of YAP and p72 at low cell density
Question 5: Does DDX20 interact with YAP and regulate it?
DDX20 interacts with YAP

**IP:DDX20**

<table>
<thead>
<tr>
<th>DDX20</th>
<th>IgG</th>
<th>IP</th>
<th>Input</th>
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<tbody>
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**IP:YAP**

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</table>

**DAPI**

**DDX20**

**YAP**

**Merge**
DDX20 positively regulates TEAD/YAP activity.

**Graph 1:**
- **X-axis:** siCtrl, siDDX20
- **Y-axis:** Relative luciferase activity (% of siCtrl)
- **Legend:**
  - siCtrl: Bar graph.
  - siDDX20: Bar graph.
  - **Statistical Significance:**
    - **siCtrl to siDDX20:** Two-tailed t-test, *p* < 0.01

**Graph 2:**
- **X-axis:** Control, SMV
- **Y-axis:** Relative luciferase activity (% of Control)
- **Legend:**
  - Control: Bar graph.
  - SMV: Bar graph.
  - **Statistical Significance:**
    - **Control to SMV:** Two-tailed t-test, *p* < 0.001
DDX20 positively regulates YAP target genes

- **p<0.05**
- **p<0.01**

**YAP target genes**

- CTGF
- CYR61
- DDX20
- β-actin

**siCtrl siDDX20#1 siDDX20#2**

- Control
- SMV

**DDX20**
**CYR61**
**CTGF**
**β-actin**

* : p<0.05  
** : p<0.01
DDX20 positively regulates YAP

- Inactive
- p-YAP (Ser127)
- β-actin

Control
SMV

- DDX20
- p-YAP
- β-actin
Question 6: What is the mechanism behind YAP regulation by DDX20?
DDX20 regulates SUMOylation of YAP

*: non-specific bands
Thank You
Simvastatin downregulates DDX20 in TNBC cells in a dose-dependent manner

**MDA-MB-231**

![Graph showing relative expression of DDX20 mRNA in MDA-MB-231 cells with varying concentrations of simvastatin.](image)

**BT549**

![Graph showing relative expression of DDX20 mRNA in BT549 cells with varying concentrations of simvastatin.](image)