Role of leptin signaling in mammary tumor progression

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Leptin (1994)

Pathological processes
- Angiogenesis
- Proliferation
- Inflammation
- Anti-apoptosis

Functions:
- Regulator of energy balance/appetite
- Reproductive process
  - Ovulation
  - Endometrial receptivity
  - Embryo implantation

Cancer
- endometriosis
- diabetes
- arthritis, etc.

2008

Elevated leptin levels are associated with increased incidence, poor outcome and worse prognosis of breast cancer
**Leptin**

- Small protein (16 kDa)
- Helical cytokine
- Product of the obese gene (ob)
- Secreted mainly by adipocytes

**Leptin receptor (OB-R)**

- Several isoforms
  - OB-Rb long isoform /functional

[Diagram of leptin receptor and leptin molecule]
Signaling pathways activated by leptin

Leptin → OB-R → cancer → JAK2-STAT3 → MAPK → PI-3K

PKC, AMPK, JNK, p38 kinase

VEGF, VEGFR2, IL-1β, IL-1R tl, LIF, LIFR, Cyclin D1, Bcl-2
Hypothesis:

Disruption of leptin signaling will negatively impact mammary tumor growth by decreasing leptin-induced expression of pro-angiogenic, proliferation and anti-apoptosis factors.
Designing Leptin Peptide Receptor Antagonists (LPrA)

G-CSF

Leptin

G-CSF R

(similar to OB-R)

(similar to G-CSF)
Designing Leptin Antagonists

LPrA-1

LPrA-2

H3

H3
Structural differences between LPrA-2 and LPrA-2Sc peptides (control)

Gonzalez, Unpublished
The LPrA effects were dose dependent.

The peptides solutions (0.15-0.3 nmoles) were assayed in the CAM assay.

Experiments were repeated 20 times.

In contrast to PBS (A) and LPrA-Sc (B) (arrows), LPrA-1 (C) and LPrA-2 (D) (arrowhead) inhibit the formation of capillaries.
Mammary cancer cells express OB-R

Human MCF-7
ER+

Human MDA-MB 231
ER-

Mouse 4T1
ER+

Negative Control

OB-R

A

B

C

D

E

F
LPrA2 inhibits leptin-induced adhesion and proliferation of breast cancer cells

<table>
<thead>
<tr>
<th>Treatments</th>
<th>4T1 Cell Adhesion (%)</th>
<th>MCF-7 Cell Adhesion (%)</th>
<th>MDA-MB231 Cell Adhesion (%)</th>
<th>4T1 Cell Proliferation (%)</th>
<th>MCF-7 Cell Proliferation (%)</th>
<th>MDA-MB231 Cell Proliferation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Leptin 3nM</td>
<td>186*</td>
<td>264*</td>
<td>133*</td>
<td>170*</td>
<td>155*</td>
<td>120*</td>
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<tr>
<td>Leptin + LPA-2 300nM</td>
<td>36</td>
<td>109</td>
<td>84</td>
<td>107</td>
<td>97</td>
<td>77</td>
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<tr>
<td>Leptin + LPA-2Sc 300nM</td>
<td>175*</td>
<td>230</td>
<td>142*</td>
<td>156*</td>
<td>168*</td>
<td>122*</td>
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</tbody>
</table>

*p<0.05; n = 5
Intraperitoneal injections

**Half-life**

UNCONJUGATED 3H-wLPrA2

Half-life ~ 1 h

PEGYLATED 3H-wLPrA2

Half-life ~ 18 h
## Pharmacokinetics of PEG-3H-LPrA-2 in female mice

<table>
<thead>
<tr>
<th>Via</th>
<th>Dose (mg)</th>
<th>Dose (mg/Kg)</th>
<th>Vd liters/Kg</th>
<th>Cpo mg</th>
<th>Cpt mg</th>
<th>Kel mg/L</th>
<th>CL mg/Kg</th>
<th>T1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>12.50</td>
<td>735.294</td>
<td>54.68</td>
<td>13.446</td>
<td>0.986</td>
<td>0.362</td>
<td>0.006</td>
<td>19.1</td>
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<tr>
<td>i.v.</td>
<td>5.00</td>
<td>151.888</td>
<td>103.23</td>
<td>1.471</td>
<td>1.570</td>
<td>0.011</td>
<td>$10^{-4}$</td>
<td>59.6</td>
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<tr>
<td>i.v.</td>
<td>2.50</td>
<td>75.396</td>
<td>255.26</td>
<td>0.295</td>
<td>0.141</td>
<td>0.010</td>
<td>$3.9 \times 10^{-5}$</td>
<td>67.7</td>
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<tr>
<td>i.v.</td>
<td>1.25</td>
<td>38.320</td>
<td>408.71</td>
<td>0.093</td>
<td>0.042</td>
<td>0.010</td>
<td>$2.4 \times 10^{-5}$</td>
<td>63.1</td>
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<tr>
<td>subdermal</td>
<td>5.00</td>
<td>250</td>
<td>561.79</td>
<td>0.004</td>
<td>0.004</td>
<td>0.00008</td>
<td>$0.1 \times 10^{-7}$</td>
<td>323.8</td>
</tr>
<tr>
<td>vaginal</td>
<td>1.50</td>
<td>53.570</td>
<td>400.37</td>
<td>0.1338</td>
<td>0.025</td>
<td>0.0054</td>
<td>$2 \times 10^{-7}$</td>
<td>128.8</td>
</tr>
</tbody>
</table>
PEG-wLPrA2 treatment does not affect food intake, body weight, glucose, insulin serum levels or HOMAX-index in mice.
**In vivo studies**

**Syngeneic mouse model of MT**

- m4T1 cells
- 100 µl twice daily
- LPrAs
- 0 - 15 - 21 days
- 2nd row mammary glands
- Right nipple
- ♀ BALB/c syngeneic sisters
LPrA2 delays the onset and reduces the growth of mouse 4T1-MT in a syngeneic model.

Treatment

Chemoprevention

PEG-LPrA2 and LPrA2 treatments decrease VEGF and VEGFR2 levels

MCF-7 tumor Onset

Ovariectomized & E2 supplemented SCID mice (8-weeks old)

MCF-7 inoculation

MT detection

Controls

MT detection

PEG-wLPrA2

PEG-wLPrA2

LPrA chemoprevention significantly delays the onset of MCF-7 MT in SCID mice.
Treatment of MT-ER+ in SCID mice

MCF-7 cells

PEG-wLPrA2 or Sc

0

7

21 days 50

2nd row mammary glands
Right nipple

♀ SCID ovariectomized mice
E2/cholesterol-capsule
PEG-wLPrA2 reduces growth of MCF-7 derived tumors in SCID mice.
PEG-wLPrA2 treatment decreases the levels of VEGF/VEGFR2 in MCF-7 mammary tumors.
PEG-wLPrA2 decreases IL-1 and IL-1R Rtl levels within MCF-7 MT

*p<0.05
PEG-wLPrA2 treatment decreases CD31, VEGFR2, CD68 (TAM), IL-1R tl, VEGF, leptin and OB-R expression within MCF-7 MT.
Human MT ER-: In vivo studies

MDA-MB231 cells

PEG-wLPrA2 or Sc

-7 0

2nd row mammary glands
Right nipple

♀ SCID mice

13 28 days
PEG-wLPrA2 decreased VEGF levels in MDA-MB231 MT

![Graph showing decreased VEGF levels in MT compared to blood with PEG-wLPrA2 treatment.](chart.jpg)

**ELISA DETERMINATIONS**
PEG-wLPrA2 decreased Bcl-2 levels in MDA-MB231 MT
PEG-wLPrA2 decreased IL-1 and IL-1R tI levels in MDA-MB231 MT
LPrA2 blocks the leptin canonical signaling pathways and SOCS3 down-regulation in 4T1 cells

Leptin activated the ERK 1/2/MAPK, PI-3K/AKT1 and JAK2/STAT3 pathways

SOCS-3 was upregulated by leptin and inhibited by LPrAs

Signaling intermediates involved in the leptin-induced levels of VEGF/VEGFR2 and Cyclin D1 in 4T1 cells

Luciferase reporter constructs of mouse VEGF promoter and 5'-end deletions

Mouse mammary cancer cells:
- 4T1
- MMT
- EM6

Leptin regulation of VEGF promoter in mouse mammary cancer cells

4T1

EM6

MMT

Full-length -HRE -HRE -HRE -HRE -HRE -SP1
-HRE -AP1 -AP1 -AP1 -AP1 -AP1
-AP2 -AP2 -AP2
-NFκB -NFκB
Leptin

4T1

HRE +
AP2 −
NFκB −
SP1 +

HRE +
AP2 −
NFκB −
SP1 +

HRE +
AP2 −
NFκB +
SP1 +

AP1 +
AP2 −
NFκB +

MMT

EM6

VEGF
Molecular mechanisms of leptin induction of pro-angiogenic/pro-inflammatory factors in breast cancer cells
LPrA decreases MT growth by inhibiting leptin-induced mitogenic, angiogenic and anti-apoptotic effects.

MT ER+ were more sensitive to LPrA effects than MT ER-
FINANCIAL SUPPORT

This work was supported in part by

CONRAD (CIG-02-87, 06-113 and 07-114)
The Susan G Komen Foundation for the Cure
The Cancer Research and Prevention Foundation
NIH/UAB SPORE Breast Cancer
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