Targeting Cell Surface Chaperone in Cancer Therapy

Amy S. Lee, Ph.D.
University of Southern California
Norris Comprehensive Cancer Center
Keck School of Medicine
Endoplasmic Reticulum

- A perinuclear, cytosolic compartment
- Synthesis of secretory and membrane proteins
- Lipid synthesis
- Major intracellular Ca\textsuperscript{2+} store

**ER Stress:** The accumulation of unfolded proteins resulting from exceeding the ER capacity to refold them or export them for degradation.
Factors that Induce ER Stress in Cancer

Intrinsic
- Genetic mutation
- Altered metabolism
- Hyperproliferation

Extrinsic
- Nutrient deprivation
- Hypoxia
- Low pH
- ROS accumulation
- Viral infection

Cells adapt to ER stress by:
✓ Inducing protective UPR (GRP78, PERK, ERAD)
✓ Preparing for apoptosis (CHOP, C-7)
What is GRP78?

- First discovered as a glucose regulated protein induced by glucose starvation
- Also referred to as BiP or HSPA5
- GRP78 50% Homology HSP70 cytosolic
- A major chaperone in the ER with ATPase activity
  a) Protein folding
  b) Degradation of misfolded protein
  c) Prevent protein aggregation
- Ca\(^{2+}\)-binding protein

✓ Regulator of ER stress signaling
✓ Potent anti-apoptotic protein
✓ Regulator of PI3K/AKT signaling
Critical Role of GRP78 as Regulator of UPR and Apoptosis

Lee AS. Nat Rev Cancer, 2014
Single Molecule Super Resolution Imaging of GRP78 on the Cell Surface

- Cell surface localization of GRP78 requires its substrate binding function
- Active promotion of GRP78 to the cell surface in cancer cells resistant to therapy
- ER stress further elevates cell surface GRP78 expression in resistant cancer cells

Tsai et al., J Biol Chem, 2015
Collaborator: Fabien Pinaud
ER Stress Enhances Localization of Endogenous GRP78 to the Cell Surface

Zhang et al., J Biol Chem, 2010
Active Promotion of GRP78 to the Cell Surface in Cancer Cells Resistant to Therapy

ER stress further elevates cell surface GRP78 expression in resistant cancer cells.

Zhang et al., PLoS One, 2013
## Estimated Number of GRP78 (HSPA5) on the Cell Surface of Tumor Cells

From proteomics (Hein et al., *Cell* 163:712-723, 2015)

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Total GRP78 molecules</th>
<th>After Tg stress</th>
<th>Cell surface GRP78 molecules</th>
<th>After Tg stress</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HeLa:</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>9,900,000</td>
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<tr>
<td><strong>NIH3T3:</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>1,370,000</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>C4-2B:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6,440,000</td>
<td>12,870,000</td>
<td>430,000</td>
<td>2,500,000</td>
</tr>
</tbody>
</table>
Cell Surface GRP78 Regulates Signaling and Viability

Ni et al., Biochem J, 2011
Cell Surface GRP78 Forms Complex with PI3K

C4-2B cells

A

sGRP78/DAPI

sGRP78

p85

Merge

HeLa cells

B

lysate

eluate

IP

Western blot

○ cells

▲ biotin

● monomeric Avidin

C

F-GRP78

IP

Input IgG FLAG

p85

p110α

F-GRP78

PI3K
Anti-GRP78 Strategy

**Anti-cancer effects of GRP78 inhibitors**

- **Induce cancer cell apoptosis**
- **Suppress xenograft growth**
- **Suppress endogenous tumorigenesis**
- **Suppress metastasis**

**Induce cancer cell apoptosis**

**Suppress xenograft growth**

**Suppress endogenous tumorigenesis**

**Suppress metastasis**

**Lee, Nat Rev Cancer, 2014**

**GRP stress-induced transcription**

- Genistein (soy)
- Versipelostatin, etc.

**GRP78 translation**

- miR-30d; -181a; -199a5p

**GRP78 proteolytic cleavage**

- Bacterial toxin SubAB

**Cell surface GRP78 targeting**

- Peptides: CTVAPGGVRVC, WIFPWQL, WDLAWMFRLPVG, GIRLRG
- Antibodies: PAT-SM6 (human IgM), MAb-159 (humanized IgG), C107 (mouse IgG)

**Protein folding**

- BAG1 peptide

**ATPase domain**

**GRP activity**

**Transcription targeting**
Cell Surface GRP78 in Anti-cancer Therapy

- Dual targeting of tumor and microenvironment
- Simultaneous suppression of multiple pathways
Inducing Apoptosis in Chemotherapy-resistant B-lineage Acute Lymphoblastic Leukaemia Cells by Targeting HSPA5, a Master Regulator of the Anti-apoptotic Unfolded Protein Response Signalling Network
Fatih M. Uckun, Sanjive Qazi, Zahide Ozer, Amanda L. Garner, Jason Pitt, Hong Ma and Kim D. Janda
Children’s Hospital Los Angeles, NCI, Scripps

Anti-GRP78 (HSPA5) peptide-conjugate induces apoptosis in ALL cell lines.
Inhibition of Established Micrometastases by Targeted Drug Delivery via Cell Surface-associated GRP78

Yu Rebecca Miao, Bedrich L. Eckhardt, Yuan Cao, Renata Pasqualini, Pedram Argani, Wadih Arap, Robert G. Ramsay and Robin L. Anderson
University of Melbourne

**BMTP78**

<table>
<thead>
<tr>
<th>GRP78 homing peptide</th>
<th>Proapoptotic moiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIFPWIQL-GG-D(KLAKLAK)₂</td>
<td></td>
</tr>
</tbody>
</table>

Arap et al., *Cancer Cell*, 2004

**MDA-MB-231 Luc cells**

Day 10 (i)  Day 17 (i)  Day 20

GRP78 binding peptide fused to a pro-apoptotic peptide (BMTP78) reduces growth of established lung metastases of MDA-MB-231 breast cancer cells.
## Anti-cancer Phage Particles Targeting Cell Surface GRP78

<table>
<thead>
<tr>
<th>Agent</th>
<th>Reference</th>
<th>Therapeutic Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRP78 targeting phage particles</td>
<td>Dobroff et al., <em>PNAS</em>, 2016</td>
<td>• Simultaneous non-invasive molecular PET/CT imaging and targeted suicide transgene therapy in aggressive breast and prostate cancer <em>in vivo</em></td>
</tr>
<tr>
<td></td>
<td>R. Pasqualini, W. Arap, University of New Mexico</td>
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</tbody>
</table>

**NIR-labeled GRP78-targeting phage particles for preclinical imaging of human inflammatory breast carcinoma xenografts**
A Murine Monoclonal Antibody Directed Against the Carboxyl-terminal Domain of GRP78 Suppresses Melanoma Growth in Mice
Gustaaf G. de Ridder, Rupa Ray and Salvatore V. Pizzo
Duke University

✓ N88 against N-GRP78 – agonist
✓ C38 against C-GRP78 – antagonist
✓ C107 against N-GRP78 – antagonist, induces apoptosis

Higher reactivity of antibodies to cell surface GRP78 from ex vivo samples.
Monoclonal Antibody Against Cell Surface GRP78 as a Novel Agent in Suppressing PI3K/AKT Signaling, Tumor Growth, and Metastasis

Ren Liu, Xiuqing Li, Wenming Gao, …, Amy S. Lee and Parkash S. Gill
University of Southern California

- Mouse IgG (MAb159) against C-terminus domain of GRP78
- Suppresses PI3K/AKT signaling and tumor growth

MAb159 specifically recognizes GRP78 and localizes preferably to tumor cells.
Grp78 Deficiency in the Prostate Epithelium Suppresses Prostate Cancer
Mutant Mouse Model: Grp78\(^{f/+}\); Pten\(^{f/f}\); probasin Cre

GRP78 haploinsufficiency in prostate epithelial cells blocks prostate cancer progression and AKT activation.

Fu et al., PNAS, 2008
Targeting Cell Surface GRP78 Suppresses Prostate Cancer

Monoclonal antibody (MAb159) blocks prostate cancer progression and AKT activation.

Liu et al., Clin Cancer Res, 2013
GRP78 haploinsufficiency in bone marrow suppresses leukemia and PI3K signaling.

Wey et al., Blood, 2012
Targeting Cell Surface GRP78 Suppresses PTEN Loss Induced Leukemia

**A** $Pten^{fl}; Mx1^{Cre}$

**B**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Ctrl IgG</th>
<th>$cpl^{lf} ($Ctrl IgG)</th>
<th>$cpl^{lf} (MAb159)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen weight (g)</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
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**C**

- White blood cells (1x10^9/L): 30, 25, 3, 2.5
- Lymphocytes (1x10^9/L): 20, 15, 10, 7.5
- Monocytes (1x10^9/L): 1.5, 1.0, 0.5, 0.25
- Granulocytes (1x10^9/L): 12, 10, 8, 6
- Hemoglobin (g/L): 160, 140, 120, 100

**D**

- Western Blot: IgG, IgG 159, 159
- p-AKT, AKT, β-Actin

**MAb159 reduces leukemic blast and suppresses AKT activation with minimal effect on normal blood cells.**

*Liu et al., Clin Cancer Res, 2013*
GRP78 Targeted Cancer Imaging

A  Human Lung Cancer

B  Human Pancreatic Cancer

Liu et al., Clin Cancer Res, 2013

Wang et al., J Nucl Med, 2015
A GRP78-directed Monoclonal Antibody Recaptures Response in Refractory Multiple Myeloma with Extramedullary Involvement
Leo Rasche, Emmanuelle Ménoret, Valentina Dubljevic, …, Andreas Rosenwald, Hermann Einsele, Stephanie Brändlein
University of Würzburg, Germany

✓ A natural human IgM antibody (PAT-SM6) reported to target a modified GRP78 isoform
✓ Blocks cell proliferation, induces lipid accumulation and apoptosis
✓ Completed Phase I/IIa clinical trial in MM showed disease stabilization

Anti-GRP78 antibody suppresses Akt/TOR signaling and delays xenograft growth.
mAbW9 specifically recognizes GRP94 in cancer cells but not normal cells and shows anti-tumor activity in single and combination therapy.
Summary

1. Molecular chaperones are emerging as novel targets for immunotherapy.

2. Under pathological conditions, Glucose Regulated Proteins can relocalize to the cell surface where it exerts control over survival and invasiveness.

3. The preferential expression of GRPs on the surface of tumor cells but not normal organs *in vivo* enables antibody-based theranostics.

4. This approach may be particularly relevant in targeting aggressive and resistant tumors which exhibit elevated levels of cell surface GRPs.
Yong Fu
Emma Hadley
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Jieli Shen
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Yi Zhang
He Zhao

Anthony Carlos
Dat Ha
John Johnson
Daisy Rangel
Yuan-Li Tsai
Chun-Chih Tseng
Pu Zhang
Vicky Yamamoto

Collaborators
Zea Borok
Louis Dubeau
Parkash Gill
Susan Groshen
Kevin Kelly
Ite Laird
Fabien Pinaud
Min Yu
Beiyun Zhou

The Lee Laboratory

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