A Translational Investigation of Metastasis

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Tianjin Medical University
Metastasis of Cancer Cells
What is Chemotaxis?

• Leukocyte trafficking
• Angiogenesis
• Wound healing
• Brain Development
• HIV infection
Correlation between Chemotaxis and Cancer Metastasis

CXCR4 mediates chemotaxis of human breast cancer cells

Zlotnik Nature 2001
EGF is a more potent chemoattractant than CXCL12

MDA-MB-231 cells
Chemotaxis

GPCR

Gi protein

PLCβII

? 

PLCγ

EGFR

Chemotaxis
PKC Family

βI, βII, γ

• Novel, DAG-dependent, such as δ, ε, θ, η.

• Atypical, doesn’t require either Ca\(^{2+}\) or DAG, such as ζ and λ.
EGF induces PKC\(\zeta\) translocation

<table>
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<th>EGF</th>
<th>Ly294002</th>
<th>PKC(\zeta)</th>
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**EGF**
- Induces PKC\(\zeta\) translocation
- **Ly294002** (PKC inhibitor) blocks EGF-induced PKC\(\zeta\) translocation
Activated PKCζ regulates directional cell migration and polarity.
PKCζ Pseudosubstrate inhibits EGF-induced chemotaxis

Medium
Non-Myristoylated 50 µM
Myristoylated 50 µM

EGF (ng/ml)
- 0 ng/ml
- 1 ng/ml
- 10 ng/ml
- 100 ng/ml

Chemotaxis Index

Cancer Research 2005
Expression of PKCζ Correlates with Lymph Node Metastasis

Normal  Carcinoma  Lymph Node
Rictor Interacts with PKCζ
PKCζ colocalizes with Rictor along plasma membrane under EGF stimulation.
PKCζ colocalizes with Rictor at the leading edge of migratory cells
Knockdown of Rictor by siRNA Decreases EGF Induced Chemotaxis

**Graph:**
- **X-axis:** Cell number/HPF
- **Y-axis:** Cell number/HPF
- **Legend:**
  - EGF (ng/ml): 0, 1, 10, 100
- **Groups:**
  - MDA-MB-231, Scr, clone 4, clone 11, clone 15
- **Bar Chart:**
  - Different concentrations of EGF (0, 1, 10, 100 ng/ml) show a decrease in cell number/HPF for each group.
  - MDA-MB-231 and Scr show a significant decrease in cell number/HPF at 10 and 100 ng/ml.
  - Clone 4, clone 11, and clone 15 show a decrease in cell number/HPF at 100 ng/ml.

**Significance:**
- *****:** Significantly different compared to the control.
Knockdown of Rictor Impairs EGF Induced PKCζ Membrane Translocation
Rictor and Raptor Define Two Distinct mTOR Containing Complexes

Kim DH, Cell, 2002
Rictor still co-immunoprecipitated with PKCζ in siSIN1 cells
EGF-induced actin polymerization was impaired in siRictor cells
Knockdown of Rictor Inhibited Spontaneous Metastasis of MDA-MB-231 Cells to SCID Mouse Lung

6 weeks after implantation

Scr: 6 weeks
siRictor: 9 weeks
Expression of Rictor is Linked with Lymph Node Metastasis of Breast Cancer tissues

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Rictor is Expressed in Lung Cancer Tissues
Expression of Rictor correlates with NSCLC lymph node metastasis and poor prognosis

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![Survival Rate Graph]

- Neglective group
- Positive group

Survival Time (months)
Rictor was Expressed in Renal Cancer
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Expression of Rictor in Renal Cancer Correlates with Poor Prognosis
Chemotaxis

GPCR

EGFR

Gi protein

PLC\(\beta II\)

PI3K\(\gamma/\delta\)

PLC\(\gamma\)

PI3K\(\alpha/\delta?\)

Rictor

PKC\(\zeta\)

LIMK1/Cofilin

Integrin\(\beta 1\)

(Actin Polymerization) (Adhesion)

Chemotaxis
Current Working Model

GPCR → Gi protein → PLCβ → Rictor → PKCζ → Substrates ?? → Chemotaxis

EGFR → PTEN → PDK1 → Akt2

PLA2 ??

Cancer Research 2005
Mol Membrane Bio 2007
Cell Signal. 2007
Cell Signal. 2008
Lung Cancer 2008
International J Cancer 2009
Mol Cancer Research 2009
J Proteome Research 2009
Cancer Research 2010
J Clinical Invest. 2010
So What?

GPCR  
EGFR  
Gi protein  
PI3Kγ/δ  
P  
P  
P  
P  
PI3Kα/δ?

PDK1  
AKT2  
PKCζ

Chemotaxis

Compound Library  
siRNA  
Biochemical  
Cell-Based  
Animal Model  
Clinical  
Novel Drug
Screening for PKCζ inhibitors

Transform of drug 5-8 plus 572625 (control)

% Activity

M

- 9021878
- 7750407
- 5119533
- 5734879
- 572625
A Cell Based Screening for the Inhibitors of PKCζ

EGF -- + + + Akt inhibitor LY294002

Anal Biochem 2007
Acknowledgements

Zhang Fei
Guo Hua
Zhang Baogang
Zhang Xiaofang
Tian Gang
Liu Yan

Sun Ronghua
Liu Ying
Wan Wuzhou
Wang Jingna
Metastasis is the Major Cause of Morbidity

"The surgery went well. It had spread, but I'm quite confident we got it all."
Knockdown of Rictor by siRNA Decreases EGF Induced Chemotaxis
Knockdown of Rictor by siRNA Decreases EGF Induced Chemotaxis
Cell adhesion was Impaired in siRictor cells

![Graph showing cell number per HPF over time with different treatments: EGF(10 ng/ml), Scr EGF (-), Scr EGF (+), siRictor EGF (-), siRictor EGF (+).](image1)

![Western blot analysis showing p-integrin β1 and integrin β1 expression over time with Scr and siRictor treatments.](image2)

![Graph showing integrin phosphorylation over time with Scr and siRictor treatments.](image3)
EGF still induces Rictor membrane translocation in siPKCζ cells
EGF induced PKCζ phosphorylation was impaired in siRictor cells
EGF Induces Co-IP of Akt2 and PKCζ

- **IP: Akt2**
  - **WCL**
  - **NC**
  - **0’**
  - **5’**
  - **30’**

  - PKCζ
  - Akt2

- **IP: PKCζ**
  - **WCL**
  - **NC**
  - **0’**
  - **5’**
  - **30’**

  - Akt2
  - PKCζ

- **IP: Akt1**
  - **WCL**
  - **0’**
  - **5’**
  - **30’**
  - **NC**

  - PKCζ
  - Akt1

- **IP: PKCζ**
  - **WCL**
  - **0’**
  - **5’**
  - **30’**
  - **NC**

  - Akt1
  - PKCζ
Akt2 Plays a Critical Role in Metastasis

EGF (ng/ml)

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<td>C102</td>
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**Graph:**
- X-axis: Control, C44, C97, C102
- Y-axis: Chemotaxis Index
- Legends: 0, 1, 10, 100

**Image:**
- Western blots for p-LIMK, p-cofilin, cofilin
- Immunohistochemistry for Akt2 and Akt1

**Table:**

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<tr>
<td>5'</td>
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PDK1 is Required for Metastasis
An Optimal Level of PTEN is required for Chemotaxis

Chemotaxis Index

EGF (ng/ml)
0
100
1000

PTEN
GAPDH

MDA
Control
Clone 6
Clone 74
Clone 100

PDK1
Control
siPTEN/MDA

MDA MB-231 V5-His-PTEN/MDA MB-231
Txl12在乳腺癌转移中起关键作用

↓ TXNL2 → ↑ ROS → ↓ GSH → ↓ NFkB → ↓ metastasis
What’s Next?

Co-immunoprecipitation

MDA-MB-231 cell

Input

IP

EGF

IgG

p32

- +

- +

WB: PKCζ

HEK293 cell

anti-p32

anti-Flag
B23

SCR

siB23

20min

SCR

SiB23

6h
cPLA2a
Treatments with Gd@C$_{82}$(OH)$_{22}$ Inhibit hepatoma growth in a mouse model.

Gd@C$_{82}$(OH)$_{22}$ inhibits tumor growth in a breast tumor model.

Nano Lett Vol 5, pg 2050
Gd@C$_{82}$(OH)$_{22}$ induced iDC maturation and TH-1 response.
Treatments with Gd@C\textsubscript{82}(OH)\textsubscript{22} cancer cell chemotaxis and metastasis

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<th>Metastasis Rate</th>
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<td>C\textsubscript{60}(OH)\textsubscript{20} (0.4 mg/kg, n = 10)</td>
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<td>Gd@C\textsubscript{82}(OH)\textsubscript{22}, 0.35mg/kg q.d. ×20 day</td>
<td>4.3%</td>
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Summary

1. Treatment with Gd@C_{82}(OH)_{22} inhibits tumor growth without detectable toxicity.

2. Gd@C_{82}(OH)_{22} doesn’t show cytotoxicity.

3. Gd@C_{82}(OH)_{22} inhibits blood supply to tumor tissues.

4. Gd@C_{82}(OH)_{22} induced tumor immunity.

5. Gd@C_{82}(OH)_{22} inhibits cancer cell chemotaxis.