Pigment Epithelium-Derived Factor (PEDF) Expression Induced by EGFRvIII Promotes Self-renewal and Infiltration of Glioma Stem Cells

박종배

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박종배
Plasticity of cancer stem cells

Environmental factors

- Stem-cell niche
- Progenitor cells
- Differentiated cells

Cancer stem cell

- Stationary Cancer stem

Differentiated cancer cell

Mobile Cancer stem

Angiogenesis
Mobile- and stationary-GSC
What is the difference between NSCs and GSCs?
The Somatic Genomic Landscape of Glioblastoma


Cell 155, 462–477, October 10, 2013
What is the difference between proneural and mesenchymal cancer stem cells?
EGFR Mutation Variant III (EGFRvIII)

- Tumor-specific oncogene expressed in one-third of primary GBM, seldom expressed with IDH mutations but not in normal tissue
- EGFRvIII(+) cells may induce growth in EGFRvIII(-) cells via paracrine signaling, membrane-derived microvesicles, and tumor stem cells1-4
- Rindopepimut consists of EGFRvIII peptide conjugated to Keyhole Limpet Hemocyanin (KLH)
  - Generates a specific immune response against EGFRvIII-expressing GBM
  - “Off the shelf”
  - Delivered as intradermal injection of 500 μg rindopepimut with 150 μg GM-CSF as an adjuvant

EGFRvIII Linked To Poor Long Term Survival5

1. Inda, et al. Genes Dev. 2010
Expression of EGFRvIII in glioma

14/46: EGRFvIII/EGFR
EGFRvIII and glioma infiltration

- Expression of EGFRvIII correlates with the expression of stem/progenitor markers, such as Nestin, Sox2 and CD133, as well as increasing ability to self-renew and initiating tumorigenesis.

- Glioma cells expressing EGFRvIII are found to recruit EGFR-WT-expressing cells and accelerate glioma tumorigenicity by upregulating the expression levels of cytokines.

- EGFRvIII is known to drive glioma infiltration in EGFRvIII overexpressing mice, wherein tumor cells infiltrate along white matter tracts and the perivascular space.
EGFRvIII drives glioma infiltration
EGFRvIII Expression Maintains Stemness of GSCs

A

Astrocyte
EGFRvIII
CSC2
X01
X03
X06
X08
X09
MD30
1123NS
83NS
X02
EX Vivo
528NS

EGFR-WT
EGFRvIII
GAPDH

RT-PCR

B

Serum (day)
EGFR
Sox2
Nestin
GFAP
α-tubulin

CSC2

siEGFRvIII
p-EGFR
EGFR
Sox2
Nestin
GFAP
α-tubulin

C

D

CSC2

siCon

siEGFRvIII

Log fraction without spheres

p=0.00966

Number of Cells

CSC2

siCon

siEGFRvIII

F

X02

ConEGFRvIII

p-EGFR

EGFR

Sox2

Nestin

GFAP

α-tubulin

X02

Con

EGFRvIII

Log fraction without spheres

p=0.0000266

Number of Cells
EGFRvIII expressed in small population of cells in vivo
Hypothesis:

Driver and passenger cancer cells?
Identification of PEDF as a Novel Autocrine Factor Regulated by EGFRvIII through STAT3 Signaling

A  

B  

C

D  

E  

F

G
Table S1. Proteins enriched in the secretomes of serum-free GSC conditioned medium (CM) from CSC2 (EGFRvIII+ GSC) compared to the secretomes of paired serum-differentiated CSC2 CM (DIF) and serum free GSC CM from Ex Vivo (EGFRvIII- GSC) by LC-MS/MS analysis.

*Proteins upregulated more than 2 folds in both CSC2/DIF and CSC2/Ex Vivo.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Accession number</th>
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Recent functions of PEDF (i) in neural stem-cell maintenance; (ii) in anti-inflammation in diabetic retinopathy; (iii) as a survival factor against oxidative stress; and (iv) as an inducer of immune-cell migration.
Identification of PEDF as a Novel Autocrine Factor Regulated by EGFRvIII through STAT3 Signaling II
PEDF Expression Maintains Stemness and Self-renewal of GSCs

A

PEDF 100ng/ml

B

PEDF (ng/ml) 0 100

Sox2

Nestin

GFAP

β-Actin

C

PEDF 100ng/ml

D

PEDF (ng/ml) 0 100

EGF/bFGF

PEDF

Sox2

Nestin

GFAP

α-tubulin
PEDF Expression Maintains Stemness and Self-renewal of GSCs II

**A**

![Graph showing the effect of PEDF on cell division](image)

**B**

**CSC2**

<table>
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<th>Condition</th>
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<td>shPEDF-1</td>
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**C**

![Graph showing the effect of PEDF on cell division](image)

**D**

![Graph showing the effect of PEDF on cell division](image)

**E**

![Graph showing the effect of PEDF on cell division](image)

**F**

![Graph showing the effect of PEDF on cell division](image)

**G**

![Graph showing the effect of PEDF on cell division](image)

**H**

![Graph showing the effect of PEDF on cell division](image)
PEDF Maintains Stemness and Self-renewal of GSCs by Activating Notch-Sox2 Pathway

**A**
 PEDF
 NICD
 α-tubulin
 Ex Vivo
 Con PEDF
 PEDF
 NICD
 α-tubulin

**B**
 PEDF
 NICD
 α-tubulin
 Con PEDF
 Con shPEDF
 PEDF
 NICD
 α-tubulin

**C**
 DAPT (1μM)
 NICD
 Sox2
 Nestin
 α-tubulin
 PEDF
 Sox2
 GAPDH

**D**
 Sphere No (>100μm)
 Con
 PEDF
 PEDF + DAPT

**E**
 PEDF
 Sox2
 GAPDH
 Con PEDF
 PEDF
 Sox2
 GAPDH

**F**
 Consensus CBF1 binding site
 -1007
 -1002
 -894
 TGGGAA
 TGGGAA
 TS
 Sox2

**G**
 PEDF
 Sox2
 Nestin
 GFAP
 NICD
 α-tubulin
 Con shPEDF
 Con shPEDF
 Con shPEDF

**H**
 Sphere No (>100μm)
 Con
 shPEDF
 Sox2
 shPEDF + Sox2
**A**

PEDF Promotes Infiltration and Tumorigenesis of GSCs

<table>
<thead>
<tr>
<th>CSC2</th>
<th>X01</th>
<th>X03</th>
<th>X04</th>
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<tr>
<td>α-tubulin</td>
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<table>
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<tr>
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<th>EGFRvIII+/PEDF&lt;sub&gt;high&lt;/sub&gt;</th>
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<td>α-tubulin</td>
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**B**

<table>
<thead>
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**C**

<table>
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<tr>
<th>X01-con</th>
<th>X01-shPEDF</th>
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**D**

![Graph](image_url)

**E**

![Graph](image_url)
SOX2

NICD

HES1

gain of function; PEDF promotes glioma infiltration
PEDF Expression Correlates with Patient Survival in Human Glioma

A

![Graph showing PEDF expression correlates with patient survival in All glioma.](image)

- **PEDF down-reg >= 3.0 (n=81)**
- **PEDF intermediate (n=254)**

*p value < 0.0001

Survival time (months)

Probability of survival

B

![Graph showing PEDF expression correlates with patient survival in GBM.](image)

- **PEDF down-reg >= 3.0 (n=22)**
- **PEDF intermediate (n=153)**

*p value = 0.0134

Survival time (months)

Probability of survival

C

![Western blot images showing PEDF, p-EGFR, EGFR, NICD, p-STAT3, and GAPDH.](image)

D

<table>
<thead>
<tr>
<th></th>
<th>EGFRvIII</th>
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| NICD       |          | 0.977355"" |            | 0.99683""

**Correlation coefficients (r)**
1. What is the role of PEDF in EGFRvIII mediated transition?
2. What is the role of PEDF in resistance of EGFRvIII targeted therapy
3. What is the receptor for the PEDF?
감사합니다...

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이승훈
유헌
곽호신
김연재
김종헌
유병철
Omics core
윤금룡
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