The splicing regulation and clinical significance of epithelial splicing regulatory protein 1 in invasion and metastasis of epithelial ovarian cancer

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Disclosure of interest: None
Introduction

Ovarian Cancer Epidemiology:

• About 240,000 women are diagnosed each year worldwide.

• The 5th most common cause of cancer death among women in the US.
Introduction

• Standard treatment: Debulking surgery and chemotherapy;

• 5-year survival rate of EOC: still less than 30%;

• Key of EOC treatment: invasion and metastasis.
Introduction

Epithelial-mesenchymal transition (EMT): an abnormally activated during cancer metastasis and recurrence.

EMT: acquisition of a migratory phenotype leading to increased invasion and metastasis.
ESRP1 (also called RBM35A):

A newly discovered epithelial-specific RNA binding protein regulating alternative splicing events in EMT process

Promoting splicing of the epithelial variant of the FGFR2, ENAH, CD44 and CTNND1 transcripts

Warzecha CC, Shen S, Xing Y, Carstens RP. The epithelial splicing factors ESRP1 and ESRP2 positively and negatively regulate diverse types of alternative splicing events. RNA biology. 2009;6(5):546.
Introduction

ESRP1:

- Play multiple roles in tumor progression
- Whether ESRP1 play positive or negative roles during tumor progression remains controversial
- Functions and roles in ovarian cancer have not been reported yet.

Methods and Results

ESRP1 in the heat map (a) and scatter plot (b) of an Affymetrix HTA 2.0 scanning in 3 EOC Vs. 3 normal tissues
Methods and Results

An validation of ESRP1 expression in normal ovary and ovarian cancer by three different GEO datasets: *** presents P<0.001
Methods and Results

The expression of ESRP1 in ovarian cancer and normal tissues measured by Western Blot (A) and RT-PCR (B)

(A) Western Blot

(B) RT-PCR
Methods and Results

ESRP1 was abundant in malignant lesions measured by IHC.

200x

400x
Methods and Results

ESRP1 was weakly in normal and benign lesions

a: normal ovary tissue; b: benign lesions; c&d: borderline ovarian tumor
## Methods and Results

ESRP1 expression was associated with clinical staging (P=0.04) and differentiation degree (P=0.002). Expression of ESRP1 was measured by HIS(IHC). Epithelial ovarian cancer (EOC) with stage III or low differentiated had a higher expression of ESRP1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case number</th>
<th>Expression of ESRP1</th>
<th>P value</th>
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<tbody>
<tr>
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<td>low (-/+ )</td>
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<td>Differentiated degree</td>
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<tr>
<td>Poorly</td>
<td>37</td>
<td>11</td>
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Methods and Results

A down-regulation of ESRP-1 in human ovarian cancer cell line HO-8910 by shRNA lentivirus, confirmed by RT-PCR and Western Blot.
Methods and Results

There was no significant change in cell proliferation between HO8910 and HO8910-shESRP1 (A, CCK-8, \( P = 0.8272 \)), but down-regulation of ESRP1 increased migration (B, Transwell, \( P < 0.0001 \)) and invasion (C, Transwell, \( P < 0.0001 \)) of EOC cells significantly.
Methods and Results

Knockdown of ESRP1 promoted EMT measured by RT-PCR
TGF-β-induced EMT (10ng/mL, 48h), Snail were continuously activated and the mRNA expression of ESRP1 was decreased (A). Down-regulation of snail and slug caused increasing of ESRP1 expression (B, C).
Methods and Results

Both FGFR2-IIIb and FGFR2-IIIc were up-regulated in ESRP1 knockdown cells while the mesenchymal IIIc isoform increased more significantly than IIIb isoform (A). Down-regulated of ESRP1 also effectively up-regulated CD44s isoform expression in EOC cells(B).
Discussion

1. Positive or negative roles during ovarian cancer progression?

“Plastic: during carcinogenesis, ESRP-1 is up-regulated relative to their levels in normal epithelium but down-regulated in invasive fronts.”


Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility via different mechanisms.

Ishih H¹, Saitoh M², Sakamoto K³, Kondo T⁴, Kato H⁴, Tanaka S⁵, Motzuki M², Masuyama K⁶, Mivazawa K⁶.
Discussion

2. What happened in ovarian cancer invasion fronts?

Epithelial Cell
- Adherens Junctions
- Tight Junctions
- Desmosomes
- Apico-basal polarity
- Stationary
- Cytokeratins
- Cortical actin
- E-Cadherin

Mesenchymal Cell
- No Junctions
- Focal adhesions
- ECM deposition
- Anoikis resistant Moticile and invasive
- Front-back polarity
- Vimentin / SMA
- FSP-1
- FN, CSPG

EMT
ESRP1 played an important role in the progression of carcinogenesis and was associated with disease prognosis in EOC. It regulated alternative splicing events during the EMT process and might provide some potential clinical application in EOC patients.
Questions?

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