The origin of endometriosis based on genome-wide DNA methylation analysis

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Pathophysiology of Endometriosis

Transplantation theory and coelomic metaplasia theory have been widely accepted.

Metaplasia

coelomic epithelium

endometriosis

sex steroid hormone
menstrual blood
inflammation

Transplantation

lymph ducts
vessels
fallopian tube
fistula
operation

endometrium
### Cell specific DNA methylation profiles

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell specific DNA methylation region</th>
</tr>
</thead>
<tbody>
<tr>
<td>heart</td>
<td>1</td>
</tr>
<tr>
<td>lung</td>
<td>1</td>
</tr>
<tr>
<td>liver</td>
<td>1</td>
</tr>
<tr>
<td>uterus</td>
<td>1</td>
</tr>
<tr>
<td>stomach</td>
<td>1</td>
</tr>
<tr>
<td>brain</td>
<td>1</td>
</tr>
<tr>
<td>kidney</td>
<td>1</td>
</tr>
</tbody>
</table>

*Images of human organs correspond to cell types.*
A stem cell will differentiate into various cells accompanying with DNA methylation patterns. DNA sequences are not changed.

Once DNA methylation profiles are fixed, DNA methylation profiles do not change easily.

Epigenetic Landscape C. Waddington (1953)

Programed cell differentiation
Specific circumstances
Disease onset

iPS change
DNA methylation profiles express the feature of choESC well.
Method 1  (Cluster analysis of Genome-wide DNA methylation profiles)

ESC from endometrium  
n=3
ESC from chocolate cyst  
n=3

endometrium  
n=3
ovary (cortex)  
n=3
peritoneum  
n=2
chocolate cyst  
n=3
peritoneal lesion (blue berry spot)  
n=1

Genomic DNA was extracted from cell and tissue

Genome wide DNA methylation profile by Illumina HumanMethylation27 / 450K

27K/450K data of Human tissue was downloaded from GEO (Gene Expression Omnibus)

Methylation data was integrated, and cluster analysis was performed.
Cluster analysis using integrated genome wide DNA methylation data

Where is the endometriosis cell come from?

Endometrial stromal cells from chocolate cysts

Endometrial stromal cells from eutopic endometrium

Endometrium

Using HumanMethylation27 data
Cluster analysis using integrated genome wide DNA methylation data

Where is endometriosis cell come from?

Using HumanMethylation450 data
Method 2 (DNA methylation analysis of SF1 gene)

In chocolate cyst, local estradiol concentration and SF1 gene expression is high. SF1 (NR5A1) is important transcription factor, and positively regulate sex steroid-producing enzymes. SF1 seems to involve in the pathogenesis of endometriosis.

Genomic DNA was extracted from tissue. The DNA methylation status of 13 CpGs in SF1 (NR5A1) gene promoter ~ first exon region was analyzed by bisulfite sequencing.

- endometrium: n=3
- ovary (cortex): n=3
- peritoneum: n=3
- chocolate cyst wall: n=3
- peritoneal lesion (blue berry spot): n=1

[Diagram of steroid production pathway: cholesterol → STAR aromatase etc. → estradiol]
NR5A

Bisulfite sequencing

-68  exon1  +83

CpG sites

Ovary (cortex)

Hypomethylated

Endometrium

Hypermethylated

Chocolate cyst

Hypomethylated

Methylated CpG

Unmethylated CpG

Black circles = Methylated CpG

White circles = Unmethylated CpG

Circle half-filled = Appear methylated

Circle half-filled = Appear not methylated

NR5A 1
NR5A

Bisulfite sequencing

Endometrium
Hypermethylated

Peritoneum
Hypermethylated

Peritoneal lesion (blue berry spot)
Hypermethylated

CpG sites
-68
exon1
+83

Methylated CpG
Unmethylated CpG
Conclusion

There is a possibility that

Ovarian endometriotic lesion is from ovarian surface.

Peritoneal endometriotic lesion is from peritoneum, however further analysis is needed.