Effects of uniaxial stretch forces and matrix stiffness on the cell behavior of desmoid-type fibromatosis

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Desmoid tumor invasiveness may be influenced by microenvironment.
Pathogenesis of desmoid

- Trauma
- Involvement of cytokine including TGF-β
- Cell stretch
- Substrate stiffness
Cell stretch

Cells
- Human dermal fibroblast (HDF)
- Desmoid cultured cell (WT, 41A, 45F)

Stimuli
- Uniaxial stretch: 10, 20%
- Frequency: 1, 10, 30, 60 times stretch / min.
STREX:STB-140 silicon chamber

stretch (+), 24hr

Perpendicular arrangement

Stretch direction

stretch (-), 24hr
MTS assay

cyclic stretch 1/min, 20%

12hr (n=8)

24hr (n=8)
Results

<table>
<thead>
<tr>
<th>伸展率</th>
<th>HDF 10%</th>
<th>HDF 20%</th>
<th>S45F 10%</th>
<th>S45F 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/min</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>10/min</td>
<td>↓\text{↘}→</td>
<td>↓\text{↘}→</td>
<td>↓\text{↘}→</td>
<td>↓\text{↘}→</td>
</tr>
<tr>
<td>30/min</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓\text{↘}→</td>
</tr>
<tr>
<td>60/min</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
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→ no change
\text{↘} tendency to decrease
\downarrow statistic significant

Cyclic stretch does not stimulate cell proliferation
β-catenin expression (westernblot)

**stimuli (-)**

<table>
<thead>
<tr>
<th></th>
<th>NHDF</th>
<th>WT</th>
<th>T41A</th>
<th>S45F</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytoplasmic</td>
<td>c</td>
<td>n</td>
<td>c</td>
<td>n</td>
</tr>
<tr>
<td>nucleus</td>
<td>c</td>
<td>n</td>
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**stimuli (+)**

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<td>n</td>
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</tbody>
</table>

c: cytoplasmic, n: nuclear
non-phospho β-catenin

stimuli (-)  
NHDF  WT  T41A  S45F  
c  n  c  n  c  n  c  n

stimuli (+)  
NHDF  WT  T41A  S45F  
c  n  c  n  c  n  c  n

c: cytoplasmic, n: nuclear
cyclin-D1

stimulate (-)

NHDF  WT  T41A  S45F

stimulate (+)

NHDF  WT  T41A  S45F

β-actin
Substrate stiffness

Cell culture
T41A mutated cells
2.0 x 10^4 cells/well (35mm dish)

Substrate stiffness:
0.5, 2, 8, 16, 32, 64kPa, normal dish

48 hr’s culture

MTS assay, α-SMA and β-catenin expression
MTS assay

0.5kPa vs others

p < 0.05
α-SMA expression

αSMA+DAPI 48hr

16kPa

32kPa

64kPa

plastic
Western blot: β-catenin

16, 32kPa, plastic vs 0.5, 2, 8kPa (p<0.05)
Results of the present study

Substrate stiffness $\uparrow$ $\rightarrow$ $\alpha$-smooth muscle actin

Cell proliferation partially $\uparrow$ $\uparrow$

Lung fibroblasts

0.4 kPa

$\alpha$-SMA

F-actin

Merge

25.6 kPa

$\alpha$-SMA

F-actin

Merge

Liu F et al. JCB. 2010

What is the mechanoreceptor in desmoid?
Fibrogenic disorder: idiopathic pulmonary fibrosis

Substrate stiffness↑

TGF-β1

fibroblast ⏬ Myofibroblast differentiation↑

α-SMA expression

mediated by TRPV4 (mechanosensor)
Hypothesis

Desmoid cells sense substrate stiffness via TRPV4

- TRPV4 expression
- Use TGF-β (10ng/ml) to stimulate cell viability
- HC-067047(1μM): TRPV4 antagonist
α-SMA expression
48 hrs culture

Control  TGF-β (+)  HC-067047
TRAPV4 mediate the substrate stiffness: not yet determined

Rahaman SO et al. (J Clin Invest 2014)
Future plan

- Effects of TRPV4 knockdown in cultures under various substrate stiffness
- Analyses for various types of CTNNB1 mutation status