Unravelling the desmoid-type fibromatosis at the cellular level: GSK-3beta, a new piece of the puzzle

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COLLECTION OF DESMOID-TYPE FIBROMATOSIS (DF) BIOPTIC SAMPLES

Melanoma and Sarcoma Unit

- Clinical data of DF patients
- Surgery of DF patients

Cell Biology Unit

- Isolation of DF cells from bioptic samples
- Cellular and molecular analyses

Pathology Unit

- Histopathological analyses of DF bioptic samples
ISOLATION AND PROLIFERATION OF DESMOID-TYPE FIBROMATOSIS (DF) CELLS

• Fragmented bioptic fresh tissue were cultured in CHANG medium (50% FBS) for 1 week
• Primary DF cell cultures were grown and expanded in CHANG medium (10% FBS)

LOW DF CELLS PROLIFERATION RATE
WNT/β-CATENIN PATHWAY IS INVOLVED IN THE PATHOGENESIS OF DESMOID-TYPE FIBROMATOSIS

β-catenin is the common marker used for desmoid-type fibromatosis diagnosis
CHARACTERIZATION OF $\beta$-CATENIN IN DESMOID-TYPE FIBROMATOSIS CELLS

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>33.5</td>
<td>53.3</td>
<td>40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CTNNB1 mut (%)}$</td>
<td>46.6</td>
<td>43</td>
<td>45.5</td>
</tr>
<tr>
<td>$\text{CTNNB1 wt (%)}$</td>
<td>53.4</td>
<td>57</td>
<td>54.5</td>
</tr>
</tbody>
</table>

- The female patients are younger than male patients
- Only 45% of the DF cases have mutations of the exon 3 of $\text{CTNNB1}$ gene, with no difference between females and males

- $\beta$-catenin is highly expressed in DF samples in comparison to the control
- The expression level of GSK-3$\beta$ is comparable in DF and control samples

There is no correlation between $\beta$-catenin mutations and its expression in desmoid-type fibromatosis cells
NUCLEAR LOCALIZATION OF GSK-3β AND ITS COLOCALIZATION WITH β-CATELIN

GSK-3β  β-Catenin  Merge

Ctrl  

DF45(#4)  DF45(#1)

β-catenin  GSK-3β  β-actin

IP β-catenin
**β-CATENIN AND GSK-3β NUCLEUS-POSITIVE DF CELLS**

<table>
<thead>
<tr>
<th>Samples</th>
<th>β-catenin</th>
<th>GSK-3β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleus %</td>
<td>Cytoplasm %</td>
</tr>
<tr>
<td>DF#1S45</td>
<td>88</td>
<td>12</td>
</tr>
<tr>
<td>DF#2WT</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>DF#3WT</td>
<td>72,7</td>
<td>27,3</td>
</tr>
<tr>
<td>DF#4S45</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td>DF#5T41</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td>Ctr</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

- The number of β-catenin nucleus-positive cells is reduced of 10% in *CTNNB1* non-mutated DF cells compared to *CTNNB1* mutated DF cells.
- The number of GSK-3β nucleus-positive cells is equivalent in *CTNNB1* non-mutated and mutated DF cells.
PHOSPHORYLATED BETA-CATENIN IS ALWAYS ABSENT IN DF CELLS

- Ctr
- DF<sup>WT</sup>(#3)
- DF<sup>S45</sup>(#4)
- DF<sup>T41</sup>(#5)

- β-catenin phosphorylation is not associated to mutations in CTNNB1 gene
- In DF cells β-catenin is not phosphorylated and consequently not degraded

Nuclear accumulation of β-catenin
APC AND AXIN COLOCALIZE IN THE CYTOPLASMIC COMPARTMENT OF DF CELLS
BETA-CATENIN AND AXIN ARE LOCALIZED IN DIFFERENT CELL COMPARTMENTS IN DF CELLS
GSK-3β and APC are localized in different cell compartments in DF cells.
β-catkenin is not the sole component of the multiprotein complex accumulated in the nucleus

GSK-3β is exclusively nuclear and it is complexed with β-catenin

Nuclear translocation of β-catenin and GSK-3β is not associated to CTNNB1 mutations

Proteins of the Wnt pathway have different cells compartmentalization

The multiprotein complex, responsible for β-catenin phosphorylation, cannot be assembled
GSK-3β binds to an altered β-catenin that cannot be phosphorylated.

GSK-3β binds to β-catenin but it cannot be phosphorylated because the complex is not assembled.

Nuclear migration of β-catenin/GSK-3β

NUCLEAR GSK-3β AS ADDITIONAL MARKER FOR DF CELLS
To identify the key molecules of the Wnt pathway leading the alteration of β-catenin and GSK-3β localization

To investigate the effect of nuclear GSK-3β
**GENE EXPRESSION OF Wnt TARGETS: AXIN2 AND C-MYC**

**AXIN2** gene expression was increased two to six-fold in DF cells

**c-myc** gene is not expressed in DF cells
**GENE EXPRESSION OF Wnt TARGETS:**

**CCND1**

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**CCND1** gene expression is downregulated in DF cells

Possible reason of low DF cells proliferation rate

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GSK-3β might play a role in Cyclin D1 degradation
Activation of Wnt pathway results in an inactivation of GSK-3β

Akt phosphorylates and inactives GSK-3β
An image of a diagram illustrating the signaling pathways involving GSK3β, β-catenin, Axin, APC, and CSNK1A1. The diagram shows the interaction between Wnt, Dkk-1, Frizzled (SFRP), and proteasome in the context of β-catenin degradation and nuclear translocation. Growth factor receptors activate PI3K, which phosphorylates Akt, leading to GSK3β phosphorylation and activation. GSK3β inactivates β-catenin, which is then degraded by the proteasome. The diagram highlights the regulatory roles of Axin, APC, and CSNK1A1 in the Wnt signaling pathway.
**CELLS VIABILITY AFTER DRUGS TREATMENT**

**LiCl**: inhibitor of GSK-3β

<table>
<thead>
<tr>
<th>Concentration</th>
<th>OD 490nm</th>
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</thead>
<tbody>
<tr>
<td>untreated</td>
<td>0.3</td>
</tr>
<tr>
<td>LiCl 5mM</td>
<td>0.4</td>
</tr>
<tr>
<td>LiCl 20mM</td>
<td>0.4</td>
</tr>
<tr>
<td>LiCl 80mM</td>
<td>0.3</td>
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**Dkk1**: antagonist of the Wnt signalling

<table>
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<th>Concentration</th>
<th>OD 490nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>0.3</td>
</tr>
<tr>
<td>Dkk1-3 ng/ml</td>
<td>0.4</td>
</tr>
<tr>
<td>Dkk1-12,5 ng/ml</td>
<td>0.4</td>
</tr>
<tr>
<td>Dkk1-50 ng/ml</td>
<td>0.3</td>
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**LY294002**: inhibitor of PI3 kinase

<table>
<thead>
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<th>Concentration</th>
<th>OD 490nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>0.3</td>
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<tr>
<td>LY294002 10uM</td>
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<tr>
<td>LY294002 40uM</td>
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<tr>
<td>LY294002 160uM</td>
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The optimal drug concentrations compatible with cells viability are:

- **LiCl**: 20mM
- **Dkk1**: 50ng/ml
- **LY294002**: 40mM
**EXPRESSION AND LOCALIZATION OF TOTAL AND PHOSPHORYLATED GSK-3β IN NORMAL AND DF CELLS**

- Normal and DF cells treated with drugs lead to nuclear GSK-3β translocation
- Substantial loss of P-GSK-3β in DF cells treated with drugs
Normal and DF cells treated with Lithium lead to nuclear APC and Axin translocation.

Substantial loss of Axin in DF cells treated with Dkk-1 inhibitor.
EXPRESSION AND LOCALIZATION OF AKT AND β-CATENIN IN NORMAL AND DF CELLS

**Normal cells**

- **Dkk-1**
- **LiCl**
- **Lv294002**

**DF cells**

- **Dkk-1**
- **LiCl**
- **Lv294002**
**SUMMARY**

- **LiCl**: inhibitor of GSK-3β
  - Nuclear translocation of β-catenin, GSK-3β, APC and Axin in normal and DF cells
  - Loss of P-GSK-3β expression in DF cells

- **Dkk1**: antagonist of the Wnt signalling
  - Nuclear translocation of GSK-3β and P-GSK-3β in normal cells
  - Nuclear translocation of Akt in normal and DF cells
  - Loss of Axin and P-GSK-3β expression in DF cells

- **LY294002**: inhibitor of PI3 kinase
  - Nuclear translocation of GSK-3β and Akt in normal cells
  - Loss of P-GSK-3β expression in DF cells
  - Increase cytoplasmic Akt in DF cells
THERE IS STILL A LOT OF WORK TO BE DONE ...

To validate of nuclear GSK-3β as a novel clinical marker for desmoid-type fibromatosis

To deeply investigate the role of inhibitor molecules on the expression and localization of the Wnt proteins in DF cells

To identify the factors responsible for the DF cells growth and aggressiveness
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