The role of SUPT6H in gliomagenesis

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## Gliomas

- 3000 cases primary CNS tumors/year
- 90% die within 2-5 years post diagnosis
- the most malignant variant - GBM

<table>
<thead>
<tr>
<th></th>
<th>Pilocytic Astrocytoma (WHO grade I)</th>
<th>Diffuse Astrocytoma (WHO grade II)</th>
<th>Anaplastic Astrocytoma (WHO grade III)</th>
<th>Glioblastoma a.k.a. Glioblastoma Multiforme (WHO grade IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of Onset</strong></td>
<td>First two decades of life</td>
<td>30 to 40 yrs</td>
<td>Early 40s</td>
<td>Mid 50–60s</td>
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<tr>
<td><strong>Typical Location</strong></td>
<td>Throughout the neuraxis. Optic pathway tumors are frequent</td>
<td>Cerebral hemispheres. Pons/brainstem, esp. in children</td>
<td>Cerebral hemispheres</td>
<td>Cerebral hemispheres</td>
</tr>
<tr>
<td><strong>Average Survival</strong></td>
<td>Years to decades</td>
<td>Five years</td>
<td>Two to five years</td>
<td>Fourteen months</td>
</tr>
</tbody>
</table>

*Angiogenesis*

*Neuroglia, 2012*
Replication stress and oxidative damage contribute to aberrant constitutive activation of DNA damage signaling in human gliomas

Bartkova*, Hamerlik*, Oncogene, 2010; *equal contribution
Cancer stem cells and radioresistance in GBM

Bao et al. 2006 Glioma stem cells promote radioresistance by preferential activation of the DNA damage response.
siRNA library: against 360 genes
3 validated independent siRNA performed as pooled siRNA screen

**CD133-positive glioma-derived cancer stem cells (GSCs):**

- search for genes whose knock-down would result in changes:
  - proliferation rates (**EdU** pulse labeling)
  - spontaneous DNA damage (**γH2AX**)

i.e. candidate genes important for GSCs maintenance, repair efficiency and self-renewal

**Readout:** ScanR microscopy screening station
Experimental setup

- Papain dissociation

  →

  CD133+

  →

  Confirmation of stem cell phenotype by qPCR (GFAP, Sox2, Oct, Musashi, CD133)

  →

  CD133−

  →

  Read-out on ScanR
Hit from siRNA screen: **SUPT6H** (suppressor of Ty 6 homolog)

- Encodes the protein Spt6
- Histone chaperone interacting with H3, H4 and H2b
- Involved in both assembly and disassembly of the DNA
- Spt6 regulates chromatin structure and gene expression

- **Involved in the differentiation of stem cells** (AH Wang et al., The EMBO journal, 2013, Kedes et al., J Cell Physiol, 2003)
- **Spt6 is required for proper activation of Notch signaling pathway genes** shown in a zebrafish model (F.O. Kok et al., Developmental Biology, 2007)

HtH, helix-turn-helix domain, binds to double-stranded DNA;
YqgFc, predicted to be a resolvase or ribonuclease, but in Spt6, catalytic residues are exchanged, thus probably not active; HhH, triple-helix-domain, binding to double-stranded DNA;
S1, RNA-binding domain
SH2-N, SH2-C, tandem SH2 domains, binds phosphorylated Ser residues
Spt6 is expressed in primary GBM

![Image of protein expression](image1)

**T1** | **T2** | **T3**
---|---|---
**Spt6** | ![Spt6 expression](image2) | ![Spt6 expression](image3)
**LaminB** | ![LaminB expression](image4) | ![LaminB expression](image5)

**Con** | **si34** | **si35** | **si36**
---|---|---|---
**Spt6** | ![Spt6 expression](image6) | ![Spt6 expression](image7) | ![Spt6 expression](image8)
**TUB** | ![TUB expression](image9) | ![TUB expression](image10)

**DAPI** ![DAPI staining](image11)

**Emb-5** ![Emb-5 staining](image12)
GBM cells show decreased proliferation rates and increased DNA damage after Spt6 knock-down
Knock-down of Spt6 leads to activation of the ATM signaling pathway and slows down the repair of damaged DNA.
SUPT6H knock-down leads to decreased cell viability

Annexin V positive cells

- si36: 70.6%
- si35: 56.5%
- si34: 55.6%
- siCon: 14.5%

Relative viability over time (days)

- s36
- s35
- s34
- con

Caspase 3
C-Caspase 3
TUB
SUPT6H knock-down arrests GBM cells in G1 and G2-M phase of the cell cycle.
Conclusion and working model

Spt6 KD

DNA damage and activation of DDR

Proliferation

- Cell cycle arrest and eventually apoptosis
Thank you for your attention

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