Understanding the Role of *Eed* Deletion in Medulloblastoma

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GERSHON LAB

CELEBRATION OF UNDERGRADUATE RESEARCH
What is Medulloblastoma?

- The most common malignant pediatric brain cancer
- Occurs in the cerebellum region of the brain
- Conventional treatment plans result in 5-year survival rate of 80%
- Treatment is highly toxic, causing long-lasting impacts

- Important to research the signaling mechanisms that drive tumor growth and apoptotic pathways that make treatment effective in some patients but not in others.

Reference: Huang, Medscape, 2017
Sonic Hedgehog (SHH) Signaling

SHH hyperactivation leads to SHH-subgroup medulloblastoma

30% of medulloblastoma cases
Polycomb Repressive Complex 2 (PRC2)

**EZH2**: catalytic subunit that trimethylates H3K27

**EED**: enhances EZH2 by binding to trimethylated H3K27
Senior Honors Thesis Aims

1) ELUCIDATE HOW CELL CYCLE DYNAMICS ARE ALTERED IN EED-DELETED MEDULLOBLASTOMAS

2) WHETHER EED DELETION ALTERS THE LEVELS OF CELL CYCLE MARKERS, CELL DEATH, AND ABERRANT EXPRESSION OF MUSCULAR PROTEINS IN SHH-DRIVEN MEDULLOBLASTOMA
Methodology #1: Fluorescence-Activated Cell Sorting (FACS)

**Gating Definitions for Each Phase**

<table>
<thead>
<tr>
<th>Phase</th>
<th>pRB Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0</td>
<td>pRB-</td>
</tr>
<tr>
<td>G1</td>
<td>pRB+ / EdU- / 2n DNA</td>
</tr>
<tr>
<td>S</td>
<td>pRB+ / EdU+</td>
</tr>
<tr>
<td>G2</td>
<td>pRB+ / EdU- / 4n DNA</td>
</tr>
<tr>
<td>M</td>
<td>pRB++</td>
</tr>
</tbody>
</table>

The pRB- cells are G0

The cells that have very high amounts of pRB are M phase

Of the cells that are pRB+, the ones that are EdU+ are S phase

The cells that are pRB+ and EdU- are separated into G1 and G2 based on the amount of DNA
Methodology #2: Immunohistochemistry (IHC)

<table>
<thead>
<tr>
<th>Cell cycle markers</th>
<th>Cell death markers</th>
<th>Muscle proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRB</td>
<td>cC3</td>
<td>Myogenin</td>
</tr>
<tr>
<td>proliferation</td>
<td>apoptosis</td>
<td></td>
</tr>
<tr>
<td>NeuN</td>
<td>pH2Ax</td>
<td>Troponin</td>
</tr>
<tr>
<td>differentiation</td>
<td>DNA damage</td>
<td></td>
</tr>
<tr>
<td>p21</td>
<td>cC3/pH2Ax</td>
<td></td>
</tr>
<tr>
<td>cell cycling</td>
<td>apoptosis and DNA</td>
<td></td>
</tr>
<tr>
<td>inhibiting CDK</td>
<td>damage co-expression</td>
<td></td>
</tr>
<tr>
<td>Cdkn1C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor suppression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Image of 3D model with blue and green sections]
Conclusions for Aim #1

*Elucidate how cell cycle dynamics are altered in Eed-deleted medulloblastomas*

**pRB Cells at P12**

- Decreased proportion of total cells are proliferating cells
- Increased proportion of proliferating cells are in S and M phase

Although there are more cells exiting the cell cycle at P12 in Eed-deleted tumors, of the ones that remain, they are cycling faster than in control tumors.

Eed deletion slows tumor growth in early development, but the tumor later develops resistance, resulting in decreased survival.

P12
- **Increased proportion of proliferating cells are in S and M phase**

P18
- **Decreased proportion of total cells are proliferating cells**
Conclusions for Aim #1

Elucidate how cell cycle dynamics are altered in Eed-deleted medulloblastomas

Quantification of Proliferating Cells at P12

Cross-Age Comparisons:
- Significant decrease in M phase cells from P12 to P18 in Eed cKO MSmo mice (p < 0.05)

Although there are more cells exiting the cell cycle at P12 in Eed cKO tumors, of the ones that remain, they are cycling faster than in control tumors.

- Decreased proportion of total cells are proliferating cells
- Increased proportion of proliferating cells are in S and M phase
Conclusions for Aim #1

*Elucidate how cell cycle dynamics are altered in Eed-deleted medulloblastomas*

**pRB Cells at P18**

- **Control MSmo**
- **EED KO MSmo**

**Cross-Age Comparisons:**
- Significant increase in proliferating cells from P12 to P18 in Eed cKO MSmo mice (p < 0.001)

**P12**
- Decreased proportion of total cells are proliferating cells
- Increased proportion of proliferating cells are in S and M phase

**P18**
- Same proportion of total cells are proliferating cells

Although there are more cells exiting the cell cycle at P12 in Eed cKO tumors, of the ones that remain, they are cycling faster than in control tumors.
Conclusions for Aim #1

**Elucidate how cell cycle dynamics are altered in Eed-deleted medulloblastomas**

### Quantification of Proliferating Cells at P18

<table>
<thead>
<tr>
<th>Cell Cycle Phase</th>
<th>Control MSmo</th>
<th>EED KO MSmo</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>S</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>G2</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

* P12
  - Decreased proportion of total cells are proliferating cells
  - Increased proportion of proliferating cells are in S and M phase

* P18
  - Same proportion of total cells are proliferating cells
  - Increased proportion of proliferating cells are in S phase

Although there are more cells exiting the cell cycle at P12 in Eed cKO tumors, of the ones that remain, they are cycling faster than in control tumors.

Eed deletion slows tumor growth in early development, but the tumor later develops resistance, resulting in decreased survival.
Conclusions for Aim #2

Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma

![Diagram showing the effect of Eed deletion on myogenin expression in SHH-driven medulloblastoma.](image)

**Eed-deleted Medulloblastoma**
- Increased myogenin expression

**Eed-deleted CGNPs**
- Increased myogenin expression

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### Graph

<table>
<thead>
<tr>
<th>Cell Count</th>
<th>Whole tumor</th>
<th>Region of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Myogenin+ at P12</td>
<td><strong>Control MSmo</strong></td>
<td><strong>EED KO Msmo</strong></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

- **Whole tumor**
  - Control MSmo: [Graph Data]
  - EED KO Msmo: [Graph Data]

- **Region of interest**
  - Control MSmo: [Graph Data]
  - EED KO Msmo: [Graph Data]
Conclusions for Aim #2

Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma

![](chart.png)

In tumors, unlike Eed-deleted cerebella, later stages of muscle differentiation were blocked.
Conclusions for Aim #2

Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma

<table>
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<th>%pRB+ at P12</th>
<th>Control MSmo</th>
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<tr>
<td>Whole tumor</td>
<td>*</td>
<td>EED KO Msmo</td>
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In tumors, unlike Eed-deleted cerebella, later stages of muscle differentiation were blocked

**Eed-deleted Medulloblastoma**
- Increased myogenin expression
- Equivalent troponin expression
- Decreased proliferation

**Eed-deleted CGNPs**
- Increased myogenin expression
- Increased troponin expression
- Decreased, prolonged proliferation

**Increased p21 expression**
Conclusions for Aim #2

*Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma*

In tumors, unlike *Eed*-deleted cerebella, later stages of muscle differentiation were blocked. Muscle differentiation may have been an alternative fate for tumor cells exiting the cell cycle.

### %NeuN+ at P12

<table>
<thead>
<tr>
<th></th>
<th>Whole tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MSmo</td>
<td>1.2</td>
</tr>
<tr>
<td>EED KO Msmo</td>
<td>0.5</td>
</tr>
</tbody>
</table>

** increased significantly

### Eed-deleted Medulloblastoma

- Increased myogenin expression
- Equivalent troponin expression
- Decreased proliferation
- Decreased differentiation
- Increased apoptosis
- Equivalent p21 expression

### Eed-deleted CGNPs

- Increased myogenin expression
- Increased troponin expression
- Decreased, prolonged proliferation
- Decreased differentiation

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![Insert Zoom Video Here](https://via.placeholder.com/150)
Conclusions for Aim #2

Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma

In tumors, unlike Eed-deleted cerebella, later stages of muscle differentiation were blocked. Muscle differentiation may have been an alternative fate for tumor cells exiting the cell cycle.

**Whole tumor**

- **%cC3+ at P12**
  - Control MSmo: 1.5% ± 0.2%
  - EED KO Msmo: 0.5% ± 0.1%

**Eed-deleted Medulloblastoma**
- Increased myogenin expression
- Equivalent troponin expression
- Decreased proliferation
- Decreased differentiation
- Decreased apoptosis

**Eed-deleted CGNPs**
- Increased myogenin expression
- Increased troponin expression
- Decreased, prolonged proliferation
- Decreased differentiation
- Increased apoptosis
Conclusions for Aim #2

Whether Eed deletion alters the levels of cell cycle markers, cell death, and aberrant expression of muscular proteins in SHH-driven medulloblastoma

<table>
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<th>%p21+ at P12</th>
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Eed-deleted Medulloblastoma
- Increased myogenin expression
- Equivalent troponin expression
- Decreased proliferation
- Decreased differentiation
- Decreased apoptosis
- Equivalent p21 expression

Eed-deleted CGNPs
- Increased myogenin expression
- Increased troponin expression
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- Decreased differentiation
- Increased apoptosis
- Increased p21 expression

In tumors, unlike Eed-deleted cerebella, later stages of muscle differentiation were blocked. Muscle differentiation may have been an alternative fate for tumor cells exiting the cell cycle.