Estrés del reticle endoplasmàtic com estratègia terapèutica contra els tumors cerebrals

Enric Xipell Badals

1 de juny de 2016
Glioma

60% of the brain tumors in adults.

High proliferation rate and invasiveness.

Standard Treatment: surgery, radiotherapy and chemotherapy.

Despite maximum therapeutic efforts, the prognostic is dismal.

High mortality rate and relapse.
Temozolamide (TMZ)

Surgery
Radiotherapy
Chemotherapy (TMZ)
Brain Tumor Stem Cells (BTSC):
Efficient DNA reparation
Temozolamide (TMZ)

Adapted from Xipell et al., 2016

Adapted from Hegi et al., 2005
Temozolamide (TMZ)

Adapted from Xipell et al., 2016

Adapted from Hegi et al., 2005
Hypothesis

Temozolamide

Accumulation of O6-MeG

BER repairation (MPG)

MGMT

O6-MeG

N7-MeG

N3-MeA

10% 60-80% 10-20%

O6-G

N7-G

N3-A

HR (RAD51)

Double-strand break (DSB)

Cell cycle arrest

Cell survival

Apoptosis cell death

Reticle endoplasmatic

Adapted from Xipell et al., 2016

Adapted from Hegi et al., 2005

Adapted from Clarke et al., 2009
SLM induces a maintained and potent ER stress/UPR

Adapted from Clarke et al., 2009
ER stress down-regulates DNA repair proteins

Adapted from Yoshimoto et al., 2012
Combination of SLM/TMZ results in low levels of proteins involved in TMZ response

Adapted from Yoshimoto et al., 2012
SLM plus TMZ increments DSB

Adapted from Yoshimoto et al., 2012
Combination of SLM with TMZ increments DSB

<table>
<thead>
<tr>
<th></th>
<th>NSC23 Vehicle</th>
<th>SLM (10^{-8}M)</th>
<th>SLM (10^{-7}M)</th>
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<tbody>
<tr>
<td>T98G</td>
<td>Vehicle</td>
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<tr>
<td>NSC11</td>
<td>Vehicle</td>
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<td>TMZ (10^{-4}M)</td>
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<td>Vehicle</td>
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<td>TMZ (10^{-4}M)</td>
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<td>Vehicle</td>
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Phospho-histone H2A.X (% Positive cells)

- NSC23
- T98G
- NSC11
SLM in combination with TMZ exerts a potent antitumor effect.
SLM in combination with TMZ exerts a potent antitumor effect.
SLM in combination with TMZ exerts a potent antitumor effect.
in vivo experiments

Treatment scheme:

Time (days) 0 10 20 30

Cell Implantation

Adapted from Lal et al., 2000
SLM plus TMZ results in a significant antitumor effect *in vivo*
SLM plus TMZ results in a significant antitumor effect \textit{in vivo}.

\begin{itemize}
  \item [\textbf{NSC11}]\begin{itemize}
    \item Vehicle \hspace{1cm} SLM \hspace{1cm} TMZ \hspace{1cm} SLM/TMZ
  \end{itemize}
  \begin{itemize}
    \item Percent survival (%)
  \end{itemize}
  \begin{itemize}
    \item Time (days)
  \end{itemize}

  \begin{itemize}
    \item \(P < 0.001\)
  \end{itemize}

\begin{itemize}
  \item [\textbf{NSC23}]\begin{itemize}
    \item Vehicle \hspace{1cm} SLM \hspace{1cm} TMZ \hspace{1cm} SLM+TMZ
  \end{itemize}
  \begin{itemize}
    \item Percent survival (%)
  \end{itemize}
  \begin{itemize}
    \item Time (days)
  \end{itemize}

  \begin{itemize}
    \item \(P < 0.05\)
  \end{itemize}
\end{itemize}
Acknowledgements:

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Human resources and mobility
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Enric Xipell Badals

1 de juny de 2016
High throughput screening on CSC

Adapted from Gupta et al., 2009
Molecular mechanism of action of Salinomycin (SLM)

Ionophore for $K^+$ and $Na^+$. 

Adapted from Gupta et. al., 2009
Salinomycin and UPR

Halting protein translation

Degrading misfolded proteins

Increasing chaperones and/or proteins related to the protein folding

Cell death induction mediated by Apoptosis

Adapted from Clarke et. al., 2009
DNA repair proteins levels in the different cell lines used

Suplemental

<table>
<thead>
<tr>
<th>Protein</th>
<th>NSC23</th>
<th>NSC11</th>
<th>NSC7-2</th>
<th>NSC6-27</th>
<th>NSC5-22</th>
<th>NSC2</th>
<th>NSC10-6</th>
<th>SF188</th>
<th>GSC229</th>
<th>U87 MG</th>
<th>U251 MG</th>
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</table>
2. SLM plus TMZ increments DSB *in vivo*

P-H2A.X

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLM</th>
<th>TMZ</th>
<th>TMZ/SLM</th>
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<tbody>
<tr>
<td>% P-H2A.X (positive pixels/negative pixels)</td>
<td></td>
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<td></td>
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<tr>
<td>SLM</td>
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<tr>
<td>TMZ</td>
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*NSC11*
Therapeutic potential of SLM as a UPR drug

Adapted from Clarke et. al., 2009
1. SLM plus NAC generates autophagy and necrosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>AIF</th>
<th>DAPI</th>
<th>Merge</th>
<th>Plot profile</th>
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<td><img src="image2" alt="Control DAPI" /></td>
<td><img src="image3" alt="Control Merge" /></td>
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<tr>
<td>NAC 7·10⁻³M</td>
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<td><img src="image6" alt="NAC DAPI" /></td>
<td><img src="image7" alt="NAC Merge" /></td>
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<td><img src="image10" alt="SLM DAPI" /></td>
<td><img src="image11" alt="SLM Merge" /></td>
<td><img src="image12" alt="SLM Plot" /></td>
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<tr>
<td>SLM 1·10⁻⁷M NAC 7·10⁻³M</td>
<td><img src="image13" alt="SLM NAC AIF" /></td>
<td><img src="image14" alt="SLM NAC DAPI" /></td>
<td><img src="image15" alt="SLM NAC Merge" /></td>
<td><img src="image16" alt="SLM NAC Plot" /></td>
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</table>
UPR modulation alters DNA repair protein levels

Adapted from Yoshimoto et al., 2012
SLM with TMZ induces a synergistic antiglioma effect

<table>
<thead>
<tr>
<th>Range of Combination Index</th>
<th>Description</th>
<th>Graded Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>Very strong synergism</td>
<td>+++++</td>
</tr>
<tr>
<td>0.1–0.3</td>
<td>Strong synergism</td>
<td>+++++</td>
</tr>
<tr>
<td>0.3–0.7</td>
<td>Synergism</td>
<td>+++</td>
</tr>
<tr>
<td>0.7–0.85</td>
<td>Moderate synergism</td>
<td>++</td>
</tr>
<tr>
<td>0.85–0.90</td>
<td>Slight synergism</td>
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</tr>
<tr>
<td>0.90–1.10</td>
<td>Nearly additive</td>
<td>±</td>
</tr>
<tr>
<td>1.10–1.20</td>
<td>Slight antagonism</td>
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</tr>
<tr>
<td>1.20–1.45</td>
<td>Moderate antagonism</td>
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</tr>
<tr>
<td>1.45–3.3</td>
<td>Antagonism</td>
<td>– – –</td>
</tr>
<tr>
<td>3.3–10</td>
<td>Strong antagonism</td>
<td>– – – –</td>
</tr>
<tr>
<td>&gt;10</td>
<td>Very strong antagonism</td>
<td>– – – – – – –</td>
</tr>
</tbody>
</table>

Adapted from Chou and Talahay, 1984.
SLM and regulated cell death

Programmed and different cell death from autophagy and apoptosis

Autophagic cell death

Apoptosis cell death

SLM

Necrosis regulated cell death

Orchestrated by caspase family

Autophagy exceed signal
Regulated Necrosis:

Regulated necrosis cell death is the least characterized cell death.

There are not determinant markers for necrosis regulated cell death, however several process have been related with this type of cell death.

Adapted from Linkerman et al., 2014
TMZ/SLM induces regulated necrosis cell death