Investigating efficacy of combinational use of Rapamycin and Valproic acid in treatment of triple negative breast cancer cells

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Introduction

Background
- Breast cancer is the most common cancer afflicting Canadian women and is the 2nd leading cause of death from cancer in Canadian women. Even though treatment for breast cancer exists, relapse is a possibility.
- Triple-negative breast cancer (TNBC) is a subset of breast cancer, possessing tumors not expressing estrogen receptors, progesterone receptors, or HER-2 proteins. TNBC is consequently resistant to standard drug therapies, has the worst prognosis of breast cancer subtypes and metastasizes more and recurs faster than other types of cancer, urgently requiring targeted therapies.

Hypothesis

The MDA-MB-231 Human breast cancer cells are more effectively treated by combinational administration of Rapamycin and Valproic acid by inhibiting both the mTOR/Akt pathway and through altering of the epigenetic landscape via the HDAC pathway.

Methodology

Cell Culture
MDA-MB-231 Human breast cancer cells were cultured in a T75 flask with media and incubated at 37°C and humidified 5% CO₂.

Treatment

<table>
<thead>
<tr>
<th>Wells</th>
<th>Label</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 2</td>
<td>C</td>
<td>2.5μL DMSO</td>
</tr>
<tr>
<td>3 &amp; 4</td>
<td>V</td>
<td>1μL 0.25μM VPA</td>
</tr>
<tr>
<td>5 &amp; 6</td>
<td>2V</td>
<td>2μL 0.25μM VPA</td>
</tr>
<tr>
<td>7 &amp; 8</td>
<td>R</td>
<td>0.5 μL of 10μM Rapamycin.</td>
</tr>
<tr>
<td>9 &amp; 10</td>
<td>VR</td>
<td>1μL of 0.25μM VPA and 0.5 μL of 10μM Rapamycin.</td>
</tr>
<tr>
<td>11 &amp; 12</td>
<td>2VR</td>
<td>2μL of 0.25μM VPA and 0.5 μL of 10μM Rapamycin.</td>
</tr>
</tbody>
</table>

Figure 1. Photomicrograph of MDA-MB-231 human breast cancer cells after drug treatment at 120h. The representative images of growing colonies 5d after treatment were taken with 588nm·52mm lens at 10X magnification. The treatments given are as follows: VPA: 1μL of 0.25μM Valproic acid, Rapamycin: 0.5 μL of 10μM Rapamycin, and VPA + Rapamycin: VPA:1μL of 0.25μM Valproic acid and 0.5 μL of 10μM Rapamycin. Cells treated with VPA + Rapamycin show a decrease in cell number compared to the control treated with 2.5μL of DMSO.

Results

Table 1. Percentage of MDA-MB-231 cells harvested compared to control population (%) at 120h compared with the harvested control population (267 750 cells). Cells of each well were stained with Trypan blue and counted using a compound microscope and hemocytometer. Compared to the control (C) treatment of 2.5μL of DMSO, the combinational therapy (VR) 1μL 0.25μM VPA and 0.5 μL 10μM Rapamycin, and (2V) 2μL 0.25μM VPA and 0.5 μL of 10μM Rapamycin showed a decrease in observed cell population. This reduction in cell population is comparably lower to the (R) treatment of 0.5 μL of 10μM Rapamycin and (V) and (2V) treatments of 1μL and 2μL of 0.25μM VPA respectively.

Discussion

- mTOR is a downstream target of Akt and inhibiting mTOR using Rapamycin results in a decrease in protein synthesis that ultimately blocks the pro-growth, proliferative and survival functions of the mTOR kinase and halts cell cycle progression.
- Histone deacetylases (HDACs) regulate the acetylation of a variety of histone and nonhistone proteins which controls the transcription and regulation of genes responsible for cell proliferation and survival. The HDAC inhibitor VPA was chosen due to its safe long-term use treating epilepsy and its practical pharmacokinetics.
- Previous studies show that HDACi have a wide range of anticancer effects including induction of tumor cell apoptosis and DNA damage repair and combined use of HDACi and Rapamycin also prevents resistance development seen in mono-drug therapy.
- Simultaneously combining VPA with Rapamycin may be more efficacious than either alone in treating TNBC. VPA may repress phosphorylation of Akt upstream of mTOR which contributes to the overall antitumor effect.

Figure 3. Pharmacological inhibition of the PI3K/Akt/mTOR pathway. mTOR is downstream of Akt and Rapamycin inhibits mTOR.

Conclusion

The combination of VPA and Rapamycin killed more of the MDA-MB-231 breast cancer cell population than either VPA or Rapamycin alone. Although further studies are needed to substantiate these results, the combined effect of the two inhibitors suggests a greater additive antitumor effect of combinational treatment and prompts future studies on possible synergistic effects between simultaneously targeting HDAC and mTOR in increasing remission time of TNBC. The exact interaction between HDACi and mTORi should be elucidated in order to understand their effect.

References


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