Disulfiram and copper reverses hypoxia induced chemoresistance in E58 and 2591 Malignant Mesothelioma cells.

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Background

- Malignant Mesothelioma (MM) is a mesothelial neoplasm with a very long latency period and low survival rates.
- Pleural Mesothelioma is the most common, usually with a strong connection to past asbestos exposure.
- Diagnosis frequently occurs by chance by which point the tumour may have grown and metastasised.

- Hypoxic environments observed in solid tumours induce NF-κB pathway whose downstream effects promote cancer hallmarks like angiogenesis and anti-apoptotic characteristics, as well as Cancer stem cells (CSCs).
- CSCs comprise a small proportion of a tumour, responsible for cancer recurrence and chemoresistance of drugs used today like Pemetrexed and Cisplatin.

- Disulfiram (trade name Antabuse) is a well established anti-alcoholism drug that has numerously been shown to effectively inhibit NF-κB and CSCs, combat cancer cells and chemoresistance (Yip et al., 2011).
- Use of Disulfiram is a promising example of drug repurposing, saving years and millions on compound and target discovery and development.
- Nanocapsulisation of Disulfiram in poly lactic co-glycolic acid (PLGA) gives longer drug t1/2, rendering it to be more effective and longer lasting in the bloodstream (Faseehi et al., 2017).

Disulfiram’s mechanism of action in cancer cells.

Cancer Research UK, 2019

Aims and Objectives

To examine to the ability of Disulfiram and copper to reverse hypoxia-induced chemoresistance in E58 and 2591 malignant mesothelioma cells using MTT cytotoxicity assay.

Method

Add 200µl of cell-media mixture into wells

Collect, centrifuge and resuspend cell pellet

Dosing

- Add serially diluted drugs:
  - PMT/Cis= 1/10mM
  - PLGA=5mM
  - CuZ= 10mM

- Add 20µl MTT reagent

Plate reading

- Aspirate wells
- Add 80µl DMSO
- Add 20µl Sorensen’s glycine buffer
- Read Plates at 540nm.

Table 1: IC50 values after 72 hours of treatment. Both E58 and 2591 cells exhibited high degree of chemoresistance, which only escalated with hypoxic cultures. Cis and PMT showed more anti-cancer activity in E58 and 2591 cells, respectively.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Normoxia</th>
<th>Hypoxia</th>
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<tr>
<td>E58</td>
<td>PMT(nM)</td>
<td>Cis(nM)</td>
</tr>
<tr>
<td>2591</td>
<td>&gt;100</td>
<td>&gt;100</td>
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Figure 1: Hypoxic cells from both cell lines show higher degree of chemoresistance than normoxic cells to PMT and Cisplatin.

Figure 2: A-D. PLGA-DS/Cu enhances Cis and PMT effect in both cell lines in hyoxia.

Figure 3. A-B. Cis and PMT enhance PLGA-DS/Cu drug effect in E58 cells in hypoxia. C-D. Cisplatin and PMT doesn’t show to have enhancing effects on PLGA-DS/Cu in 2591 hypoxic cells.

Discussion

- Hypoxic E58 and 2591 cultures show a higher degree of chemoresistance than normoxic cells when treated with PMT and Cis, which correlates with traits of CSCs in low oxygen partial pressure tumour microenvironments.
- This is supported by IC50 values for hypoxic cultures which exceeded the concentrations tested, and agrees with the idea of chemoresistance being governed by hypoxia-activated pathways such as NF-κB (Liu et al., 2014).
- Cis has not greatly potentiated the effect of PLGA-DS/Cu on hypoxic 2591 cells (ED values >0.9), the only weak synergistic interaction in hypoxia being that of PMT+PLGA-DS/Cu (ED50=0.73).
- E58 combination indices at ED75 and ED90 show strong synergistic interaction of both agents in combination with PLGA-DS/Cu (≤0.5). This is supported by cell viability curves.
- Differences in CI and IC50 values observed between cell lines can be inferred by variations in genetic/epigenetic acquisitions.

Acknowledgments

I want to thank Professor Wang and his whole research team for their invaluable help in accomplishing this project.

References


PLGA-DS/Cu is a promising stand alone, as well as combination, therapy for solid malignancies due to its CSC eradication ability.

- PLGA-DS/Cu enhances synergistically the effect of anti-cancer therapies currently used clinically.
- Synergy between PLGA-DS/Cu and conventional anticancer therapies is cell line-dependent.
- Further MTT assay repeats should be conducted to ensure optimal accuracy in identifying inhibitory concentrations in order to ensure minimal cytotoxicity of non-cancerous tissues.