Energy Restriction Synergizes Doxorubicin Activity via Targeting Breast Cancer Stem Cells

Hany A. Omar, PhD

Department of Pharmacology - College of Pharmacy
University of Sharjah, UAE &
Department of Medicinal Chemistry - College of Pharmacy
The Ohio State University, USA
Introduction

The resistance of human malignancy to multiple chemotherapeutic agents remains a major obstacle in cancer therapy.

Cellular factors that cause drug resistance:

- Increased efflux (ATP-dependent efflux pumps)
- Decreased influx
- Activation of DNA repair
- Blocked apoptosis (e.g. decreased ceramide levels)
- Activation of detoxifying systems (cytochrome P450)
Antitumor drug efflux caused by ATP-driven pumps is the primary reason for chemoresistance.

The increased expression of ATP-binding cassette (ABC) transporters gene family contributes to multidrug resistance (MDR) via pumping out many anti–tumor drugs, thereby resulting in low intracellular drug concentrations.

\[ \text{ABC transporter} \]

ABC transporters are membrane transporters that can pump various structurally unrelated cytotoxic drugs out of cells at the expense of ATP hydrolysis.

ABC superfamily includes:
- Multidrug resistance proteins (MRPs/ABCC)
- Breast cancer resistance protein (BCRP/ABCG2)
- P-glycoprotein (P-gp/ABCB1)
Cancer stem cells (CSC) show high expression levels of ABC transporters which play a major role in their chemoresistance and great capacity for tumorigenesis.

The activity of these transporters can be followed through the transport of fluorescent dyes like rhodamine and Hoechst.

This property has allowed CSC to be separated from non-stem cells on fluorescence-activated cell sorters (FACS).
Protein expression of cancer CSC markers (CD24, CD44 and ALDH1) and classic prognostic factors (tumour size, histological grade and lymph node metastasis) in the series of 466 invasive breast carcinomas.
Carcinomas with **more than 10%** of $\text{CD}44^+\text{CD}24^{-/low}$ cells showed increased risk of disease-free survival (DFS), when compared with tumours with less than 10% of $\text{CD}44^+\text{CD}24^{-/low}$ cells.
**Introduction**

**Targeting ATP-driven efflux transporters**

- Several pharmacological agents that can interact with ABC transporters have been developed to inhibit multidrug resistance (MDR) such as **verapamil**, **MRP1**, **MS-209** and **VX-710**.

- An alternative strategy targeting ABC transporters involves regulating the protein expression levels of these transporters.

However, none of them is clinically successful because of the dose limiting toxic effect of these modulators.

Modified from Katzung
Hypothesis

Limit the availability of energy

Energy restriction mimetic agents (ERMA)

Classical anticancer agents (Doxorubicin)

ERMA selectively* target cancer cells

Synergistic anticancer effect

Antagonizes ATP-driven pumps

Limits Chemo-resistance

*Unlike non-neoplastic cells, exhibit a high demand for glucose with very limited flexibility for modifying their means of ATP generation (Warburg Effect)

Cancer cells lack the metabolic flexibility and shift cellular metabolism to aerobic glycolysis as a cellular source of energy.

The antitumor effects of ERMAs and/or doxorubicin and the therapeutic combination were assessed by MTT assay, caspase activation, PARP cleavage, colony formation assay, immunofluorescence, and Western blot analysis.

Percentage of ALDH1+/CD44+/CD24-/low in MCF-7 & MDA-MB-231 cells was assessed by fluorescence-activated cell sorting (FACS)
CSC (CD44+/CD24-/low) content in MCF-7, MCF-7/ADR, MDA-231, MDA-231/ADR cells determined by flow cytometry

Un-published data
Results

Breast CSC exhibit greater therapeutic resistance

MTT assay for cell viability analysis of cells treated with doxorubicin.

Un-published data
Results

Breast CSC exhibit greater therapeutic resistance

Caspase 3/7 activity in cells treated with doxorubicin.

Un-published data
Results

Breast CSC exhibit greater therapeutic resistance

In vitro confocal images of subcellular doxorubicin fluorescence distribution
The combination of OSU-CG5 and Dox restored the sensitivity

MTT Assay

Caspases 3/7 Assay

Un-published data
Results

The presence of CSC in breast cancer cell lines. Cells were stained with Hoechst33342 in the presence or absence of resveratrol, and were analyzed using FACS Vantage. The trapezia in each panel indicate the SP area.

Un-published data
Results

<table>
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<tr>
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<th>Control</th>
<th>+Verapamil</th>
<th>+OSU-CG5</th>
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<td>MCF-7</td>
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<td>MCF-7/ADR</td>
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Verifying the mechanism of synergism of the combination of both drugs.

- Over expression of AMPK
- Effect on glucose transport
- Oxidative stress

In vivo combination study (safety and efficacy)

- MTD
- Ectopic animal
Conclusions

- The results demonstrated the effectiveness of ERMAs such as **OSU-CG5** to **overcome** the chemoresistance to doxorubicin via targeting breast CSCs population.

- Our findings raised the possibility that OSU-CG5 might be a promising drug in **combinatorial therapy** with the existing chemotherapeutic agents that fail to eliminate CSCs.

- This combination strategy, therefore, may open a **new avenue** for more effective therapies for treatment-resistant breast carcinomas.
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