Energy Restriction Synergizes Doxorubicin Activity via Targeting Breast Cancer Stem Cells

Hany A. Omar, PhD

جامعة الشارقة
UNIVERSITY OF SHARJAH

Department of Pharmacology -College of Pharmacy

University of Sharjah, UAE &

Department of Medicinal Chemistry -College of Pharmacy

The Ohio State University, USA

THE OHIO STATE UNIVERSITY 1870

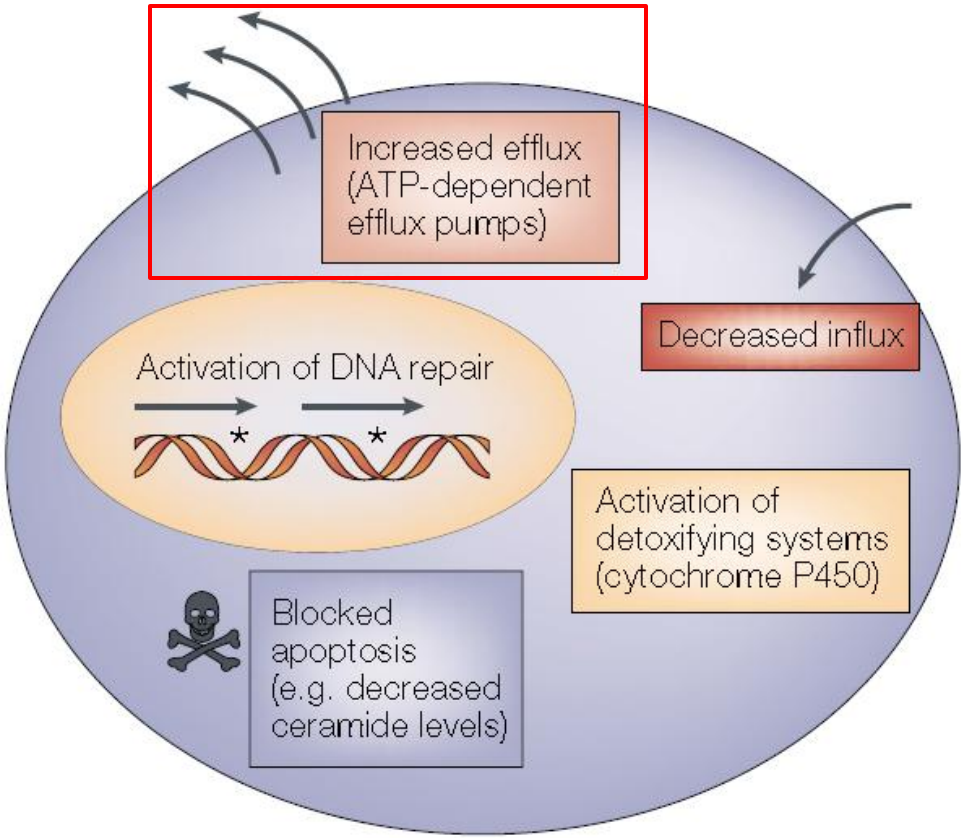
OUTLINE

- Introduction
- Hypothesis
- Approach
- Results
- Conclusions

Introduction

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

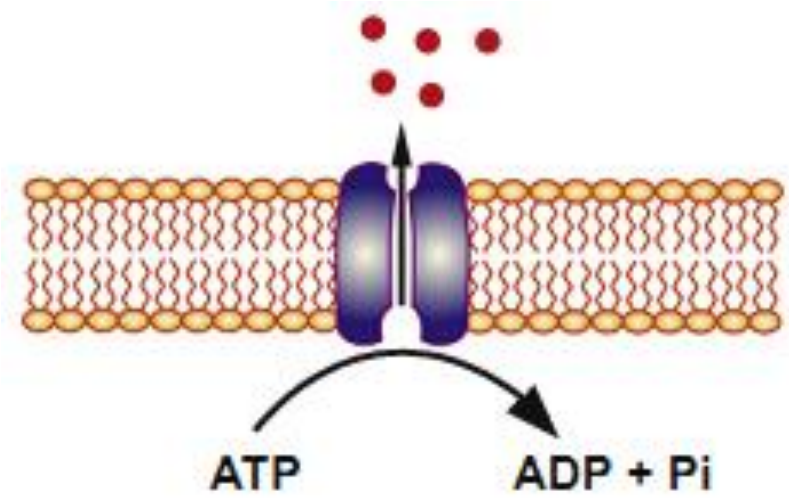
Cellular factors that cause drug resistance



Nature Reviews Cancer **2**, 48-58

Introduction

- 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

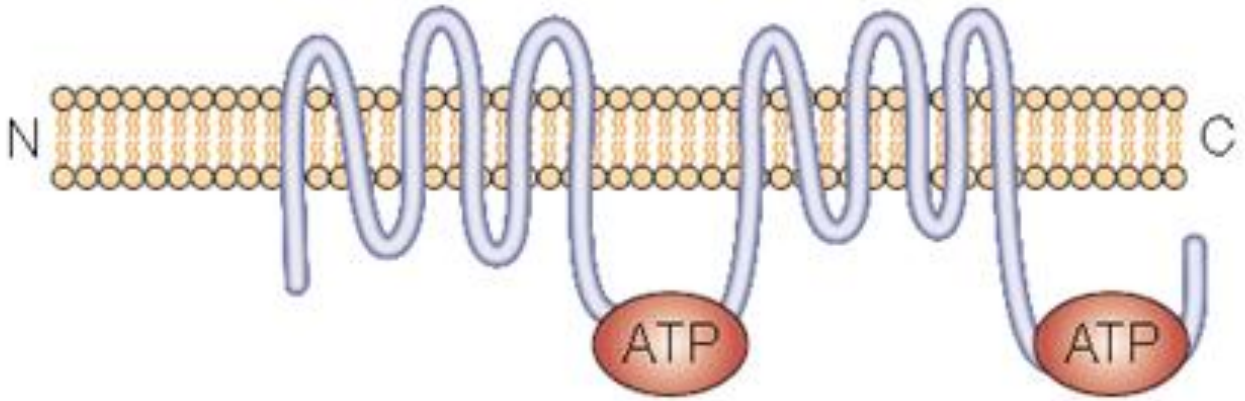


ABC transporter

Oncogene (2007) **26,** 1357–1360.

Introduction

- 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)



Nature Reviews Cancer **2**, 48-58

Introduction

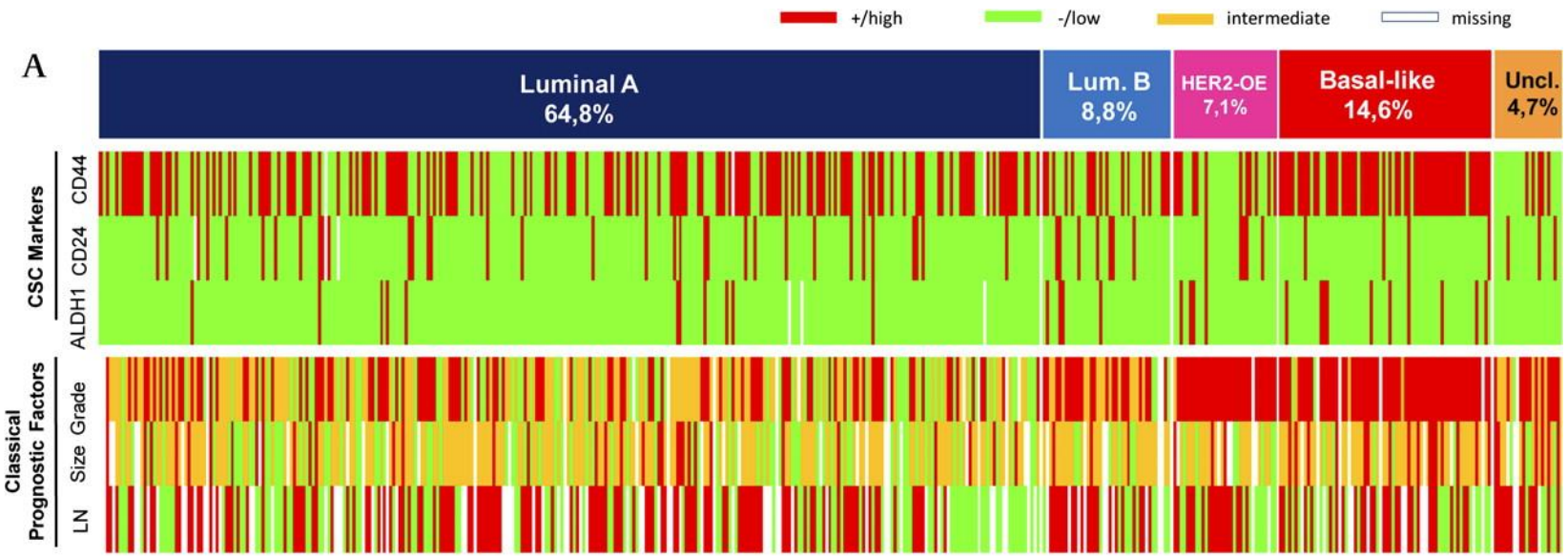
- Cancer stem cells (CSC) show high expression levels of ABC transporters which play a major role in their **chemoresistance** and great capacity for **tumorigenesis**.
- The activity of these transporters can be followed through the transport of fluorescent **dyes** like rhodamine and Hoechst.
- This property has allowed CSC to be separated from non-stem cells on fluorescence-activated cell sorters (FACS).

Oncogene (2007) 26, 1357–1360.

Introduction

Breast Cancer Signature

(Immunohistochemistry array)



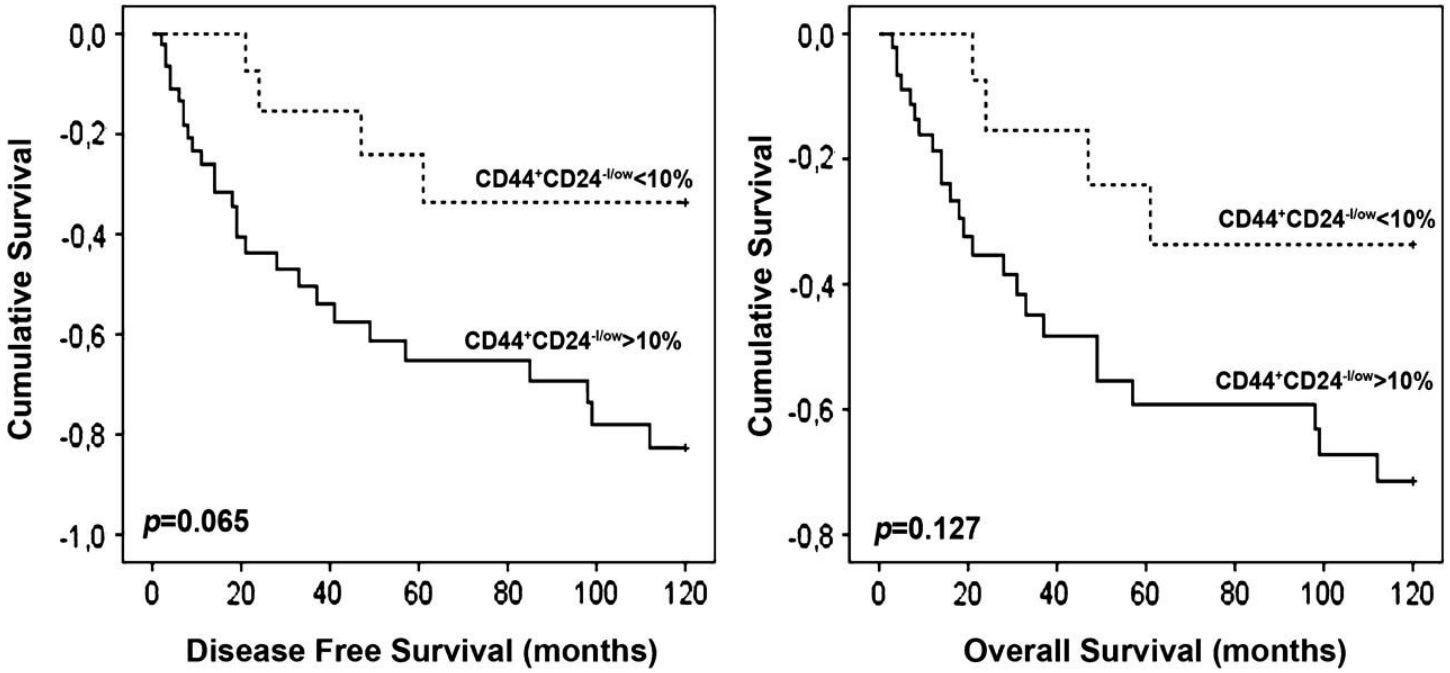
Protein expression of cancer CSC **markers** (CD24, CD44 and ALDH1) and classic prognostic factors (tumour size, histological grade and lymph node metastasis) in the series of 466 invasive breast carcinomas

J Clin Pathol 2011;**64**:937-946

Introduction

Disease-free survival & overall survival in carcinomas

(Defined according to the CD44/CD24 pattern of expression)



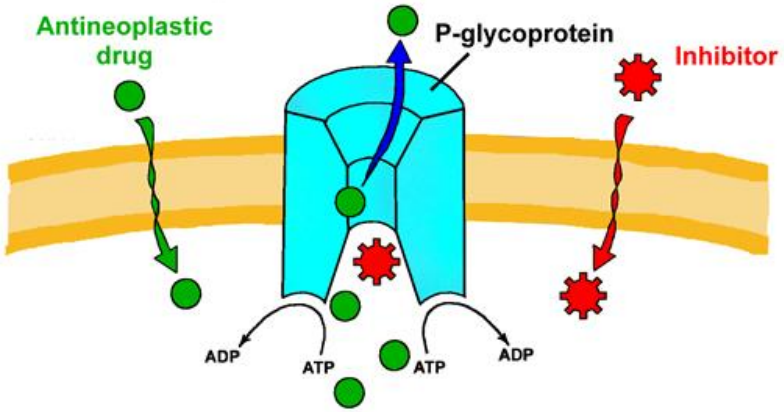
Carcinomas with **more than 10%** of $CD44^{+}CD24^{-/low}$ cells showed increased risk of disease-free survival (DFS), when compared with tumours with less than 10% of $CD44^{+}CD24^{-/low}$ cells.

J Clin Pathol 2011;**64**:937-946

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.**

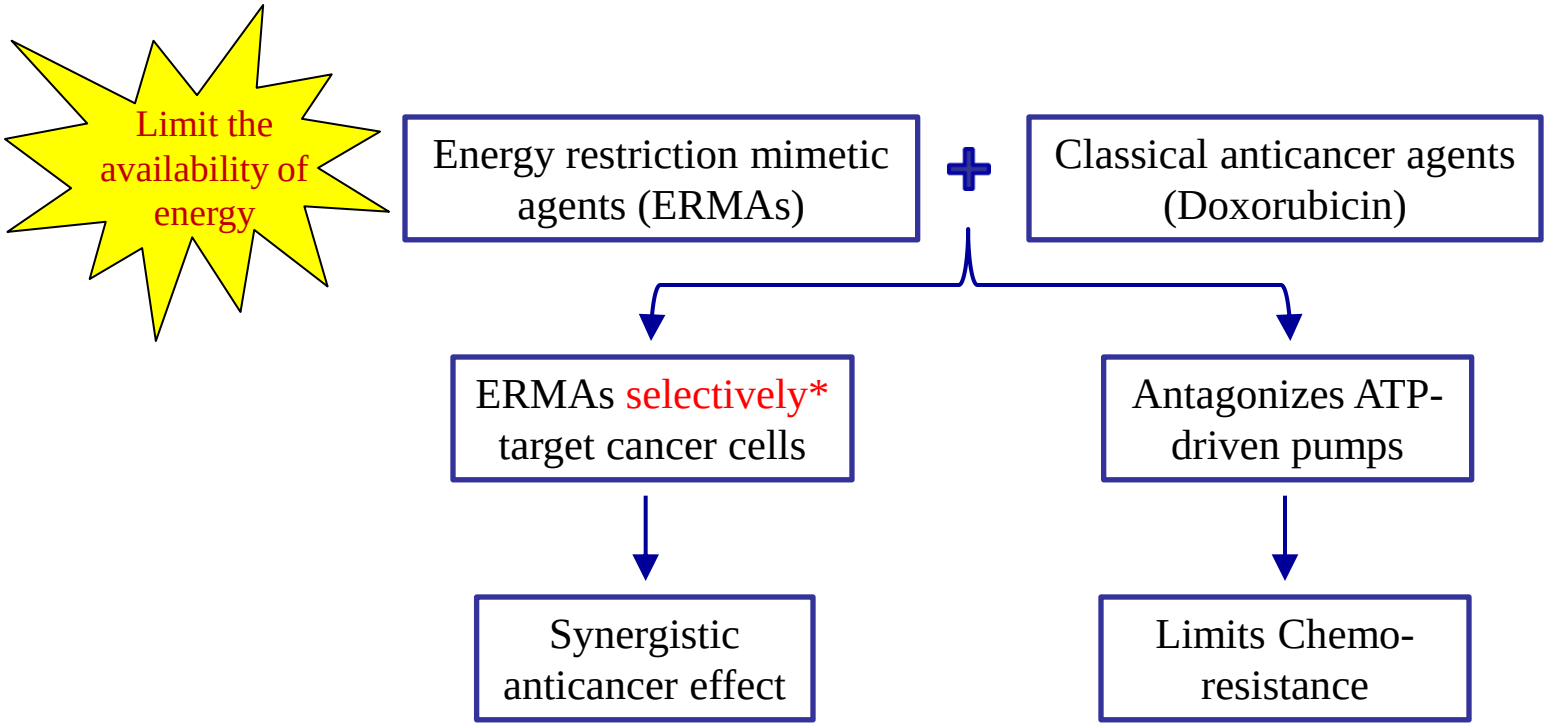


- An alternative strategy targeting ABC transporters involves regulating the protein expression levels of these transporters.

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

Modified from Katzung

Hypothesis

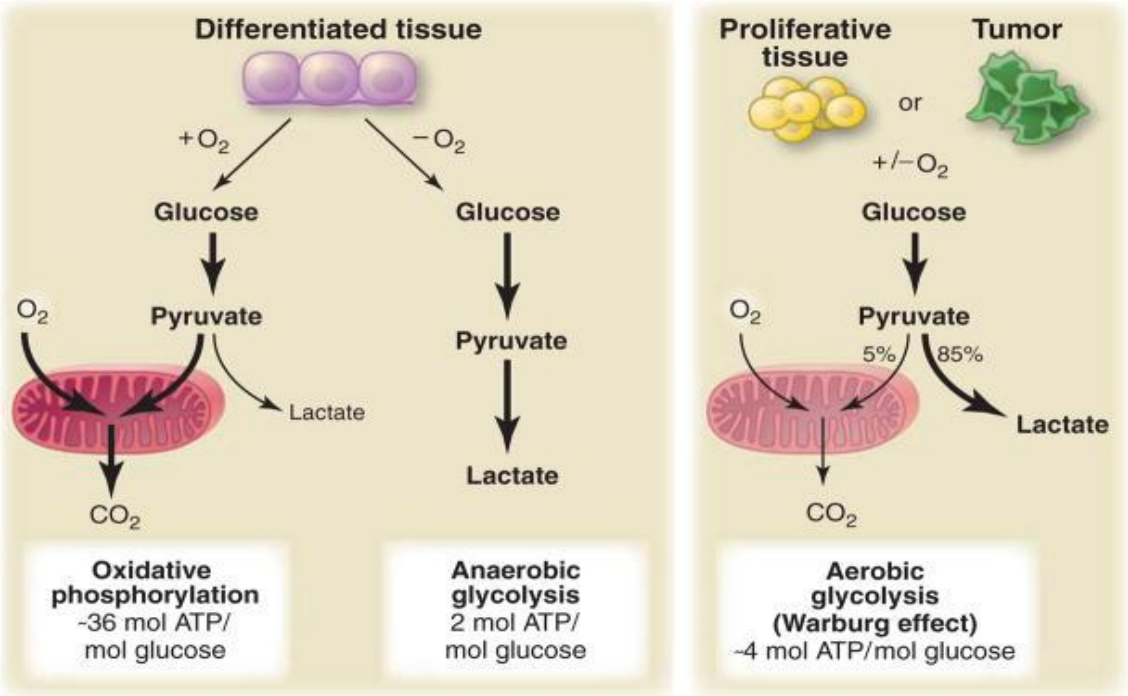


*Unlike non-neoplastic cells, exhibit a high demand for glucose with very limited flexibility for modifying their means of ATP generation (Warburg Effect)

Science. 2009; 324(5930):1029-33.

Hypothesis

Warburg Effect



Normal Cell

Cancer Cell

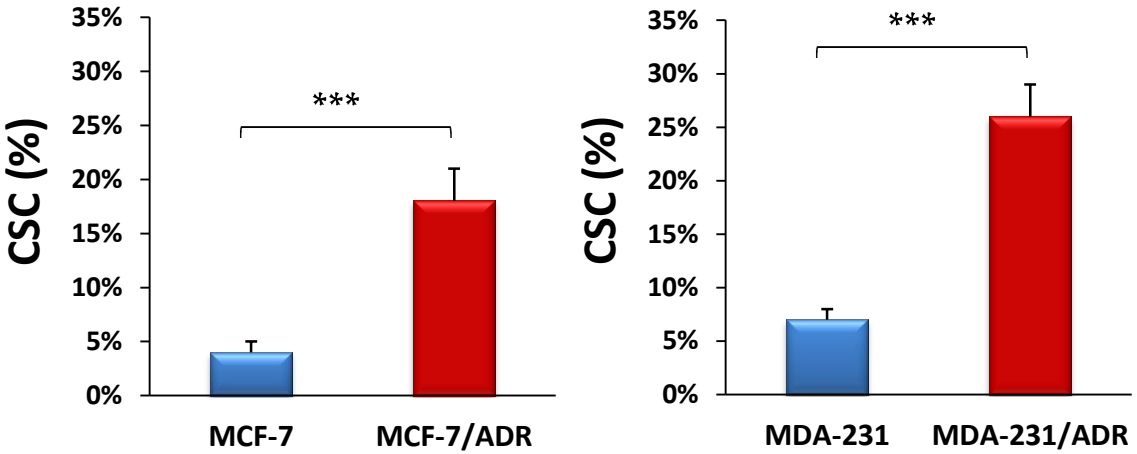
Cancer cells lack the metabolic flexibility and shift cellular metabolism to aerobic glycolysis as a cellular source of energy

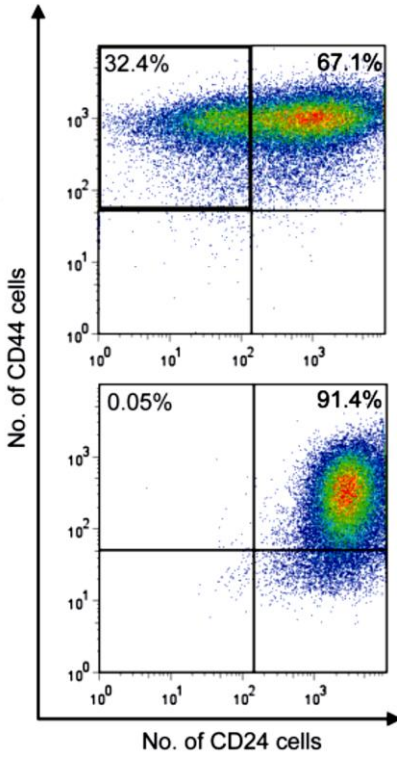
Science. 2009; 324(5930):1029-33.

Approach

- 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)

Results



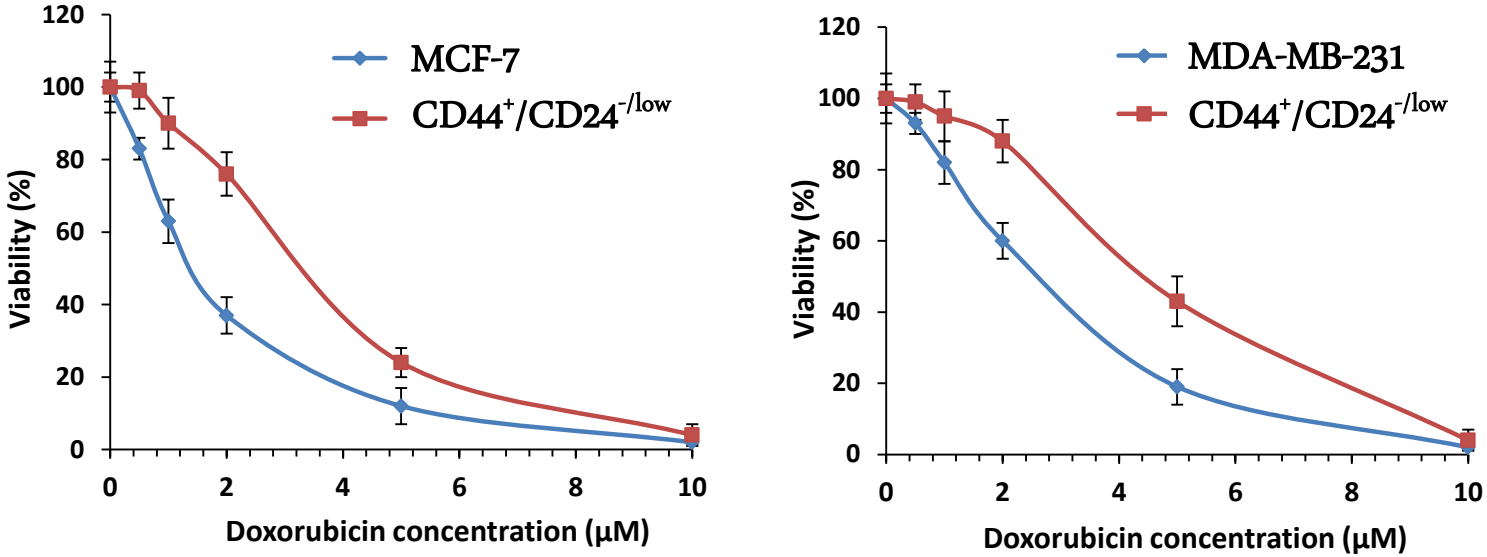


CSC ($CD44^{+}/CD24^{-/low}$) content in MCF-7, MCF-7/ADR, MDA-231, MDA-231/ADR cells determined by flow cytometry

Un-published data

Results

Breast CSC exhibit greater therapeutic resistance

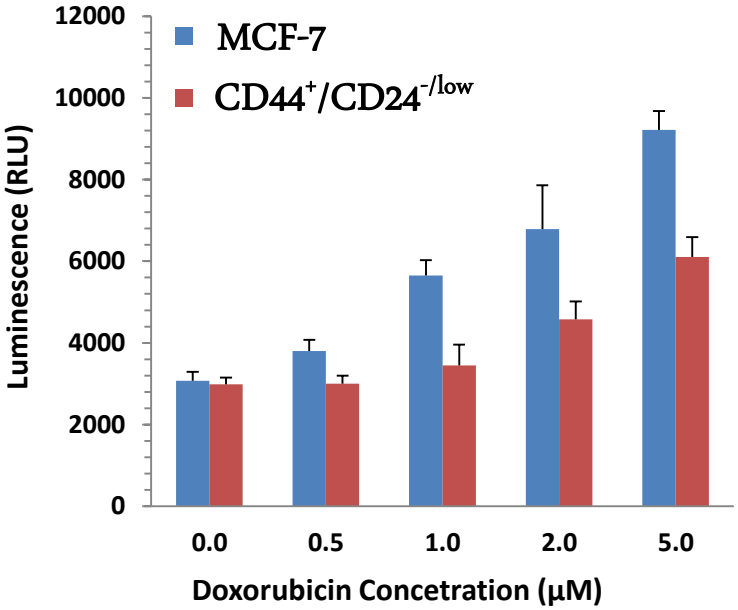


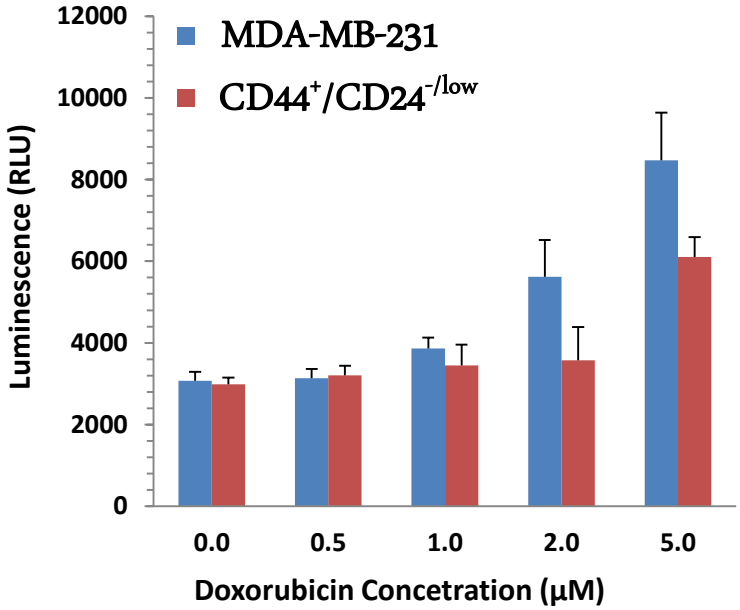
MTT assay for cell viability analysis of cells treated with doxorubicin.

Un-published data

Results

Breast CSC exhibit greater therapeutic resistance



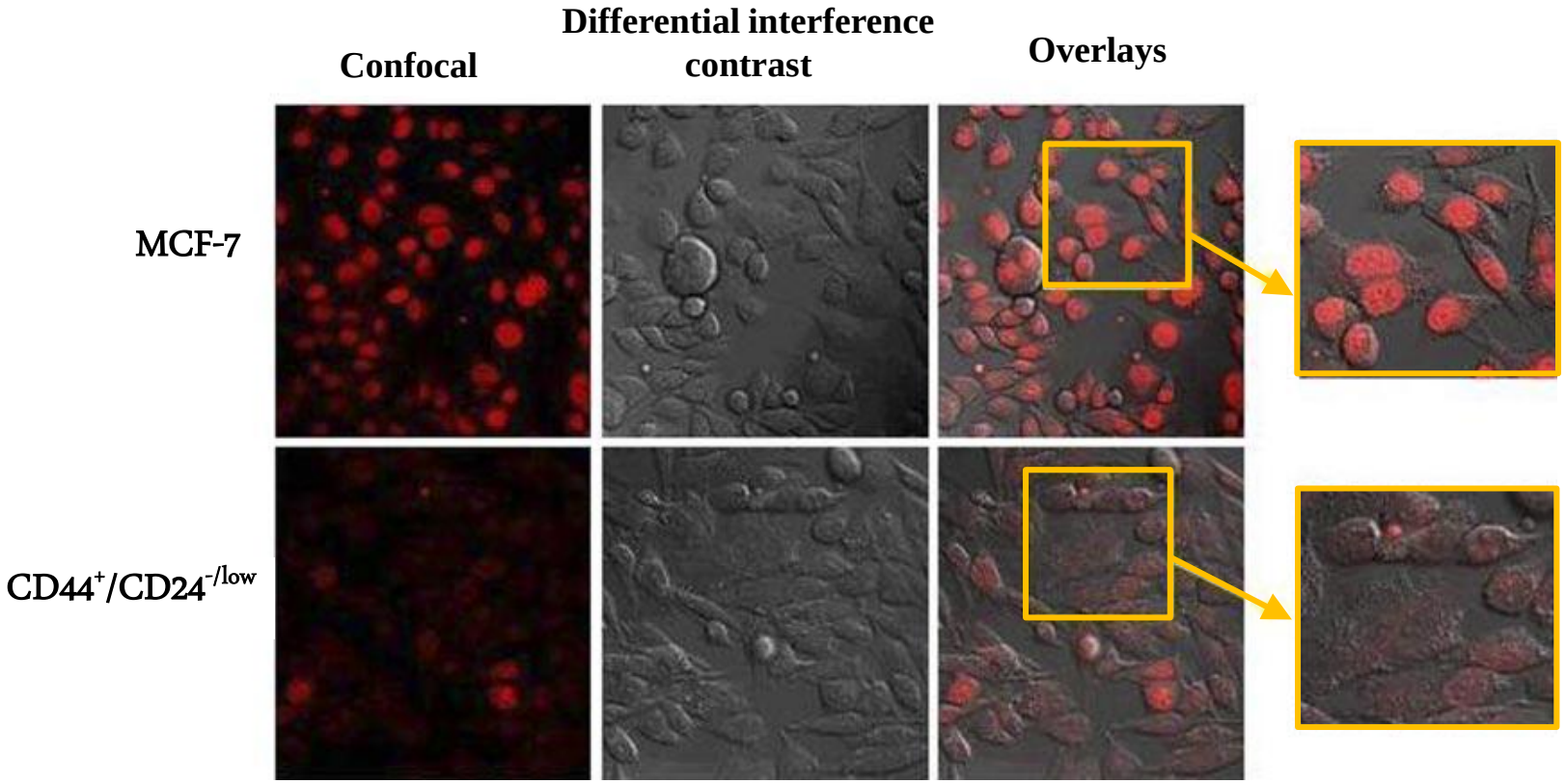


Caspase 3/7 activity in cells treated with doxorubicin.

Un-published data

Results

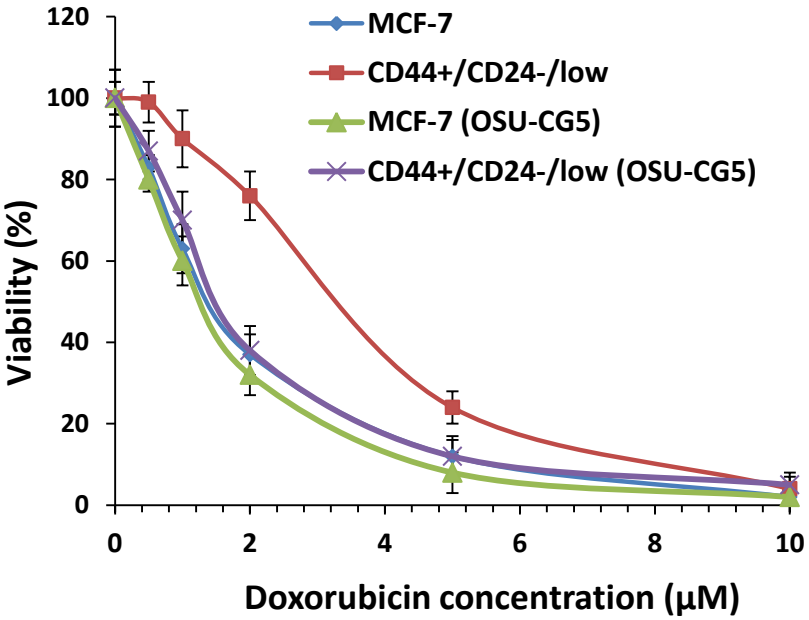
Breast CSC exhibit greater therapeutic resistance



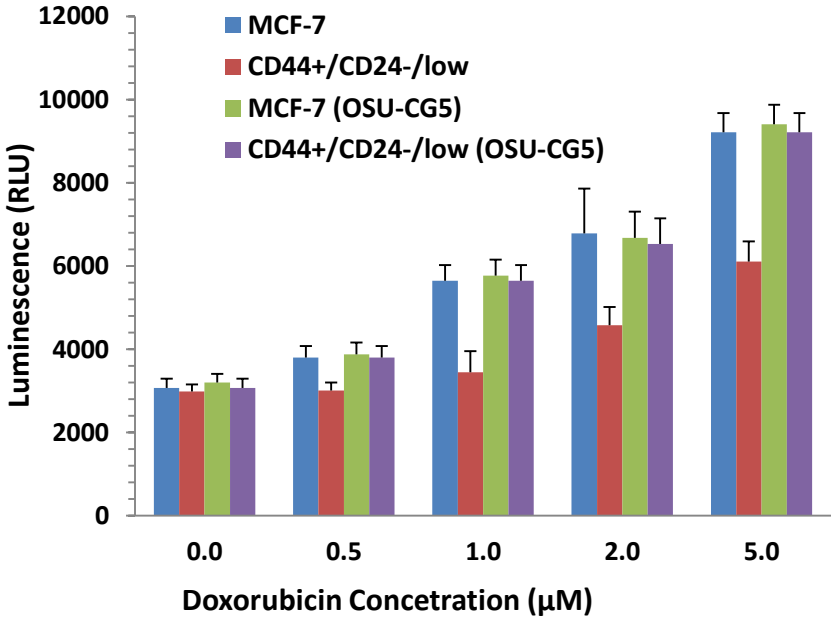
In vitro confocal images of subcellular doxorubicin fluorescence distribution

Results

The combination of OSU-CG5 and Dox restored the sensitivity



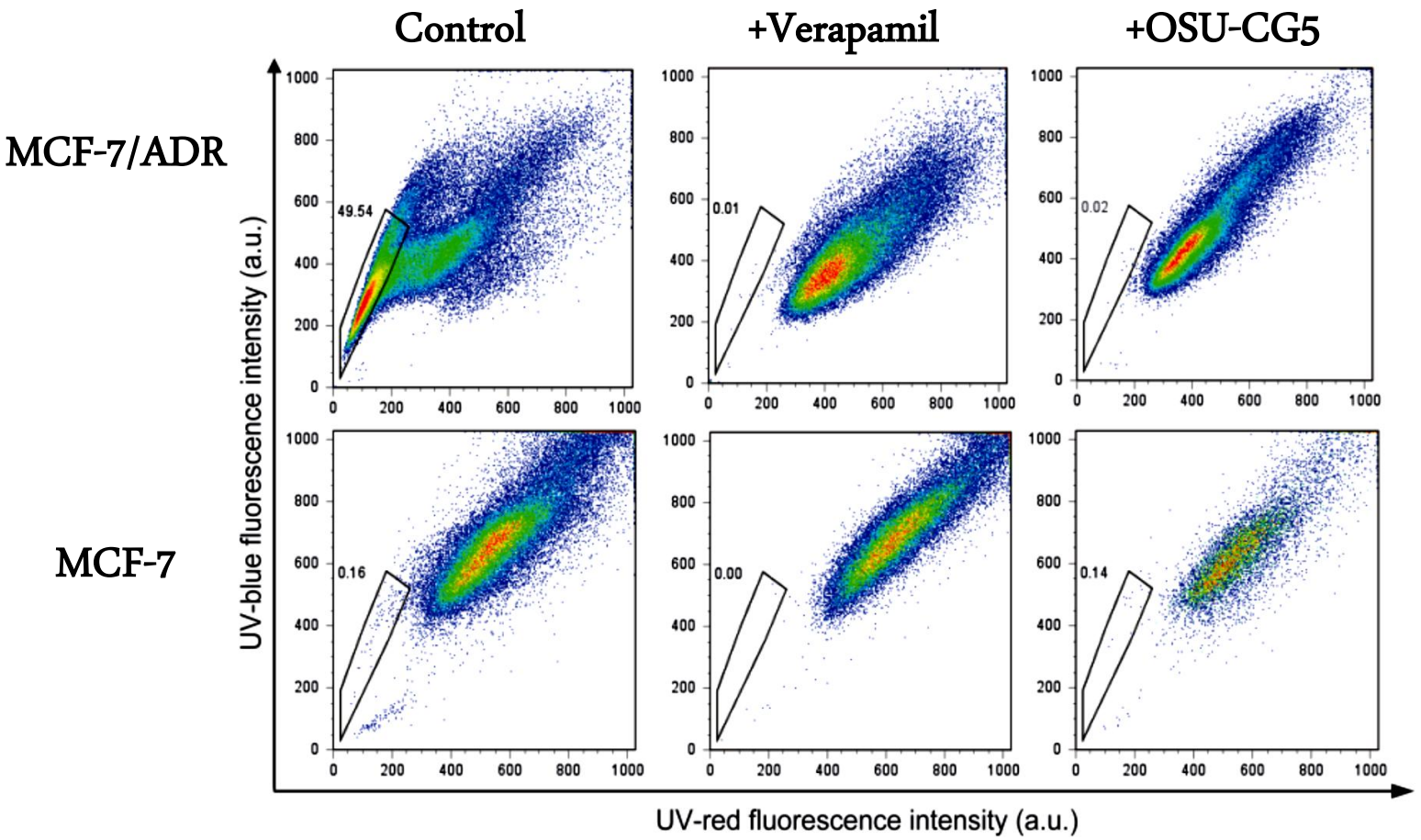
MTT Assay



Caspases 3/7 Assay

Un-published data

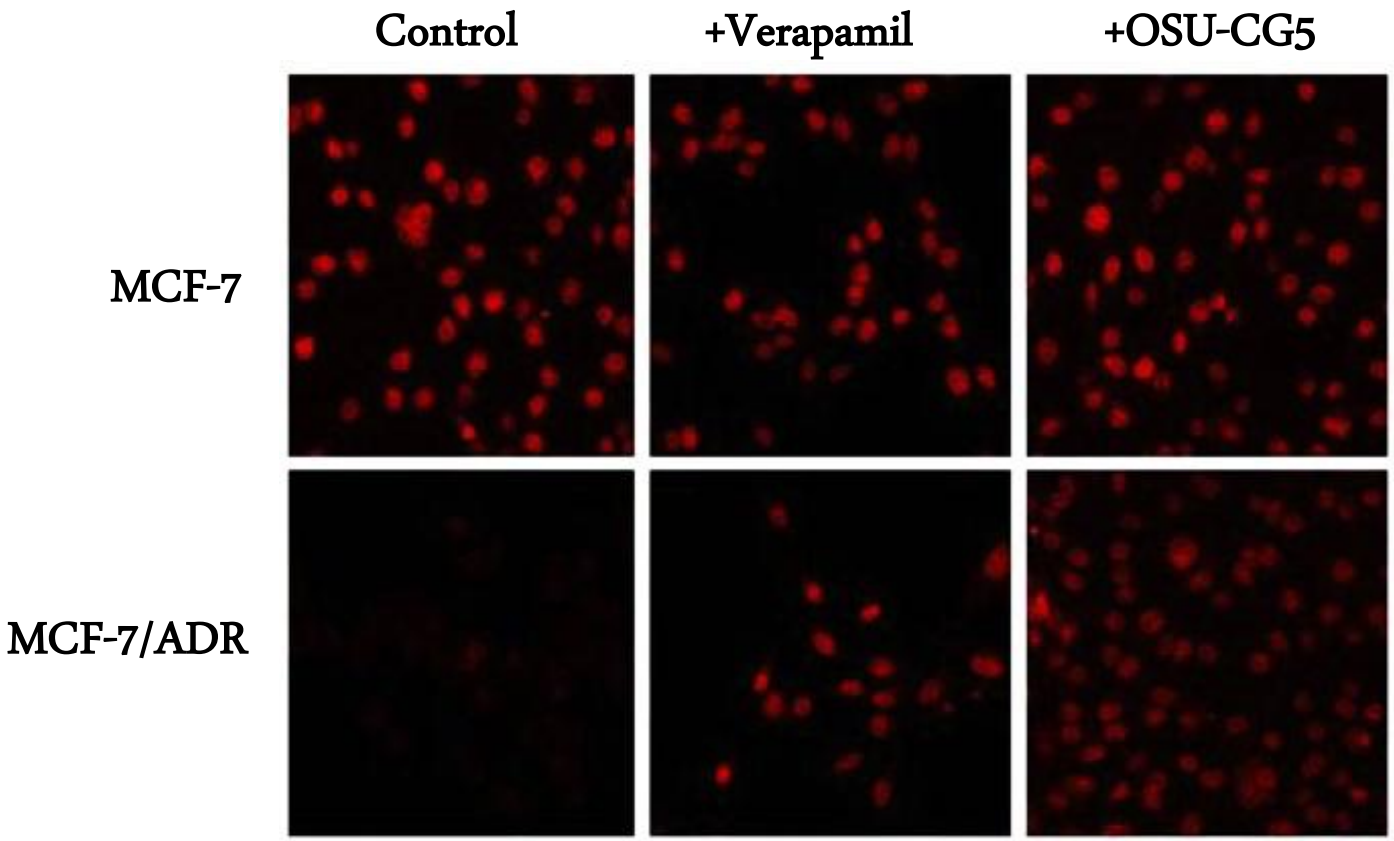
Results



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



Ongoing Work

- **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.

Acknowledgements

Chen Lab

Ching-Shih Chen, PhD

Samuel Kulp, DVM, PhD

Dasheng Wang, PhD

Jui-Hsiang Hung, PhD

Aaron M. Sargeant, DVM, PhD

Lisa D. Berman-Booty DVM, PhD

Yihui Ma, MS

Patel Lab

Tushar Patel , MD, ChB

Takayuki Kogure , PhD

Brueggemeier Lab

Robert Brueggemeier, PhD

Matthew D. Ringel, MD

Taleb Lab

Taleb Al-Tal, PhD

Rafat El-Awady , MD



Thank you