Anti-inflammatory combinatorial therapy to enhance killing efficacy with patient-derived preclinical models

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Cancer stem cells (CSCs)

- **Drug-resistant** cancer phenotypes is a challenge for anti-cancer therapy.
- CSCs are relatively quiescent cells capable of **self-renewal**.
- CSCs are identified as one of the ways by which chemoresistance develops.

Cancer metastasis

- Cancer metastasis is the major cause of cancer morbidity and mortality
- accounts for about 90% of cancer deaths
- a process in which cancer cells disseminate from the primary tumor, settle and grow at a site other than the primary tumor site.

66% of patients who have advanced metastatic or terminal cancer

COX-2 and cancer

- An inhibition of redox-responsive cyclooxygenase (COX) enzymes is often attributed to the mechanism of aspirin.
- COX-2 regulates tumour growth, invasion and metastasis in breast cancer.
- the pro-neoplastic effects of COX-2 action.

ROS and DNA damage

- Reactive oxygen species (ROS) are a group of short-lived, highly reactive, oxygen-containing molecules

- induce DNA damage and affect the DNA damage response (DDR).

- COX-2 expression could be triggered by ROS

- ROS favors the expression of an inflammatory phenotype that leads to the induction of COX-2.

Circulating tumor cells (CTCs) cluster assay

- CTCs are a rare subset of cells found in the blood of patients with solid tumors.
- We demonstrate an efficient approach to evaluate drug response using patient-derived CTC cultures obtained from liquid biopsy.

1: a gradient generator
2: open channels of the middle layer
3: custom-designed tapered microwells

- microenvironmental mimic
- three-dimensional
- multilayered
- under low shear

Combinatorial therapy

DA treatment: In this proof of concept study, a range of therapeutic drug concentrations for 0–500 mg/ml aspirin (A) and 0–1 µM doxorubicin (D) were screened with a microfluidic culture and drug-screening assay validated for primary cell cultures.

Schematics of the gradient generator

Distribution of two drugs in combination with the effect of gradient generator

Significance of liquid biopsy and CTCs

- Cancer is a **dynamic** disease. During the course of disease, cancers generally become more **heterogeneous**.
- **Heterogeneous response** of therapy highlights the importance of **patient-derived** preclinical models.
- Lack of a robust anticancer **drug screening** system to monitor patients during treatment.
- Our CTC assay obtained from liquid biopsy is **efficient, non-invasive, inexpensive** and **drug screening** for personalized treatment.

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DA therapy improved killing efficacy and apoptosis

- Viability ratio breast cancer cell line MDA-MB-231 cultures decreases after prolonged exposure to combinatorial DA treatment.

- Killing efficacy of DA treatment surpassed that of treatment with doxorubicin alone after 7 days of exposure, as determined by the live and apoptotic cell proportions.

DA therapy reduced cancer stem cells

- Increase in CSCs post treatment have a high potential to form secondary metastases, leading to cancer relapse.

- CD44⁺/CD24⁻ phenotype corresponded to the proportion of CSC-like cells

- DA treatment with 500 mg/ml aspirin resulted in a significant reduction of CSC proportion.
DA therapy in clinical cohorts

- Representative images of microwell array with clusters (left) and without clusters (right). Scale bar is 50 µm.

- Patient samples that responded in terms of killing efficacy are marked in red.

- Heterogeneity of patient profiles and the reliable patient-derived models are important for screening similar anti-inflammatory and anti-cancer strategies.

DA treatment acts via a COX-independent pathway mediated by anti-inflammatory mechanisms

- COX-2 protein expression was reduced under combinatorial DA treatment.
- COX-2 reduction is directly associated with malignancy and resistance.
- Reduction in COX-2 protein expression was significantly greater under low-dose aspirin (200 mg/ml).
- DA treatment is more effective in its anti-cancer effects.

Immunostaining for COX-2 protein. Scale bar is 10 µm.

Relative intensity levels of COX-2 protein after 7 days of drug exposure.

DA: combinatorial treatment with 0.5 D and 500 mg/ml aspirin.
Doxorubicin treatment: treatment with 0.5 µM dosage of doxorubicin

DA treatment reduces intracellular oxidative activity

- Calcein AM is also an indicator of intracellular oxidative activity.

- The differential intensity of Calcein AM suggests a reduction in intracellular oxidative activity in samples under both single drug aspirin and combinatorial DA therapy.

- Intracellular oxidative activity is a factor in metabolism and a key regulating process for several core functions including cell proliferation and transcription.

DA treatment revert cells to a less metastatic phenotype

- **Increased ROS levels** causes cancer development and **metastasis** during or after chemotherapy.
- Extracellular **ROS** help to facilitate the formation of multifoci tumors and act as a springboard for **metastatic tumor cells**.
- Cancer cells usually present as a hybrid phenotype and express intermediate levels of **epithelial (E)** and **mesenchymal (M)** characteristics.
- With DA treatment, the proportion of cells with **epithelial-like phenotypes** were **increased** within 72 h and more apparent after 7 days.

**Immunostaining for CK and Vim**

Cytokeratin (CK): a cytoplasmic marker for **epithelial** phenotypes

Vimentin (Vim): a cytoplasmic marker for **mesenchymal** phenotypes

Scale bar is 20 µm

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Discussion

• Compared with the treatment with doxorubicin alone, the intracellular oxidative activity in the sample under combinatorial DA treatment was reduced, as demonstrated by the intensity of Calcein AM. We demonstrated that the treatment outcomes were mediated by the reduction of COX-2, which was associated with inflammation triggered by ROS.

• Overall, the preclinical model could be used as a proof of concept to demonstrate the efficacy of anti-inflammatory combinatorial therapies by influencing oxidative stress. Similar research could provide a basis for more DNA-related cancer treatment research in the future.
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