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Computational analysis of image-based drug profiling predicts synergistic drug combinations: Applications in triple-negative breast cancer

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ABSTRACT

An imaged-based profiling and analysis system was developed to predict clinically effective synergistic drug combinations that could accelerate the identification of effective multi-drug therapies for the treatment of triple-negative breast cancer and other challenging malignancies. The identification of effective drug combinations for the treatment of triple-negative breast cancer (TNBC) was achieved by integrating high-content screening, computational analysis, and experimental biology. The approach was based on altered cellular phenotypes induced by 55 FDA-approved drugs and biologically active compounds, acquired using fluorescence microscopy and retained in multivariate compound profiles. Dissimilarities between compound profiles guided the identification of 5 combinations, which were assessed for qualitative interaction on TNBC cell growth. The combination of the microtubule-targeting drug vinblastine with KSP/Eg5 motor protein inhibitors monastrol or ispinesib showed potent synergism in 3 independent TNBC cell lines, which was not substantiated in normal fibroblasts. The synergistic interaction was mediated by an increase in mitotic arrest with cells demonstrating typical ispinesib-induced monopolar mitotic spindles, which translated into enhanced apoptosis induction. The antitumour activity of the combination vinblastine/ispinesib was confirmed in an orthotopic mouse model of TNBC. Compared to single drug treatment, combination treatment significantly

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reduced tumour growth without causing increased toxicity. Image-based profiling and analysis led to the rapid discovery of a drug combination effective against TNBC *in vitro* and *in vivo*, and has the potential to lead to the development of new therapeutic options in other hard-to-treat cancers.

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1. Introduction

Multi-drug regimens are the leading treatment for cancers since single agent chemotherapies often have limited antitumour activity and have been linked to drug resistance (Ramaswamy, 2007; Zimmermann et al., 2007). The development of these treatments has been increasingly laborious due to the plethora of potential combinations available (Zinner et al., 2009). Thus, the majority of existing combination therapies were developed empirically based on clinical experience (Borisy et al., 2003; Zimmermann et al., 2007). The discovery of new drug combinations from a system-oriented angle has only recently received increasing attention. Mathematical models and computational approaches, such as optimisation and search algorithms, were successfully applied to hasten the identification of effective drug combinations (Calzolari et al., 2008; Wong et al., 2008; Zinner et al., 2009). In addition, functional interaction models reliably predicted perturbation effects in signalling pathways and changes required to obtain a favourable outcome (Nelander et al., 2008). However, in-depth assessment of identified combinations and translation to clinical trials remain to be realised.

The development of new and effective combination treatments is crucial to improve the outcome of patients with aggressive malignancies such as triple-negative breast cancer (TNBC). This breast cancer subtype is associated with young patient age and reduced progression-free and overall survival (Haffty et al., 2006; Liedtke et al., 2008). The dismal clinical outcome is mainly attributed to intrinsic and acquired drug resistance, and the absence of key molecular markers of breast cancer, which provide valuable therapeutic targets for other breast cancer subtypes (Reis-Filho and Tutt, 2008). TNBC was found to be generally more responsive to chemotherapy than other subtypes (Carey et al., 2007; Liedtke et al., 2008); however, these therapies remain suboptimal as patients without complete response have significantly shorter overall survival (Carey et al., 2007). Hence, the development of novel combination chemotherapies for treatment of TNBC is essential in order to increase patient survival rates.

The presented study identifies novel drug combinations based on the integration of high-content screening (HCS), computational, and experimental biology. HCS facilitated the automatic extraction of compound-induced phenotypes from fluorescence images of TNBC cells. Determination of compound profiles allowed for the identification of compound pairs with either very similar or distinct phenotypic outcomes, which were further assessed for synergistic interactions in *in vitro* and *in vivo* models of TNBC.

2. Materials and methods

2.1. Cell culture

MDA-MB-231-luc-D3H2LN cells (Caliper, Hopkinton, MA, USA) were cultured in DMEM medium supplemented with 10% FBS, 1% L-glutamate, 1% Na-pyruvate, 1% penicillin/streptomycin, 1% non-essential amino acid and 0.1% zeocin. MDA-MB-231 cells were maintained in DMEM (Invitrogen, Mount Waverley, Australia) supplemented with 10% FCS, 1% L-glutamate, 1% Na-pyruvate, 1% penicillin/streptomycin, and 1% non-essential amino acid. MDA-MB-468 and BT-549 cells were grown in RPMI (Invitrogen) containing 10% FCS, which was additionally plied with 0.023 IU/ml insulin for BT549 cells. MRC-5 lung fibroblasts were grown in MEM (Invitrogen) supplemented with 10% FCS, 2% sodium bicarbonate, 1% NEAA, 1% sodium pyruvate, and 1% L-glutamine. Cell lines were grown as monolayers in a humidified atmosphere at 37 °C and in 5% CO_2. The ratio of cells to well surface and compound volume was kept constant in all experiments.

2.2. Compounds

Compounds for HCS (10 mM in DMSO) were purchased from Sigma (St. Louis, MO, USA) and Tocris bioscience (Ellisville, MO, USA). Stock solutions of 50 mM monastrol (Tocris Bioscience, Bristol, UK) and 10 mM ispinesib (Selleck, Scoresby, VIC, Australia) were prepared in DMSO, and stored at −20 °C. Clinical grade vinblastine sulphate (1.1 mM) (David Bull Laboratories, Melbourne, VIC, Australia) was stored at 4 °C. For further use, compounds were diluted in the respective media.

2.3. Fluorescence staining and image acquisition

MDA-MB-231-luc-D3H2LN cells ($5*10^3$ cells/well) were grown on poly-D-lysine coated black wall 96-well plates prior to compound exposure (0.1, 1.0 and 10 μM). After 24 h immunostaining with multifluorescent markers for DNA, microtubule and actin following standard methods using the Cellomics HCS Cytoskeleton Rearrangement Kit (Thermo, Rockford, USA) was conducted. Briefly, cells were first fixed and permeabilized using formaldehyde (3.7%), before incubation with primary antibody solution containing DY554-phalloidin and tubulin primary antibody, followed by incubation with the secondary antibody solution containing DyLight 649 Goat Anti-Mouse and DAPI. Fluorescence images were captured using an Olympus IX81 microscope and a 40X objective. Images of channels for DAPI (DNA), DY554 (F-actin), and DyLight 649 (tubulin) were acquired for at least 4 different positions in wells.

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