High content analysis (HCA) approaches in development of differentially human melanoma cell lines as tools for screening and characterization of therapeutic drug candidates

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Malignant melanoma is a highly aggressive tumour that frequently resists chemotherapy [1] and consequently the search for better agents for its treatment is of great importance. Efficacy in therapeutics is measured by the rate of success in the management of cancer patients. Thus, the search for new agents that can reduce the drug resistance associated with cancer cells is of vital importance. HCA technology was exploited to develop differentially conditioned melanoma cell lines as models for elucidating drug resistance mechanisms.

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Abstract

A persistent challenge in developing effective therapeutic agents targeted at solid tumors is the acquisition of drug resistance, and hence continued survival of a sub-population of tumor cells. Evidence indicates that, under high glucose conditions, many tumors acquire drug resistance by circumventing oxidative phosphorylation as a mechanism of protection [2]. Under these conditions, glycolysis becomes the pre-eminent route of ATP production in preference to oxidative phosphorylation. Such metabolic changes are likely to contribute to the drug resistance phenotype. Cells dependent on glycolysis are more resistant to drugs that target mitochondria, but are also more vulnerable to drugs that exploit the metabolic dependency. To better understand the mechanisms behind drug resistance in melanoma cells, and possibly exploit differences related to their adaptation to the environment encountered in vivo, we have established cell models representative of both high and low glucose conditions.

The cell models were first characterized using IN Cell Analyser 2000 and a biochemical assay before challenging with several candidate drugs known to have mitochondrial liabilities. Additionally, the effect of siRNA knockdown was assessed using a panel of selective drugs directed against specific proteins including the ATP-binding cassette (ABC) transporters, known to contribute to drug resistance by mediating the efflux of drugs from cells [3].

Introduction

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Materials and Methods

Human malignant melanoma cell line WM115 was maintained in complete growth medium, Minimum Essential Medium Eagle (MEM) supplemented with 5% fetal bovine serum (FBS). The cell line was then cultured with an additional 10 mM galactose to produce two daughter cell lines WM115-Glu and WM115-Gal respectively. Once exponential cell growth had resumed, cells were maintained for a further 3 weeks to allow characterisation.

Results

We hypothesized that melanoma cells conditioned into glucose-rich medium (the WM115-Gal cell line) would be resistant to the glycolytic cell line (WM115-Glu) that is resistant to the mitochondrial poison Antimycin A, but would be more vulnerable to the mitochondrial poison Antimycin A. Consistent with this prediction, a rapid preview scan of one of the test plates (Figure 4) revealed a marked difference in susceptibility of the cells to Antimycin A; the mitochondrial poison that acts on respiratory complex II of the electron transport chain. Antimycin A worked on WM115-Gal cells in a dose-dependent manner. In contrast, WM115-Glu, grown in a glucose-rich environment, remained relatively resistant to Antimycin A, consistent with glycolysis being the primary mechanism for ATP production in this cell line.

Conclusion

We hypothesized that melanoma cells conditioned into glucose-rich medium (the WM115-Gal cell line) would be resistant to the glycolytic cell line (WM115-Glu) that is resistant to the mitochondrial poison Antimycin A, but would be more vulnerable to the mitochondrial poison Antimycin A. Consistent with this prediction, a rapid preview scan of one of the test plates (Figure 4) revealed a marked difference in susceptibility of the cells to Antimycin A; the mitochondrial poison that acts on respiratory complex II of the electron transport chain. Antimycin A worked on WM115-Gal cells in a dose-dependent manner. In contrast, WM115-Glu, grown in a glucose-rich environment, remained relatively resistant to Antimycin A, consistent with glycolysis being the primary mechanism for ATP production in this cell line.

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Visualisation of the data for the WM115-Gal cells using hierarchical clustering, based on multiple nuclear and mitochondrial parameters, revealed distinct cell phenotypes that clustered according to treatment conditions (Figure 6).

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References


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Conclusions

HCA technology was exploited to develop differentially conditioned melanoma cell lines as models for elucidating drug resistance mechanisms.

Conclusive results were obtained in our study. The Warburg Effect was observed in the WM115-Gal cell line, which was sensitive to the mitochondrial poison Antimycin A. Consistent with this prediction, a rapid preview scan of one of the test plates (Figure 4) revealed a marked difference in susceptibility of the cells to Antimycin A; the mitochondrial poison that acts on respiratory complex II of the electron transport chain. Antimycin A worked on WM115-Gal cells in a dose-dependent manner. In contrast, WM115-Glu, grown in a glucose-rich environment, remained relatively resistant to Antimycin A, consistent with glycolysis being the primary mechanism for ATP production in this cell line.