Epithelial to Mesenchymal Transition Confers Sensitivity to Cytotoxic Agent Ophiobolin A via Alterations in Mitochondrial Function and Metabolic Pathways

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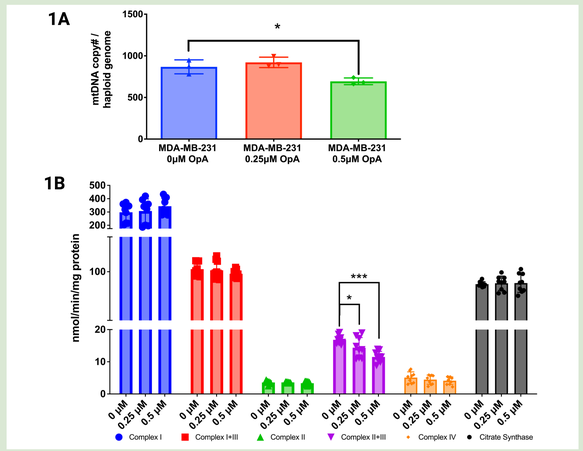
ABSTRACT

Metastatic progression in patients with triple negative breast cancer (TNBC) occurs in approximately half of all patients, reducing median overall survival. Metastasis may be facilitated through the epithelial to mesenchymal transition (EMT), which generates cancer cells with enhanced self-renewal and resistance to chemotherapeutics, which are partially mediated by alterations in metabolic pathways and mitochondrial function. Here we show that a drug-like small molecule possesses EMT-specific cytotoxic activity through effects on metabolic and mitochondrial functions. The fungus-derived sesterterpenoid, Ophiobolin A (OpA), possesses nanomolar cytotoxic activity and a high therapeutic index, though its target and mechanism of action remain unknown. Our analysis indicates that OpA acts in a mitochondria-specific manner to cause a loss of membrane potential in EMT-positive, but not EMT-negative, cells with specific effects on complex III of the electron transport chain, mitochondrial DNA copy number, and the TCA cycle. Therefore, we conclude that EMT imparts alterations in mitochondrial function and metabolic pathways, conferring sensitivity to the cytotoxic effects of OpA.

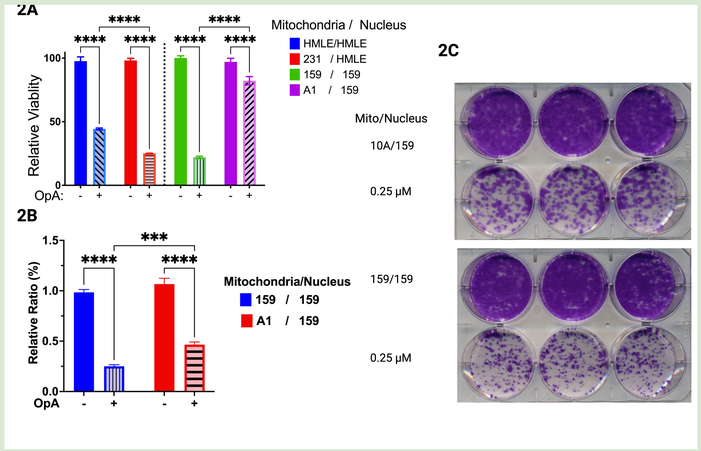
CONCLUSIONS

- OpA decreases mitochondrial DNA copy number and dose dependently decreases the activity of complexes II & III in MDA-MB-231 cells.
- Placing a mitochondria from a cancerous cell into a non-cancerous cell confers enhanced OpA sensitivity. Likewise, placing a mitochondria from a healthy cell into a cancerous cell results in decreased OpA sensitivity.
- OpA induces a loss in membrane potential and a shift toward non-functional mitochondria.
- OpA significantly deregulates specific metabolites involved in glycolysis, TCA cycle and the glutamine pathway.

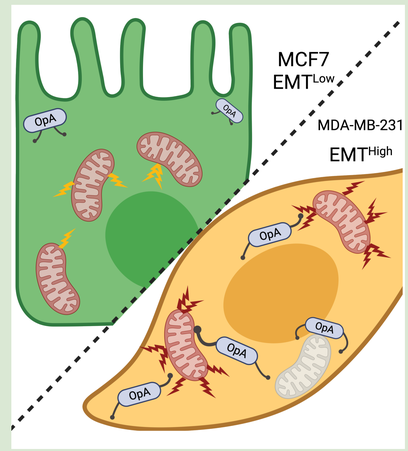
RESULTS



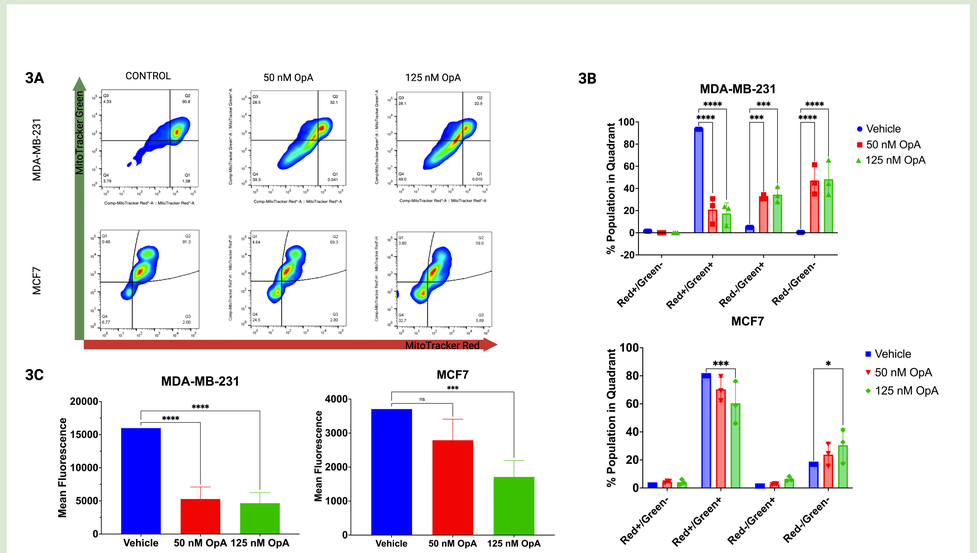
1A Mitochondrial DNA copy number measured in MDA-MB-231 cells treated with 0.25 μM or 0.5 μM OpA for one day. Bars represent the mean of three replicates in mtDNA copy#/haploid genome.**1B** Activity of electron transport chain complexes in MDA-MB-231 cells treated with 0.25 μM or 0.5 μM OpA for one day. Bars represent the mean of three assays in nMol/min/mg protein.



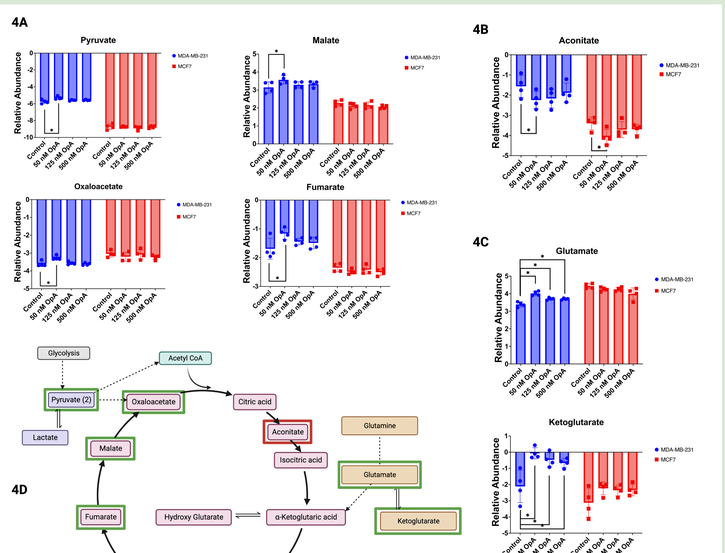
2A Relative viability of HMLE and SUM159 cells depleted of mitochondria and supplemented with either HMLE or SUM159 isolated mitochondria. Cells were treated with 0.5 μM OpA for three days. Bars represent the mean of three replicates. **2B** Quantification of clonogenic assay (right). Bars represent the mean of three replicates. **2C** Clonogenic assay in SUM159 cells with either SUM159 (bottom) or MCF10A (top) mitochondria treated with 0.25 μM OpA. Bars represent the mean of three replicates.



MCF7/EMT^{Low} cells are affected less at the same concentrations of OpA compared to MDA-MB-231/EMT^{High}cells. OpA acts in a mitochondria-targeted manner, decreasing membrane potential and viability and altering metabolic pathways.



3A Flow cytometry analysis of MDA-MB-231 and MCF7 cells treated with 50 nM or 125 nM OpA for three hours. Cells were stained with mitochondria marker MitoTracker Green, membrane potential marker MitoTracker Red and viability stain Sytox Blue. Y-axis labeled as MitoTracker Green and X-axis labeled as MitoTracker Red, generating quadrants describing mitochondrial functionality.**3B** Quantification of percent population per quadrant. Bars represent average of three replicates in mean fluorescence**3C** Mean membrane potential of MDA-MB-231 and MCF7 cells treated with either 50 nM or 125 nM OpA for three hours. Bars represent the average of three replicates mean fluorescence.



4A, B, C Relative abundance of specific metabolites involved in TCA cycle, measured in MDA-MB-231 and MCF7 cells treated with either 50 nM or 125 nM OpA for three hours. Bars represent four replicates measured by targeted metabolomics, high pressure liquid chromatography and mass spectronomy.**A** Upregulated metabolites involved in the TCA cycle.**B** Down regulated metabolite involved in TCA cycle**C** Upregulated metabolites involved in glutamine pathway**C** Digram of TCA cycle with OpA-driven up regulated (green) and down regulated (red) metabolites.

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