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Differential susceptibility of breast cancer cells to mitochondrial dysfunction induced by Wnt inhibitors

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INTRODUCTION

A better understanding of cancer cell metabolism can provide valuable information about the harsh tumor microenvironment, which can profoundly impact the efficacy of emerging immune therapies for solid tumors. Recent research demonstrates that at least certain cancers are heavily reliant on mitochondrial respiration, suggesting that mitochondria can be an effective therapeutic target. [1,2]

The Agilent Seahorse XF Mito Tox assay is a rapid and robust way to directly detect and measure mitochondrial inhibitors and uncouplers. [3,4] The drug-induced mitochondrial modulation can be quantitatively interpreted via the Mito Tox Index (MTI), which enables the identification of effective drug doses for *in-vitro* applications.

This study investigates the impact of Wnt-signaling modulators on mitochondrial dysfunction. It evaluates the effects of selected drug treatments on cellular energy metabolism and cytotoxicity by comparing two distinct breast cancer cell lines: one highly oxidative and the other glycolytic.

EXPERIMENTAL

Mitochondria-targeted drug screening and evaluation workflow

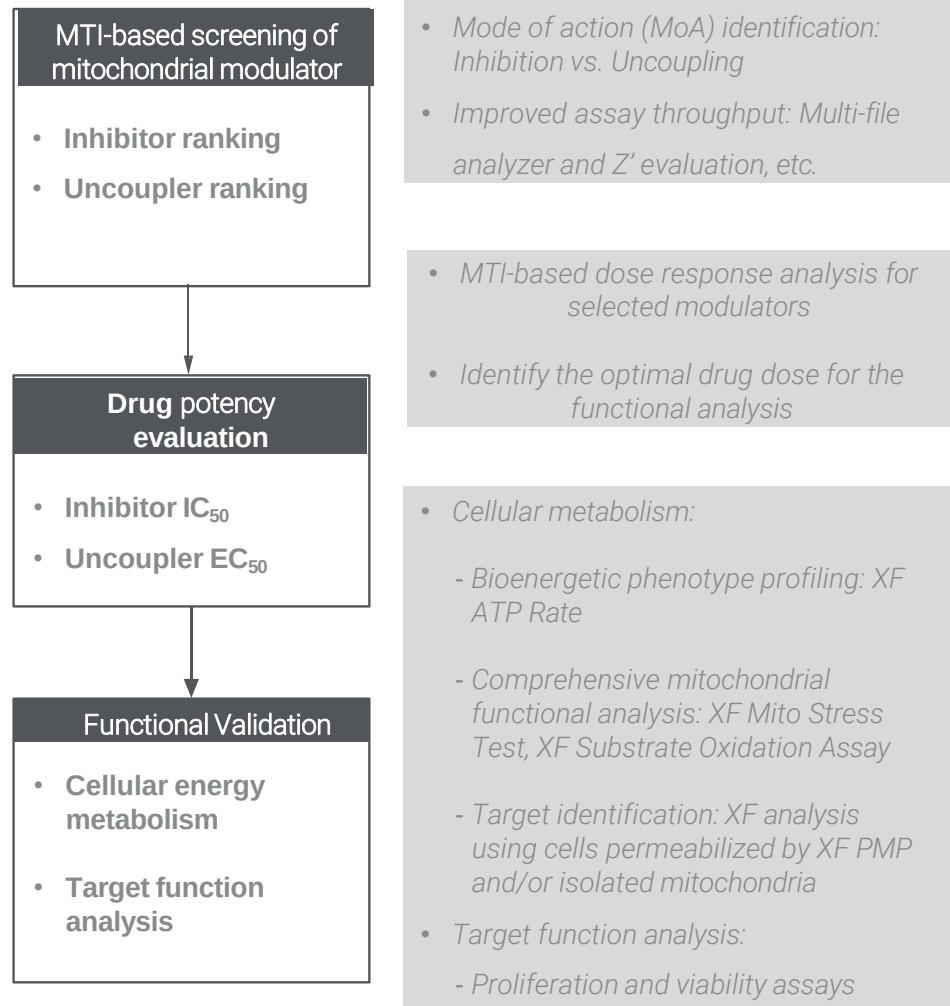


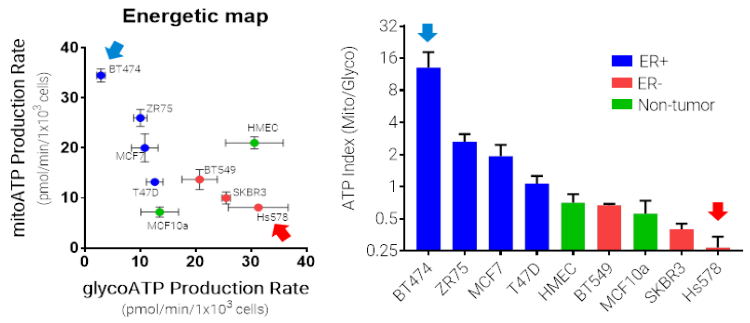
Figure 1. Mitochondria-targeting drug screening and evaluation workflow.

XF Seahorse Mito Tox screening assay detects potent mitochondrial-targeting drug candidates, and identifies the mode of action. The appropriate dosing information can be obtained from an MTI-based dose-response assay. The information collected from screening and dose-response analysis can then be used in further detailed functional analyses, including cellular energy metabolic profiling.

RESULTS AND DISCUSSION

MTI-based screening of Wnt modulators targeting mitochondria

A



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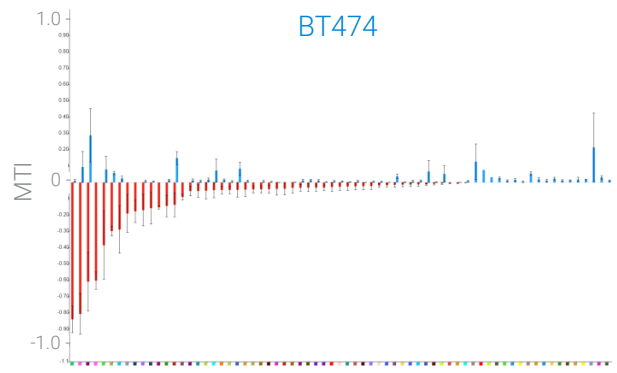
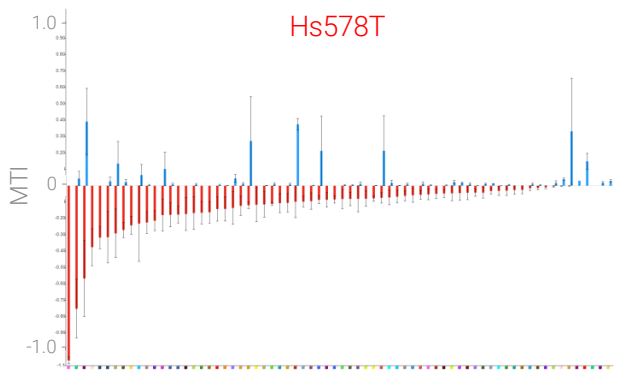


Compound	$MTI_{Inhibitor}$	$MTI_{Uncoupler}$
Niclosamide	-0.84	0.01
Pyrvinium pamoate	-0.81	0.09
Hexachlorophene	-0.61	0.29
Terreic acid	-0.60	0.00



Compound	$MTI_{Inhibitor}$	$MTI_{Uncoupler}$
Niclosamide	-0.75	0.04
Pyrvinium pamoate	-1.07	0.00
Hexachlorophene	-0.57	0.39

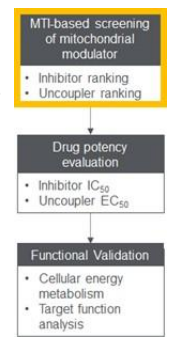
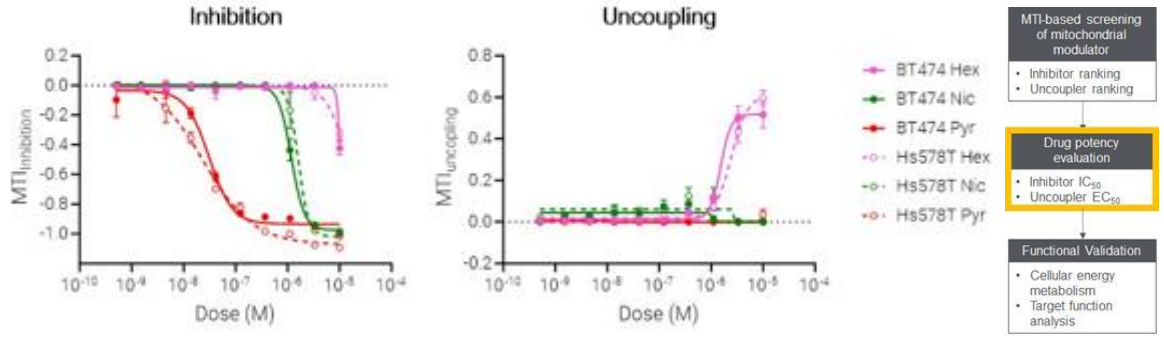


Figure 2. Screening of mitochondrial inhibitor or uncoupler among Wnt signaling modulators using two metabolically distinct

A. Metabolic phenotype of breast cancer cell lines determined by the XF Real-time ATP Rate Assay.

B. BT474 and Hs578T cells were treated with 10 μM Wnt signaling modulators (SCREEN-WELL® Wnt Pathway, Enzo Life Sciences) for 24 hours, and the effect of the drugs on their mitochondrial activities was measured and analyzed using the XF Seahorse Mito Tox assay kit and the Seahorse XF Pro analyzer with Seahorse Analytics software, respectively. The compounds were ranked by the inhibitory impact based on the Mito Tox Index (MTI). The most potent inhibitors (MTI < -0.5) were selected, as shown in the tables above.

Drug Potency Evaluation



Compound	Inhibition IC_{50} (μM)		Uncoupling EC_{50} (μM)	
	BT474	Hs578T	BT474	Hs578T
Niclosamide	1.40	1.96	NA	NA
Pyrvinium	0.03	0.02	NA	NA
Hexachlorophene	8.61	7.30	1.82	2.80

Figure 3. Dose response of breast cancer cells to selected mitochondrial modulators BT474 and Hs578T cells were cultured in the presence of three mitochondrial modulators (Hex, Hexachlorophene; Nic, Niclosamide; Pyr, Pyrvinium) for 24 hours at various concentrations as indicated. The dose-response pattern of Mito Tox Indices (MTIs) for inhibitor (left graph) and uncoupler (right graph) were compared, and the respective IC_{50} and EC_{50} were assessed.

Functional Validation (I): Cellular Energy Metabolism

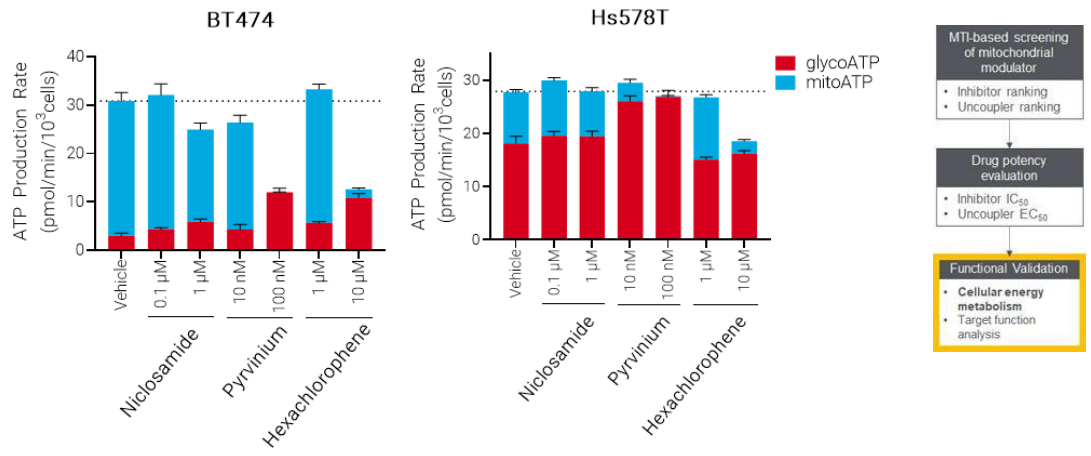
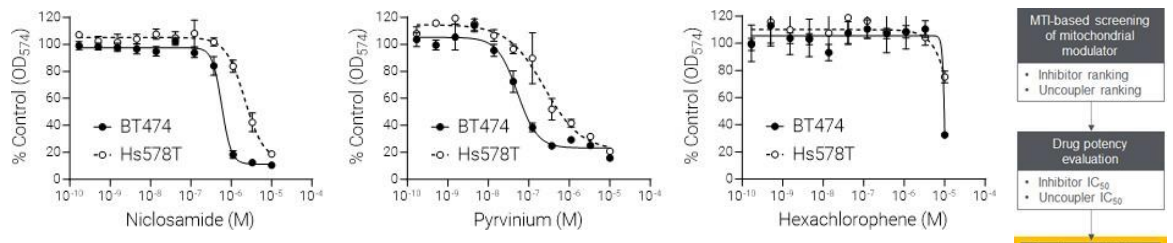


Figure 4. Changes in cellular energy metabolism induced by mitochondria-targeting Wnt modulators.

BT474 (left) and Hs578T (right) were cultured for 24 hours in the presence of the selected drugs at the indicated concentrations. The total ATP production rates were measured using the XF Seahorse Real-Time ATP Rate Assay kit. The contribution of glycolytic and mitochondrial ATP production is indicated by red and blue colors, respectively.

Functional Validation (II): Cytotoxicity



Treatment	Cytotoxicity LC_{50} (μM)	
	BT474	Hs578T
Niclosamide	0.58	2.16
Pyrvinium	0.052	0.24
Hexachlorophene	12.2*	41.1*

Figure 5. Differential cytotoxicity of mitochondria-targeting Wnt modulators between metabolically distinct breast cancer cell lines.

BT474 and Hs578T cells were cultured in the presence of the drug at the indicated concentration for 72 hours. The viable cells were stained with Crystal Violet, and the absorbance was measured at 570 nm. **The LC_{50}s of Hexachlorophene are estimated value due to the incomplete dose-response curve.*

CONCLUSION

- Wnt signaling modulators, specifically Niclosamide and Pyrvinium, inhibit mitochondrial respiration.
- Hexachlorophene acts differently by uncoupling the electron transport chain even after a 24-hour exposure period.
- The potency of these modulators as inhibitors or uncouplers does not significantly vary between metabolically distinct cell lines.
- The cellular energy metabolic responses, however, are notably different between the two cell lines.
- BT474 cells, which rely heavily on mitochondrial respiration, are more vulnerable to ATP energy crisis induced by Wnt modulators compared to the more glycolytic Hs578T cells.
- The cell-killing efficacy of the modulators across the two cell types correlates to ATP energy crises.
- Mitochondrial Targeting Index (MTI)-based drug screening offers a straightforward and reliable method for identifying potent mitochondrial-targeting drugs.
- This screening allows for comparison of mitochondrial inhibition and uncoupling effects of drug candidates.
- These methods can be complemented with further functional validations using other cellular energy metabolism analysis tools, such as the XF ATP Rate assay.[5]

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