

# Bioenergetic differences in breast cancer cell lines

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## Abstract

With Otto Warburg's discovery of the inverse relationship in tumor cells between the oxidative and glycolytic metabolism in comparison to normal tissue counterparts, understanding the differences in bioenergetics in cancer became a new area in cancer research. However, so far our understanding of these different metabolic pathways is minimal. In the present study, we tried to identify bioenergetic differences in breast cancer subtypes, at baseline and in response to various mitochondrial metabolic inhibitors in vitro. Using an Extracellular Flux Analyzer, we analyzed glycolysis and mitochondrial respiration in ten different breast cancer cell lines (MCF7, HCC202, HCC1395, T47D, MDA-MB-231, MDA-MB-468, Hs578T, BT-474, BT-549, and UACC-893) representing four subtypes (HER2- ER+, HER2+ ER-, HER2- ER-, HER2+, ER+) and identified possible bioenergetic differences among these cell lines.

## Methods

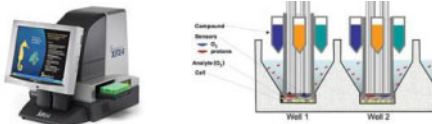
**Cell culture**  
ten different breast cancer cell lines (table 1) taken in to study and cultured as suggested by ATCC. These cell lines cultured in 10cm plates and frequently changed media and subcultured as confluency reached greater than 75%.

Table 1. Cell lines and responding groups

Cell line	HER-2	ER
MCF-7	-	+
HCC-1395	-	+
T-47D	-	+
MDA-MB-231	-	-
MDA-MB-468	-	-
Hs 578T	-	-
BT 549	-	-
BT 474	+	+
HCC 202	+	-
UACC 893	+	-

## XF metabolic assay

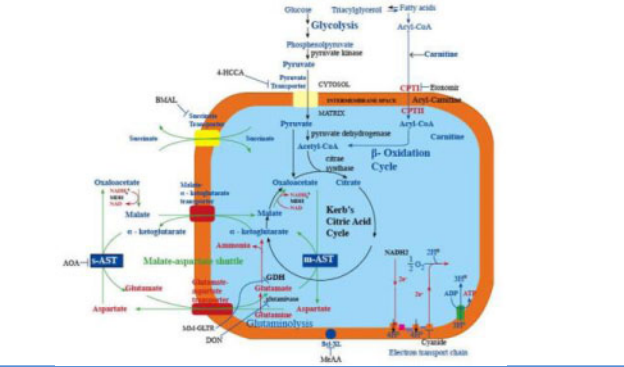
Measurements taken from Seahorse XF 24 instrument. Adherent cells seeded according to its growth rate in 24 well cell culture microplates and cultured overnight. Approximately 30min prior to assay, culture medium exchanged to unruflled Seahorse assay medium and Oxygen Rate Consumption (OCR) and Extracellular Acidification Rate (ECAR) measurements taken as response to metabolic inhibitors.



## Metabolic Inhibitors

Appropriate amount of concentrated stock solutions of chemical inhibitors diluted in Seahorse assay medium. KCN, Butyl malonate (BMAL), 2MeO-Antimycin A (MEEA), Amino-oxyacetate (AOA), Etomoxir, 6-Diazo-5-oxo-L-norleucine (DON), mono-Methyl glutarate (MM-GLTR), α-Cyano-4-hydroxycinnamic acid (4HCCA) and carbonyl cyanide m-chloro phenyl hydrazone (CCCP) used as inhibitors for metabolic pathways.

## Metabolic Inhibitor chart



## Results

Basal OCR and ECAR values of the cell lines

Cell line	Basal value		OCR/ECAR
	OCR	ECAR	
MCF-7	183.3	13.38	13.7
HCC-1395	26.58	1.5	17.63
T-47D	117.2	5.92	19.8
MDA-MB-231	61.58	15.4	4
MDA-MB-468	112.3	14.41	7.79
BT 549	136.7	24.07	5.68
BT 474	190.1	7.33	25.93
HCC 202	106.8	9.05	11.8
UACC 893	219.7	12.08	18.18

triple-negative cell lines (MDA-MB-231, MDA-MB-468 and BT-549) cell lines have lowest OCR/ECAR rate

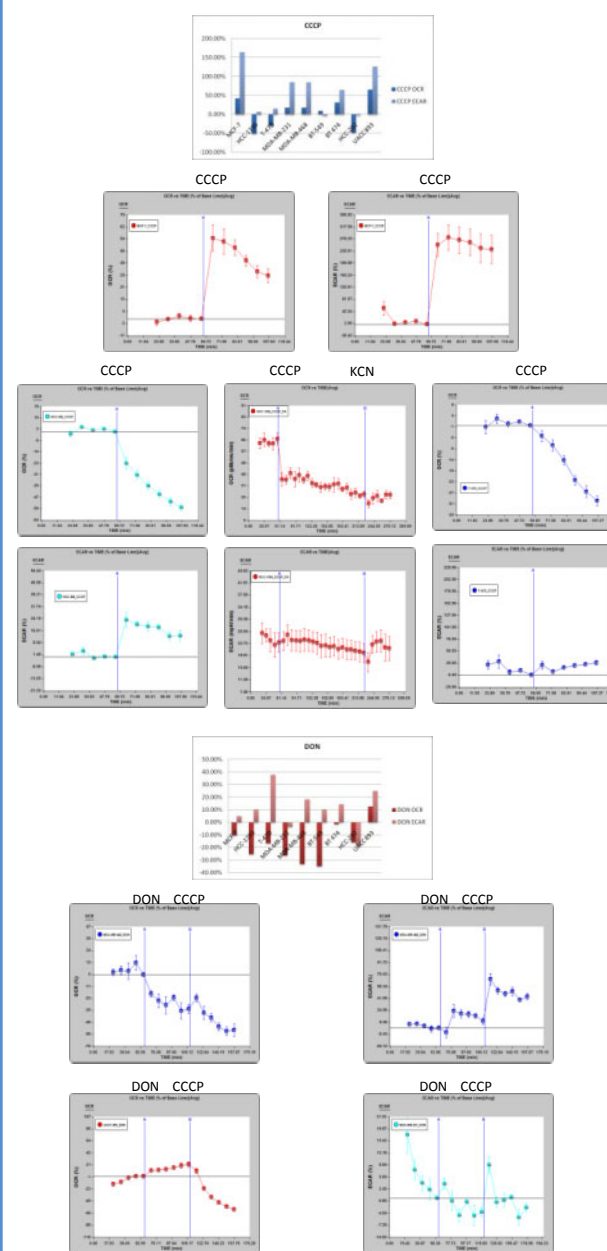
Cell line	4-HCCA		BMAL		2-methylglutarate		Etomoxir	
	OCR	ECAR	OCR	ECAR	OCR	ECAR	OCR	ECAR
MCF-7	-10.4	0.1	0.0	22.0	-87.0	-134.0	-59.6	2.3
HCC-1395	-30.2	44.5			-50.4	15.0	-63.8	-3.4
T-47D	-3.0	246.5	-7.0	64.0	-42.0	12.0	-61.0	38.0
MDA-MB-231	2.9	55.0	-10.0	12.0	-83.0	-141.0	-62.8	4.8
MDA-MB-468	-13.0	14.0	-20.8	25.1	-49.0	48.0	-75.5	57.6
BT-549	-18.0	39.2	-16.0	43.1	-51.0	75.1	-64.0	12.6
BT 474	7.0	55.7	9.0	117.6	-41.0	51.9	-60.5	-42.3
UACC 893	-33.4	28.2	20.2	29.0	-67.1	33.6	-46.2	18.9
HCC-202							-1.4	-51.0

Percentage of rate increase/decrease after addition of metabolic inhibitor

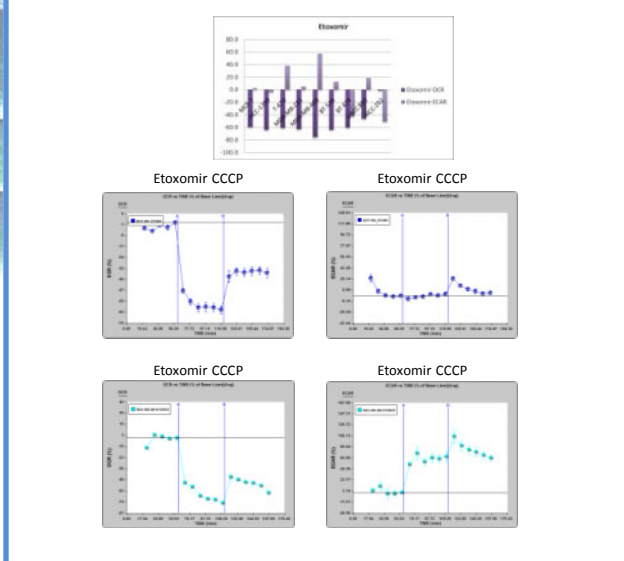
Cell line	CCCP		Cyanide		2MEEA		DON		AOA	
	OCR	ECAR	OCR	ECAR	OCR	ECAR	OCR	ECAR	OCR	ECAR
MCF-7	42.4	163.9	-99.3	41.8	-2.0	-4.0	-10.3	4.8	-6.0	-3.0
HCC-1395	-50.9	6.4	-6.2	12.3	-12.8	-3.8	-25.4	10.3	-12.1	-2.5
T-47D	-29.7	15.3	-3.4	57.6	-2.0	-2.1	-16.8	37.7	-3.0	60.0
MDA-MB-231	51.5	-21.5	-29.7	29.7	-4.0	7.0	-26.2	-3.4	-7.3	-4.7
MDA-MB-468	17.3	84.6	-65.0	83.0	-22.0	32.0	-33.3	18.1	-25.2	13.3
BT-549	9.5	-3.3	-73.0	43.0			-35.0	9.9	-12.0	-4.7
BT 474	30.7	64.4	-87.6	69.9	-11.5	1.2	-1.5	14.2	-8.2	-1.2
HCC-202	-48.6	-3.2	-69.0	34.0			-16.0	-18.5	-4.0	-2.1
UACC 893	64.9	126.1	-64.6	42.7	1.0	-1.0	12.6	24.9	-2.8	-2.5

Percentage of rate increase/decrease after addition of metabolic inhibitor

## Results



## Results



## Conclusions

Our results demonstrate that triple-negative cell lines (MDA-MB-231, MDA-MB-468 and BT-549) cell lines which do not express the genes for estrogen receptor (ER), progesterone receptor (PR) or Her2 are more glycolytic than other subtypes. We also noted that 2 ER+ cell lines (HCC1395 and T47D) and a HER2+ ER- cell line (HCC202) exhibit a paradoxical decrease in respiration when treated with a mitochondrial respiration coupler, CCCP (carbonyl cyanide m-chloro phenyl hydrazone), in contrast to the expected increase in uncoupled respiration observed in other cell lines. This suggests that these cell lines utilize a mitochondrial fuel requiring a higher level of membrane energization, possibly glutamine. Moreover, we observed that a second HER2+ ER- cell line, UACC893, increases oxygen consumption rate (OCR) when treated with DON (6-Diazo-5-oxo-L-norleucine), an inhibitor of glutaminase. In summary, our results indicate that there are differences in metabolic pathways between cell lines, even in the same type of cancer, with an overlap between ER+ and HER2+ ER- cell lines, distinct from the metabolic profile in triple-negative cell lines. Better understanding of the mechanistic links between cellular metabolism and cell survival may ultimately lead to improved treatments for human cancer.

## Acknowledgements

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