

## **Mitochondria Organelle Transplantation: The Mitochondrion, “An Intracellular Organelle for Cell-Based Therapy” Opinion Commentary**

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### **Abstract**

*Mitochondria are powerful intracellular organelles involved in many vital cellular functions especially cellular respiration and energy production. Mitochondrial dysfunction is associated with many human diseases including cancer and neurodegenerative disease. Warburg showed in the 1920s that defective mitochondrial respiration altered tumor cell metabolism with a shift to glycolysis, an initiating step in tumorigenesis. We have recently supported Warburg’s mitochondrial dysfunction theory of cancer by transplanting normal isolated mitochondria into cancer cells. This organelle transfer decreased proliferation, lactate production and increased drug sensitivity of the cancer cells. Confirmation of organelle transfer was done by confocal and fluorescent microscopy. In conclusion, this early evidence supported transplantation of normal exogenous mitochondria as possible therapy for cancer and neurodegenerative diseases. The mitochondrion could be a biologic intracellular organelle for cell based therapy.*

**Keywords:** Mitochondrial dysfunction, Warburg Effect, Aerobic Glycolysis, Organelle transplantation

The above title is controversial and to some probably impossible, however, it is our prediction and opinion that in the near future mitochondria organelle transplantation or transfer will be a mechanism used in cell based therapy. We believe that normal mitochondria will be targeted to cells to treat cancer and neurodegenerative diseases. Thus, allowing mitochondria to become a powerful organelle for cell based therapy. Our recent research supports this prediction and will be discussed later in this communication.

Mitochondria are dynamic intracellular organelles involved in many vital cellular functions. Some of these functions are: (1) energy conversion with production of ATP, (2) signaling through reactive oxygen species, (3) regulation of membrane potential, (4) apoptosis (programmed cell death), (5) calcium signaling, (6) cellular metabolism, (7) iron metabolism and heme synthesis reactions, and (8) steroid synthesis. Mitochondria are active, mobile intracellular organelles that undergo constant fission and fusion.

They are probably descended from bacteria that survived endocytosis by another cell, and became incorporated into the cytoplasm. This endosymbiotic relationship of mitochondria with their host cells was popularized by Lynn Margulis (Sagon, 1967) and probably developed around 1.5-2.5 billion years ago. These bacteria had the ability to conduct respiration in host cells that had relied on glycolysis and fermentation. This would have conferred a tremendous evolutionary advantage.

Many serious health problems, such as, aging, metabolic, cancer, cardiovascular and neurodegenerative diseases are associated with mitochondrial dysfunction. Our mitochondrial research has been done in cancer; stimulated by revisiting the marvelous work of Otto Warburg. Warburg postulated in the 1920’s that altered tumor cell metabolism was caused by a defect in cellular respiration with a shift to glycolysis, which was the initiating step in tumorigenesis (Warburg, Posener, & Negelein, 1924). Cancer cells had a marked increase in glycolysis and lactate production in the presence of oxygen without an increase in oxidative phosphorylation. This phenomenon of aerobic glycolysis became known as the “Warburg Effect.”

We have studied the ultrastructure of numerous human breast cancers and recently reported on the ultrastructural observation of mitochondria in breast carcinoma cells from 778 patients (Elliott, & Barnett, 2011). These observations revealed three groups: (1) mitochondria present and normal, (2) mitochondria present but sparse and (3) mitochondria absent. The mitochondria absent group were ultrastructurally more anaplastic and aggressive, while in the mitochondria present group cells were more differentiated with normal appearing mitochondria.

The mitochondria in the present but sparse group were very abnormal. They were swollen, ovoid, vacuolated, less dense with fractured cristae. These findings, in our opinion, supported mitochondrial dysfunction in breast carcinoma altering cancer cell metabolism. The fact that absence of mitochondria in breast carcinoma cells contributes to treatment resistance suggested this could be a therapeutic target for mitochondrial transplantation.

These results led us to hypothesize that isolated normal mitochondria might enter cancer cells and restore mitochondrial function, reverse aerobic glycolysis, inhibit cell growth, and reverse chemo resistance. Amazingly, this is what occurred, and to the best of our knowledge had not been reported before that communication (Elliott, Jiang, & Head, 2012), (Fig. 1-2). These observations were interesting and very exciting but also generated many questions that needed to be answered. Some of these questions are as follows: (1) How do the mitochondria get into the cancer cells? (2) Are mitochondria organ and tissue specific? (3) Can we sustain or grow isolated mitochondria in culture? (4) Is inhibiting proliferation and increasing drug sensitivity mitochondrial dose dependent? (5) Can we develop an in vivo model? (6) How can we target isolated normal mitochondria to tumor cells in vivo? (7) Are mitochondria species specific?

We have been diligently trying to answer these important questions, and believe some have been answered; however, more work needs to be done. Live cell imaging by confocal microscopy suggest the mitochondria enter the cancer cells by phagocytosis. The evidence is strong that mitochondria are tissue and organ specific. This is supported by our experiment where normal mitochondria from human skin fibroblast were stained, isolated and cocultured with cancer cells. The fibroblast mitochondria easily entered the cancer cells, however, there was no inhibition of proliferation and no increase in drug sensitivity. This result supports the premise that mitochondria are probably organ and tissue specific. This is further supported in a great article by Nunnari and Suomalainen (Nunnari, & Suomalainen, 2012). They emphasize that the critical functions of mitochondria depend on their external structure, cellular location and highly regulated activities of mitochondrial fission, mobility and tethering. They admit that little data is available, but make it clear that the contributions of these activities and molecular events that control them are highly tissue specific. Initial evidence is that inhibition of proliferation and reversal of drug resistance by transplanted normal mitochondria are probably similar. Our evidence is that there is a reversal of glycolysis, decreased expression of glucose transporter III, and promotion of apoptosis. (Fig. 3-4). Xu et al. (Xu, Pelicano, & Zhou, 2005) have shown that inhibition of glycolysis in cancer cells is a novel strategy to overcome drug resistance in cancer cells. Gogvadze et al. (Gogvadze, Orrenius, & Zhivotovskiy, 2009) reported on mitochondria as targets for chemotherapy. They showed that ATP depletion by reversing glycolysis promotes apoptosis and combination of glycolytic inhibitors with conventional chemotherapeutic drugs could be a novel strategy to overcome drug resistance under hypoxic conditions. We believe these effects are dose dependent.

A recent and very interesting finding is that although mitochondria are probable tissue specific, they may not be species specific. In the process of trying to develop an in vivo model in the mouse the normal mouse mammary epithelial cell did not contain many mitochondria. The stained isolated mitochondria from this cell line did, however, get into the mouse mammary carcinoma cells. However, they were sparse and did slightly inhibit proliferation. Frustrated we transferred normal human mammary epithelial mitochondria into the mouse mammary cancer cells, and they entered the cells with ease. They were not abundant, but early studies show they probably do inhibit proliferation, however, not to the same degree as in human cancer cells. More work is in progress, and our in vivo model is almost ready and soon will be approved for our experimental protocol.

Our most exciting finding is that we may be able to keep mitochondria viable and possibly expand them in culture. Mitochondria in our culture media (proprietary) were viable at 2 ½ weeks. Viability was confirmed by staining with the vital JC-1 stain and fluorescent mitochondria observed with the fluorescent microscope. The fact that they stain at all means some viability and at least an adequate membrane potential. Isolated normal mammary epithelial mitochondria of the untransformed human mammary epithelial MCF-12 A cell line were cultured for 8 days, stained with JC-1 and co cultured with the human mammary cancer MCF-7 cell line. The mitochondria entered the cancer cells with ease and were abundant at 2 hours. (Fig. 5).

If mitochondria are to be an organelle for cellular biotherapy, we must overcome many obstacles. Our greatest challenge is how do we target mitochondria to tumor cells in vivo? For mitochondria to be a powerful biologic organelle for cancer therapy this must be accomplished! It may be very difficult but possibly easier than expected; because mitochondria may have a predilection for stressed cells, such as, cancer cells. It could be that cancer cells are phagocytic or possibly just a simple physical phenomenon.

We might be able to coat the outer mitochondrial membrane with transferrin or a transferrin receptor antibody for targeting. Selectivity for cancer cells is because cancer cells express numerous transferrin receptors. Another consideration is to load mitochondria into liposomes which tend to localize in the leaky, abnormal tumor vasculature. Isolated normal mitochondria might have a tendency to accumulate in the tortuous and abnormal tumor vasculature, similar to nanoparticles and the Albumin bound drug Abraxane. To promote extravasation of mitochondria into the tumor microenvironment we may have to address adhesion molecules on endothelial cells in the tumor vasculature.

Pasquieretal. (Pasquier,Guerrouahen, & Thawanli, 2013) have published an interesting paper describing the preferential transfer of mitochondria from endothelial cells to cancer cells through tunneling nanotubes, which could mediate cytoplasmic exchange and phenotype transfer between stromal and cancer cells. They noted that transfer of mitochondria from endothelial to cancer cells resulted in the acquisition of chemoresistance. This is opposite of the effect we saw with mitochondrial transfer of normal mitochondria of the same cell of origin as the cancer cell. We observed, as stated, increased drug sensitivity and inhibition of proliferation. These conflicting results suggest that for mitochondrial transfer to be effective in treating cancer it may have to be tissue specific. It may take a combination of methods to target mitochondria to tumor cells in vivo especially when delivering them intravenously.

Recently Kitani et al. (Kitani, Kami, & Kawasaki, 2014) have published a paper entitled “Direct Human Mitochondrial Transfer: A Novel Concept Based on the Endosymbiotic Theory”. They cited our paper on mitochondria organelle transplantation, and confirmed that isolated human mitochondria can be internalized into isogenic mesenchymal cells. They used different cell lines and isolation techniques and flow cytometry as a proof of transfer. Their work is strong confirmation of our work on mitochondrial transfer.

Their work and ours is evidence that transfer of exogenous mitochondria into human cells is now envisioned as a mechanism of cell-based therapy. Our work supports that Warburg was correct about mitochondrial dysfunction playing a role in tumorigenesis. In our opinion, it opens new areas of research on tumorigenesis and the development of new therapies for cancer and possibly neurodegenerative diseases.

Our demonstration that normal mitochondria from the same epithelial cell of origin as the cancer cell can enter cancer cells, reverse drug resistance and inhibit proliferation is tremendous support that mitochondria might be powerful biologic organelles for cell based therapy. To the best of our knowledge, we are the first to report that mitochondrial organelle transplantation to cancer cells can promote cell death and increase drug sensitivity. This technique alone or in combination with other therapies could significantly improve our armamentarium of cancer therapies.

Whole organ transplantation has been offered for many years. The improved surgical technique and prevention of organ rejection has spared many lives. It is now time to enter into a new era of cellular organelle transplantation. This has the potential to revolutionize treatment for cancer and other mitochondrial diseases, especially neurodegenerative disease. We are beginning experiments on fibroblast and neuronal cells. It is worth the effort, if in some way we could impact the treatment of a disease like amyotrophic lateral sclerosis (ALS). In order for mitochondrial transfer to be feasible, we probably need to be able to culture, grow, expand and bank them for use when needed.

The time for cellular organelle transplantation is now. It will be a difficult trip and we invite our colleagues in mitochondrial research to take the trip with us. It will be challenging, but we look forward to an exciting journey. The destination could alleviate much suffering and death from terrible diseases, making the trip worth it.

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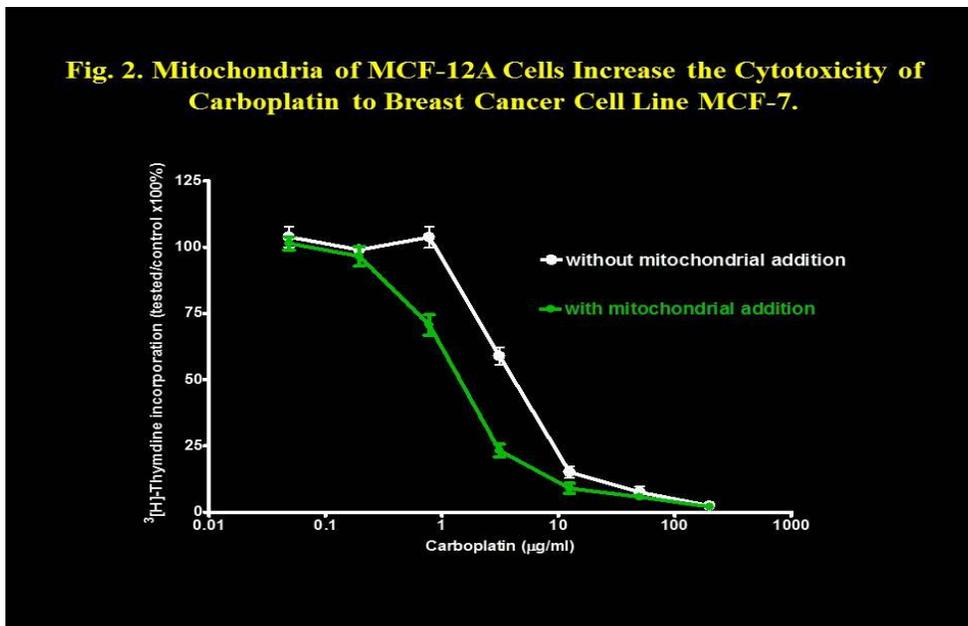
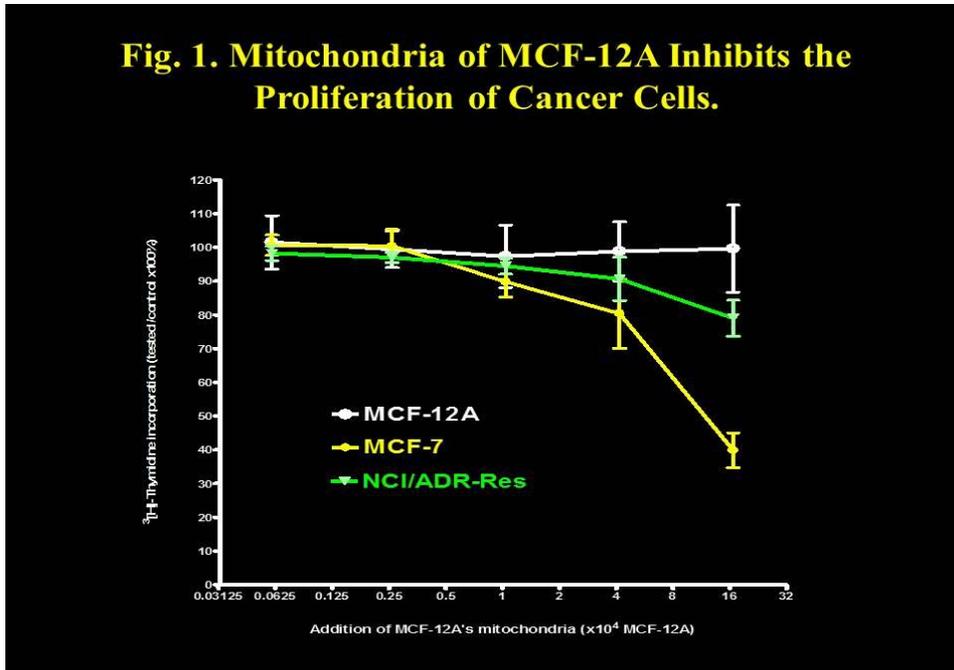
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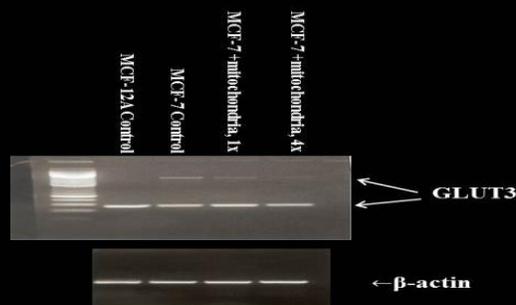


**Fig. 3. Concentration of lactate in culture media.**

Cell Line	Lactate ( $\mu\text{m}$ ) mean $\pm$ SD (n)	P value
MCF-7 medium control	4894 $\pm$ 1105 (3)	
MCF-7 + mitochondria of MCF-12A	3635 $\pm$ 2358 (3)	0.22 <sup>1</sup>
T47D medium control	5836 $\pm$ 712 (2)	
T47D + mitochondria of MCF-12A	2437 $\pm$ 512 (2)	<0.05, Significant
MDA-MB-231 medium control	2722 $\pm$ 767 (3)	
MDA-MB + mitochondria of MCF-12A	1838 $\pm$ 189 (3)	0.17

<sup>1</sup>Compare to control, paired t-test, p<0.05 is statistical significant

**Fig. 4. Effect of exogenous mitochondria of MCF-12A on mRNA expression of Glucose Transporter 3 in breast cancer cells (RT-PCR).**



**Fig. 5. JC-1-stained isolated MCF-12 cells' mitochondria which were cultured 8 days *in vitro* enter into MCF-7 breast cancer cells.**

