**Transfer of Mitochondrial DNA from Cancer-Associated Fibroblasts Can Rescue Hormone-Therapy Cancer Cells from Dormancy**

**Abstract**

The transfer of genomic material between mammalian cells has become a hot topic in recent research. With the collaboration of multiple research teams, it has been suggested that genetic material can be transferred between cells via extracellular vesicles (EV) from cancer-derived cell lines, and that mitochondrial DNA (mtDNA) can be transferred between cells via intracellular pathways. With these implications in place and with the recent discoveries of their own research, Sansone et al. have taken these discoveries a step farther and hypothesized that (i) EVs can contain the full mitochondrial genome, (ii) hormone-therapy dormant cancer cells uptake mtDNA via EV as a means to exit dormancy and regain oxidative phosphorylation (OXPHOS) function, allowing metastasis, and (iii) the mtDNA is transferred via EVs from cancer-associated fibroblasts (CAFs) to the dormant cancer cells. With their extensive research, Sansone et al. have provided compelling evidence for these aforementioned postulations. Although there is compelling evidence for these theories, the mechanisms for these postulations are unknown and could serve as topics for future cancer research.

**Introduction**

It is known that extracellular vesicles are pertinent mediators of intercellular communication, specifically juxtacrine and paracrine signaling mechanisms. Multiple studies have shown that EVs, also known as exosomes, can transfer proteins, lipids, and different types of RNA to local cells that have been damaged or become metabolically inactive due to mutations or loss of vital OXPHOS machinery. It is also known that quiescent, or dormant, cancer cells can be “re-awakened” by the acquisition of mitochondrial DNA. Mitochondrial DNA is a type of extra-nuclear genetic material that consists of many vital genes for the oxidative phosphorylation system. When cancer cells lose mtDNA, they do not die, but rather become dormant. During this dormancy, these cells usually rely on other mechanisms for energy production such as aerobic glycolysis (Warburg Effect). These energy mechanisms are not as efficient as OXPHOS, thus due to their low energy production, these cancer cells do not metastasize or become tumorigenic. Furthermore, it has also been identified that cancer-associated fibroblast’s EVs can contain the full mitochondrial genome. Fibroblasts are descendants of mesenchyme and aid in the structural support of connective tissue by secreting components that make up the extracellular matrix. When these cells become carcinogenic, they begin to promote tumorigenesis and limit the immune response, resulting in tumor progression or metastasis.

With all of these factors in play, Sansone et al. have determined that these postulations are interrelated and are a vital force in the metabolism and mechanism of cancer growth. Their research has connected these principles to form a theory of their own. Using ER+ breast cancer, they hypothesized that (1) mtDNA were present in circulating EVs, (2) Hormone therapy-resistant (HTR) and hormone therapy-sensitive (HTS) breast cancer cells have different metabolic pathways, (3) HTR cancer cells carry wild-type mtDNA and thus have an increased OXPHOS potential, (4) cancer-associated fibroblasts transfer mtDNA to HTS cancer cells, promoting the transition to HTR cancer cells, and (5) cancer stem cell-like cells (CSCs) can uptake mtDNA to promote increased proliferation despite hormone therapy.

To determine the presence of mitochondrial DNA in extracellular vesicles, plasma from patients were centrifuged sequentially and then treated with DNase. The samples were then analyzed using electron microscopy and NanoSight analysis which determined the size and shape of the extracellular vesicles. (i.) To set up the xenograft models, metastatic tissues were collected and grown in vitro. Cancer cells were obtained using cell-sorting purification and then injected into the tissue where half of the models were also treated with hormone therapy. (ii.) To determine the OXPHOS potential, cells were treated with glucose and oligomycin, which inhibits the electron transport chain, using Seahorse technology. (iii.) To quantify the amount of mitochondrial DNA present in the cells, standard PCR was used to amplify the human and murine mitochondrial DNA and then extracted from agarose gels using Nucleospin Gel and PCR Clean-Up kit, and quantified using Agilent 2100 Bioanalyzer. DNA copy number was calculated using a specific algorithm. (iv.) The mtDNA of the samples was then analyzed using long-range and qPCR to determine the presence of certain genes. They were then visualized on agarose gel.

**Recent Progress**

Sansone et al. have provided compelling evidence and data to contribute and assist in the research of cancer, specifically in the metabolism and metastasis. Through the efforts of their research, they have determined that (1) mtDNA is present in the circulating EVs in breast cancer patients and at a high concentration, (2) HTR tumor-derived cells had a three-fold greater OXPHOS potential than HTS cancer cells, indicating that their metabolisms are different, (3) high levels of mtDNA were extracted from EVs originating from CAF in breast cancer metastases, indicating that hormone-resistance can be conferred from CAFs, and (4) HTS cancer cells can regain metabolic function after injection with CAF-derived VEs, indicating that mtDNA can be horizontally transferred from CAF VEs.

Mitochondria are a well- known energy producer in eukaryotic cells. Along with supplying normal functioning cells with energy, mitochondria also fuel cancer cells, allowing them to metastasize. Along with their oxidative phosphorylation activity, mitochondria also play a role in signaling and biosynthesis. Some cancer biologists have been targeting mitochondrial metabolism in hopes of finding a treatment for some cancers. Sansone, et al. may be able to contribute to this area of cancer research.

**Discussion**

Cancer research is an ongoing field of study with many steps forward, and multiple steps back. Although much is known about cancer, even more is left unknown. With the collaboration of research teams, recent discoveries and ideas can be brought together to formulate overarching theories. Such as what authors Sansone, et al. have accomplished. Their research and discoveries have greatly contributed to cancer research. Although there was a lot of focus on the metabolism of cancer, such as metabolic reprogramming and metastasis, little was known about extracellular vesicles and their role in the disease. Sansone et al. used available information concerning mtDNA, metastasis, metabolism, and cancer to investigate how these themes were interrelated. With these findings, other research teams can propel their research and even further the advancement of cancer biology, and potential cancer treatments.

**Resources**

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