Mitochondrial DNA in Tumor Initiation, Progression, and Metastasis: Role of Horizontal mtDNA Transfer

Michael V. Berridge¹, Lanfeng Dong², and Jiri Neuzil²,³

Abstract

Mitochondrial DNA (mtDNA), encoding 13 out of more than 1,000 proteins of the mitochondrial proteome, is of paramount importance for the bioenergetic machinery of oxidative phosphorylation that is required for tumor initiation, propagation, and metastasis. In stark contrast to the widely held view that mitochondria and mtDNA are retained and propagated within somatic cells of higher organisms, recent in vitro and in vivo evidence demonstrates that mitochondria move between mammalian cells. This is particularly evident in cancer where defective mitochondrial respiration can be restored and tumor-forming ability regained by mitochondrial acquisition. This paradigm shift in cancer cell biology and mitochondrial genetics, concerning mitochondrial movement between cells to meet bioenergetic needs, not only adds another layer of plasticity to the armory of cancer cells to correct damaged mitochondria, but also points to potentially new therapeutic approaches. Cancer Res; 75(16). 3203-8. ©2015 AACR.

Introduction

Genes of somatic cells in higher organisms are thought to be constrained within cells except during specialized processes such as syncytia formation that occurs during cell differentiation, and during embryogenesis and morphogenesis. However, this view was challenged a decade ago when it was shown that membrane connections or cytoplasmic bridges form between cells in culture and that cellular organelles traverse these nanotube connections (1). Since then, considerable in vitro evidence has confirmed organelle transfer, including mitochondria, to and between tumor cells (2–8) as well as between nontumor cells (9–15). Additionally, recent phylogenetic evidence suggests that mtDNA can “move” between normal cells and tumor cells in canine venereal transmissible cancer (16, 17), while pathophysiological models of lung injury (18, 19), lung inflammation (19), and tumor formation by respiration-deficient tumor cells (19) have added weight to intercellular mitochondrial transfer being a new (patho) physiological phenomenon.

In this review, we briefly summarize the role of mtDNA damage in cancer and highlight recent research on horizontal transfer of mitochondria between cells with particular emphasis on tumor models. The cellular mechanisms underlying mitochondrial transfer to tumor cells with defective mtDNA and the metabolic consequences of these transfer events are discussed in relation to metabolic remodeling in cancer. We also discuss cancer plasticity in relation to mtDNA acquisition by malignant cells and highlight the importance of respiration for tumor initiation and progression. We contend that physiological transfer of mitochondria between cells is largely unexplored because it is a “silent” phenomenon, without a strong rational driver (at least given our current understanding) or hypothesis, and a paucity of experimental tools for exploring such transfer. We suggest that mitochondrial respiration should be revisited as a promising target for cancer therapy.

Energy Requirements of Tumor Cells

Considerable evidence now suggests that energy requirements of rapidly proliferating tumor cells and primary cells are met by a predominantly glycolytic metabolism and that differentiated cells with high energy requirement maximize the use of mitochondrial respiration (20). In addition, stem cells and cancer stem cells that divide infrequently yet have high proliferative potential preferentially use glycolysis that is thought to be a safer form of energy metabolism that protects DNA integrity (21). Nevertheless, many tumor cell lines have very high respiration rates, and increasing evidence supports the complexity relationship between energy metabolism of tumor cells and tumor formation and progression (20–26). Indeed, one of the hallmarks of cancer appears to be metabolic flexibility as well as remodeling toward glycolytic metabolism as originally postulated by Warburg (20). Interestingly, metastatic tumor cells without mitochondrial DNA (p⁰ cells) that are unable to use their mitochondria for respiration, grow in culture medium supplemented with uridine and pyruvate, although their growth is usually considerably slower than that of parental cells. These cells exhibit elevated cell surface oxygen consumption via plasma membrane electron transport,
a compensatory pathway thought to correct for the buildup of intracellular reductants in the absence of respiration (25), a factor that needs to be taken into account in assessing the contribution of glycolysis to overall energy metabolism.

**Mitochondrial Damage in Cancer**

Although mitochondrial damage was postulated by Warburg to be a cause of cancer and this view is still upheld by some researchers, overwhelming evidence points to complex nuclear genetic changes, some of which will impinge on mitochondrial function, being the primary cause of cancer with environmentally dictated epigenetic changes contributing in ways that have yet to be fully understood. Alterations in mtDNA occur in most cancers due to high mutational rate relative to nuclear DNA and poor repair mechanisms, but the contribution of these mutations to cancer development has been established in only a modest number of cancers (26–28). Other mitochondrial damage, e.g., due to oxidative damage resulting from sustained inefficient respiration and oxygen radical production, not directly related to genome damage may also occur, but how this damage plays out in terms of cancer initiation and progression is unclear at present. Nevertheless, even small changes that contribute to rebalancing energy metabolism toward glycolysis may play a role in tumor formation and progression.

Several articles document a role for mtDNA mutations in cancer initiation as well as metastasis. For example, HeLa cells that do not form tumors following xenotransplantation were converted into malignant cells capable of carcinoma initiation by introducing mtDNA with specific mutations (29). Mutated mtDNA was also shown to be important for the metastropic propensity of these cells (30). Intriguingly, Ishikawa and colleagues swapped around mtDNA between metastatic and nonmetastatic breast cancer cells, after which the original nonmetastatic cells formed metastatic tumors and vice versa (31). This strongly indicates that some mtDNA mutations, in addition to nuclear mutations, can be important for metastasis.

**Table 1. Transfer of mitochondria to cancer cells**

<table>
<thead>
<tr>
<th>Recipient cell</th>
<th>Donor cell</th>
<th>Function</th>
<th>Note</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human lung adenocarcinoma A549</td>
<td>Human MSCs Human fibroblasts</td>
<td>Transfer of mitochondria from MSCs rescues cancer cells devoid of mtDNA</td>
<td>In vitro</td>
<td>2</td>
</tr>
<tr>
<td>Rat pheochromocytoma PC12</td>
<td>Rat pheochromocytoma PC12</td>
<td>Cytochalasin B blocks formation of TNTs and mitochondrial transfer between PC12 cells while not affecting endocytosis/phagocytosis</td>
<td>In vitro</td>
<td>40</td>
</tr>
<tr>
<td>Human osteosarcoma 143B</td>
<td>Human MSCs</td>
<td>Transfer of mitochondria via TNTs to 143B cells but not to cancer cells with mtDNA</td>
<td>In vitro</td>
<td>3</td>
</tr>
<tr>
<td>Human mesothelioma cell lines and patient-derived cells</td>
<td>Human mesothelioma cell lines and patient-derived cells</td>
<td>Bidirectional transfer of mitochondria between malignant or between normal cells, not between malignant and normal cells</td>
<td>In vitro</td>
<td>4</td>
</tr>
<tr>
<td>Human ovarian carcinoma cells</td>
<td>Human MSCs and endothelial cells</td>
<td>Transfer of mitochondria from endothelial cells to cancer cells via TNTs is preferential to transfer from MSCs</td>
<td>In vitro</td>
<td>5</td>
</tr>
<tr>
<td>Human breast carcinoma</td>
<td>Human MSCs and endothelial cells</td>
<td>Transfer of mitochondria from endothelial cells to cancer cells via TNTs is preferential to transfer from MSCs</td>
<td>In vitro</td>
<td>5</td>
</tr>
<tr>
<td>Primary human laryngeal squamous cell carcinoma</td>
<td>Primary human laryngeal squamous cell carcinoma</td>
<td>Long-distance (up to 1 mm) transfer of mitochondria via TNTs</td>
<td>In vitro</td>
<td>6</td>
</tr>
<tr>
<td>UV-treated rat pheochromocytoma PC12</td>
<td>Untreated rat pheochromocytoma PC12</td>
<td>Unidirectional transfer of mitochondria to damaged cancer cells via TNTs rescues them from demise as a consequence of apoptosis</td>
<td>In vitro</td>
<td>7</td>
</tr>
<tr>
<td>Human osteosarcoma cells 143B</td>
<td>Human Wharton's jelly-derived MSCs</td>
<td>Restores mitochondrial complexes and function</td>
<td>In vitro</td>
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</tr>
<tr>
<td>Various cancer cell lines</td>
<td>Mesenchymal stem cells</td>
<td>Transport of mitochondria to cancer cells to enhance mitochondrial function</td>
<td>In vitro</td>
<td>8</td>
</tr>
<tr>
<td>Mouse metastatic melanoma B16</td>
<td>Unknown*</td>
<td>Acquisition of mtDNA by p53 melanoma cells is essential for respirasome assembly and tumor initiation</td>
<td>In vivo</td>
<td>22</td>
</tr>
<tr>
<td>Mouse metastatic breast cancer 4T1</td>
<td>Unknown*</td>
<td>Acquisition of mtDNA by p53 breast cancer cells is essential for respirasome assembly and tumor initiation</td>
<td>In vivo</td>
<td>22</td>
</tr>
</tbody>
</table>

*In theory, any cell of the tumor stroma can serve as a donor of mitochondria.
pathogenic mtDNA mutations (3). Other recent publications support mitochondrial transfer between tumor cells including human malignant pleural mesothelioma but not normal and malignant mesothelioma cells (4), and from normal human endothelial and MSCs to triple-negative human breast carcinoma cells (MDA-MB-231; refs. 5, 8) and ovarian carcinoma cell lines (SKOV3 and OVCAR3; ref. 5). Surprisingly, cancer cells acquiring mitochondria displayed chemoresistance, indicating functional aspects of mitochondrial acquisition beyond respiration recovery (5). In another study, mitochondrial transfer between cells from human laryngeal squamous cell carcinoma cultures was demonstrated (6).

**In vivo studies**

Analysis of mtDNA in 37 transmissible venereal tumors in dogs and comparable mtDNA regions from 15 host animals and 43 published canine mtDNA sequences suggested that these tumors have periodically acquired mitochondria from their hosts, perhaps over a period of 11,000 years since this tumor originated (16, 17). It has been estimated that transfer of mitochondria into malignant cells with heavily mutated mtDNA occurs once in about 100 years. It was also speculated that mitochondrial transfer may occur in many cancers as they seek to optimize or repair their metabolic machinery (16).

Although mitochondrial respiration is not essential for the survival and growth of eukaryotic cells as demonstrated by the ability of cells lacking mtDNA to grow in culture medium supplemented with uridine and pyruvate, recent studies found that melanoma (22, 32) and breast carcinoma cells (22) without mtDNA (p0 cells) form tumors in syngeneic mice. Tumor growth was observed after a long lag period of 20 to 30 days after grafting the p0 cells, suggesting adaptation to one of the auxotrophic requirements. Using prolonged cell culture in medium lacking uridine and pyruvate, and in the breast cancer model, a drug-resistance marker, cell lines were isolated from the tumors. Contrary to expectation, these cell lines contained the gene for cytochrome b, a mitochondrionally encoded respiratory protein. Extensive characterization of the cell lines, including analysis of mtDNA polymorphisms, established that the origin of mtDNA was from the host experimental animal. These results show not only that mitochondrial respiration is required for tumor growth in these models, but also that the mitochondrial genome can move from normal cells in the local environment to tumor cells lacking mtDNA in animal models of cancer, as shown for two different types of cancer. While these studies do not directly show mitochondrial transfer between cells, when considered together with the in vivo mitochondrial transfer results summarized above, a strong case can be made for mtDNA transfer between cells being explained by intercellular mitochondrial trafficking.

These findings by our two groups (laboratories of Berridge and Neuzil) raise many questions about the physiological prevalence and relevance of mitochondrial trafficking between cells, and are the focus of ongoing studies. We show that in order to initiate and propagate tumors, cancer cells with defective mtDNA acquire mtDNA, assemble mitochondrial respiratory complexes and supercomplexes (in particular the respirasome), and recover mitochondrial respiration (22). Our unpublished data suggest that recovery of a threshold level of respiration is a prerequisite for tumor initiation, and that this threshold varies in different cancers. Our recent work (22) sets a precedent in demonstrating horizontal transfer of genetic information between mammalian cells in vivo.

### Mechanisms of Intercellular Mitochondrial Transfer

Transfer of mtDNA between cells is most likely facilitated by intercellular mitochondrial transfer as there are no known mechanisms of transferring the mitochondrial genome across the inner and outer mitochondrial membranes and plasma membranes of donor and acceptor cells. A number of distinct mitochondrial transfer mechanisms are possible including endocytosis of vesicles containing mitochondria, fusion of whole cells, cell fragments or exosomes containing mitochondria, or transfer of mitochondria via the well-characterized membrane-bound cell-bridging structures originally described by Rustom and colleagues and referred to as tunneling nanotubes (TNT; ref. 1). Because endocytosis is normally associated with pathways involved in phagocytosis and macromolecular processing, and transfer of functional mitochondria via this pathway would involve movement across a double membrane into the cytoplasm, this mechanism is highly unlikely. Also, mitochondria transferred in this way would have to escape from the early endosome before further acidification that characterizes the late endosome/lysosome compartment where biomaterials are degraded. Such a mechanism has not been reported to date. While fusion of whole cells, apoptotic bodies or exosomes containing mitochondria cannot be completely excluded, it is unlikely because it was ruled out in one study by analysis of nuclear DNA polymorphisms (2). Likewise, the possibility that transfer structures are remnants of cytokinesis is excluded, at least in short-term coculture experiments involving MitoTracker dyes (see Table 1).

A number of in vitro studies have associated mitochondrial movement between cells with membrane structures frequently referred to as TNTs, or sometimes, cytoplasmic bridges. These structures are usually 50 to 150 nm in diameter and often up to 100 μm or several cell diameters in length. They contain actin filaments and sometimes microtubules, along which cellular organelles such as mitochondria can travel (1, 3, 6, 7, 19). While the nature of these membrane-bound cytoplasmic connections between cells varies enormously depending on the cell type and culture conditions, visualization of similar structures in vivo (19, 33, 34) and ex vivo (18) lends support to the view that specialized structures that transport organelles, including mitochondria, can form between cells.

Several studies have described bidirectional movement of organelles, particularly between identical or similar cells, while others have observed unidirectional movement. This includes mitochondrial trafficking from human MSCs to damaged human umbilical vein endothelial cells in an ischemia-reperfusion model (14), and to lung epithelia following LPS-induced acute lung injury (18), although in this model, transfer was mediated by microvesicles. Studies with human tumor cells devoid of mtDNA and with damaged mtDNA have shown unidirectional mitochondrial transfer, and we and others have observed bidirectional transfer of mitochondria between wild-type tumor cells and between their p0 counterparts, and between bone marrow-derived MSCs and p0 tumor cells. This raises questions about intercellular mitochondrial transfer being involved in both replacement of defective organelles and recycling damaged organelles. In this context, a recent study showed that mitochondria
from the optic nerve head are packaged into exosomes that are taken up and processed by surrounding astrocytes (35).

The molecular mechanisms facilitating mitochondrial transfer between cells are not clear at present. Reports from in vitro studies suggest that mitochondria “ratchet” between cells along microfilaments and/or microtubules. In the case of microtubule involvement, propulsion would likely be mediated by kinesin, to which the "cargo" is attached via Milton and Miro-1 adaptor proteins (19). Whether this mechanism also governs intercellular trafficking of mitochondria in vivo has yet to be established. The relevant experiments are challenging due to the difficulty of real-time confocal imaging in vivo combined with specific fluorescent staining to visualize the donor and acceptor cells, the mobilized mitochondria and the TNTs bridging the two cells. This also raises the question of how TNTs are established, what signals initiate the process of TNT "construction," and what are the critical components of the transfer process. Undoubtedly, these questions and many more will be addressed by future research.

**Metabolic Consequences of Intercellular Mitochondrial Trafficking in Tumor Progression**

While literature detailing the bioenergetics and metabolic consequences of mitochondrial movement between cells in a tumor setting is very limited, both in vitro (2, 3) and in vivo (22) studies establish that restoration of mitochondrial respiratory function is a major cellular outcome, while other in vitro reports of tumors acquire from apoptotic cell death (7) and modulation of chemoresistance (5).

The study by Tan and colleagues (22) is of particular interest because it documents a quite unexpected metabolic remodeling following mtDNA acquisition in the 4T1 breast carcinoma model. In this work, stable cell lines derived from primary subcutaneous tumors that grew from 4T1P0 cells showed partial recovery of mitochondrial respiration and an intermediate lag to tumor growth. Cell lines from circulating tumor cells and from lung metastases showed further and staged recovery of mitochondrial respiration, and tumor growth more similar to that of parental 4T1 cells. More detailed analysis showed that cell lines from primary tumors and from circulating tumor cells had low ability to form the respirasome, the supercomplex responsible for mitochondrial respiration, while cell lines from lung metastases were similar to parental 4T1 cells. In contrast, all cell lines derived from tumors that grew from B16P0 cells showed respirasome assembly, respiration, and tumor growth properties comparable to parental B16 cells (unpublished results). Thus, respiration recovery and tumor-forming ability of P0 tumor cells is not only dependent on mitochondrial acquisition from the local microenvironment, but with the breast tumor model requires additional local input for step-wise remodeling of respiration.

Our finding that the recovery of respiration differs in primary tumors derived from breast cancer P0 cells (22) and melanoma P0 cells indicates that different tumors require different “threshold” levels of respiration. Whether this is the case across the landscape of neoplastic diseases has yet to be determined. Notwithstanding this, it is clear that respiration is important (if not absolutely required) for the ability of cancer cells to initiate and propagate tumors. This is of great interest not only from the perspective of the basic properties of cancers but also concerning prospective tumor therapy. In the case that tumors are dependent on respiration (at least to its “threshold” level), it should be plausible to design novel anticancer agents specifically targeting components of mitochondrial respiration, i.e., individual complexes selective for cancer cells. This would then result in novel, unprecedented anticancer strategies effective also against untreatable cancers.

**Biological Constraints, Technical Limitations, and Challenges of Exploring Mitochondrial Transfer between Cells**

Convention and dogma constrain scientific progress, and it is often serendipity that opens Pandora’s box by generating the framework for a paradigm shift. The idea of horizontal gene transfer between somatic cells of higher organisms is conceptually challenging and pushes the boundaries of basic genetic and biological knowledge. While nothing has changed regarding cellular constraints on nuclear genes, a few recent publications, both in vitro and in vivo demonstrate that mitochondria with their mtDNA payload not only can, but do break the rules surrounding cellular genetic integrity. MitoTracker dyes have been central in establishing cell-to-cell mitochondrial transfer in cell culture systems. However, there are few fluorescence markers or genetic approaches that can be used to track intercellular mitochondrial transfer in vivo, with outbred animal systems suffering from complications of mtDNA heteroplasmy. Furthermore, a strong rationale for progressing in vitro studies into animal models and human systems, where mitochondrial transfer between cells will be physiologically "silent," has been lacking.

Unexpected results regarding the ability of tumor cells devoid of mtDNA to grow as tumors, and the equally unexpected observation that these tumors apparently contained mitochondrially encoded cytochrome c head, serve to provide a sound scientific basis for intercellular mitochondrial transfer. A key factor was that most inbred mice are homoplastic and contain informative mtDNA polymorphisms and that the tumors chosen for study contained useful mtDNA polymorphic markers that we utilized in our recent report that definitively documents mtDNA acquisition by horizontal transfer by means of using next generation sequencing (22). In the future, methodological approaches where nuclear-encoded green and red fluorescent proteins decorated with mitochondrial import sequences will facilitate exploration of the prevalence of intercellular mitochondrial transfer as a normal mitochondrial maintenance mechanism in eukaryotic organisms. These anticipated advances will be made possible by novel, cutting-edge technology, such as intravital confocal imaging, digital PCR, or single-cell DNA/RNA profiling.

**Concluding Remarks**

Recent research clearly documents the existence of the elusive phenomenon of intercellular transfer of mitochondria with functional consequences. This area of research started with the pioneering publication by the Gerdes group (1), followed by several articles reporting intercellular mitochondrial trafficking between cancer and noncancerous cells, as well as normal cells with mitochondrial damage (refer to recent review in ref. 36). A report published by Islam and colleagues (18) documented mitochondrial transfer from bone marrow-derived MSCs exogenously injected into the lungs of mice in which the mitochondria of alveolar epithelial cells had been damaged. Recovery of mitochondrial function and pathology ensued. Our article on grafting...
p0 cancer cells into syngeneic mice showed in vivo acquisition of mtDNA from the host. In this context, mtDNA acquisition enabled regrowth of the tumor, pointing to unprecedented metabolic plasticity of tumor cells in overcoming adverse bioenergetic conditions. In this case, mitochondria were "stolen" from healthy stromal cells (37). The notion that respiration is important for tumor metastasis and for therapy of hard-to-treat cancer cells has been highlighted in recent reports (38, 39). Collectively, these findings indicate that mitochondrial transfer may be a more frequent event in various tissue types than appreciated at present. Further investigation of this phenomenon and its functional consequences will add to our understanding of the mechanisms involved. Importantly, it is likely that this new knowledge will lead to the development of novel and efficient therapies for neoplastic diseases as well as for other mitochondria-associated bioenergetic pathologies. It remains to be established whether horizontal transfer of mtDNA with a functional consequence is a rare event or a relatively common phenomenon that, for reasons highlighted above, has not been appreciated to date.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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