MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN CUTANEOUS MELANOMA

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ABSTRACT

The Warburg effect in tumour cells is associated with the upregulation of glycolysis to generate ATP, even under normoxic conditions and the presence of fully functioning mitochondria. However, scientific advances made over the past 15 years have reformed this perspective, demonstrating the importance of oxidative phosphorylation (OXPHOS) as well as glycolysis in malignant cells. The metabolic phenotypes in melanoma display heterogeneic dynamism (metabolic plasticity) between glycolysis and OXPHOS, conferring a survival advantage to adapt to harsh conditions and pathways of chemoresistance. Furthermore, the simultaneous upregulation of both OXPHOS and glycolysis (metabolic symbiosis) has been shown to be vital for melanoma progression. The tumour microenvironment (TME) has an essential supporting role in promoting progression, invasion and metastasis of melanoma. Mesenchymal stromal cells (MSCs) in the TME show a symbiotic relationship with melanoma, protecting tumour cells from apoptosis and conferring chemoresistance. With the significant role of OXPHOS in metabolic plasticity and symbiosis, our review outlines how mitochondrial transfer from MSCs to melanoma tumour cells plays a key role in melanoma progression and is the mechanism by which melanoma cells regain OXPHOS capacity even in the presence of mitochondrial mutations. The studies outlined in this review indicate that targeting mitochondrial trafficking is a potential novel therapeutic approach for this highly refractory disease.

KEY WORDS

Melanoma, Skin Cancer, Metastasis, Tumour Microenvironment, Oxidative Phosphorylation, Mitochondrial transfer

INTRODUCTION

Melanoma is the most aggressive, deadly form of skin cancer (1) — despite accounting for only 5% of cases, it constitutes the main cause of deaths from skin cancer (2). It is also one of the fastest growing cancers worldwide (2), with the UK reporting 16,000 new cases every year (3). Along with the long-standing global trend of incidence rise (4), worldwide mortality rates are expected to increase from 61,850 in 2016 to 108,630 by 2040 (5).

Melanoma is highly curable when limited to the primary site (6); metastatic melanoma, however, confers a poor prognosis of a median survival of six months (7). Current systemic therapies in patients with metastatic melanoma have a varied response rate, and tumour resistance develops rapidly in the majority of patients (6, 8-10). Further research is therefore required to understand the pathophysiology of this highly refractory disease, in the context of the role of metabolism (oxidative phosphorylation and/or glycolysis) in melanoma, and the interaction of melanoma with the tumour microenvironment (TME), which supports its survival and proliferation, and contributes to drug resistance.

Primary cutaneous melanoma comprises a distinctly heterogeneous population of both cancerous and non-cancerous cells (11, 12), including fibroblasts, adipocytes and other niche cells such as mesenchymal stromal cells (MSCs), which make up the extracellular matrix, endothelial cells of the microvasculature, and immune cells (11-13). In addition to the cellular component of the tumour microenvironment (TME), the non-cellular component consists of several growth factors, chemokines and cytokines (14). Melanoma cells can manipulate the close association between themselves and the TME to facilitate tumour progression, invasion and metastasis (15, 16). Currently, immune cells in the TME have been the focus of much

interest in an attempt to understand how an immunosuppressive microenvironment that allows for proliferation, growth and invasion of melanoma is created (10), while, by contrast, relative little research has been carried out on the role of MSCs in the TME in melanoma growth.

In this review, we explore the symbiotic relationship between melanoma and MSCs and the ensuing metabolic advantage conferred on melanoma. We begin by describing the metabolism of melanoma and metabolic plasticity in melanoma cells before introducing metabolic symbiosis with MSCs and outlining potential mechanisms of transfer of mitochondrial DNA from MSCs to melanoma to facilitate oxidative phosphorylation.

MSCs IN THE TME

MSCs — spindle-shaped cells that are present in bone marrow, adipose, skin, umbilical cord, blood and various other tissues (17-20) — are highly proliferative and can differentiate into various cells such as osteoblasts, chondrocytes and adipocytes (17-19). These properties, in addition to their ability to home towards injured tissue, can be exploited by melanoma, which, like many other solid cancers, behaves like tissues that do not heal (21, 22): increasing evidence has shown that, like a chronic, non-healing wound, melanoma secretes chemoattractants (23), similar to those used in inflammatory signalling pathways (24), to attract and direct MSCs towards the tumour sites and form part of the TME to contribute towards tumour progression, invasion and metastasis (23, 25-28).

MSCs and melanoma growth

A positive effect for MSCs on tumour incidence was first demonstrated by co-injecting allogeneic mice with B16 melanoma cells and MSCs: not only was the incidence of tumour formation 100% when the melanoma cells and MSCs were injected together versus 0% in the control group (29, 30), but the onset of tumour formation was faster when soluble MSC-derived factors were added (31). Kucerova *et al.* demonstrated this increased tumour incidence and growth using the human melanoma cell line A375 and human MSCs, as well as showing that this increase was dependent on the dose of MSCs (32). MSCs also abrogated tumour latency *in vivo* for low numbers of cells that would otherwise not normally produce tumours if injected alone (32)**.** Furthermore, MSCs were shown to protect melanoma cells from sustaining cellular stress in response to systemic treatment, such as doxorubicin, and cytotoxicity by inhibiting apoptosis. Notably, the effect of MSCs on tumour initiation was reported in experiments using low volumes of A375 melanoma cells, mimicking minimal residual disease that is common following radiotherapy treatment. Together, these data demonstrate the pro-oncogenic role of MSCs on melanoma growth.

Additional pro-oncogenic roles of MSCs

MSCs also display various other pro-oncogenic behaviours, which are outlined here but not covered in detail as they are not the focus of this review. MSCs have been reported to increase the motility and invasiveness of melanoma by communicating with melanoma-derived exosomes, to manipulate melanoma cells towards a more metastatic phenotype via the process of epithelial–mesenchymal transition (EMT) (33) and by increasing the porosity of blood vessels, thereby facilitating tumour migration (33). Current reports have demonstrated the ability of MSCs to support neovascularisation in a mouse model of melanoma through the

secretion of pro-angiogenic factors (27, 28, 34). Kucerova *et al.* demonstrated enhanced melanoma growth as a result of this proangiogenic cellular milieu created by the mutual crosstalk between melanoma and MSCs (32). In addition to the secretion of various factors, Vartanian *et al.* provided direct evidence that melanoma can educate MSCs to engage in vasculogenic mimicry, a process in which MSCs adopt certain endothelial-cell-like properties to directly contribute to the formation of the tumour vasculature (28). Several studies have also demonstrated the ability of MSCs to differentiate into carcinoma-associated fibroblasts (CAFs) (28, 34), a key cellular component of the growth-supporting TME, aiding the formation of the stem-cell niche and promoting stemness in the tumour (23, 35). Not only do these CAFs and MSCs promote tumour growth, but they have also been shown to have immunomodulatory functions — for example, reducing T-cell proliferation and the number of tumour-infiltrating T and B cells *in vivo*, and producing cytokines — thereby creating a highly effective immunosuppressive TME for melanoma proliferation (23, 36).

MSC–melanoma symbiosis confers metabolic advantage

The processes of MSC-mediated tissue repair, which involves activation, migration and homing to TME, and MSC differentiation and subsequent secretion of factors (by both melanoma cells and MSCs) produce a strong pro-oncogenic symbiotic relationship between MSCs and melanoma cells (21). This symbiotic relationship provides a metabolic advantage that is effective for melanoma proliferation and metastasis (37-39). Given the significance of metabolism in melanoma, supported by the growing evidence of its impact on the efficacy of current systemic therapies for this highly refractory disease (38), below we explore the symbiotic relationship between MSCs and melanoma, and how it might arise.

METABOLISM OF MELANOMA

Due to its significant influence on all aspects of tumorigenesis, metabolic reprogramming has been widely accepted as one of the hallmarks of cancer (40). Determining the biochemical pathway that melanoma cells use for energy production allows researchers to understand the influence of metabolism on the symbiotic relationship between melanoma and MSCs and its corresponding pro-oncogenic role (41).

Glycolysis in melanoma

In the 1920s, Warburg reported that, even in presence of oxygen, cancer cells take up glucose for glycolysis (42). This preferential method for energy production adopted by cancers was termed 'aerobic glycolysis' (also known as the Warburg effect) and was demonstrated to provide ATP necessary for survival and proliferation of the tumour (43).

Melanoma has been demonstrated to be associated with a glycolytic phenotype (44, 45). Aerobic glycolysis in melanoma cells is driven by a multitude of factors, including activation of oncogenes, the presence of a hypoxic TME and an absence of tumour suppressors (46). Approximately 50–60% of melanomas contain a *BRAF* gene mutation (47), the most frequent of which (BRAFV600E, accounting for 90% of BRAF mutations (48) and rendering the gene product B-Raf constitutively active (49)) has been shown to be associated with higher glucose uptake and subsequent glycolysis (50). B-Raf activates the mitogen-activated protein kinase (MAPK) pathway, which promotes hypoxia-inducible factor 1α [HIF1α (master regulator of glycolysis)], resulting in an increase in glycolysis (50). Furthermore, B-Raf inhibits microphthalmia-associated transcription factor (MITF) and peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), thereby inhibiting oxidative phosphorylation

(OXPHOS) (43, 51, 52). OXPHOS is the main pathway for energy production in mitochondria via aerobic respiration. Providing direct evidence for this B-Raf mediated upregulation of aerobic glycolysis, Hall *et al.*(44) demonstrated a 14–16-fold higher extracellular acidification rate (ECAR, resulting from respiratory and glycolytic acidification) in melanoma cells compared to melanocytes. Furthermore, treatment with the glycolysis inhibitor 2-Deoxy-D-glucose (2-DG) induced a significant drop in ATP production by melanoma cells, causing them instead to revert to OXPHOS for energy production. Analysis of the ECAR (a surrogate marker for glycolysis) and oxygen consumption rate (OCR; a surrogate marker for OXPHOS) in these cells uncovered a lower OCR/ECAR ratio, indicating the upregulation of glycolysis rather than low oxygen consumption or lower OXPHOS capacity. In fact, the absolute OCR values were higher in melanoma cell lines compared to melanocytes, with corresponding high OXPHOS enzyme activity. Therefore, although glycolysis is upregulated in melanoma, OXPHOS also plays a role.

Oxidative phosphorylation in melanoma

Whether a specific metastatic lesion relies on either glycolysis or OXPHOS depends upon the heterogeneity of individual tumour types (53). Tumours behave individually, with each cancer demonstrating its own metabolic properties (46, 53). To add further complexity, even within an individual tumour, the constituent cells can be heterogeneous, displaying different energy metabolic phenotypes (46). For example, large B cell lymphomas can be split into a low OXPHOS subset and a high OXPHOS subset; the latter subset show an upregulation of mitochondrial electron transport chain components (54). Whilst many melanomas have an aerobic glycolytic phenotype, a subset has been shown to present with higher OXPHOS phenotype (38, 52). Fischer *et al.* have identified that 35–50% of BRAF-mutant and wild-type

cell lines and patient samples can be categorised into this subset (38), indicating that a significant proportion of melanoma cells present with a higher OXPHOS phenotype. PGC-1α is a member of a family of transcriptional coactivators that play a central role in the regulation of cellular energy metabolism and mitochondrial biogenesis (55). Regulatory mechanisms to suppress OXPHOS mediated via the PGC-1α pathway fail to occur in high OXPHOS melanomas (38). Higher PGC-1α levels are correlated with poorer survival in melanoma patients (52). The PGC-1α-driven high OXPHOS subset demonstrated an improved tolerance to the damaging effects of reactive oxygen species (ROS), indicating their increased ability to survive under conditions of oxidative stress (52). *In vivo* experiments in mice demonstrated that PGC-1α knockdown resulted in reduced metastasis of melanoma (56), highlighting the pro-oncogenic role of OXPHOS in melanoma progression and metastasis.

OXPHOS and glycolysis in melanoma

Ho *et al.*(57) suggested that both OXPHOS and glycolysis play a significant role in the progression of melanoma and generation of ATP. They discovered two patient populations within their melanoma cohorts: one with high serum levels of lactate dehydrogenase (LDH) and one with normal serum LDH levels. The high serum LDH population had a corresponding high ECAR, suggesting that glycolysis was the predominant metabolic pathway. By contrast, in the normal serum LDH population, the tumours demonstrated elevation of several OXPHOS enzymes and higher OCR, indicating that OXPHOS was the predominant metabolic pathway. However, it is important to note that, although high serum LDH levels are associated with poor prognosis in metastatic melanoma patients (58), serum LDH levels might not necessarily always be a marker of tumour-associated increased cell turnover, as patients can have high LDH levels and perform poor clinically due to other factors such as tissue damage, severe

infections and renal failure (59). The OCR rates were higher in both populations of melanoma patients, as well as in melanoma samples from patient tumour biopsy samples and cell lines in culture, compared to normal melanocytes. Thus, OXPHOS and glycolysis both play a significant role in melanoma metabolism (60, 61).

METABOLIC PLASTICITY

Although it is simpler to categorise melanoma into a glycolytic or OXPHOS phenotype, an increasing body of evidence suggests that the nature of metabolic phenotypes in melanoma is dynamic — this is termed 'metabolic plasticity' (38, 40). Jose *et al.*(40) demonstrated that the metabolic phenotype of melanoma is not fixed during tumorigenesis and, in fact, melanoma has a 'hybrid' glycolysis/OXPHOS metabolic phenotype, intuitively conferring selective advantages on tumour cells (45). Importantly, this hybrid phenotype provides tumour cells with the flexibility to use different energy sources to meet their bioenergetic needs in the different and changing TME (62). In a glucose-deprived environment, tumour cells are metabolically reprogrammed towards elevated levels of OXPHOS with decreased glycolysis, whereas in hypoxic conditions, the melanoma cells preferentially use glycolysis, uncoupling from the TCA cycle and attenuating mitochondrial respiration (45).

Metabolic plasticity confers on melanoma cells not only the ability to adapt to harsh TME conditions but also a degree of chemoresistance, thereby providing a survival advantage in treatment-induced conditions (38). The use of targeted systemic therapy such as BRAF inhibitors (e.g. vemurafenib) to target BRAFV600E is associated with a switch from glycolysis to OXPHOS (63). Similarly, Haq *et al.* demonstrated that treatment with MAPK inhibitors resulted in increased PGC-1α-driven OXPHOS (51). Notably, an increase in PGC-1α-driven

OXPHOS is observed in 30–50% of *BRAF*-mutant melanomas with *de novo* and acquired resistance to MAPK inhibitors (64)and, in these circumstances, PGC-1α knockdown resulted in reduced tumour growth (64). This metabolic switch from glycolysis to OXPHOS and the corresponding adaptive resistance was observed in patients treated with inhibitors of BRAF or MEK (MAPK and ERK kinase; an upstream activator of MAPK), alongside increased mitochondrial content, mitochondrial activity and mitochondrial oxidative capacity (37, 65-68). These observations highlight the ability of melanoma to redirect the metabolic phenotypes to confer multiple pathways of chemoresistance. Collectively, it is clear that metabolic plasticity confers a significant survival advantage on cancer cells.

METABOLIC SYMBIOSIS

Within melanoma tumours, the extent of tissue perfusion and oxygenation depends on the location and physical distance of the tumour cells from the local vasculature (57). Accordingly, melanoma cells located in the poorly perfused centre of tumour masses are more likely to be predominantly dependent on glycolysis, whereas tumour cells closer to the vasculature at the periphery are more likely to be dependent on OXPHOS (57). It has, however, been proposed that these two spatially distinct cell populations might be linked, such that the end products from glycolysis (e.g. lactate) are utilised to feed into the TCA cycle for OXPHOS (57).

Indeed, Ho *et al.* (57) reported that, although melanoma patients showed high levels of serum LDH, monocarboxylate transporters MCT4, (the principal transporter for lactate efflux (69) and a downstream effector of hypoxia-inducible factor (HIF)-1a (70)), indicating that the melanoma cells predominantly used glycolysis for energy production, the serum lactate levels remained constant. Although it is plausible that the lactate levels might not be affected by

the tumour, the above data demonstrating upregulation of glycolysis make it more likely that the lactate levels remain unchanged due to other processes. Ho *et al.* suggested that increased levels of lactate resulting from glycolysis are taken up by the metabolically symbiotic melanoma cells that use OXPHOS as their primary mechanism of energy production. When enzymes associated with OXPHOS and glycolysis were both expressed at higher levels, it was evident that OXPHOS and glycolysis were both upregulated in melanoma, compared to normal tissues, demonstrating a further stage to metabolic plasticity (57). This co-operation of both OXPHOS and glycolysis occurring at the same time has been coined 'metabolic symbiosis' (Figure 1A). Several papers (71) have reported this phenomenon and demonstrated its importance in melanoma initiation, growth and metastasis.

THE REVERSE WARBURG EFFECT

In vivo work carried out over the past decade has demonstrated that metabolic reprogramming involves not only cancer cells but also the MSCs and CAFs in the TME (62, 72). Whereas the Warburg effect refers to glycolysis being the preferential method of energy production in tumour cells, according to the 'Reverse Warburg' effect, tumour cells, by secreting ROS (by-product of OXPHOS melanoma cells), stimulate cells in the surrounding TME to undergo aerobic glycolysis, resulting in the secretion of metabolites, such as lactate, into the TME via MCT4 (73)**.** These metabolic intermediates can be taken up by tumour cells, via MCT1, to feed into the TCA cycle for OXPHOS-mediated energy production (74), similar to the situation in metabolic symbiosis outlined above. Loss of Cav-1, a protein involved in endocytosis and vesicular transport, in TME cells results in a positive-feedback loop of oxidative stress in these cells, consequently increasing OXPHOS in tumour cells (62). This Reverse Warburg effect was initially reported in a variety of cancers (38) but is as yet to be

fully elucidated in melanoma. However, taking together the use of OXPHOS in the periphery of melanomas, the metabolic symbiosis reported earlier, and Ho *et al.*'s findings of increased expression of MTC1 and MTC4 in melanoma (57), it is likely that the Reverse Warburg effect occurs in the TME of melanoma (Figure 1B).

The above research has demonstrated that the oncological hallmark of altered metabolism is not only due to the regulation for growth, but can be primary cause for tumour initiation, progression, metastasis and chemoresistance. Due to the heterogeneic dynamism (metabolic plasticity) between glycolysis and OXPHOS of melanoma, the effective blockade of OXPHOS (e.g. using inhibitors of mTORC1) as well as glycolysis (e.g. MAPK pathway inhibitors) has been shown to resensitise melanomas that are resistant to inhibitors of BRAF and other MAPK pathway components **[AU: OK?]**, and thus to be a promising form of treatment (64, 75). Previous work has shown that upregulation of aerobic glycolysis in tumour cells is due to the presence of mitochondrial DNA (mtDNA) mutations, which were assumed to impair OXPHOS capacity. However, several papers have demonstrated that these mtDNA mutations do not necessarily equate to a compromise in OXPHOS capacity. Conversely, although cancer cells retain OXPHOS capacity, they can also possess mtDNA mutations due to damaging effects of higher ROS secretion in mitochondria from inefficient repair mechanisms, close proximity and vulnerability of mtDNA (43, 46, 50, 76). Consequently, further research was carried out to discover why melanoma cells with mtDNA mutations still possessed the capacity to use OXPHOS for energy production, as well as to develop more effective OXPHOS therapies against melanoma.

MITOCHONDRIAL TRANSFER

In 2010, Berridge and Tan (77) designed a model of B16 melanoma cell lines with severe mtDNA damage caused by the mitochondrial gene deletion ρ0 to investigate mitochondrial OXPHOS. The authors observed that not only did the ρ0 cells grow at half the rate of their parental cells *in vitro*, but they also underwent delayed primary subcutaneous melanoma growth and reduced lung metastasis formation in mouse models *in vivo*, compared to B16 parental cells (77). At the time these studies were carried out, this delay was suggested to be due to the time taken to adapt to auxotrophic requirements and local microenvironmental conditions. However, a series of *in vitro* experiments demonstrating mitochondrial trafficking in other cancers (Table 1) as a prerequisite for aerobic respiration, tumour growth, metastasis and chemoresistance (78-88) implied that the delay might be the result of mitochondrial trafficking from MSCs in the TME to tumours. Additional investigations in other cancers into the mechanisms and stimuli behind mitochondrial trafficking, such as NAPDH-oxidase-2-driven and CD38-driven in acute myeloid leukaemia and multiple myeloma, respectively, have led to the development of effective therapeutic agents targeting mitochondrial trafficking, with demonstrated effective tumour regression (83, 84, 89).

Mitochondrial transfer in melanoma

Consistent with the results obtained in other tumours, Tan *et al.* (88) subsequently demonstrated, in 2015, that the delay in melanoma tumour growth when B16ρ0 cells were injected in NOD/SCID mice was due to the time taken for these cells to acquire mtDNA from the TME *in vivo*. In 2017, Dong *et al.* (90) demonstrated that the tumours that grew from injected B16ρ0 cells *in vivo*, after a delay, contained host TME mtDNA (confirmed via single-cell droplet PCR methods), and that the B16ρ0 cells had acquired mitochondria from host

MSCs by the presence of double-positive cells when B16ρ0 cells with nuclear-targeted blue fluorescent protein were injected into C57BL/GN mice with red fluorescent mitochondria in mouse MSCs.

The primary role of mitochondria is to produce energy via OXPHOS (91), and mtDNA encodes peptides that are essential for this task (92). Accordingly, Dong *et al.* demonstrated that the injected B16ρ0 cells that gained mtDNA subsequently contained mtDNA-encoded proteins and fully assembled respirasomes, with a higher OCR and increased ATP production than injected B16ρ0 cells that failed to gain mtDNA. These results demonstrated that the mtDNA transferred to the B16ρ0 cells was functional and conferred similar OXPHOS respiration rates and respiration recovery to those of their parental B16 cells.

Finally, Dong *et al.* (90) provided direct evidence for the requirement of OXPHOS respiration mediated by mtDNA transfer from MSCs to melanoma cells in tumour formation. B16ρ0 cells with OXPHOS respiration suppressed (via inhibition of the catalytic subunits of CI and CII) formed tumours with an even longer lag period of 15–40 days compared with B16ρ0 cells without OXPHOS suppressed, *in vivo*. A similar pattern was observed for parental B16 cells with OXPHOS knocked down (90). This indicates a shift in viewpoint regarding cancer metabolism, with mitochondrial DNA mutations not compromising OXPHOS capacity. These results collectively demonstrate that melanoma cells gain mtDNA from the MSCs and their subsequent rapid OXPHOS recovery is a prerequisite for driving efficient tumour formation. Although mitochondrial trafficking in melanoma has only been shown in the murine B16 cell line, the extensive experiments mentioned above, coupled with the importance of mitochondrial trafficking demonstrated in several other human cancers, make it very

plausible that mitochondrial trafficking plays a vital role in human melanoma progression. A major gap in the literature therefore exists, and further experiments are required to demonstrate role of mitochondrial trafficking in other cell lines and human melanoma.

Despite intracellular transfer of mtDNA being the most likely transfer mechanism, other possible explanations for mtDNA acquisition and respiration recovery have been suggested. First, it is plausible that a few tumour cells with mitochondria and mtDNA replicate their mtDNA and proliferate at much faster rate than tumour cells without mtDNA, and that the tumour cells without mtDNA might then be progressively removed by autophagy. However, markers for autophagy, such asLC3A11 protein, were lower in B16ρ0 cells compared with B16 cell lines, suggesting that this is not the case (90). Another possible explanation is the presence of B16ρ0 cells that contained residual undetectable mtDNA. However, this theory was rejected by Dong *et al.*, who used assays that were able to detect heteroplasmy down to 0.5%. The absence of mtDNA in ρ0 cells was further reinforced by confocal microscopy analysis, and the absence of any latent respirasomes/supercomplexes prior to mtDNA acquisition was shown via native blue gel electrophoresis. Thus, the only plausible mechanism of mtDNA acquisition in tumour cells is thought to be transfer from host TME.

MECHANISM OF MTDNA TRANSFER

Studies carried out over the past 15 years have demonstrated that mitochondria can cross cell boundaries and be transferred horizontally between cells (93). The main mechanisms of mtDNA transfer from MSCs to tumour cells are tunnelling nanotubules (TNTs), microvesicles and gap junctions, although other plausible mechanisms exist that require further research, such as cell fusion and direct mtDNA secretion into extracellular media (93-95) (Figure 2).

Tunnelling nanotubules

Tunnelling nanotubes (TNTs) are filopodial extensions (bundles of rod-like shaped parallel actin filaments) of cell cytoplasm that connect two cells via open-ended channels (96, 97) (Figure 2A). TNTs have been shown to facilitate the transfer of biomaterial such as cellular organelles, cytoplasmic molecules and membrane molecules between cells (97). Koyangi *et al.* were the first to document (in 2005) whole mitochondrial transfer through TNTs from cardiomyocytes to endothelial progenitor cells (98); mitochondrial transfer into melanoma via TNTs was not demonstrated until 2017 (90).

Extracellular vesicles (EVs)

mtDNA can also be horizontally transferred through the movement of mitochondrial-derived products or intact mitochondria in EVs — exosomes or microvesicles, respectively (93) (Figure 2B). Exosomes are small (30-100nM in diameter) membrane-encompassed vesicles formed in the endosomal pathway (94, 99, 100). During the endosomal pathway, segments of endosomal membrane bud inside the endosome as a collection of intraluminal vesicles (ILVs) to form multivesicular bodies (MVBs) (94). These MVBs move to the cell's surface plasma membrane release ILVs (exosomes) externally into the extracellular media (94, 101). In

contrast, microvesicles, largest EVs (50-1000nM in diameter) (99), are formed directly from external budding and fission of the plasma membrane of the cell into the extracellular media (94, 102, 103). Guescini *et al.* demonstrated the potential of exosomes as vectors for mitochondrial transfer in glioblastoma and astrocyte cells, which routinely secrete EVs into the intercellular space (104). Isolation of these purified EVs demonstrated the presence of mtDNA and absence of nuclear DNA. Furthermore, high mtDNA levels and mitochondrial proteins were shown to be present in exosomes released into the intercellular media by skeletal muscle cells (105). Although these papers implied that EVs could function as mtDNA carriers **[AU: OK?]**, Islam *et al.* (106) were the first to demonstrate mitochondrial transfer from MSCs to lung alveolar epithelial cells via EVs *in vivo*. Furthermore, Sinclair *et al.* (95) demonstrated that mitochondrial trafficking was reduced by 34.7% after inhibition of endocytosis of EVs into lung epithelial cells. These results provide *in vivo* evidence for the transfer of mitochondria from MSCs to tumour cells via EVs, although transfer from MSCs to melanoma by this means has not yet been reported.

Gap junctions

Gap junctions are intercellular channels composed of two connexons, joined together in the intercellular space, that directly connect the cytoplasm of two different cells (107) (Figure 2C). Whereas TNTs facilitate long-distance communication, gap junctions promote close cell-to-cell communication (107). Islam *et al.* (106) demonstrated gap-junction-mediated mitochondrial transfer from MSCs and a subsequent increase in ATP production for tissue repair in alveolar epithelial cells in an *in vivo* mouse model of acute lung injury. These results were reproducible in other models comprising MSCs with haematopoietic stem cells (108) or epithelial cells (95), with a increase in mtDNA transfer and mitochondrial content in recipient

cells. Most current literature agrees that gap junctions are one of the main mechanisms for mitochondrial transfer from MSCs to the target cell (93), although this method of mitochondrial transfer has so far not been demonstrated in melanoma.

Alternative mechanisms

In the three main transfer mechanisms outlined above, mitochondrial transfer is quick and unidirectional. However, Sinclair *et al.* (95) demonstrated that, although inhibition of all these mechanisms reduced mitochondrial transfer, it did not completely prevent it, indicating the possibility that additional mechanisms exist.

Cell fusion, whereby the plasma membranes of two cells fuse and merge together whilst retaining their nuclei (94), is a contentious form of intercellular communication (Figure 2D). Evidence for mitochondrial transfer via cell fusion remains scarce, as it is difficult to ascertain whether the host cell remains as a host cell after fusion (93). Wada *et al.* modelled cell fusion *in vitro* by developing microfluidic devices that fused paired single cells together through a microslit to form a cytoplasmic connection (109). They demonstrated that this cell fusion system enabled whole mitochondria to be transferred from parental osteosarcoma cells to ρ0 osteosarcoma cells and that, after transfer, the fused cells would spontaneously disconnect and recover in normal culture. Further data are required to substantiate cell fusion as a method of transfer from MSCs to cancer.

Although Guescini *et al.* demonstrated the transfer of mtDNA via EVs, they also showed that a significant proportion of mtDNA was free in the intercellular media (104). Other studies have identified the release of endogenous mtDNA, as 'damage'-associated molecular

patterns (DAMPs), into the intercellular media in response to injury and inflammation (110). As carcinogenesis mimics a chronic inflammatory state (111), it is likely that tumours secrete mtDNA into the media, identifying mtDNA secretion into the media as another potential method of mitochondrial transfer (Figure 2E).

CONCLUSIONS AND DISCUSSION

In melanomas, the TME is known to be important for conferring treatment resistance to the tumour cells. The bulk of the TME is formed by MSCs and the cells they give rise to. In contrast with melanoma cells, MSCs have stable genomes, and so offer themselves as a better potential for therapeutic targeting. OXPHOS plays a significant role in metabolic plasticity, metabolic symbiosis and the homeostasis of the high OXPHOS subset in melanoma, allowing the development of treatment resistance. MSCs ensure that melanoma cells can retain an independent OXPHOS capacity via mitochondrial trafficking to melanoma cells. Mitochondrial trafficking has been shown to be a prerequisite for continued aerobic respiration, subsequent tumour growth, metastasis and the development of chemoresistance and, consequently, inhibition of this process has been integrated into the treatment pathway for other cancers (112, 113).

In this review, we have highlighted mitochondrial trafficking as a potential target to combat the prevalent resistance to current therapies in melanoma. We also outline the need for further research into the different potential mechanisms of mitochondrial trafficking. As mentioned above, only TNT-mediated transfer has definitively been demonstrated as a means for mitochondrial transfer to melanomas; the fact that EVs and gap junctions are commonly employed for mtDNA transfer by most cancers highlights the need for further research to elucidate if these important mechanisms occur in melanoma as well, to facilitate the development of targeted therapeutics against this highly refractory disease.

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2. Study concepts and design: Prakrit Kumar, Stuart Rushworth, Marc Moncrieff
3. Literature research: Prakrit Kumar, Stuart Rushworth
4. Figure preparation: Prakrit Kumar, Jamie Moore, Stuart Rushworth
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REFERENCES

1. Laikova KV, Oberemok VV, Krasnodubets AM, Gal'chinsky NV, Useinov RZ, Novikov IA, et al. Advances in the Understanding of Skin Cancer: Ultraviolet Radiation, Mutations, and Antisense Oligonucleotides as Anticancer Drugs. Molecules. 2019;24(8).

2. Liu Y, Sheikh MS. Melanoma: Molecular Pathogenesis and Therapeutic Management. Mol Cell Pharmacol. 2014;6(3):228.

3. UK CR. Melanoma skin cancer statistics. 2019.

4. Hartman RI, Lin JY. Cutaneous Melanoma-A Review in Detection, Staging, and Management. Hematol Oncol Clin North Am. 2019;33(1):25-38.

5. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. Lancet. 2018;392(10159):2052-90.

6. Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther. 2019;20(11):1366-79.

7. Crosby T, Fish R, Coles B, Mason M. WITHDRAWN: Systemic treatments for metastatic cutaneous melanoma. Cochrane Database Syst Rev. 2018;2:CD001215.

8. Polkowska M, Ekk-Cierniakowski P, Czepielewska E, Kozlowska-Wojciechowska M. Efficacy and safety of BRAF inhibitors and anti-CTLA4 antibody in melanoma patients-real-world data. Eur J Clin Pharmacol. 2019;75(3):329-34.

9. Spagnolo F, Ghiorzo P, Orgiano L, Pastorino L, Picasso V, Tornari E, et al. BRAF-mutant melanoma: treatment approaches, resistance mechanisms, and diagnostic strategies. Onco Targets Ther. 2015;8:157-68.

10. Wang P, Zhang X, Sun N, Zhao Z, He J. Comprehensive Analysis of the Tumor Microenvironment in Cutaneous Melanoma associated with Immune Infiltration. J Cancer. 2020;11(13):3858-70.

11. Cacho-Diaz B, Garcia-Botello DR, Wegman-Ostrosky T, Reyes-Soto G, Ortiz-Sanchez E, Herrera-Montalvo LA. Tumor microenvironment differences between primary tumor and brain metastases. J Transl Med. 2020;18(1):1.

12. Pieniazek M, Matkowski R, Donizy P. Macrophages in skin melanoma-the key element in melanomagenesis. Oncol Lett. 2018;15(4):5399-404.

13. Brandner JM, Haass NK. Melanoma's connections to the tumour microenvironment. Pathology. 2013;45(5):443-52.

14. Chitty JL, Filipe EC, Lucas MC, Herrmann D, Cox TR, Timpson P. Recent advances in understanding the complexities of metastasis. F1000Res. 2018;7.

15. Falletta P, Sanchez-Del-Campo L, Chauhan J, Effern M, Kenyon A, Kershaw CJ, et al. Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. Genes Dev. 2017;31(1):18-33.

16. Kim IS, Heilmann S, Kansler ER, Zhang Y, Zimmer M, Ratnakumar K, et al. Microenvironment-derived factors driving metastatic plasticity in melanoma. Nat Commun. 2017;8:14343.

17. Augello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. Hum Gene Ther. 2010;21(10):1226-38.

18. Keating A. Mesenchymal stromal cells. Curr Opin Hematol. 2006;13(6):419-25.

19. Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. Acta Biomater. 2011;7(2):463-77.

20. Lindner U, Kramer J, Rohwedel J, Schlenke P. Mesenchymal Stem or Stromal Cells: Toward a Better Understanding of Their Biology? Transfus Med Hemother. 2010;37(2):75-83.

21. Nwabo Kamdje AH, Kamga PT, Simo RT, Vecchio L, Seke Etet PF, Muller JM, et al. Mesenchymal stromal cells' role in tumor microenvironment: involvement of signaling pathways. Cancer Biol Med. 2017;14(2):129-41.

22. Wang H, Cao F, De A, Cao Y, Contag C, Gambhir SS, et al. Trafficking mesenchymal stem cell engraftment and differentiation in tumor-bearing mice by bioluminescence imaging. Stem Cells. 2009;27(7):1548-58.

23. Mirzaei H, Sahebkar A, Avan A, Jaafari MR, Salehi R, Salehi H, et al. Application of Mesenchymal Stem Cells in Melanoma: A Potential Therapeutic Strategy for Delivery of Targeted Agents. Curr Med Chem. 2016;23(5):455-63.

24. Chulpanova DS, Kitaeva KV, Tazetdinova LG, James V, Rizvanov AA, Solovyeva VV. Application of Mesenchymal Stem Cells for Therapeutic Agent Delivery in Anti-tumor Treatment. Front Pharmacol. 2018;9:259.

25. Reagan MR, Kaplan DL. Concise review: Mesenchymal stem cell tumor-homing: detection methods in disease model systems. Stem Cells. 2011;29(6):920-7.

26. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol Cancer. 2017;16(1):31.

27. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med. 2011;17(7-8):579-87.

28. Vartanian A, Karshieva S, Dombrovsky V, Belyavsky A. Melanoma educates mesenchymal stromal cells towards vasculogenic mimicry. Oncol Lett. 2016;11(6):4264-8.

524 29. Djouad F, Bony C, Apparailly F, Louis-Plence P, Jorgensen C, Noel D. Earlier onset of
525 syngeneic tumors in the presence of mesenchymal stem cells. Transplantation.
526 2006;82(8):1060-6.
527 30. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, et al. Immunosuppressive
528 effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood.
529 2003;102(10):3837-44.
530 31. Han Z, Tian Z, Lv G, Zhang L, Jiang G, Sun K, et al. Immunosuppressive effect of bone
531 marrow-derived mesenchymal stem cells in inflammatory microenvironment favours the
532 growth of B16 melanoma cells. J Cell Mol Med. 2011;15(11):2343-52.
533 32. Kucerova L, Matuskova M, Hlubinova K, Altanerova V, Altaner C. Tumor cell behaviour
534 modulation by mesenchymal stromal cells. Mol Cancer. 2010;9:129.
535 33. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, et al.
536 Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic
537 phenotype through MET. Nat Med. 2012;18(6):883-91.
538 34. Kucerova L, Zmajkovic J, Toro L, Skolekova S, Demkova L, Matuskova M. Tumor-driven
539 Molecular Changes in Human Mesenchymal Stromal Cells. Cancer Microenviron. 2015;8(1):1-
540 14.
541 35. Kucerova L, Skolekova S, Demkova L, Bohovic R, Matuskova M. Long-term efficiency
542 of mesenchymal stromal cell-mediated CD-MSC/5FC therapy in human melanoma xenograft
543 model. Gene Ther. 2014;21(10):874-87.
544 36. Ling W, Zhang J, Yuan Z, Ren G, Zhang L, Chen X, et al. Mesenchymal stem cells use
545 IDO to regulate immunity in tumor microenvironment. Cancer Res. 2014;74(5):1576-87.

37. Avagliano A, Fiume G, Pelagalli A, Sanita G, Ruocco MR, Montagnani S, et al. Metabolic Plasticity of Melanoma Cells and Their Crosstalk With Tumor Microenvironment. Front Oncol. 2020;10:722.

38. Fischer GM, Vashisht Gopal YN, McQuade JL, Peng W, DeBerardinis RJ, Davies MA. Metabolic strategies of melanoma cells: Mechanisms, interactions with the tumor microenvironment, and therapeutic implications. Pigment Cell Melanoma Res. 2018;31(1):11-30.

39. Lyssiotis CA, Kimmelman AC. Metabolic Interactions in the Tumor Microenvironment. Trends Cell Biol. 2017;27(11):863-75.

40. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta. 2011;1807(6):552-61.

41. Bellance N, Benard G, Furt F, Begueret H, Smolkova K, Passerieux E, et al. Bioenergetics of lung tumors: alteration of mitochondrial biogenesis and respiratory capacity. Int J Biochem Cell Biol. 2009;41(12):2566-77.

42. Warburg O, Wind F, Negelein E. The Metabolism of Tumors in the Body. J Gen Physiol. 1927;8(6):519-30.

43. Abildgaard C, Guldberg P. Molecular drivers of cellular metabolic reprogramming in melanoma. Trends Mol Med. 2015;21(3):164-71.

44. Hall A, Meyle KD, Lange MK, Klima M, Sanderhoff M, Dahl C, et al. Dysfunctional oxidative phosphorylation makes malignant melanoma cells addicted to glycolysis driven by the (V600E)BRAF oncogene. Oncotarget. 2013;4(4):584-99.

45. Scott DA, Richardson AD, Filipp FV, Knutzen CA, Chiang GG, Ronai ZA, et al. Comparative metabolic flux profiling of melanoma cell lines: beyond the Warburg effect. J Biol Chem. 2011;286(49):42626-34.

570 46. Zheng J. Energy metabolism of cancer: Glycolysis versus oxidative phosphorylation
571 (Review). Oncol Lett. 2012;4(6):1151-7.
572 47. Kim A, Cohen MS. The discovery of vemurafenib for the treatment of BRAF-mutated
573 metastatic melanoma. Expert Opin Drug Discov. 2016;11(9):907-16.
574 48. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF
575 gene in human cancer. Nature. 2002;417(6892):949-54.
576 49. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, et al. The role of
577 BRAF V600 mutation in melanoma. J Transl Med. 2012;10:85.
578 50. Hosseini M, Kasraian Z, Rezvani HR. Energy metabolism in skin cancers: A therapeutic
579 perspective. Biochim Biophys Acta Bioenerg. 2017;1858(8):712-22.
580 51. Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC, et al. Oncogenic
581 BRAF regulates oxidative metabolism via PGC1alpha and MITF. Cancer Cell. 2013;23(3):302-
582 15.
583 52. Vazquez F, Lim JH, Chim H, Bhalla K, Girnun G, Pierce K, et al. PGC1alpha expression
584 defines a subset of human melanoma tumors with increased mitochondrial capacity and
585 resistance to oxidative stress. Cancer Cell. 2013;23(3):287-301.
586 53. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim
587 Biophys Acta. 2010;1805(1):105-17.
588 54. Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, et al. Metabolic
589 signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma.
590 Cancer Cell. 2012;22(4):547-60.
591 55. Bost F, Kaminski L. The metabolic modulator PGC-1alpha in cancer. Am J Cancer Res.
592 2019;9(2):198-211.

593 56. LeBleu VS, O'Connell JT, Gonzalez Herrera KN, Wikman H, Pantel K, Haigis MC, et al.
594 PGC-1alpha mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells
595 to promote metastasis. Nat Cell Biol. 2014;16(10):992-1003, 1-15.
596 57. Ho J, de Moura MB, Lin Y, Vincent G, Thorne S, Duncan LM, et al. Importance of
597 glycolysis and oxidative phosphorylation in advanced melanoma. Mol Cancer. 2012;11:76.
598 58. Petrelli F, Cabiddu M, Coinu A, Borgonovo K, Ghilardi M, Lonati V, et al. Prognostic role
599 of lactate dehydrogenase in solid tumors: a systematic review and meta-analysis of 76
600 studies. Acta Oncol. 2015;54(7):961-70.
601 59. Farhana A, Lappin SL. Biochemistry, Lactate Dehydrogenase (LDH). StatPearls.
602 Treasure Island (FL)2020.
603 60. Barbi de Moura M, Vincent G, Fayewicz SL, Bateman NW, Hood BL, Sun M, et al.
604 Mitochondrial respiration--an important therapeutic target in melanoma. PLoS One.
605 2012;7(8):e40690.
606 61. Xu K, Mao X, Mehta M, Cui J, Zhang C, Xu Y. A comparative study of gene-expression
607 data of basal cell carcinoma and melanoma reveals new insights about the two cancers. PLoS
608 One. 2012;7(1):e30750.
609 62. Jia D, Park JH, Jung KH, Levine H, Kaipparettu BA. Elucidating the Metabolic Plasticity
610 of Cancer: Mitochondrial Reprogramming and Hybrid Metabolic States. Cells. 2018;7(3).
611 63. Trotta AP, Gelles JD, Serasinghe MN, Loi P, Arbiser JL, Chipuk JE. Disruption of
612 mitochondrial electron transport chain function potentiates the pro-apoptotic effects of
613 MAPK inhibition. J Biol Chem. 2017;292(28):11727-39.
614 64. Gopal YN, Rizos H, Chen G, Deng W, Frederick DT, Cooper ZA, et al. Inhibition of
615 mTORC1/2 overcomes resistance to MAPK pathway inhibitors mediated by PGC1alpha and
616 oxidative phosphorylation in melanoma. Cancer Res. 2014;74(23):7037-47.

617 65. Figarola JL, Singhal J, Singhal S, Kusari J, Riggs A. Bioenergetic modulation with the
618 mitochondria uncouplers SR4 and niclosamide prevents proliferation and growth of
619 treatment-naive and vemurafenib-resistant melanomas. Oncotarget. 2018;9(97):36945-65.
620 66. Ruocco MR, Avagliano A, Granato G, Vigliar E, Masone S, Montagnani S, et al.
621 Metabolic flexibility in melanoma: A potential therapeutic target. Semin Cancer Biol.
622 2019;59:187-207.
623 67. Corazao-Rozas P, Guerreschi P, Andre F, Gabert PE, Lancel S, Dekiouk S, et al.
624 Mitochondrial oxidative phosphorylation controls cancer cell's life and death decisions upon
625 exposure to MAPK inhibitors. Oncotarget. 2016;7(26):39473-85.
626 68. Audrito V, Manago A, Gaudino F, Deaglio S. Targeting metabolic reprogramming in
627 metastatic melanoma: The key role of nicotinamide phosphoribosyltransferase (NAMPT).
628 Semin Cell Dev Biol. 2020;98:192-201.
629 69. Dimmer KS, Friedrich B, Lang F, Deitmer JW, Broer S. The low-affinity monocarboxylate
630 transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochem J.
631 2000;350 Pt 1:219-27.
632 70. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4,
633 but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J Biol
634 Chem. 2006;281(14):9030-7.
635 71. Ma L, Zong X. Metabolic Symbiosis in Chemoresistance: Refocusing the Role of Aerobic
636 Glycolysis. Front Oncol. 2020;10:5.
637 72. Porporato PE, Filigheddu N, Pedro JMB, Kroemer G, Galluzzi L. Mitochondrial
638 metabolism and cancer. Cell Res. 2018;28(3):265-80.
639 73. Pavlides S, Tsirigos A, Vera I, Flomenberg N, Frank PG, Casimiro MC, et al.
640 Transcriptional evidence for the "Reverse Warburg Effect" in human breast cancer tumor

641 stroma and metastasis: similarities with oxidative stress, inflammation, Alzheimer's disease,
642 and "Neuron-Glia Metabolic Coupling". Aging (Albany NY). 2010;2(4):185-99.
643 74. Gonzalez CD, Alvarez S, Ropolo A, Rosenzvit C, Bagnes MF, Vaccaro MI. Autophagy,
644 Warburg, and Warburg reverse effects in human cancer. Biomed Res Int. 2014;2014:926729.
645 75. Ashton TM, McKenna WG, Kunz-Schughart LA, Higgins GS. Oxidative Phosphorylation
646 as an Emerging Target in Cancer Therapy. Clin Cancer Res. 2018;24(11):2482-90.
647 76. Krishnan KJ, Reeve AK, Samuels DC, Chinnery PF, Blackwood JK, Taylor RW, et al. What
648 causes mitochondrial DNA deletions in human cells? Nat Genet. 2008;40(3):275-9.
649 77. Berridge MV, Tan AS. Effects of mitochondrial gene deletion on tumorigenicity of
650 metastatic melanoma: reassessing the Warburg effect. Rejuvenation Res. 2010;13(2-3):139-
651 41.
652 78. Ahmad T, Mukherjee S, Pattnaik B, Kumar M, Singh S, Kumar M, et al. Miro1 regulates
653 intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy.
654 EMBO J. 2014;33(9):994-1010.
655 79. Burt R, Dey A, Aref S, Aguiar M, Akarca A, Bailey K, et al. Activated stromal cells transfer
656 mitochondria to rescue acute lymphoblastic leukemia cells from oxidative stress. Blood.
657 2019;134(17):1415-29.
658 80. Caicedo A, Fritz V, Brondello JM, Ayala M, Dennemont I, Abdellaoui N, et al.
659 MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell
660 mitochondria on cancer cell metabolism and function. Sci Rep. 2015;5:9073.
661 81. Cho YM, Kim JH, Kim M, Park SJ, Koh SH, Ahn HS, et al. Mesenchymal stem cells
662 transfer mitochondria to the cells with virtually no mitochondrial function but not with
663 pathogenic mtDNA mutations. PLoS One. 2012;7(3):e32778.

82. Lin HY, Liou CW, Chen SD, Hsu TY, Chuang JH, Wang PW, et al. Mitochondrial transfer from Wharton's jelly-derived mesenchymal stem cells to mitochondria-defective cells recaptures impaired mitochondrial function. Mitochondrion. 2015;22:31-44.

83. Marlein CR, Piddock RE, Mistry JJ, Zaitseva L, Hellmich C, Horton RH, et al. CD38-Driven Mitochondrial Trafficking Promotes Bioenergetic Plasticity in Multiple Myeloma. Cancer Res. 2019;79(9):2285-97.

84. Marlein CR, Zaitseva L, Piddock RE, Robinson SD, Edwards DR, Shafat MS, et al. NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. Blood. 2017;130(14):1649-60.

85. Moschoi R, Imbert V, Nebout M, Chiche J, Mary D, Prebet T, et al. Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. Blood. 2016;128(2):253-64.

86. Pasquier J, Guerrouahen BS, Al Thawadi H, Ghiabi P, Maleki M, Abu-Kaoud N, et al. Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. J Transl Med. 2013;11:94.

87. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci U S A. 2006;103(5):1283-8.

88. Tan AS, Baty JW, Dong LF, Bezawork-Geleta A, Endaya B, Goodwin J, et al. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab. 2015;21(1):81-94.

89. Mistry JJ, Moore JA, Kumar P, Marlein CR, Hellmich C, Pillinger G, et al. Daratumumab inhibits acute myeloid leukaemia metabolic capacity by blocking mitochondrial transfer from mesenchymal stromal cells. Haematologica. 2020.

687 90. Dong LF, Kovarova J, Bajzikova M, Bezawork-Geleta A, Svec D, Endaya B, et al.
688 Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial
689 DNA-deficient cancer cells. Elife. 2017;6.
690 91. Friedman JR, Nunnari J. Mitochondrial form and function. Nature.
691 2014;505(7483):335-43.
692 92. Desler C, Marcker ML, Singh KK, Rasmussen LJ. The importance of mitochondrial DNA
693 in aging and cancer. J Aging Res. 2011;2011:407536.
694 93. Torralba D, Baixauli F, Sanchez-Madrid F. Mitochondria Know No Boundaries:
695 Mechanisms and Functions of Intercellular Mitochondrial Transfer. Front Cell Dev Biol.
696 2016;4:107.
697 94. Murray LMA, Krasnodembskaya AD. Concise Review: Intercellular Communication Via
698 Organelle Transfer in the Biology and Therapeutic Applications of Stem Cells. Stem Cells.
699 2019;37(1):14-25.
700 95. Sinclair KA, Yerkovich ST, Hopkins PM, Chambers DC. Characterization of intercellular
701 communication and mitochondrial donation by mesenchymal stromal cells derived from the
702 human lung. Stem Cell Res Ther. 2016;7(1):91.
703 96. Bukoreshtliev NV, Wang X, Hodneland E, Gurke S, Barroso JF, Gerdes HH. Selective
704 block of tunneling nanotube (TNT) formation inhibits intercellular organelle transfer between
705 PC12 cells. FEBS Lett. 2009;583(9):1481-8.
706 97. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for
707 intercellular organelle transport. Science. 2004;303(5660):1007-10.
708 98. Koyanagi M, Brandes RP, Haendeler J, Zeiher AM, Dimmeler S. Cell-to-cell connection
709 of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for
710 cell fate changes? Circ Res. 2005;96(10):1039-41.

711 99. Lai RC, Tan SS, Yeo RW, Choo AB, Reiner AT, Su Y, et al. MSC secretes at least 3 EV
712 types each with a unique permutation of membrane lipid, protein and RNA. J Extracell
713 Vesicles. 2016;5:29828.
714 100. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends.
715 J Cell Biol. 2013;200(4):373-83.
716 101. van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a
717 specialized function. J Biochem. 2006;140(1):13-21.
718 102. Tricarico C, Clancy J, D'Souza-Schorey C. Biology and biogenesis of shed microvesicles.
719 Small GTPases. 2017;8(4):220-32.
720 103. Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, et al. Therapeutic Effects of Human
721 Mesenchymal Stem Cell-derived Microvesicles in Severe Pneumonia in Mice. Am J Respir Crit
722 Care Med. 2015;192(3):324-36.
723 104. Guescini M, Genedani S, Stocchi V, Agnati LF. Astrocytes and Glioblastoma cells
724 release exosomes carrying mtDNA. J Neural Transm (Vienna). 2010;117(1):1-4.
725 105. Guescini M, Guidolin D, Vallorani L, Casadei L, Gioacchini AM, Tibollo P, et al. C2C12
726 myoblasts release micro-vesicles containing mtDNA and proteins involved in signal
727 transduction. Exp Cell Res. 2010;316(12):1977-84.
728 106. Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, et al. Mitochondrial transfer
729 from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung
730 injury. Nat Med. 2012;18(5):759-65.
731 107. Eugenin EA, Gaskill PJ, Berman JW. Tunneling nanotubes (TNT): A potential
732 mechanism for intercellular HIV trafficking. Commun Integr Biol. 2009;2(3):243-4.

108. Mistry JJ, Marlein CR, Moore JA, Hellmich C, Wojtowicz EE, Smith JGW, et al. ROS-mediated PI3K activation drives mitochondrial transfer from stromal cells to hematopoietic stem cells in response to infection. Proc Natl Acad Sci U S A. 2019;116(49):24610-9.

109. Wada KI, Hosokawa K, Ito Y, Maeda M. Quantitative control of mitochondria transfer between live single cells using a microfluidic device. Biol Open. 2017;6(12):1960-5.

110. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010;464(7285):104-7.

111. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.

112. (NICE) TNIfHaCE. Daratumumab with bortezomib and dexamethasone for previously treated multiple myeloma. 2019.

113. Excellence NIfHaC. Daratumumab monotherapy for treating relapsed and refractory multiple myeloma. 2018.

TABLES

TABLE 1: Mitochondrial transfer from the TME to cancer cells

FIGURE LEGENDS

Figure 1: Metabolism in melanoma. A) The smooth co-operation of OXPHOS and glycolysis in the two spatially distinct melanoma cell populations (melanoma cells in the centre that use glycolysis versus melanoma cells in periphery that use OXPHOS mainly for energy production) promotes melanoma initiation, growth and metastasis of melanoma through metabolic symbiosis, whereby the waste products from glycolysis are used to feed into the TCA cycle for OXPHOS in melanoma cells in the periphery. **B)** Mesenchymal stromal cells (MSCs) migrate from the bone marrow and liver towards the melanoma, where they are then manipulated by tumour cells to produce lactate and other macromolecules via glycolysis, for use by melanoma cells that mainly use OXPHOS in the peripheral part of the tumour (Reverse Warburg).

Figure 2: Mechanisms of mtDNA transfer A) tunnelling nanotubules (TNTs), **B)** microvesicles and **C)** gap junctions, as well as other plausible mechanisms that require further research, such as **D)** cell fusion and **E)** direct mtDNA secretion into extracellular media.