Warburg Effect Reshapes Tumor Immunogenicity

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Tumor cells rewire their metabolism to fulfill the demands of highly proliferative cells. This changes cellular metabolism to adapt to fuel and oxygen availability for energy production and to increase the synthesis capacity of building blocks for cell division and growth. In addition, the metabolic shift modulates the immunogenicity of the tumor cells. Recently, Mahmood and colleagues reported a connection between mitochondrial DNA mutations in cancer cells and their response to immunotherapy in a mouse model of melanoma.

Mitochondria, a key regulator of anabolism and catabolism, control not only immune function and response but also the immunogenicity of tumors and their susceptibility to immune attack (1, 2). The oxidative phosphorylation (OXPHOS) system is central in metabolism and is composed of roughly 90 structural subunits encoded by nuclear and mitochondrial DNA (mtDNA). mtDNA is a peculiar chromosome, and its life cycle diverges substantially from the rest of the eukaryotic chromosomes. Among the more relevant specificities are its location outside the nucleus, polyploid nature, and uniparental transmission. These characteristics prevent recombination, heterozygosity, Mendelian behavior, and other features that define the genes encoded in the rest of the chromosomes. Thus, in normal circumstances, the mtDNA of an organism is generated by the clonal expansion of the oocyte mtDNA, rising to a cell type–defined copy number range (from hundreds to thousands). Thus, all copies of the mtDNA of an organism tend to be identical in sequence (homoplasmy). Alternatively, heteroplasmy refers to the coexistence of heterogeneous sequences of mtDNA in a single cell. Heteroplasmy arises naturally from replication errors and expansion of mutated mtDNA species to a significant proportion. An additional singular characteristic of mtDNA is its relatively higher mutability with respect to the nucleus-located chromosomes (3). This leads to the level of heteroplasmy tending to increase over an individual's lifespan and has been associated with cancer and other age-related diseases.

The relevance of mtDNA to the tumorigenic process has been extensively studied in the past (4). Variability in the OXPHOS system can dictate the nature of metabolites, the overall mitochondrion-derived factors, and the profile of cytokines released by the tumor cells and microenvironment. This in turn exerts a direct influence on the recruitment and engagement of cytotoxic T cells. Very importantly, the mitochondrial location of T-cell stimulatory proteins significantly enhances priming of antigen-specific CD8 T cells *in vivo*. Thus, the chances of the prototype antigens NY-ESO-1 and OVA to be recruited for antigen presentation is higher when they are localized in mitochondria than in the cytoplasm of tumor cells (5). Several studies confirmed that mitochondrial localization of proteins favors its MHC presentation and therefore their immunogenicity. Moreover, not only nucleus-encoded, mitochondrion-located proteins but also mtDNA-encoded proteins are immunogenic (6). Although the mechanism whereby mitochondrial localization confers increased immunogenicity remains to be clarified, it has two critical consequences. The ability of immune cells to capture mitochondria and/or mitochondrial components from cancer cells together with the ability of tumor cells to accumulate mutant mtDNA can provide a source of variable neoantigens that can lead to exhaustion of the immune system. To complete the picture, the response of the immune cells is also modulated by their mtDNA.

Even though the accumulation of mtDNA mutations in tumors has been a constant observation for more than 25 years (4), the debate on their relevance for the tumorigenic process is still unsolved. This is the case for melanoma, in which approximately 16% to 19% of tumors exhibit truncating mutations in mtDNA-encoded complex I (CI) genes. To directly assess its relevance in the tumorigenic process, Mahmood and colleagues used the TALE cytosine base editor DdCBE to induce mtDNA truncating mutations in the *mt-Nd5 CI* gene in melanoma cells (1). Using this strategy, the authors obtained melanoma cell populations bearing 40%, 60%, and 80% mutation heteroplasmy and accordingly, heteroplasmy-dependent defects on the assembly of CI. These mutations disrupt cellular redox balance, inducing a Warburg effect characterized by elevated lactic fermentation increasing the levels of metabolites such as malate, lactate, and fumarate adducts, coupled with alterations in the malate–aspartate shuttle. This metabolic rewiring activates IFNα and IFNγ responses, IL6–JACK–STAT3 signaling, and TNF signals influencing tumor susceptibility to immune attack. By utilizing syngeneic allografts in immune-competent mice and single-cell RNA sequencing (scRNA-seq), the authors observed that the immune cell populations that infiltrate tumors with CI mtDNA mutations are unique, with a reduction in S100A9 neutrophils and changes in other myeloid subsets. Functionally, mice bearing melanomas with these mutations respond better to immune checkpoint blockade. Consequently, point mutations in melanoma CI mtDNA–encoded genes impact the response of the immune system to the tumor and its progression after immunotherapy.

Remarkably, Mangalhara and colleagues highlight the relevance of the balance between electron entrance [CI vs. complex II (CII)] and

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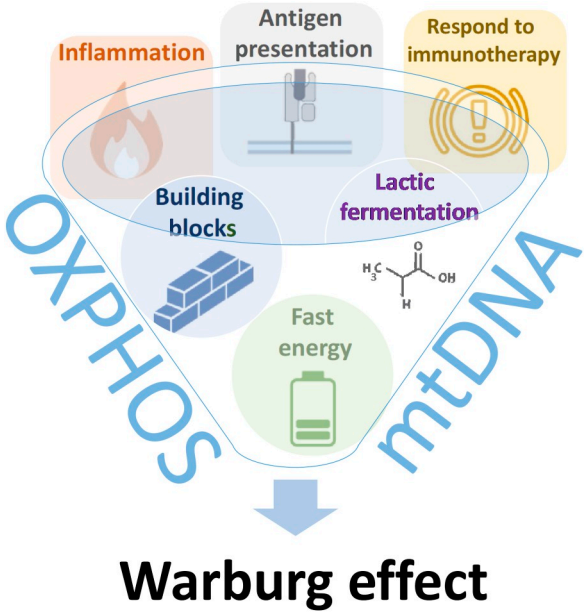

Figure 1.
Tumor cells rewire their metabolism to meet the demands of highly proliferative cells. This metabolic shift, known as the Warburg effect, is characterized by promoting lactic fermentation. It is necessary to adapt to fuel and oxygen availability for energy production and to increase the synthesis capacity of building blocks for cell division and growth. Additionally, the metabolic shift modulates the immunogenicity of tumor cells and their vulnerability to immune checkpoint blockade.

the OXPHOS system in shaping immunogenicity in melanoma (7). The authors used two strategies to modulate electron flux in melanoma mitochondria. In one approach, they eliminate CII in these tumors. In a second strategy, they promoted electron entry by CI at the expense of CII, using genetic depletion of the CI regulator methylation-controlled J protein. By both strategies, they found that favoring CI increases the expression of MHC molecules and genes related to antigen presentation, increasing tumor antigenicity and facilitating T-cell attack (7). In addition, they observed that succinate accumulation inhibits histone demethylases, increasing the trimethylation of histone 3 lysine 4 (H3K4) and H3K36 on genes involved in antigen processing and presentation, independent of IFN signaling (7).

Metabolites that accumulate during mitochondrial stress, such as succinate or fumarate, can also trigger inflammatory responses by different molecular mechanisms. Fumarate inhibits the histone demethylase activity of lysine-specific demethylase 5A, and inhibition of lysine-specific demethylase 5A increases the levels of H3K4me3, a marker of active gene transcription at the promoters of TNF and IL6 (8). Furthermore, elevated levels of succinate, resulting from mitochondrial dysfunction, may contribute to chronic inflammation by activating the HIF1α pathway through stabilization of the hypoxic transcription factor HIF1α. Succinate inhibits prolyl hydroxylases, which are enzymes responsible for the degradation of HIF1α. Stabilized HIF1α translocates into the nucleus and initiates the transcription of inflammatory cytokines, such as IL1β (9).

Additionally, mitochondria constitute a latent trigger of inflammation because they retain features that resemble characteristics of infectious agents. Under stress conditions, mitochondrion-derived molecules, including mtDNA, cardiolipin, formyl peptides, ATP, and mitochondrial metabolites, can act as damage-associated molecular patterns (10). They can be released from mitochondria and activate several inflammatory pathways (10). The mtDNA released in the cytosol can trigger a cascade of inflammatory innate responses, such as cGAS–STING, Toll-like receptor 9 pathway activation, and cytosolic inflammasome formation. Formyl peptides released from mitochondria can also trigger an inflammatory response as they are interpreted as bacteria-derived proteins causing the so-called sterile inflammation.

Although there is still much to understand about the impact of electron transport chain activity on tumor antigenicity, it is evident that metabolic rewiring of melanoma tumor cells impacts their immunogenicity and their response to immune checkpoint blockade (**Fig. 1**). Exploring whether this extends to other tumor types should be a new area of study in the fight against cancer.

Authors' Disclosures

No disclosures were reported.

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