



Hanging in the balance: mitochondrial uncoupling protein-2 and the tumor microenvironment

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Tumor microenvironment is an ecosystem in which cancer initiation, progression and dissemination take place through complex cell-cell interactions, more or less successfully countered by innate and adaptive immunity of the host (1,2). Outcomes of anti-cancer immune responses greatly depend on recognition of tumor differentiation antigens and destruction of cancer cells by cytotoxic CD8⁺ T cells recruited into the tumor tissue and activated by dendritic cells. To prevent indiscriminate activation of immunosurveillance, these mechanisms are further regulated by checkpoints involving a variety of molecules such as the programmed cell death protein 1 (PD-1) receptor and its ligand PD-L1 (3). Cancer cells often co-opt immune checkpoints to evade host surveillance, which explains why inhibition of this regulatory system is a promising chapter of cancer research (4). Lack of intratumoral infiltration by CD8⁺ T cells is a predictor of primary resistance to immune checkpoint blockade therapy in difficult-to-treat malignancies such as melanoma (5). However, the molecular mechanisms by which anti-cancer immune responses can be enhanced are incompletely understood.

In a recent work, Cheng *et al.* identified the mitochondrial uncoupling protein UCP2 as one of the molecular regulators of anti-cancer immune response (6). Uncoupling proteins belong to the SLC25 group of solute carrier family of transporters (7). UCP1, the prototype uncoupling protein, is restricted to brown adipose tissue where it is abundant and regulates non-shivering thermogenesis by increasing the permeability of mitochondrial inner membrane for protons and dissipating

heat from the metabolic energy of electron transport (8). By contrast, UCP2 is a scarce but ubiquitous protein with prominent presence in the immune system exerting biological functions that remain debated (9). There is evidence that UCP2 mediates proton leak when activated by increased levels of intracellular reactive oxygen species (ROS), acting therefore as a sensor and regulator of intracellular oxidative stress in a variety of cell types (10). UCP2 may also interfere with the efficiency of oxidative phosphorylation and modulate the rate of mitochondrial ATP synthesis (11). Moreover, UCP2 has been implicated in mitochondrial utilization of fatty acids and pyruvate (12) and in mitochondrial calcium uptake (13), transport activities that do not necessarily involve corresponding proton leak.

By analyzing tumor-associated gene expression scores in a Swiss cohort of patients who underwent resection of primary cutaneous melanoma, Cheng *et al.* found that tumor tissue UCP2 expression positively correlated with intratumoral inflammation, CD8⁺ T cell infiltration and responsiveness to anti-cancer immunotherapy, translating into prolonged survival rates (6). Tumor tissue UCP2 expression was not related to the number of mutations, which led the authors to conclude that augmented T cell responses in these patients were unlikely to result from increased neo-antigen burden (6). Since earlier studies of single-cell mRNA sequencing in melanoma indicated that tumor tissue UCP2 is most abundant in lymphocytes (14), UCP2 may plausibly serve as a marker of intratumoral T cell infiltration. Importantly, Cheng *et al.* also found

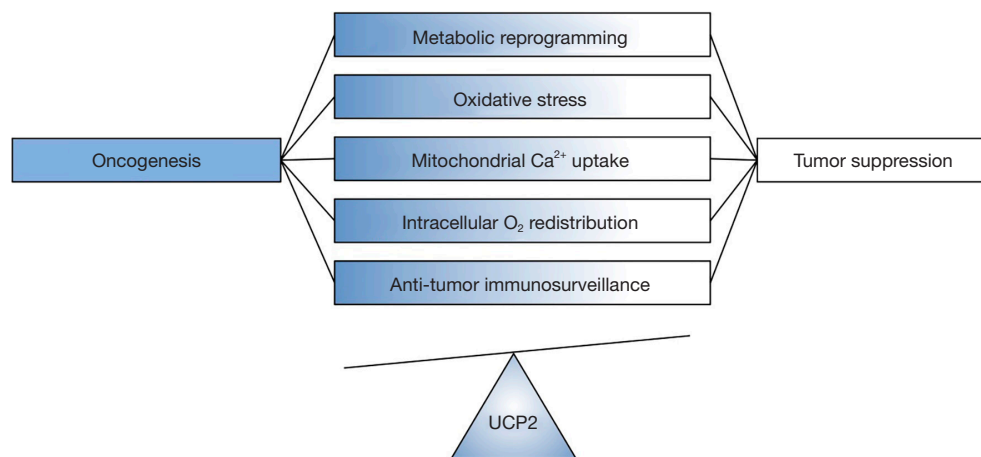


Figure 1 Impact of mitochondrial UCP2 on tumorigenesis. The mitochondrial inner membrane transporter uncoupling protein-2 (UCP2) has been implicated in both promoting and hindering the development and progression of cancer, with the precise molecular mechanisms underlying these opposing effects being debated. UCP2 is centrally positioned to fine-tune critical pathways of energy metabolism and mitochondrial signaling that regulate cellular homeostasis by balancing cell death with uncontrolled proliferation. Reprogramming the tumor microenvironment in a way that supports the efficiency of immune checkpoint blockade in melanoma is the latest biological function assigned to UCP2. Please see details in the main text.

that UCP2 abundance in melanoma correlated with the activation of genes controlling IFN- γ signaling, migration of dendritic cells and T cell recruitment as well as with higher PD-L1 expression, all consistent with increased efficiency of immune checkpoint blockade therapy (6).

Based on these correlative observations, Cheng *et al.* set out to study the impact of UCP2 on tumor progression in allografts generated by using a doxycycline-inducible expression system in B16-OVA and YUMM1.7 melanoma cell lines (6). They found that enforced UCP2 expression thwarted *in vivo* tumor growth, promoted intratumoral infiltration with CD8⁺ T cells and NK cells, and resulted in increased pro-inflammatory cytokine production and normalization of tumor microvasculature (6). Absent effect of UCP2 overexpression on tumor growth seen in *Rag*^{-/-} and *Batf3*^{-/-} mice respectively confirmed that UCP2-mediated benefits require the presence of dendritic cells and lymphocytes (6). Induction of UCP2 in mice by the commercially available antidiabetic drug PPAR- γ agonist rosiglitazone also sensitized tumor allografts to anti-PD-1 therapy, indicating that pharmacologic stimulation of UCP2 was able to recapitulate the effects of genetically induced UCP2 overexpression (6). Importantly, UCP2 overexpressing allografts showed no change in their intracellular and mitochondrial ROS content or hypoxia-inducible factor 1 α (HIF-1 α) expression (6), while these

molecular events were previously associated with UCP2 action (15,16). Moreover, Cheng *et al.* found that anti-tumor effects of UCP2 did not involve the β -catenin pathway or PGE2 production, mechanisms known to promote immune evasion of tumor cells (6).

The work of Cheng *et al.* adds an exciting and potentially game-changing facet to the biology of UCP2 (6). However, the findings seem to generate more questions than provide answers. Effects of UCP2 overexpression in the experimental models utilized were independent of several common oncogenic pathways and apparently did not involve previously established biological functions of mitochondrial uncoupling proteins. Thus, the molecular mechanisms by which UCP2 may enhance anti-tumor immune responses remain unclear. To be fair, controversies about the role of UCP2 in health and disease abound since its identification over 20 years ago. The primary substrate of UCP2-mediated transport (protons, fatty acids, pyruvate, calcium or their combination) remains unknown (9,17-19). Moreover, outcomes of UCP2 actions are sometimes difficult to categorize since it is not always clear how UCP2 would contribute to pathology (Figure 1).

The relationship between UCP2 and mitochondrial ROS is a case in point. ROS are known to have Janus-type biological effects, which range from promoting aberrant cell growth to promoting cell destruction (20,21). Accordingly,

cellular responses to ROS levels altered by too much or too little UCP2 may widely differ depending on the clinical or experimental setting. A similar argument can be made for UCP2-induced changes in mitochondrial membrane potential and rates of oxidative phosphorylation (22,23). It is also noteworthy that some of the earliest observations about the biology of UCP2 were made on immune cells which, once made UCP2-deficient, developed enhanced anti-infection and immune properties via increased intracellular ROS levels (24,25). How changes in the ROS content of tumor-infiltrating CD8⁺ T cells may affect their activity in relation to their UCP2 expression is not clear.

The findings of Cheng *et al.* are consistent with reports on cell lines derived from highly aggressive cancers such as melanoma, pancreas adenocarcinoma and glioblastoma whereby UCP2 overexpression resulted in repressed malignant phenotypes, reversed metabolic reprogramming and reduced HIF stabilization with no impact on ROS levels (16). However, current findings are more difficult to reconcile with some other studies on the role of UCP2 in cancer. Clinical and experimental studies described higher UCP2 expression in several types of cancer cells (26,27). Also, increased UCP2 mRNA levels have been associated with lower mitochondrial membrane potential, lower intracellular ROS levels, and chemoresistance in melanoma and leukemia cells (28). Moreover, UCP2 overexpression promoted chemoresistance in leukemia and colon cancer cells (29,30). By contrast, inhibition of UCP2 by genetic or pharmacologic approaches made many types of cancer cells (albeit not melanoma) less resistant to different anti-cancer drugs (31). Potential molecular mechanisms implicated in UCP2-mediated protection of cancer cells include reduced intracellular ROS production and reduced apoptosis rates, altered post-translational modification of p53 resulting in weaker tumor surveillance, augmented intracellular oxygen gradients between cytosol and mitochondria promoting HIF stabilization, and induction of the Warburg effect and metabolic reprogramming (29,30,32).

Differences in the biology of cancer cells according to stages of neoplastic development add another layer of complexity when we consider the balance between pro- and anti-tumorigenic effects of UCP2. Low UCP2 expression is associated with increased invasiveness in lung cancer cell lines *in vitro* and predicts poor response to chemotherapy in lung cancer patients (33). Interestingly, low UCP2-expressing lung cancer cells respond with increased ROS generation in response to the anti-cancer drug paclitaxel (33). Increased intracellular ROS levels in cancer

cells stimulate oncogenic pathways, increase the rate of p53 mutations, and promote autophagy (33,34). Thus, chronic oxidative stress due to relative lack of UCP2 action, rather than protection by UCP2 abundance, may represent a selection pressure to activate novel oncogenic mechanisms and account for chemoresistance.

Finally, it is important to note that studies using biological engineering to overexpress a membrane protein such as the SLC25 transporter UCP2 have inherent challenges as proper targeting is essential to draw proper conclusions on protein functions (35). Thus, making sure that overexpressed UCP2 is inserted into the inner mitochondrial membrane may be necessary before analyzing its biological activities, although mitochondrial fractionation studies to validate correct localization are rarely performed (36). For this reason, the fact that rosiglitazone-induced UCP2 was able to sensitize melanoma cells to anti-PD-1 therapy has particular importance in validating the findings based on enforced UCP2 expression in the study of Cheng *et al.* (6).

In summary, we have now a novel line of evidence for the biological activity of UCP2 as a regulator of anti-tumor immune response and an enabler of immune checkpoint inhibition therapy. What is the molecular mechanism by which UCP2 exerts these beneficial actions? Can we validate these findings by using genetic and pharmacological inhibitors (i.e., knockout models, genipin, or chromanes)? Are there distinct roles of UCP2 expressed in various types of cells within the tumor microenvironment (i.e., tumor cells *vs.* immune cells)? Can we achieve similar impact on immune checkpoint blockade in other types of difficult-to-treat cancer? What specific UCP2 activators (beyond rosiglitazone) can we consider to support the anti-tumor immune cycle? Future research will hopefully provide answers to these practical and theoretical questions before stimulation of UCP2 could find its way into clinical applications.

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