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REVIEW



# Mitochondrial calcium uniporter complex modulation in cancerogenesis

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**ABSTRACT**

Aberrations in mitochondrial $Ca^{2+}$ homeostasis have been associated with different pathological conditions, including neurological defects, cardiovascular diseases, and, in the last years, cancer. With the recent molecular identification of the mitochondrial calcium uniporter (MCU) complex, the channel that allows $Ca^{2+}$ accumulation into the mitochondrial matrix, alterations in the expression levels or functioning in one or more MCU complex members have been linked to different cancers and cancer-related phenotypes. In this review, we will analyze the role of the uniporter and mitochondrial $Ca^{2+}$ derangements in modulating cancer cell sensitivity to death, invasiveness, and migratory capacity, as well as cancer progression in vivo. We will also discuss some critical points and contradictory results to highlight the consequence of MCU complex modulation in tumor development.

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## Mitochondrial calcium and cancer

In the last century, Otto Warburg postulated that a tumorigenic event initiates when a non-cancerous cell adopts an anaerobic metabolism as a consequence of damaged respiration, thus depicting a metabolic profile of the neoplastic condition [1,2]. The so-called "Warburg effect" *de facto* revolutionized the field of oncology, to the extent that the high rate of glucose uptake by tumors provided the rationale behind the development of the 2-[$^{18}$F]fluoro-2-deoxy-D-glucose (18F-FDG) positron emission tomography (PET) imaging technique, which is nowadays used in clinics for the detection and monitoring of neoplastic formations. However, it is now clear that (i) elevated glycolytic rates of cancers do not originate from defects in oxidative respiration and (ii) mitochondria are essential for tumor cell viability and proliferation [3]. Indeed, mitochondria contribute to malignant transformation at multiple levels, which included generation of radical oxygen species (ROS), accumulation of specific mitochondrial metabolites, or alterations in programmed cell death response [4–6]. Most (if not all) of these processes are tightly regulated by calcium ($Ca^{2+}$) ions.

Historically, the first evidence that connected cancer-related molecular mechanisms to mitochondrial $Ca^{2+}$ aberrations dates back to the early 2000s, when the activities of the oncoprotein Bcl-2 and the pro-apoptotic factors Bax and Bak were associated with a drastic alteration of the endoplasmic reticulum (ER)-mitochondria $Ca^{2+}$ connection [7–9]. These observations demonstrated that (i) $Ca^{2+}$ transfer from the ER to mitochondria is required for initiation of the cell death program by specific pro-apoptotic agents, and (ii) proteins with oncosuppressive or pro-oncogenic properties could exert their pro- or anti-apoptotic functions by altering $Ca^{2+}$ homeostasis. The ER is the major store of $Ca^{2+}$ inside the cell [10], and mitochondria can accumulate large amounts of $Ca^{2+}$ through the formation of transient contacts with the ER membranes. Numerous apoptotic stimuli or chemotherapies promote a slow but continuous ER $Ca^{2+}$ discharge, followed by $Ca^{2+}$ entry inside mitochondria, which in turn decode $Ca^{2+}$ signals, such as death messages, favoring the release of cytochrome c and other apoptotic factors [10]. Thus, the general idea is that a reduced

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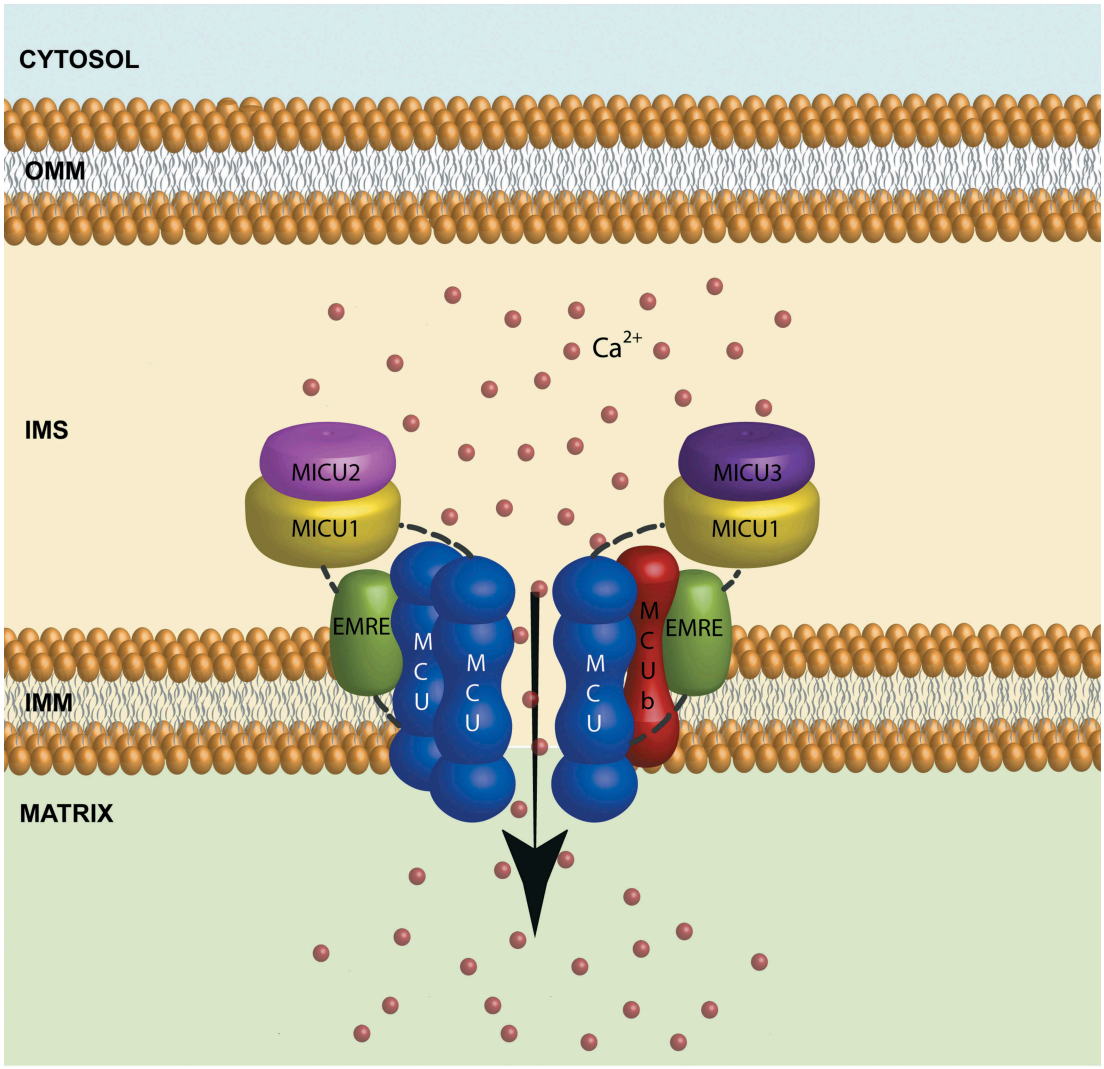
ER-mitochondria $Ca^{2+}$ transfer could help the tumorigenic cells evade apoptosis and resist cancer therapeutics [11,12]. The $Ca^{2+}$-dependent regulation of apoptosis is mainly related to the opening of the mitochondrial permeability transition pore (mPTP), a complex of proteins located in the inner mitochondrial membrane (IMM) [13,14]. $Ca^{2+}$ entering the mitochondrial matrix enhances mPTP opening due to the presence of $Ca^{2+}$-binding sites, triggering a series of events that lead to IMM permeabilization and culminating with the release of pro-apoptotic factors and execution of programmed cell death [15].

In recent years, several oncogenes, including H-RAS12V [16], fetal and adult testis expressed 1 (FATE1) [17], the signal transducer and activator of transcription 3 (STAT3) [18],and AKT [19–21], or tumor-suppressors, such as the promyelocytic leukemia protein PML [22,23], p53 [24], the redox-sensitive oxidoreductase TMX1 [25,26] and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [27], have been described as modulators of ER-mitochondria $Ca^{2+}$ interplay and cell death through their localization at the mitochondria-associated membranes (MAMs), a specialized area formed by the close apposition between the ER and mitochondrial membranes (for reviews on this topic, see [28–30]). These observations, although they validate the pivotal role played by the ER-mitochondria interface in cancer cells, lack direct evidence on the mechanistic relevance of mitochondrial $Ca^{2+}$ uptake in tumor progression. Some cancer-related proteins, such as Bcl-$X_L$ or the fragile histidine triad (FHIT), have been described as modulating mitochondrial $Ca^{2+}$ entry without affecting ER $Ca^{2+}$ release [31–33]. However, aside from their effect on mitochondrial $Ca^{2+}$, additional Bcl-$X_L$/FHIT targets also contributed to their pro-/anti-malignant activities. The identification of the molecular nature of the mitochondrial calcium uniporter (MCU) complex, the channel that allows $Ca^{2+}$ entry inside mitochondria [34], has led to better characterization of the role of mitochondrial $Ca^{2+}$ in a wide range of pathological conditions, including cancer [35]. Aberrations in the composition of the MCU complex, with consequent $Ca^{2+}$ derangements, affect not only the apoptotic response but also other features ascribed to a malignant phenotype, such as uncontrolled proliferation, migration, invasion, and capacity to metastasize.

## The mitochondrial calcium uniporter (MCU) complex

The $Ca^{2+}$ derived from the extracellular milieu or released by the intracellular stores (ER, Golgi or lysosomes) passes across the outer mitochondrial membrane (OMM) through the voltage-dependent anion channel (VDAC) and reaches the internal matrix by the MCU complex, located at the IMM [36]. The entire uniporter structure (480 kDa) is composed of nuclear-encoded channel-forming elements (MCU, EMRE, MCUb) and regulatory components (MICUs) [34,35] (Figure 1). The MCU complex is a highly $Ca^{2+}$-selective channel, displays a relative conductance to divalent cations ($Ca^{2+} \approx Sr^{2+} \gg Mn^{2+} \approx Ba^{2+}$) [37], and is inhibited by $Mg^{2+}$ [38]. Due to the lack of amino acidic sequence analogies with other $Ca^{2+}$ channels, MCU can be conceived as a new class of $Ca^{2+}$ transporters [39]. X-ray and electron microscopy structures showed that the pore-forming subunit MCU (previously known as CCDC109a) [40,41], similarly to other classical $Ca^{2+}$ channels, assembles as a tetramer [42–45]. The uniporter functions are pharmacologically inhibited by the polycationic dye Ruthenium Red (RuR) and its derivatives (such as the oxygen-bridged dinuclear amine complex Ru360), which abolish $Ca^{2+}$ uptake in isolated mitochondria through direct binding with the intermembrane space (IMS)-exposed region of MCU, known as DIME motif [41]. The proper architecture of the complex is ensured by the essential MCU regulator (EMRE), a single-pass membrane protein that is necessary for MCU activity [46], whereas MCUb, a paralog of MCU, acts as a dominant negative subunit and its expression largely varies among different tissues [47]. The fine regulation of the channel occurs in the IMS, where the components of the MICU family are located [48,49]. Through their EF-hand motifs, which are typical domains found in different calcium-binding proteins, MICU members sense the cytoplasmatic $Ca^{2+}$ and regulate the gating of the pore [50,51]. Loss-of-function




| | BASAL MITOCHONDRIAL [$Ca^{2+}$] | MITOCHONDRIAL [$Ca^{2+}$] UPON STIMULATION |
|---|---|---|
| *MCU* loss | ⇩⇩⇩ | ⇩⇩⇩ |
| *MCUb* loss | ⇧ | ⇧⇧ |
| *EMRE* loss | ⇩⇩⇩ | ⇩⇩⇩ |
| *MICU1* loss | ⇧⇧⇧ | ⇩⇩ |
| *MICU2* loss | ⇧⇧ | ⇧⇧ |
| *MICU3* loss | -- or ⇧ | ⇩⇩ |

**Figure 1.** The MCU complex.

Schematic representation of the Mitochondrial Calcium Uniporter (MCU) complex. $Ca^{2+}$ entry into the mitochondrial matrix is mediated by the pore-forming subunit MCU, the negative regulator MCUb, and the essential MCU regulator EMRE. All these components, located at the inner mitochondrial membrane (IMM), represent the core of the channel. In the intermembrane space (IMS) resides the MICU (Mitochondrial Calcium Uptake) family members (MICU1-2–3), which regulate the closure/opening of the channel depending on the cytosolic [$Ca^{2+}$]. The effects on both basal $Ca^{2+}$ levels and [$Ca^{2+}$] upon agonist stimulation caused by the genetic loss of the indicated subunit have been listed.

studies revealed that they control both the threshold and cooperative activation of the uniporter. Specifically, when the $Ca^{2+}$ concentration ($[Ca^{2+}]$) in the IMS is low, MICU1-2 help to preserve the closure of the channel, avoiding detrimental $Ca^{2+}$ entry and maintaining an extremely low $[Ca^{2+}]$ inside the matrix [52–54]. However, when the $Ca^{2+}$ increases, MICU1-2 control the channel's opening, with MICU1 acting as a positive regulator [55,56] and MICU2 limiting the activation of the channel and downregulating the mitochondrial $Ca^{2+}$ uptake [52,56]. MICU1 also contributes to the $Ca^{2+}$ selectivity of the uniporter, since it helps to discriminate between $Ca^{2+}$ and $Mn^{2+}$ [57,58]. MICU3 is mainly expressed in the brain [49,59] and does not display gatekeeping functions but works as a genuine activator of the uniporter, facilitating $Ca^{2+}$ entry more efficiently compared to MICU1 [59]. Structurally, MICU1 can bind MICU2 or MICU3 through the formation of disulfide bonds [56,59,60], and the resulting MICU dimers are anchored to the channel core by the interaction of MICU1 with EMRE [61] and the IMS-spanning portion of MCU [62] (Figure 1). This sophisticated regulation ensures low mitochondrial $Ca^{2+}$ levels at resting conditions and prompt $Ca^{2+}$ responses upon agonist stimulation. Importantly, large and transient mitochondrial $Ca^{2+}$ increases, generated by ER $Ca^{2+}$-mobilizing agents (histamine, carbachol, etc.) are associated with activation of the metabolic machinery, resulting in a fast synthesis of ATP [63]. Low concentration of calcium ions activates four Ca2+-sensitive mitochondrial enzymes, including the FAD-glycerol phosphate dehydrogenase (FAD-GPDH), located on the cytosolic surface of the IMM, and the mitochondrial matrix-resident pyruvate dehydrogenase phosphatase (PDHP), NAD-isocitrate dehydrogenase (NAD-ICDH) and oxoglutarate dehydrogenase (OGDH), which in turn furnish an increased supply of reducing equivalents and boost ATP synthesis [64]. Therefore, cancer cells can strategically take advantage of increased mitochondrial $Ca^{2+}$ to supply the energy demand required for faster proliferation. Alternatively, a lower $Ca^{2+}$ uptake may help them to overcome cell death and resist a multitude of apoptotic stimuli. In the sections below, we will discuss this dual, and apparently antithetic, role of mitochondrial $Ca^{2+}$ in the context of cancer as well as how the different members of the MCU complex can affect such cancer-related features and tumor growth.

## MCU complex and cell death

The first observations that directly linked the activity of the MCU complex to increased sensitivity to cell death were obtained in HeLa cells overexpressing the pore-forming subunit MCU. The cells displayed higher levels of apoptosis once treated with $Ca^{2+}$-dependent apoptotic stimuli, such as C2-ceramide or hydrogen peroxide [40]. The concept of mitochondrial $Ca^{2+}$ overload as a key factor of cell death has been confirmed in noncancerous cell lines [65–67]. Accordingly, genetic MCU downregulation [65,66,68,69] or MCU-targeted pharmacological interventions [70,71] protect from apoptosis by reducing mPTP opening and release of pro-apoptotic factors. These findings have been extended to a specific form of cell death, called paraptosis, induced by Celastrol, an active compound extracted from the Chinese medical plant "Thunder of God Vine" [72]. Of note, our group first demonstrated that MCU is a target of miRNA-25, which decreased the sensitivity of both prostate and colon cancer cells to multiple $Ca^{2+}$-dependent apoptotic stimuli by reducing MCU protein levels and mitochondrial $Ca^{2+}$ uptake [73]. These data have been confirmed in the process of pulmonary arterial hypertension (PAH) cancer-like phenotypes, characterized by excessive proliferation and apoptosis resistance [74]. In this context, high MCU levels decrease cell migration, proliferation, and apoptosis resistance, whereas MCU silencing has the opposite effects. However, other observations suggested a more marginal role for MCU in the control of cell survival [75], or an augmented, rather than inhibited, caspase-independent cell death in the MCU-silenced MDA-MB-231 breast cancer cell line [76]. Nonetheless, by comparing mouse embryonic fibroblasts (MEFs) derived from MCU-null and WT mice, no difference in the sensitivity to multiple $Ca^{2+}$-related pro-apoptotic and necrotic stimuli was detected, although MCU knock-out (KO) mitochondria exhibited a permanent mPTP closure upon exposure to high levels of calcium [77].

Additional information on the role of mitochondrial $Ca^{2+}$ in cell viability originates from studies on the regulatory components of the MCU complex. Loss of MICU1 increases basal mitochondrial $Ca^{2+}$ content and predisposes cancer and noncancer cells to apoptosis, which is strictly related to a high $Ca^{2+}$-dependent ROS generation inside the matrix [50,62]. The molecular mechanisms linking mitochondrial $Ca^{2+}$ entry and ROS production have not been fully understood, although the general idea is that the high mitochondrial $Ca^{2+}$ stimulating respiratory chain activity could lead to increased amounts of ROS [78]. MICU1-loss promotes hepatocytic death, which is prevented by pharmacological inhibition of mPTP opening [79], and significantly aggravated cardiomyocyte death during ischemia/reperfusion injury [80]. Interestingly, reducing the protein expression of both the enhancer of zeste homolog 2 (EZH2) [81] or Homeobox (HOX) transcript antisense RNA (HOTAIR) [82] in head and neck squamous cell carcinoma cells, as well as ribosomal protein S3 (RPS3) in melanoma [83], contributes to apoptosis, at least in part, by decreasing MICU1 levels. Moreover, histidine triad nucleotide-binding (HINT2) increases the susceptibility of pancreatic cancer cells to death by decreasing MICU1 and upregulating the MCU associate subunit EMRE [84] (Figure 2). Thus, higher mitochondrial $[Ca^{2+}]$ at resting conditions, induced by either MICU1-silencing or MCU-overexpression (which increase the quota of unregulated/hyperactive MCU complexes) sensitizes cancer cells to apoptosis [85]. However, for MICU1 functions, some contradictory findings have been described.

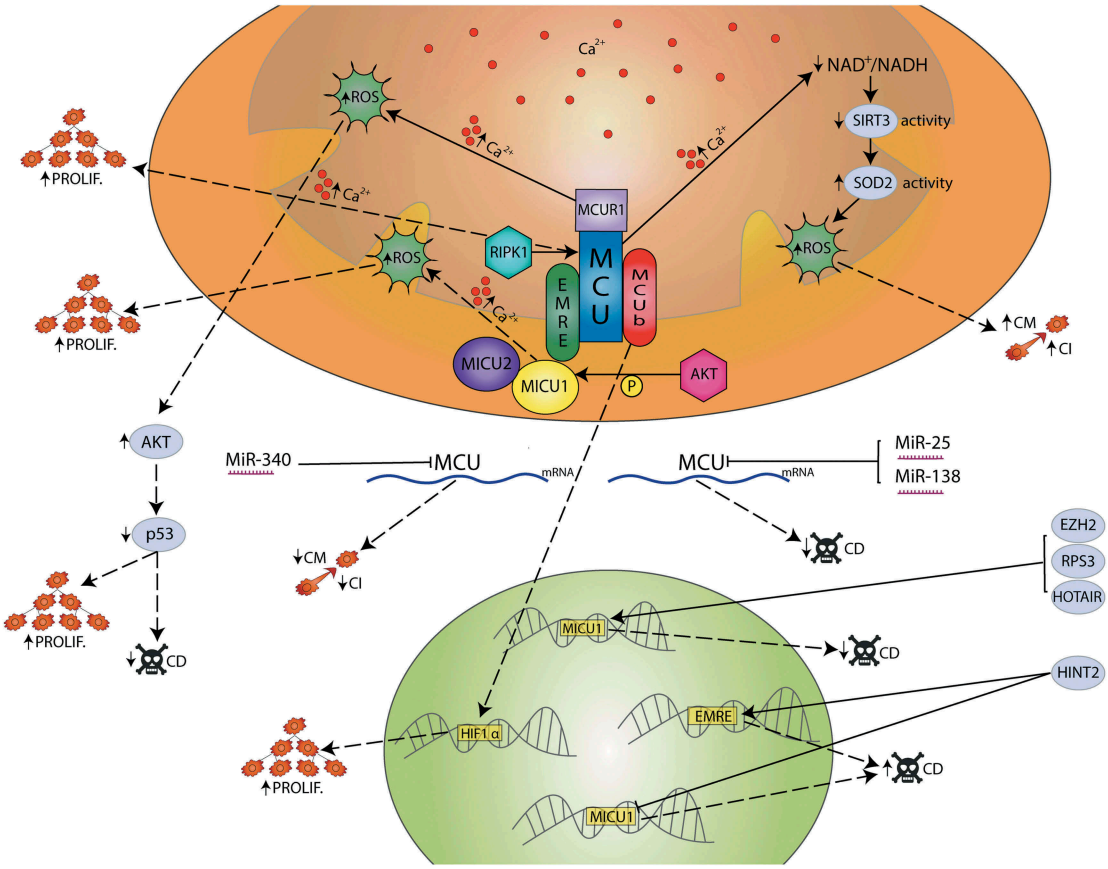



**Figure 2.** Oncogenic regulation of the MCU complex.

Representation of the different molecular pathways that converge on the MCU complex to exert their pro- or anti-neoplastic functions. Some factors accumulate into mitochondria and directly target the MCU complex, whereas other oncogenic proteins reasonably affect cancer-related features by regulating the expression of uniporter's subunits at the transcriptional level. MCU-targeting cancer-related miRNAs have been also reported. See text for further details. ROS: Radical Oxygen Species; Prolif.: Proliferation; CI: Cell Invasion; CM: Cell Migration; CD: Cell Death

In MDA-MB-231 cells, MICU1 silencing does not affect apoptosis [75], whereas in ovarian cancer cells MICU1 downregulation indeed potentiates gold nanoparticle-induced cytotoxicity, but this effect is related to higher cytosolic [$Ca^{2+}$] and ER stress, not to an augmented mitochondrial $Ca^{2+}$ entry [86]. Similar results have been obtained using the potent MCU inhibitor Ru360, thus confounding the interpretation of the data [86].

Other observations that strongly questioned the link between high mitochondrial $Ca^{2+}$ entry and cell death induction can be found in the role of mitochondrial calcium uniporter regulator 1 (MCUR1) in hepatocellular carcinoma (HCC). MCUR1 has been proposed as a key interactor of the MCU channel and to promote massive mitochondrial $Ca^{2+}$ entry [87], by ensuring the correct assembly of the entire uniporter complex [88]. In HCC cells, MCUR1 silencing induces high levels of apoptosis, whereas its overexpression has the opposite effects. The molecular mechanism includes a $Ca^{2+}$- and ROS-dependent degradation of p53 and modulation of other intrinsic apoptotic pathways [89] (Figure 2). However, a role of MCUR1 outside the uniporter has also been proposed, suggesting that the mitochondrial $Ca^{2+}$ uptake aberrations ascribed to modulation of MCU function is secondary to the mitochondria membrane depolarization caused by the respiratory chain defects [90]. Thus, multiple factors should be evaluated before drawing conclusions on the putative role of mitochondrial $Ca^{2+}$ in modulating the cell death response. In particular, it should be taken into consideration that (i) not all death stimuli exert their toxic effects by inducing mitochondrial $Ca^{2+}$ overload, (ii) if MCU loss reduces mitochondrial $Ca^{2+}$ both at the basal state and after agonist stimulations, MICU1 downregulation increases the organelle $Ca^{2+}$ uptake at low cytosolic [$Ca^{2+}$], but decreases it when cytosolic $Ca^{2+}$ rises, (iii) loss of a specific subunit could affect the expression of other MCU complex members, and iv) the composition of the MCU complex may greatly vary among different cancer cell lines.

## MCU complex and cancer cell migration

If mitochondrial $Ca^{2+}$ overload has been generally associated with cell death induction, numerous observations reported that inhibition of mitochondrial $Ca^{2+}$ entry through downregulation of the MCU channel subunit could delay cancer cell migration. Indeed, in vitro studies showed that MCU silencing in HeLa and Hs578T breast cancer cells drastically inhibits cell motility, migration, and invasion without affecting basal apoptosis levels or proliferation rates [91]. Similar data have been obtained in triple-negative breast cancer and HCC cells [92–95]. Other evidence arises from the inhibition of MCU functions through chemical compounds [93,94], although these data should be taken with caution, as RuR is mainly impermeable and cannot be used in intact cells.

Different molecular mechanisms have been proposed to justify the positive role of mitochondrial $Ca^{2+}$ accumulation in cell migration, including increased ATP production and regulation of cytosolic $Ca^{2+}$ levels through store-operated $Ca^{2+}$ entry (SOCE). However, MCU loss does not seem to affect global ATP levels [91], and the contribution of MCU in the control of SOCE and SOCE-dependent cell motility is highly debated (for a review on this topic, see [96]). Conversely, a greater consensus has been reached for the mechanisms that link cancer cell migration to $Ca^{2+}$-dependent mitochondrial ROS generation. Indeed, higher, but subtoxic, levels of ROS (particularly hydrogen peroxide) have been associated with increased migration, invasion, and metastatic potential [97]. Several indirect observations demonstrate the positive link between elevated mitochondrial $Ca^{2+}$ uptake, ROS and cell migration, including the mechanism of action of the induced myeloid leukemia cell differentiation protein Mcl-1, which promotes mitochondrial $Ca^{2+}$ entry [98] and favors cell migration in a ROS-dependent manner [99]. Accordingly, loss of MCU minimizes ROS generation, which in turn leads to a reduction of the amount of the hypoxia-induced factor 1a (HIF1a) transcription factor and an attenuated hypoxic response [92]. Similar results have been obtained in HCC, where MCU depletion increased superoxide dismutase 2 (SOD2) activity by upregulating the NAD+/NADH ratio and deacetylase activity of SIRT3 [95] (Figure 2). Importantly, treatment with mitoTEMPO, a mitochondrial ROS scavenger, produces the same effects as MCU silencing [92,95].

When ROS levels drastically increase and the antioxidant systems are unable to maintain them below a cytotoxic threshold, multiple cell death pathways can be triggered in response to oxidative damage. This

series of events may explain the impaired cell migration observed in MICU1 knock-down HeLa cells, which displayed high levels of apoptosis at resting conditions [50]. Moreover, MICU1 silencing in ovarian cancer cells predisposes the cells to cisplatin-driven cell death and impairs cell migration and invasion [100]. Thus, when increased mitochondrial [$Ca^{2+}$] does not result in cell death induction, for example, when a concomitant pro-survival pathway is activated, a key advantage for the proliferation of cancer cells may arise. To test this hypothesis, we analyzed the effect of high basal mitochondrial $Ca^{2+}$ levels, either by overexpressing MCU in prostate cancer cells or silencing MICU1 in HeLa, on cancer phenotypes, and we observed that upon pharmacological inhibition of mPTP functions, increased mitochondrial $Ca^{2+}$ entry strongly activates cancer cell proliferation, migration, invasion and colony formation [85]. Overall, these data suggest that increased mitochondrial [$Ca^{2+}$] could drastically boost the aggressiveness of cancer cells upon inhibition of key effectors of cell death. Moreover, a careful analysis of other signaling pathways is necessary before establishing whether mitochondrial $Ca^{2+}$ accumulation could be a toxic or tumor-promoting agent. Importantly, several proto-oncogenes or tumor suppressors, including AKT and p53, could affect mPTP opening by direct interaction [101,102] and simultaneously alter $Ca^{2+}$ homeostasis [24,103], thus regulating cancer development at two different stages.

## MCU complex and tumor progression

Recently, our group showed that a pool of activated AKT could localize at the IMS, where AKT phosphorylates MICU1 on a specific serine residue at its N-terminal region. The AKT-mediated MICU1 phosphorylation abolishes MICU1's gatekeeping function on MCU, leading to higher mitochondrial $Ca^{2+}$ content at resting conditions and ROS production [103] (Figure 2). We observed that phosphor-MICU1 accumulates in a non-mature form that is rapidly degraded (potentially by the ubiquitin-proteasome system, as recently proposed [104]), leading to concomitant loss of the binding partner MICU2 and a disordered MCU complex composition [103]. The expression of a nonphosphorylatable MICU1 mutant restores normal mitochondrial $Ca^{2+}$ and ROS levels and inhibits AKT-mediated tumor growth in vivo, suggesting that the MICU1-dependent regulation of mitochondrial $Ca^{2+}$ and ROS homeostasis is a crucial element in AKT-driven tumorigenesis. These data correlate with other examples demonstrating the pro-malignant role of MCU-mediated $Ca^{2+}$ entry. The receptor-interacting protein kinase 1 (RIPK1) stabilizes MCU by direct interaction and promotes colon cancer growth by upregulating mitochondrial Ca2+ uptake [105] (Figure 2). In MDA-MB-231 xenograft models, MCU depletion inhibits primary tumor growth, lymph node infiltration, and lung metastasis [92], whereas in an experimental metastatic model (obtained by injecting cancer cells into the tail vein of nude mice), MCU-overexpressing MCF7 breast cancer cells produce more lung metastatic lesions compared to control conditions [94]. Similar results have been obtained in HCC xenograft models, where MCU depletion reduced the incidence of both intrahepatic and distal lung metastases. Interestingly, expression of the $Ca^{2+}$ buffer parvalbumin targeted to mitochondria counteracts the MCU pro-tumorigenic role [95]. In the same cancer context (HCC), MCUR1 promotes $Ca^{2+}$ entry and in vivo tumor growth by inhibiting p53-mediated apoptosis [89], as previously described (see "MCU complex and cell death" section).

Analysis of mRNA expression levels of the MCU complex subunits in human patients, revealed as alterations in the MCU complex composition that led to increased mitochondrial $Ca^{2+}$ entry, correlates with a poorer prognosis or high risk of recurrence and death (Table 1). Thus, mitochondrial $Ca^{2+}$ accumulation appears to promote cancer growth and progression, a hypothesis that has been expressed since the 1970s, based on preliminary in vivo results [106]. However, in other cancer scenarios, an opposite role for $Ca^{2+}$ in tumor development emerges. In ovarian cancer, high levels of MICU1 contribute to chemoresistance by inhibiting the mitochondrial $Ca^{2+}$ response to cisplatin [100]. MICU1 inhibition reduces tumor growth by re-establishing chemosensitivity and apoptosis induction [100]. In gliomas, the expression levels of MCUb, the negative regulator of MCU, were inversely correlated with overall survival, and MCUb knock-down suppresses the proliferation, migration, and invasion of glioma cells, as well as glioma progression in vivo [107]. Such MCUb-mediated effects have not been

**Table 1.** Role of the MCU complex in cancer-related phenotypes.

| Cancer-related process | Effect on mitochondrial $Ca^{2+}$ | Genetic perturbation | Cell type/organism | Description | Ref. |
|---|---|---|---|---|---|
| Cell death | $Ca^{2+}$ up-regulation | MCU overexpression | HeLa cells | Increased apoptosis upon $H_2O_2$ and C2-ceramide | [40, 65] |
| | | | HPMVECs (human pulmonary microvascular endothelial cells) | Increased cell death upon $H_2O_2$. Expression of a MCU C97A mutant, which constitutively triggered MCU-mediated [$Ca^{2+}$] uptake, increased sensitivity to cell death. | [67] |
| | | | Pathological pulmonary artery smooth muscle cell (PASMC) | Increased apoptotic levels at basal state | [74] |
| | | | PC3 prostate cancer cells | Increased apoptosis upon C2-ceramide | [85] |
| | | MICU1 silencing | MDA-MB-231 breast cancer cells | No effect on apoptosis induced by C2-ceramide | [63,75] |
| | | | HMEC (Primary human mammary endothelial cells),<br>HeLa cells | Reduced apoptosis induced by C2-ceramide | [63,75] |
| | | MCUR1 overexpression | MHCC97H and MHCC97L HCC cells | Reduced apoptosis induced by C2-ceramide or thapsigargin acting on a ROS-p53 axis | [76] |
| | Basal $Ca^{2+}$ up-regulation | MICU1 KD | Endothelial cells,<br>HeLa cells | Increased apoptosis upon C2-ceramide | [50, 85] |
| | | | Murine hepatocytes | Permeabilized MICU1 KD hepatocytes were sensitized to PTP opening | [79] |
| | | MICU1 KO | Hek293T cells | Increased apoptosis upon C2-ceramide | [62] |
| | | MICU1 silencing | Cardiomyocytes | Increased death during ischemia/reperfusion injury | [80] |
| | $Ca^{2+}$ up-regulation in response to cisplatin | MICU1 silencing | CP20 and OV90 ovarian cancer cells | Increased apoptosis induced by cisplatin | [100] |
| | $Ca^{2+}$ down-regulation | MCU KD | Murine Hippocampal neurons,<br>Murine Cortical neurons | Reduced mitochondrial $Ca^{2+}$ overload and resistance to excitotoxicity | [66] |
| | | Expression of a dominant-negative MCU construct | HAEC (primary human airway epithelial cells) | Reduced apoptosis upon exposure with the pleiotropic Th2 cytokine IL-13 | [68] |
| | | MCU KO | MTBEC (primary murine tracheal epithelial cells) | Reduced apoptosis upon addiction of ovalbumin (OVA) | [68] |
| | | | MEFs (Mouse embryonic fibroblasts) | No effect on apoptotic response | [77] |
| | | MCU silencing | Rat ventricular myocardial H9c2 cell line | Reduced cell death in hypoxia/reoxygenation injury | [69] |
| | | | Murine Podocytes | Reduced palmitic acid-induced apoptosis | [71] |
| | | | MDA-MB435S breast cancer cells | Reduced celastrol-induced paraptosis | [72] |
| | | | MDA-MB-231 breast cancer cells,<br>HeLa cells | No effect on apoptosis induced by C2-ceramide | [75] |
| | | | HMEC (Primary human mammary endothelial cells) | Reduced apoptosis induced by C2-ceramide | [75] |
| | | | MDA-MB-231 breast cancer cells | No effect on apoptosis induced by ABT-263.<br>Reduced apoptosis induced by ionomycin | [76] |

(*Continued*)

**Table 1.** (Continued).

| Cancer-related process | Effect on mitochondrial $Ca^{2+}$ | Genetic perturbation | Cell type/organism | Description | Ref. |
|---|---|---|---|---|---|
| | | | Primary CGNs, HeLa cells | Increased apoptosis upon $H_2O_2$ | [65] |
| | | MCUR1 silencing | BEL7402 and SMMC7721 hepatocellular carcinoma (HCC) cells | Increased apoptosis induced by C2-ceramide or thapsigargin acting on a ROS-p53 axis | [89] |
| | – | MICU1 overexpression | head and neck squamous cell carcinoma (HNSCC) cell line Hep-2 | Decreased apoptotic levels at basal state | [81] |
| | | MICU1 silencing | SCC25 and Cal27 HNSCC cells | Increased apoptotic levels at basal state | [81] |
| | | | A2780 ovarian cancer cells | Potentiates gold nanoparticle-induced cytotoxicity | [86] |
| Cell migration/ cell invasion | $Ca^{2+}$ up-regulation | MCU overexpression | MCF7 breast cancer cells | Increased cell migration and invasion | [94] |
| | | | MHCC97H HCC cells | Increased migration and invasion by augmenting ROS levels | [95] |
| | | | Pathological pulmonary artery smooth muscle cell (PASMC) | Decreased cell migration by lowering cytosolic $[Ca^{2+}]$ | [74] |
| | Basal $Ca^{2+}$ up-regulation | MCU overexpression | PC3 prostate cancer cells | Increased cell migration and invasion only upon mPTP inhibition | [85] |
| | | MICU1 KD | Endothelial cells<br>HeLa cells | Reduced cell migration by affecting apoptosis at basal state | [50] |
| | | | HeLa cells | Increased cell migration and invasion only upon mPTP inhibition | [85] |
| | $Ca^{2+}$ up-regulation in response to cisplatin | MICU1 silencing | CP20 and OV90 ovarian cancer cells | Reduced cell invasion and migration | [100] |
| | $Ca^{2+}$ down-regulation | MCU silencing | HeLa cells<br>Hs578T breast cancer cells | Reduced cell invasion, migration and motility. | [78] |
| | | | Triple negative breast cancer cell lines | Reduced cell migration by decreasing ROS production | [92] |
| | | | MDA-MB-231 breast cancer cells | Reduced cell migration | [93, 94] |
| | | | SMMC7721 HCC cells | Reduced migration and invasion by lowering ROS levels | [95] |
| Human cancer samples | $Ca^{2+}$ up-regulation | Higher MCU expression + lower MCUb expression | Breast cancer | Positive correlation with tumour progression | [92] |
| | | Higher MCU expression + lower MICU1 expression | Breast cancer | Increased cancer aggressiveness | [75] |
| | | Higher MCU expression | HCC | Poorer overall survival | [95] |
| | | Lower MICU1 expression | | | [95] |
| | | Higher MCUR1 expression | | | [89] |
| | $Ca^{2+}$ down-regulation (?) | Higher MICU1 expression | Ovarian cancer | Poorer overall survival | [100] |
| | | Higher MCUb expression | Glioma | | [107] |
| *In vivo* tumour growth | $Ca^{2+}$ up-regulation | MCU overexpression | MHCC97H HCC cell xenograft model | Increased intra-hepatic and lung metastases | [82] |

(*Continued*)

**Table 1.** (Continued).

| Cancer-related process | Effect on mitochondrial $Ca^{2+}$ | Genetic perturbation | Cell type/organism | Description | Ref. |
|---|---|---|---|---|---|
| | | | MCF7 breast cancer metastatic model | Increased metastases and lymph node infiltration | [94] |
| | | MCUR1 overexpression | BEL7402 HCC cancer cell xenograft model | Increased tumour growth | [89] |
| | $Ca^{2+}$ up-regulation in response to cisplatin | MICU1 KD | Ovarian cancer cell xenograft model | Reduced tumour growth and increased sensitivity to cisplatin | [100] |
| | $Ca^{2+}$ down-regulation | MCU KO | MDA-MB-231 breast cancer cell xenograft model | Reduced tumour growth and lung metastasis | [92] |
| | | MCU KD | MDA-MB-231 breast cancer metastatic model | Reduced metastases and lymph node infiltration | [94] |
| | | | SMMC7721 HCC cell xenograft model | Reduced intra-hepatic and lung metastases | [95] |
| | | Expression of a MICU1 mutant that cannot be phosphorylated by Akt | Akt-positive transformed cell xenograft model | Reduced tumour growth | [103] |
| | | MCUR1 KD | MHCC97H HCC cancer cell xenograft model | Reduced tumour growth | [89] |
| | $Ca^{2+}$ down-regulation (?) | MCUb KD | U87MG glioma xenograft model | Reduced glioma progression | [107] |

associated with the remodeling of mitochondrial $Ca^{2+}$ homeostasis or alteration in the apoptotic response, thus the putative pro-tumorigenic MCUb activity awaits further clarifications.

## Concluding remarks

From the evidence described here, it is difficult to definitively affirm that mitochondrial $Ca^{2+}$ entry can (i) inhibit cancer growth by increasing sensitivity to apoptosis and (ii) boost tumor progression by promoting ATP and ROS production or modulating cytosolic $Ca^{2+}$. The functional role of $Ca^{2+}$ may vary depending on the type and stage of cancer or whether the $Ca^{2+}$ signaling profile is assessed in primary tumors or metastatic cells. Moreover, mitochondrial $Ca^{2+}$ could function differently based on the tumorigenic pathway or genetic alterations that are mainly involved in conferring the aggressiveness of a specific type of cancer. Once the pro- or anti-malignant role of mitochondrial $Ca^{2+}$ is established in a well-defined cancer context, a $Ca^{2+}$-based pharmacological strategy could be exploited alone or in combination with chemotherapy. Today, most solid therapeutic approaches do not directly target the MCU complex but consist of compounds that modulate mitochondrial $Ca^{2+}$ transfer by acting on ER $Ca^{2+}$ release. These strategies include photodynamic therapy [108] and G-202 (a thapsigargin-based prodrug specific for prostate cancer), which promote apoptosis by favoring ER $Ca^{2+}$ depletion and mitochondrial $Ca^{2+}$ overload [109], or Xestospongin B, which selectively kills cancer cells by blocking ER $Ca^{2+}$ release and inducing a bioenergetic crisis [110]. Recently, new cell-permeable pharmacological agents targeting uniporter activity have been proposed, including the synthetic anthracenediones mitoxantrone and pixantrone, two analogs of doxorubicin, originally developed to minimize its cardiotoxic effects [111], the small-molecule DS16570511 [112], and the new ruthenium complex Ru265 [113]. Although their biological activities could not be exclusively dependent on MCU inhibition (the antineoplastic effects of mitoxantrone have been attributed to topoisomerase II impairment [114], and DS16570511 could affect mitochondrial membrane potential [115]), the employment of

these novel compounds may provide additional elements to understand whether mitochondrial $Ca^{2+}$ and the MCU complex may represent reliable targets in cancer therapy.

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No potential conflict of interest was reported by the authors.

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