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INVITED REVIEW

The role of the mitochondrial calcium uniporter (MCU) complex in cancer

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Abstract

The important role of mitochondria in cancer biology is gaining momentum. With their regulation of cell survival, metabolism, basic cell building blocks, and immunity, among other functions, mitochondria affect not only cancer progression but also the response and resistance to current treatments. Calcium ions are constantly shuttled in and out of mitochondria; thus, playing an important role in the regulation of various cellular processes. The mitochondrial calcium uniporter (MCU) channel and its associated regulators transport calcium across the inner mitochondrial membrane to the mitochondrial matrix. Due to this central role and the capacity to affect cell behavior and fate, the MCU complex is being investigated in different cancers and cancer-related conditions. Here, we review current knowledge on the role of the MCU complex in multiple cancer types and models; we also provide a perspective for future research and clinical considerations.

Keywords Mitochondria · Cancer · MCU · Calcium · ROS · Metabolism · Redox · MICU

Mitochondrial Ca^{2+} uptake: functional relevance and mechanism of activation

Studies reporting that mitochondria can take up large quantities of Ca^{2+} were published more than 50 years ago [28]. Since then, mitochondrial Ca^{2+} signals have been shown to be responsible for many essential cellular functions such as proliferation, cell death, the production of cell building blocks, and energy production, e.g., ATP synthesis [4, 71]. However, most of these findings were generated in the absence of information regarding the molecular identity of the proteins responsible for Ca^{2+} transport across the inner mitochondrial membrane (IMM), a fact that limited the efforts to fully understand the impact of mitochondrial Ca^{2+} signaling in physiology and pathology. The long quest for the elusive mitochondrial Ca^{2+} importer was over when first a regulator and then the channel itself were identified at the beginning of this decade [5, 26, 77]. These groundbreaking studies inaugurated a new era in the field of mitochondrial Ca^{2+} research.

In this review article, we will briefly summarize the current knowledge on mitochondrial Ca^{2+} uptake, mechanisms of activation, and the molecular components belonging to the mitochondrial Ca^{2+} uniporter (MCU) complex. For more detailed information, we refer the interested reader to a number of comprehensive reviews on the subject [27, 34, 49, 59, 61, 64, 76]. The main goal of this review is to recapitulate the studies that link the MCU complex with cancer. Given the short period since the discovery of the MCU complex components, the number of such studies is relatively low; nevertheless, it is our belief that summarizing the current knowledge will promote and assist future efforts in understanding mitochondrial Ca^{2+} signaling and how it can be harnessed to curb diseases, including cancer.

In most studies that refer to the physiological and pathological roles of mitochondrial Ca^{2+} signaling, it was the free unbound Ca^{2+} within the mitochondrial matrix that was first linked to a functional outcome. The functional role of the Ca^{2+} dynamics within the mitochondrial intermembrane space (IMS) on the other hand was less well understood. To reach the mitochondrial matrix, Ca^{2+} originating from the

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endoplasmic reticulum (ER), cytosol, plasma membrane, or any other cellular source has to cross two membranes. The outer mitochondrial membrane (OMM), which expresses VDAC (voltage-dependent anion channels), is Ca^{2+}-permeable; however, the IMM is not. Thus, channels and transporters are needed to carry Ca^{2+} between the IMS and the mitochondrial matrix. This matrix-directed Ca^{2+} transport is initiated when Ca^{2+} levels in the IMS reach concentrations in the low micromolar range or higher and largely depends on the electrochemical Ca^{2+} gradient that arises from the negative IMM potential (about − 180 mV) [20, 27, 51, 78, 86]. Accordingly, for mitochondria to take up Ca^{2+}, Ca^{2+} hotspots are needed (i.e. microdomains in their vicinity); this is because at rest, the cytosolic free Ca^{2+} levels in most cells are in the lower nanomolar range. The formation of Ca^{2+} hotspots can be controlled by different factors, such as organellar architecture and dynamics, cell shape, signaling mechanisms, and the involvement of other Ca^{2+} handling proteins.

One of the major signaling mechanisms that control mitochondrial Ca^{2+} dynamics is the store-operated Ca^{2+} entry (SOCE), which is physiologically initiated by the stimulation of Gq protein-coupled receptors on the outer cell surface. Via phospholipase C and phosphatidylinositol 4,5-bisphosphate (PIP_2), this signaling cascade leads to the generation of inositol 1,4,5-trisphosphate (IP_3), the activation of the ER-based IP_3-receptors (IP_3R), the release of Ca^{2+} from the ER, and the subsequent opening of plasma membrane Ca^{2+} channels, which ultimately cause an increase in the cytosolic Ca^{2+} concentration [18, 35, 70]. The main molecular components that allow this final increase in cytosolic Ca^{2+} consist of the CRAC channels (or Ca^{2+} release activated Ca^{2+} channels), which are formed by the ER-based stromal interaction molecules (STIM1 or STIM2) and the plasma membrane-bound ORAI-channels (ORAI1, 2, or 3) [81]. During SOCE, mitochondria can take up Ca^{2+} originating from the ER, from the plasma membrane, or both, and how mitochondria buffer Ca^{2+} from the different sources can determine cellular function [69]. For example, constant low IP_3R-mediated Ca^{2+} release from the ER and transfer to the mitochondria is crucial for the maintenance of basal levels of oxidative phosphorylation (OXPHOS), whereas mitochondrial buffering of Ca^{2+} ions crossing the plasma membrane via the CRAC channels is required for proper T cell activation [55, 82].

Accumulating evidence also suggests an important interaction between Ca^{2+}-driven signals and that of reactive oxygen species (ROS), with profound implications on multiple cell activities [7, 65, 78, 90]. ROS, such as superoxide ($^{\cdot}O_2^-$) and hydrogen peroxide (H_2O_2), are generated by mitochondria and are a group of molecules which are no longer considered simply as cytotoxic byproducts of aerobic metabolic activity [106]. On the contrary, ROS are presently well established as important second messengers and signaling molecules that link mitochondrial activity to cell biology [23]. More comprehensive information regarding the functional relevance of the Ca^{2+}-ROS interplay for different cellular systems and organs is available elsewhere [7–9, 19, 30, 32, 38, 43, 45, 91].

The mitochondrial Ca^{2+} uniporter complex

The major Ca^{2+} route across the IMM is via the MCU complex, formed by the MCU protein and its regulators, found in the IMM and the IMS (see Fig. 1) [34, 49, 61, 71]. The MCU complex is a relatively bulky structure (ca. 480 kDa) composed of channel-forming subunits and regulatory elements [93]. It has been reported that the channel is organized as a pentamer, although tetrameric assembly was also proposed [66, 83]. The channel-forming subunits consist of the membrane-spanning proteins MCU and MCUb (MCU isoforms "a" and "b") and EMRE (essential MCU regulator). The MCU isoforms have different functions; MCU enhances Ca^{2+} transport, while MCUb limits it; and one molecule of MCUb within the complex is sufficient to significantly decrease Ca^{2+} transport compared to a MCU homomer [83]. Because the expression of MCU and MCUb differs across tissues and cancer types, changes in the MCU:MCUb ratio could indicate variable Ca^{2+} uptake potentials for each MCU complex; however, this is a research area that still requires attention. EMRE contributes to the structural integrity of the complex; additionally, EMRE facilitates the interaction with other regulatory proteins within the MCU complex [89]. Accordingly, following EMRE depletion, mitochondrial Ca^{2+} uptake is inhibited, causing an effect comparable to MCU knockdown [89]. This outcome is not observed upon the depletion of other regulatory proteins, suggesting that EMRE is more than just a MCU regulator.

The activity of the MCU complex is also regulated by the mitochondrial Ca^{2+} uptake family of proteins (consisting of three isoforms: MICU1, 2, 3). The best characterized isoform, MICU1, functions as a channel gatekeeper and contains two Ca^{2+}-binding EF hands (helix-loop-helix domain), which sense Ca^{2+} levels in the IMS. At low Ca^{2+} concentrations, the EF hands of MICU1 keep the MCU complex inactive. However, when the Ca^{2+} concentration in the IMS exceeds a threshold (in most cells this IMS Ca^{2+} concentration lies in the low micromolar range), the EF hands in MICU1 bind Ca^{2+} and cause a conformational change that diminishes the MCU-MICU1 interaction and opens the MCU channel [21, 58, 77, 78]. Depletion of MICU1 results in mitochondrial Ca^{2+} uptake even at low IMS Ca^{2+} concentrations, causing elevated basal Ca^{2+} levels in the mitochondrial matrix; it also results in dysregulated Ca^{2+} uptake at higher IMS Ca^{2+} levels, indicating a dual function for MICU1 (gatekeeper and enhancer) [21]. The absence of MICU1 and the resulting Ca^{2+} overload can lead to increased oxidative stress and the induction of apoptosis [58].

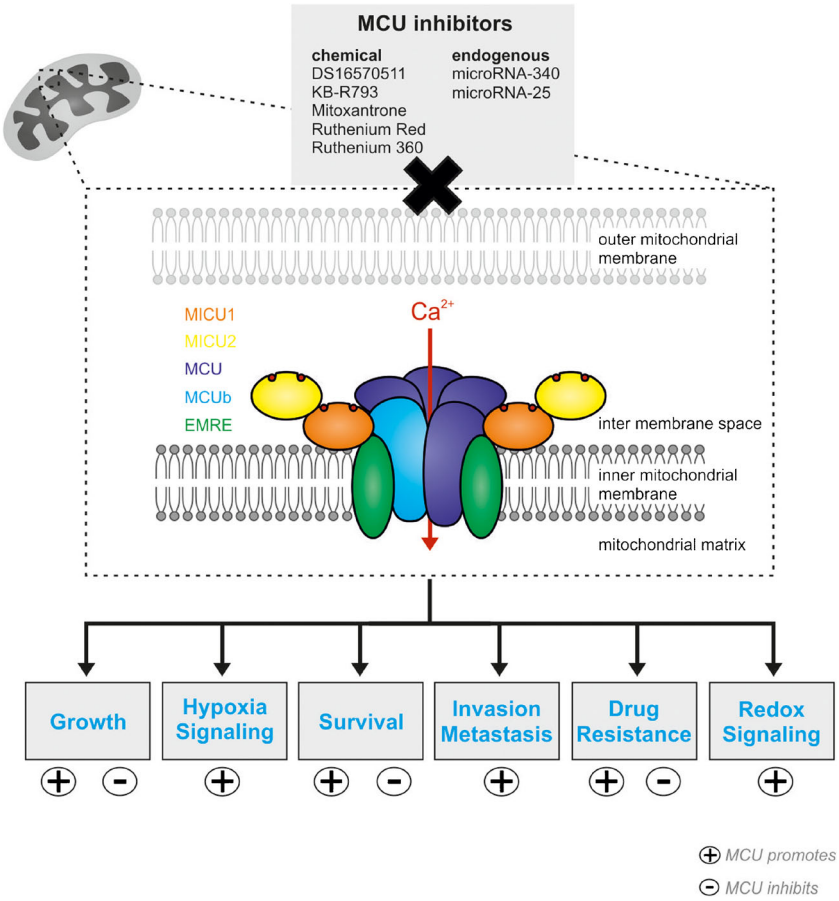

Fig. 1 The mitochondrial Ca^{2+} uniporter (MCU) complex and its role in cancer. The core of the MCU channel is formed by the membrane-spanning proteins MCU and MCUb (mitochondrial Ca^{2+} uniporters isoforms "a" and "b") and EMRE (essential MCU regulator). The MCU isoforms form a pentamer (or a tetramer) within the IMM that creates a Ca^{2+} conducting pore. The activity of this pore is further regulated by the MICU (mitochondrial Ca^{2+} uptake) family of proteins. The effects of the MCU complex on several cancer hallmarks and cancer-related signaling pathways are indicated with "+" for promoting effects and "–" for inhibiting effects (for details see Table 1). Chemical and endogenous inhibitors of the MCU complex are also listed

MICU2 was shown to inhibit the MCU complex [1, 48, 72, 80]. Overexpression of MICU2 reduces mitochondrial Ca^{2+} uptake, while its depletion has the opposite effect. It has to be noted that MICU1 and MICU2 form a disulfide-linked dimer that binds to the MCU complex; thus, depletion of MICU1 also results in the loss of MICU2 from the complex [72, 78]. This finding indicates that careful and well-designed experiments and interpretation of Ca^{2+} measurements must be undertaken since outcomes cannot be attributed to the loss of a single paralog. Additionally, MICU1 and MICU2 appear to have higher turnover rates than MCU, which hints at an additional level of MCU complex regulation and likely changes in the sensitivity of mitochondria to different Ca^{2+} levels. However, this also needs further investigation.

Much less is known about the function of MICU3; however, similarly to MICU1 and MICU2, MICU3 contains a cysteine in its C-terminal region, suggesting that it undergoes heterodimerization with other MICU isoforms. Indeed, a recent study examined the functional role of MICU3 in cortical neurons and showed that MICU3 forms heterodimers with MICU1, but not with MICU2. Moreover, MICU3 promoted mitochondrial Ca^{2+} uptake, similar to MICU1, thus regulating neuronal synaptic activity [73]. Interestingly, expression of MICU3 appears to be less pronounced compared to MICU1 and MICU2 across most tissues and cell lines, with several exceptions such as neural tissues and skeletal muscle [67, 73, 80]. Two additional IMM-based proteins, MCUR1 (MCU regulator 1) and SLC25A23, were identified as regulators of mitochondrial Ca^{2+} uptake [44, 57, 96, 98]. It was demonstrated that MCUR1 interacts with MCU but not with MICU1 and that silencing of MCUR1 strongly inhibits mitochondrial Ca^{2+} uptake. Two studies reported additional MCU-independent roles for MCUR1: as a regulator of Ca^{2+} sensitivity for the mitochondrial permeability transition pore and as an assembly factor for the electron transport chain (ETC) complex IV [15, 74]. Meanwhile, SLC25A23 physically interacts with MCU but also with MICU1. Interestingly, SLC25A23, similarly to the MICUs, possesses an EF-hand, which determines its role in the mitochondrial Ca^{2+} uptake. Further work is needed to fully understand the complex roles of MCUR1 and SLC25A23 as regulators of mitochondrial Ca^{2+} homeostasis. For additional comprehensive information on the MCU complex and its components, we suggest the following references (27, 34, 49, 59, 61, 64, 71).

Additional mitochondrial Ca^{2+} transporters

While MCU plays a major role in controlling mitochondrial Ca^{2+} uptake, it is not the only molecular transporter that has

been proposed to do so; thus, understanding the function and contribution of additional Ca^{2+} regulators could better help predict cellular response to Ca^{2+} changes. For example, studies showed that mitochondrial Ca^{2+} influx and efflux rates, as well as ATP generation, are impaired in LETM1 (leucine zipper-EF-hand-containing transmembrane protein 1) knockdown or mutant cells and also in fibroblasts derived from patients with Wolf-Hirschhorn syndrome (WHS). LETM1 levels were lower in WHS-derived fibroblasts, but the MCU components MCU, MCUR1, and MICU1 were unaltered, so this suggests their independent activity (at least with regard to changes driven by LETM1) [31, 47]. Another transporter to consider is NCLX (mitochondrial Na^{+}-Ca^{2+} (lithium) exchanger), which under normal conditions removes Ca^{2+} from the matrix and was found to be dominant to LETM1 in terms of mitochondrial Ca^{2+} export [25, 68]. Samanta et al. recently showed that after physiological stimulation, NCLX can also operate in reverse mode and instead transport Ca^{2+} into the matrix, thus generating Ca^{2+} oscillations [88]. The mitochondrial uncoupling proteins (UCPs) have also been proposed as regulators of the mitochondrial Ca^{2+} uptake in intact cells [39]. However, it has to be noted that the role of UCPs and LETM1 as Ca^{2+} transporters is under debate and additional work will help resolve this issue [10]. Finally, we have showed that both coenzyme Q10 (CoQ10) and its analogue CoQ1 undergo structural changes that allow them to bind and transport Ca^{2+} across biomimetic membranes in a redox-controlled manner [6, 40]. Additional studies are, nevertheless, needed to assess if these hydroxylated CoQ analogs regulate mitochondrial Ca^{2+} homeostasis in vivo.

Summarized, it is apparent that the exact function of all mitochondrial Ca^{2+} transporters is not yet fully understood and may in some cases change depending on the surrounding ion levels or co-regulators. Nevertheless, the crucial question is whether their aberrant expression alone or in combination with other transporters can be associated with aggressive disease or treatment resistance; additional efforts need to be invested in this direction. Novel observations in this direction could focus scientific investigations towards more clinically relevant findings in the future.

Mitochondria and cancer

Cancer cells display remarkable adaptability and robustness in order to survive, metastasize, and resist therapies. To do so, they rely on multiple molecular processes, some of which involve mitochondrial activity [87]. Mitochondria are not only sources of energy and producers of intermediates for lipid, nucleic acid, and protein synthesis but also hubs for signaling and mechanisms that control cell fate [59]. Since mitochondria play such an important yet varied role, the understanding of their function could provide useful information to treat aggressive disease. Early observations into the role of mitochondria in cancer involved unexpected metabolic switches. Almost a hundred years ago, Otto Warburg observed that cancer cells metabolize glucose differently from that of normal tissue cells and he posited that cancer cells rely on glycolysis for their ATP production even in the presence of oxygen [100]. Later studies confirmed the metabolic switch, but showed it to be reversible, and mitochondria proved to remain functional and mutation-free in many cases following cancerous transformation. We now know that mitochondria can play an important role in promoting cancer [99], and part of this mitochondrial function relies on a fine-tuned and well-localized transport and buffering system for calcium ions.

The MCU complex and cancer

According to The Human Protein Atlas (www.proteinatlas.org), a majority of cancer tissues show moderate to strong MCU immunostaining. The strongest protein expression was found in colorectal and ovarian cancers; high expression was also detected in many other cancers, such as pancreatic, stomach, and prostate cancer. For MICU1, moderate to strong immunostaining was also observed in most malignant tissues, while for MICU2, weak to moderate immunostaining was detected, with the strongest signal being observed in thyroid cancer. MICU2 was negative for several cases of malignant lymphoma, urothelial, and skin cancers (excluding melanoma). MCUb expression was weak to moderate and was found in most colorectal cancers and in breast, cervical, and ovarian cancers; most other cancers were negative. MCUR1 results are pending for human cancer tissue, but lymphoid, skin-, and breast-derived cells have the highest expression from the cell lines tested. EMRE immunostaining is highest in thyroid cancer as well as in liver, stomach, and prostate cancer. Weak to moderate expression was detected in most examined cancers with exclusion of lymphoma, testis, and skin cancer (excluding melanoma).

According to The Cancer Genome Atlas (TCGA; www.cancergenome.nih.gov), the highest rate of MCU genetic alterations was found in prostate and breast cancers, observed as gene amplifications. A few datasets show deletions (for example in malignant peripheral nerve sheath tumors (MPNST)), while the highest rate of MCU mutations was observed in uterine carcinosarcoma. MICU1 amplifications are also commonly identified in many cancers, with the highest rates detected in prostate and breast cancer. MICU1 deletions were also found in MPNST, while mutations were highly featured in melanoma. MICU2 was also most amplified in prostate and breast cancer and most highly mutated in breast and stomach cancer. Frequent MCUb amplifications were detected in prostate cancer, while mutations and deletions were found in pancreatic

cancer (deletions were also found in MPNST). MCUR1 amplifications are also prominent and are found in breast, prostate, melanoma, ovarian, and bladder cancers, but mutations and deletions are also detected in melanoma and other cancers. Genetic modifications of EMRE mostly occur as amplifications and deep deletions in breast, pancreas, prostate, and melanoma cancers.

How these MCU complex genetic modifications affect cancer biology and why their patterns differ across cancer types is currently not understood, and this could be addressed in the future. Next, we describe the current knowledge on the role of the MCU complex in different cancers. A compact summary of the publications discussed below is presented in Table 1.

Breast cancer

Breast cancer and breast cancer models appear to be the most extensively studied so far with respect to MCU complex function. Acquired knowledge in this field not only demonstrates the involvement of MCU in cancer but also highlights its complexity and context-dependent activity. In a recent study, Tosatto et al. examined the role of MCU in cancer migration and invasion and showed that MCU expression correlated with tumor size and lymph node infiltration in triple negative breast cancer (TNBC) [97]. MCU knockdown decreased cell motility and invasion potential, as well as tumor growth in xenograft models of TNBC. Meanwhile, MCU-silencing weakened the production of mitochondrial ROS and reduced the expression of HIF1-α. Using breast cancer mRNA samples, a positive correlation of MCU levels with HIF1-α signaling was revealed. In sum, the MCU-ROS interplay was identified as an important regulator of TNBC growth and metastasis [97].

In a study featuring patient data analysis, Curry et al. investigated MCU expression in 180 human breast cancer samples using microarray analysis [22]. Results not only showed expression variability among patients but also indicated that high MCU levels can be detected in estrogen receptor (ER)-negative tumors, with the strongest enrichment found in the basal-like subtype. To further explore the contribution of MCU to breast cancer, the authors silenced MCU in the triple negative MDA-MB-231 breast carcinoma cell line and examined the effects on cell number, cell cycle, and survival. Their siRNA approach did not show significant changes in either proliferation or viability; however, MCU silencing potentiated breast cancer cell death in the presence of submaximal levels of the Ca^{2+} ionophore ionomycin. This cytotoxicity was not replicated with submaximal or high levels of the Bcl-2 (B cell lymphoma 2) inhibitor ABT-263, suggesting a distinct role for MCU in caspase-independent (ionomycin) vs -dependent (ABT-263) cell death. The authors also determined that MCU does not play a major role in regulating bulk cytoplasmic Ca^{2+} levels in MDA-MB-231 cells and suggested instead that cell outcome is influenced by either changes in mitochondrial Ca^{2+} uptake or alterations in localized Ca^{2+} signals in special domains, such as mitochondrial-associated-membranes (MAMs).

The importance of MCU in breast cancer was further confirmed by Hall et al.; their study however also warns that not all carcinomas are sensitive to mitochondrial Ca^{2+} uptake-based therapies [41]. In the first part of the study, the authors searched the web-based BreastMark algorithm and observed that a significantly poorer prognosis was associated with MCU overexpression and MICU1 downregulation; additionally, those with MICU1 overexpression had a better prognosis. They also used the MDA-MB-231 breast carcinoma cell line model to conduct functional studies; however, they reported that this cell line harbors mitochondrial DNA mutations and decreased oxidative metabolism, which could alter its survival mechanisms compared to other cell types. Using siRNA-mediated knockdown studies and adenoviral overexpression techniques, as well as the mitochondrial targeted genetically encoded Ca^{2+} sensor ratiometric-pericam, Hall et al. first showed that MCU is functional in MDA-MB-231 and that ATP-induced mitochondrial Ca^{2+} uptake was inhibited by MCU knockdown. Inhibition of MCU was also confirmed with the overexpression of a dominant-negative MCU mutant. Meanwhile, Ca^{2+} uptake was enhanced by wild-type MCU overexpression and MICU1 knockdown. On the other hand, both MCU and MICU1 silencing had minor effects on mitochondrial ROS production. Minor effects were also detected using the clonogenic survival of MDA-MB-231 cells in response to stress such as radiation, paclitaxel treatment, starvation, and ceramide treatment (which according to the authors, promotes Ca^{2+} leak from the ER). These findings do not fully agree with observations in HeLa cervical cancer cells, where overexpression of MCU or knockdown of MICU1 caused constitutive mitochondrial Ca^{2+} influx, sensitization to H_2O_2, and ceramide-induced cell death [58]. Hall et al. in fact proposed a spectrum of responses following MCU manipulation. This is supported by their clonogenic survival assay results using human mammary epithelial cells (HMEC), HeLa, and MDA-MB-231 cells; where HMECs were the most sensitive to MCU channel manipulation and ceramide treatment, where HeLa had an intermediate phenotype, and where MDA-MB-231 remained largely unresponsive [41]. How MDA-MB-231 cells favor survival is not understood yet, but it appears that they separate mitochondrial Ca^{2+} transport from other mitochondrial functions (e.g., ROS production or metabolic changes upon starvation). Another interesting observation from this study showed that knockdown of MCU reduced MICU1 protein levels but not at the mRNA level (the reverse however, was not observed for MICU1 knockdown), and the authors suggest a possible MCU-dependent post-

Table 1 Summary of publications featuring MCU in cancer

Cancer type	Highlights	Ref.
Breast cancer	MCU promotes metastasis via Warburg effect MCU is a direct target of microRNA-340 MCU expression is higher in metastatic breast cancer patients	[104]
Triple negative Breast cancer	MCU correlates with tumor size and lymph node infiltration MCU knockdown (KD) reduces motility and invasion, tumor growth, lymph node infiltration, and lung metastasis in TNBC xenografts. MCU controls mROS and HIF-1α	[97]
Breast cancer, MDA-MB-231 cells	MCU mRNA levels correlate with metastasis and invasion MCU is critical for SOCE-dependent breast cancer cell migration	[95]
Breast cancer	Disease outcome negatively correlates with high MCU and low MICU1 expression MCU or MICU1 KD does not affect ROS production and cell viability after irradiation, chemotherapeutics, or starvation MCU overexpression does not affect ceramide-mediated toxicity MCU is dispensable for breast cancer cell line viability	[41]
Breast cancer, MDA-MB-468 cells	EGF-induced EMT in MDA-MB-468 is associated with alterations in ER calcium homeostasis; MCU expression is not affected	[24]
Breast cancer	High MCU expression is observed in ER-neg and basal-like breast cancer samples MCU silencing in MDA-MB-231 causes no change in proliferation or viability Caspase-dependent cell death by the Bcl-2 inhibitor ABT-263 is not affected by MCU Caspase-independent cell death by ionomycin is potentiated by MCU silencing	[22]
Breast and colon cancer cell lines	IP_3R-mediated release of Ca^{2+} from ER and MCU-mediated mCa^{2+} influx is needed for celastrol-induced paraptosis in cancer cells	[103]
Hepatocellular carcinoma	MCUR1 is upregulated in HCC cells and enhances mCa^{2+} uptake in MCU-dependent manner, promoting cell survival MCUR1 causes elevated mROS and AKT/MDM2-induced p53 degradation Overexpressed MCUR1 decreases cell death and increases proliferation in a xenograft model	[85]
Hepatocellular carcinoma	MCU expression correlates with metastasis and poor prognosis High MCU levels increase mCa^{2+} uptake and promote mROS via NAD+/SIRT3/SOD2 pathway High MCU levels promote MMP2, cell motility, and intrahepatic and distal lung metastasis in vivo	[84]
Hepatocellular carcinoma	In sublethal heat-treated SMMC-7721 HCC cells, MCU mRNA is downregulated	[29]
Colon cancer	microRNA-25 decreases mCa^{2+} uptake by MCU downregulation, causing resistance to apoptosis In human colon cancer, microRNA-25 is overexpressed and MCU levels are low	[62]
Pancreatic cancer	HINT2 triggers cancer cell death via MCU HINT2 overexpression is associated with MICU1 and MICU2 downregulation and EMRE upregulation	[16]
Head and neck squamous cell carcinoma (HNSCC)	EZH2 is a negative prognostic factor; its inhibition in vitro causes cell death and cell cycle arrest EZH2 inhibition causes MICU1 downregulation EZH2 and MICU1 are required to maintain mitochondrial membrane potential	[105]
Multiple myeloma	CYPD, SOD2, and MCU are differentially expressed in KMS cells and control mitochondrial activity and bortezomib sensitivity Membrane potential, oxygen consumption rate, ATP, and mCa^{2+} concentrations correlate with KMS drug resistance	[92]

Table 1 (continued)

Cancer type	Highlights	Ref.
$p53^{-/-}$ MEFs; HCT-116 (colorectal carcinoma) HeLa H1299 (non-small cell lung cancer)	Links p53 to Ca^{2+} signaling in cell death regulation In vivo Ca^{2+} imaging in 3D tumor masses and photodynamic stress reveal high mCa^{2+} as a cause of p53-dependent cell death	[36]
MDA-MD-468 (breast cancer) HeLa and EA.hy926 cells	ER-mitochondria tethering increases cancer cells sensitivity to resveratrol/piceatannol MCU and LETM1 control mCa^{2+} uptake MCU and LETM1 control resveratrol/piceatannol-induced cancer cell death	[56]
Oncogene-induced senescence in HEC cells	MCU loss enables escape from oncogene-induced senescence	[101]
Prostate cancer	Mitochondrial swelling induced by metformin is due to enhanced mCa^{2+} and is reversed by MCU inhibition	[54]
Melanoma	Drug resistant and tumor maintaining melanoma cells display elevated mCa^{2+}	[87] + (own unpublished observations)
Melanoma and osteosarcoma	Ca^{2+} protects osteosarcoma and melanoma cells from tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) cytotoxicity. Ca^{2+} chelators and the MCU inhibitor ruthenium 360 decrease mCa^{2+} and sensitize tumor cells to TRAIL cytotoxicity	[94]
Cardiotoxicity in cancer patients	Inhibition of Gβ5 could prevent cardiotoxicity in cancer patients treated with anthracyclines, taxanes, or fluoropyrimidines Gβ5-loss maintains membrane potential, basal MCU expression, and mCa^{2+} levels, allowing functional myocyte activity	[14]
Vasculopathy, (cancer-like phenotype) causing pulmonary arterial hypertension (PAH)	microRNA-138 and microRNA-25 downregulate MCU Impaired MCU function (MCU downregulation and MICU1 upregulation) control PAH's pathogenesis. microRNA-mediated MCU dysfunction can be targeted in PAH	[46]

Abbreviations: mROS, mitochondrial ROS; mCa^{2+}, mitochondrial calcium

transcriptional feedback loop. Additionally, similar to MICU1, MCUb and EMRE were shown to have favorable hazard ratios; therefore, searching patient data for co-expression patterns could provide more predictive responses than looking at MCU or MICU1 alone. The answer to understanding the spectrum of responses to MCU manipulation in different cancer samples could be partially addressed in the future by examining the expression and activity of multiple components of the MCU complex as recently reported [67].

In another breast cancer cell line model, this time featuring MDA-MB-468, Davis et al. examined whether epidermal growth factor (EGF)-induced epithelial-mesenchymal transition (EMT) involved gene expression changes for Ca^{2+}-related channels, pumps, or exchangers. This endeavor was based on the observation that EMT is associated with altered SOCE and with changes in Ca^{2+} signals mediated by purinergic receptors [24]. The study concluded that MCU mRNA levels remain unchanged during EMT; however, the ER Ca^{2+} channels and pumps were most highly affected, notably the ryanodine receptor RYR_2. We note here that the EMT process involves multiple transition states and intermediates over time, and this process is also reversible, so the role of MCU in EMT cannot be fully discounted [102]. In addition, given the heterogeneity found among breast cancer tumors, the contribution of MCU and its regulators to this process could still be further explored using additional models.

While the role of MCU in cancer cell survival appears cell line-specific, it could also be drug-specific as shown by Yoon et al. [103]. In their study, the authors showed that celastrol (an anticancer agent isolated from a Chinese vine) kills breast and colon cancer cell lines via paraptosis. Paraptosis is characterized by vacuolation, dilatation of the ER and mitochondria, and a caspase-independent cell death. Celastrol treatment caused an increase in mitochondrial Ca^{2+} levels and ER stress, and this was inhibited by MCU knockdown or MCU inhibition via ruthenium red pretreatment in MDA-MB-435S breast

cancer cells. Similar to the study by Curry et al. [22], this study suggested that drugs that harness caspase-independent cell death mechanisms could benefit from MCU targeting.

Focusing on the role of MCU in metastatic breast cancer, Tang et al. examined the expression of MCU mRNA in the Oncomine database and observed a correlation between MCU, metastasis, and invasive breast cancer [95]. Similar to previous studies, mechanistic follow-up on these observations was conducted with the MDA-MB-231 cell line. In this cell model, MCU inhibition halted serum-induced migration and serum- or thapsigargin-induced SOCE. Interestingly, SOCE inhibitors were also able to inhibit serum-induced MDA-MB-231 cell migration. Thus, it appears that MCU's role in MDA-MB-231 cells includes SOCE activity with consequences on cell migration.

Given the accumulated evidence for the presence of high levels of MCU in aggressive disease, Yu et al. investigated the effects of MCU knockdown and overexpression on cell migration, invasion, and glucose metabolism using the human breast carcinoma lines ZR-75-30, MDA-MB-231, MCF7, and BT-474. Highlights of the study indicate that MCU silencing in MDA-MB-231 cells decreased migration and invasion in vitro and reduced lung metastasis in vivo; conversely, overexpression in the less aggressive MCF7 potentiated these effects in vitro and in vivo. These findings increasingly support the role of MCU in invasive and metastatic processes. Additionally, Yu et al. showed enhanced glycolysis following MCU overexpression in MCF7 and they proposed a novel mechanism whereby this MCU-driven process is negatively regulated by microRNA-340. Finally, MCU expression was evaluated in 60 human breast cancer samples using immunohistochemistry techniques, and data showed that MCU protein levels are significantly increased in metastatic samples [104].

Using breast and prostate cancer cell lines, as well as transformed primary human fibroblasts, Cardenas et al. demonstrated that Ca^{2+} communication between the ER and mitochondria is essential for tumor cell survival, but this is not the case for normal cells, i.e., some cancer cells are addicted to mitochondrial Ca^{2+} [13, 55]. Briefly, the authors' findings showed that inhibition of mitochondrial Ca^{2+} uptake by knockdown of MCU or its regulator MCUR1 caused a decrease in ATP levels, caused AMPK activation, and engaged autophagy in normal cells, permitting cell survival. Additionally, genetic or drug inhibition of MCU decreased TCA cycle activity, ATP levels, and metabolic products, also leading to autophagy. However, only normal cells survived this process, while tumor cells died by necrosis. Interestingly, all the effects induced by interference with the ER-mitochondria Ca^{2+} transfer (in both normal and cancer cells) were reversed by the addition of pyruvate or alpha-ketoglutarate, indicating that a lack of metabolic intermediates was the main cause of cell death.

Together, the previously described studies suggest that the role of the MCU complex in breast cancer is important yet complex and so far, cell-type dependent; they also advocate that certain cells can acquire MCU-independent survival properties. However, the MCU complex works as a multi-component channel and it is not yet fully understood how mitochondria adapt and compensate for the knockdown of its single components. Additionally Ca^{2+} buffering systems, aside from the MCU complex, can also contribute a survival advantage. For example, MCU functionally depends on VDAC channels, and according to Liao et al., it also mediates VDAC overexpression-induced cell death in cerebellar granule neurons (CGNs). Thus, understanding the MCU complex and VDAC status could better predict mitochondrial Ca^{2+} homeostasis and possibly stress-induced apoptosis [53]. Finally, the MCU complex is not likely to be targeted alone therapeutically; thus, this highlights the need to study this Ca^{2+} regulator in the context of a broader signaling landscape. We note here that the studies listed above do not fully recapitulate the heterogeneity of breast cancer or the response in a more in vivo environment; however, they provide a useful base on which future studies can build and can help narrow the focus as we move forward.

Hepatocellular carcinoma

Similar to breast cancer, expression studies of the MCU complex in hepatocellular carcinoma (HCC) tissues (by microarray and immunohistochemical analyses) indicated that MCU is frequently upregulated, MICU1 is downregulated, while no significant differences were observed for MICU2, MCUb, or EMRE [84]. In this study, high MCU or low MICU1 expression were also associated with poor overall survival, recurrence-free survival, as well as with metastatic tissue. Using HCC cell lines, the authors demonstrated that mitochondrial Ca^{2+} uptake was enhanced in a MCU-dependent manner, also that MICU2 expression was not affected by MICU1 knockdown or overexpression in this cancer type and that both MICU isoforms played non-redundant roles in Ca^{2+} regulation. Alterations in MCU expression did not appear to have significant effects on the expression levels of sarcoendoplasmic reticulum Ca^{2+} transport ATPase pumps (SERCA) or IP_3R (both ER-related Ca^{2+} regulators). On the other hand, MCU changes affected mitochondrial ROS production via a nicotinamide adenine dinucleotide (NAD+), sirtuin 3 (SIRT3), and superoxide dismutase 2 (SOD2)-driven pathway; this enhanced metastasis (as also observed in breast cancer models). More precisely, high MCU levels induced matrix metalloproteinase 2 (MMP2) production and cell motility, confirmed by increased intrahepatic and distal lung metastases in vivo.

In a second publication from the same group, the authors showed that aside from MCU, the regulator MCUR1 was also often upregulated in HCC cells and caused increased mitochondrial Ca^{2+} uptake, tumor cell survival, and proliferation [85]. In their model, the authors suggested that following increased Ca^{2+} uptake, higher mitochondrial ROS were produced, which caused an increase in AKT/MDM2-mediated p53 degradation, and subsequent changes in the expression of apoptosis and cell-cycle related proteins. In vivo, overexpressed MCUR1 decreased cell death and increased proliferation; conversely, MCUR1 reduction impaired tumor growth in nude mice. The role of both p53 and MCU in driving cancer biology was also explored by Giorgi et al. [36, 37]. In these studies featuring colon, breast, and non-small cell lung cancer cell lines, the authors showed that upon adriamycin or H_2O_2 exposure, wild type p53 localized to the ER and to the mitochondrial associated membranes. Herein, p53 was shown to directly bind SERCA pumps, thereby altering the redox state and thus causing increases in Ca^{2+} load, mitochondria Ca^{2+} overload, and cell death. The authors also showed that this does not occur in cell lines harboring p53 mutations. Together, these studies on multiple cancer cell types suggest the need to monitor p53 status when manipulating Ca^{2+} flux and MCU for therapeutic purposes.

Another study flagged MCU as being relevant to HCC and this followed microarray analyses of sublethally heat-treated HCC cells. This sublethal heat treatment model is suggested to reflect a transition zone found in radiofrequency ablation (RFA) tumor treatment; treatment conditions in this transition zone are insufficient to kill tumor cells and are expected to cause local recurrence. While the primary goal of this study was to measure long non-coding RNAs involved in treatment resistance, differentially expressed mRNAs were also investigated and flagged MCU as being important in the SMMC-7721 cell line [29]. While more work is required to validate the role of MCU in this model, it highlights the potential contribution of MCU to acquired treatment resistance and initial response to overcoming stress. Studies exploring the role of the MCU complex in dynamic processes, such as drug resistance, would be interesting to explore in the future.

Colon cancer

Marchi et al. identified in silico a cancer-related MCU-targeting microRNA-25 and showed that its overexpression in HeLa cells reduced MCU levels and mitochondrial Ca^{2+} uptake, thus increasing cell survival following apoptotic challenges (such as H_2O_2 and C2-ceramide) [60, 62]. Additional members of the microRNA-25 family, such as microRNA-92a and microRNA-363, were proposed to have similar effects on MCU and Ca^{2+} signaling. The authors also demonstrated that the effect of microRNA-25 is focused on mitochondrial Ca^{2+} uptake alone and that no changes occurred in mitochondrial membrane potential, mitochondrial volume, number, or ER contact sites. However, aside from its effect on mitochondrial Ca^{2+}, it is not excluded that additional microRNA-25 targets, such as PTEN (phosphatase and tensin homolog), the pro-apoptotic protein Bim, and TRAIL (tumor necrosis factor related apoptosis inducing ligand), also contributed to the antiapoptotic effects. The authors then showed that multiple colon and prostate cancer cell lines displayed high microRNA-25 levels and low MCU expression compared to primary normal cells. This observation was confirmed in human samples of colonic adenocarcinoma using immunohistochemistry and microarray analyses. Finally, the authors used the PC3 prostate cancer cell line to show that increased MCU activity reduced soft agar colony formation, while overexpressed anti-microRNA-25 in HCT116 and PC3 cells led to increased sensitivity to H_2O_2 and C2-ceramide. To summarize, the authors suggested that microRNA-25, through MCU downregulation, reduced the sensitivity of cancer cells to apoptosis-inducing agents. This study sets the stage for further studies on the role of microRNAs in Ca^{2+} homeostasis and opens the possibility of targeting microRNAs instead of MCU directly in order to regulate its activity.

Pancreatic cancer

Previous studies have shown that the histidine triad nucleotide-binding protein (HINT2) sensitizes HepG2 HCC cells to mitochondrial apoptosis following cytotoxic drug treatment [63]. In pancreatic cancer, HINT2 also promoted cell death and this was proposed to involve MCU regulation and Ca^{2+} influx [16]. In this study, HINT2 expression was shown to be reduced in pancreatic cancer tissue compared to adjacent normal tissue (assessed via immunohistochemistry and microarray analyses). This downregulation was not surprising given that HINT2 was shown to inhibit tumor growth and invasion in pancreatic carcinoma models. Functional studies revealed that HINT2-mediated apoptosis could be blocked using the MCU inhibitor ruthenium red. Meanwhile, HINT2 overexpression using an adenoviral vector increased mitochondrial Ca^{2+} and changed the expression profile of MCU regulators such as MICU1, MICU2 (downregulation), and EMRE (upregulation), which could contribute to this Ca^{2+} overload. The role of MCU cannot be discounted in the anticancer effects of HINT2; however, the study by Chen et al. also highlighted 1240 differentially expressed genes following HINT2 overexpression, indicating MCU changes alone are not responsible for the anticancer effects. Of interest in the future is to potentially use the expression levels of MCU and its regulators to predict patient survival and combine them with additional cancer biomarkers.

Head and neck squamous cell carcinoma (HNSCC)

Unlike HINT2, the enhancer of zeste homolog 2 (EZH2) is overexpressed or activated in many human cancers including head and neck squamous cell carcinoma (HNSCC), where it was shown to be associated with high tumor grade and poor prognosis (TCGA data). Zhou et al. confirmed such observations in a Chinese HNSCC cohort; in addition to showing that the EZH2 inhibitor DZNep and siRNA against EZH2 decreased MICU1 expression in human oral cancer cell lines. Additionally, siRNA against MICU1 decreased cell viability in the SCC25 and Cal27 cell lines, while western blot analyses showed that Bcl-2 was decreased and BAX and cleaved caspase-3 were increased. Finally, a HNSCC xenograft model using the Cal27 cell line was used to test the in vivo effects of the EZH2 inhibitor DZNep. Immunohisochemistry analysis showed decreased MICU1 and Bcl-2 expression, increased proapoptotic BAX expression and cleaved caspase-3, and showed decreased tumor volume [105]. The authors mentioned that EZH2 inhibition triggers cytoplasmic Ca^{2+} accumulation, loss of membrane potential, and changes in mitochondrial proteins involved in cell death; however, more focused mitochondrial studies could shed light on the role of MICU1 in HNSCC and whether it should be targeted in combinatorial treatment strategies.

Multiple myeloma

The role of mitochondria in drug-induced cytotoxicity or resistance was explored in multiple myeloma (MM) cell lines displaying varied sensitivity to the proteasome inhibitor bortezomib by Song et al. [92]. Findings demonstrated that all cells increased mitochondrial Ca^{2+} levels in response to bortezomib, but what differentiated sensitive from resistant cells was the ratio between the basal and induced mitochondrial Ca^{2+} (the higher the fold increase in mitochondrial Ca^{2+}, the more cytotoxicity was observed). Differences were also observed in membrane potential, mitochondrial ROS levels (which were suggested to contribute to cytotoxicity), oxygen consumption, and mitochondrial ATP; all suggested to contribute to the drug response in MM cells. The authors next explored regulators of mitochondrial activity and showed that MCU expression was highly upregulated following bortezomib treatment, but only in the sensitive cells. Additional genes, such as cyclophilin D (CYPD) and SOD2, were highlighted as important regulators of cell death, thus suggesting the involvement of mitochondrial ROS in the process. Concluding remarks suggested the combined use of mitochondria-targeting agents with bortezomib to obtain maximal MM cell apoptosis. These findings support those of Curry et al. in breast cancer and that of Giorgi et al. in multiple cancer cell types, in that MCU status alone as a biomarker of drug response may not be sufficient to predict cell outcome; however, in a wider signaling or genetic context, it could be of use clinically [22, 37].

Supporting the role of MCU in drug-induced cytotoxicity, Madreiter-Sokolowski et al. showed that siRNA against MCU and LETM1 could prevent resveratrol/piceatannol-induced cancer cell death [56]. This study featured HeLa cells, human umbilical vein endothelial cells (HUVEC), and EA.hy926 cells (established by fusing HUVEC with a thioguanine-resistant clone of the A549 human lung carcinoma line); however, additional human cancer models could be used to confirm observations. Moreover, the authors proposed that enhanced mitochondrial Ca^{2+} sequestration within ER-mitochondrial contact sites in cancer cells made them susceptible to resveratrol/piceatannol SERCA pump inhibition, resulting in enhanced mitochondrial Ca^{2+} uptake, overload, and ultimately cell death. This would suggest that ER-mitochondrial contact sites might control cancer cell drug sensitivity. Thus, understanding the role of contact sites, or proteins involved in organellar tethering, could provide additional indicators of response if drugs affect Ca^{2+} homeostasis. For additional reading on the role of mitochondria-ER contact sites in cancer, please refer to [2, 11, 12, 33, 50, 75, 79].

Prostate cancer, osteosarcoma, and melanoma

In a study predominantly featuring prostate cancer cell lines, Loubiere et al. proposed that the antidiabetic drugs and metabolic disruptors metformin and phenformin regulate intracellular Ca^{2+} flux. In brief, these drugs were shown to induce ER stress, ER Ca^{2+} release, mitochondrial Ca^{2+} uptake, organelle swelling, and apoptosis; however, this was reversed when MCU was inhibited. Supporting the mitochondrial Ca^{2+} uptake increase, MCU mRNA was also increased following metformin treatment (among other Ca^{2+} handling proteins). In vivo, using different mouse models, metformin efficiently reduced tumor growth and increased mitochondrial areas in tumor cells [54]. Our own work in melanoma showed that phenformin blocked the emergence of a subpopulation of tumor-maintaining drug-resistant cells during anti-melanoma treatment and these cells displayed upregulated mitochondrial ETC proteins, consumed more oxygen, and generated more ATP [87]. Moreover, we found that these aggressive cells had significantly upregulated mitochondrial Ca^{2+} levels (unpublished observations). Whether a phenformin-induced increase in mitochondrial Ca^{2+} was sufficient to push this subpopulation of cells towards apoptosis was not examined. In a different study, we also demonstrated that increased mitochondrial activity and enhanced ROS production could be used as an Achilles' heel for these drug-resistant tumor-maintaining

melanoma cells [17]. Although it is evident that mitochondrial Ca^{2+} can tune the effects of ETC inhibitors such as phenformin, it must be considered that anti-metabolic drugs have activities beyond that of regulating Ca^{2+} homeostasis; thus, careful dissection of multiple biological processes, including metabolic changes, needs consideration.

A recent study by Takata et al. examined the role of mitochondrial Ca^{2+} dynamics in osteosarcoma and melanoma and showed that Ca^{2+} protects cancer cells from TRAIL cytotoxicity. The authors also showed that acute TRAIL treatment increased cytosolic and mitochondrial Ca^{2+} concentrations. Calcium chelators, the MCU inhibitor ruthenium 360, the mitochondrial permeability transition pore opener atractyloside, capsazepine, and AMG9810, all decreased mitochondrial Ca^{2+} and sensitized tumor cells to TRAIL treatment in an apoptotic and non-apoptotic way. The study points to the important role of mitochondrial Ca^{2+}, and thus MCU activity, in overcoming cancer cell resistance to TRAIL cytotoxicity [94].

Alternative, cancer-related, and cancer-like pathologies

To be of use clinically, targeting MCU does not have to be exclusively focused on cancer cells per se. For example, a cancer-like disease where excessive pulmonary artery smooth muscle cells proliferate, migrate, and resist apoptosis also relies on MCU activity; this vasculopathy leads to pulmonary arterial hypertension (PAH) and was shown to display impaired MCU complex function, downregulation of MCU and upregulation of MICU1 protein [46]. MCU dysfunction not only increased cytosolic Ca^{2+} that then stimulated proliferation and migration, but also decreased mitochondrial Ca^{2+}, thus inhibiting pyruvate dehydrogenase and glucose oxidation. Encouragingly, microRNA-mediated MCU complex regulation could be targeted. It would be interesting to examine if other pathologies with cancer-like properties, or early stages of disease, rely on MCU activity for progression. An indication of such potential comes from the study of Wiel et al. who identified MCU as a regulator of oncogene-induced senescence (OIS) through a loss-of-function genetic screen in human endothelial cells. In this study, loss of MCU or the Ca^{2+} channel ITPR2 (a member of the IP_3 receptor family) allowed cells to escape OIS by reducing mitochondrial Ca^{2+} accumulation, or Ca^{2+} release from the ER, respectively. The authors also suggested that this mechanism could be involved in replicative senescence [101].

Another cancer-related challenge arises in the form of cardiotoxicity in cancer patients treated with chemotherapeutics, such as anthracyclines, taxanes, or fluoropyrimidines. Chemotherapeutics can cause the upregulation of an atypical G protein Gβ5 in the myocardium. If targeted, Gβ5 loss can maintain membrane potential, basal MCU expression, and mitochondrial Ca^{2+} levels; it can also reduce drug-induced proinflammatory cytokines, hypertrophic, and profibrotic factors [14]. Thus, while treatment strategies revolving around targeting MCU and its regulators directly hold merit, understanding the upstream and downstream signals can also reveal novel treatment strategies, some of which may not eradicate the cancer fully, but can certainly improve patients' responses and quality of life.

The advantage of dealing with less aggressive or advanced disease when targeting the MCU complex is that the genetic landscape of these cells may be easier to navigate therapeutically. On the other hand, little is known about long-term MCU targeting, especially as it appears to have different functions in different tissues; thus, long-term adaption of cells to MCU complex manipulation is a research area still in need of attention.

Pharmacology of MCU

From a clinical point of view, an essential prerequisite to pharmacologically target a specific molecule or a signaling cascade in order to impair cancer growth and invasion is having a specific drug with minimal side effects. Based on the data summarized in this review, inhibiting rather than activating MCU should be beneficial for, at least some, cancer patients. However, all known inhibitors of MCU are currently non-specific, do not cross cellular membranes, and have a plethora of side effects. Ruthenium red (RuR) and its derivative ruthenium 360 (Ru360) were, until recently, more or less the only choices to directly manipulate MCU activity (for details see [27]). In addition, compounds such as the NCX inhibitor KB-R7943 or the antibiotic minocycline have been reported to also block MCU. Similar to RuR however, these drugs were shown to be unspecific and displayed a number of disadvantages. Accordingly, the clinical use of any of these drugs as inhibitors of MCU does not seem feasible.

A recent study by Arduino et al. characterized Mitoxantrone, an anthracenedione-derived cytostatic agent used in hematological malignancies, as a specific inhibitor of MCU [3]. However, Mitoxantrone also acts cytostatically by DNA intercalation, which may limit its use in in vivo disease models even though the antineoplastic and anti-MCU properties are supposed to be independent. Chemical modifications of Mitoxantrone that will separate the MCU-blocking properties from the antineoplastic ones might provide a new generation of specific MCU inhibitors with distinct anticancer properties [3]. An additional recent study utilized a screen of 120,000 small molecule compounds to identify DS16570511 as a specific membrane-permeant MCU inhibitor [52]. Besides inhibiting agonist-induced mitochondrial Ca^{2+} uptake at a cellular level, DS16570511 enhanced cardiac contractility in perfused rat hearts. A potential utility of DS16570511 or

one of its derivatives for cancer treatment needs to be explored in the future. A list of chemical and endogenous MCU inhibitors is displayed in Fig. 1.

Conclusion

As we have learned from studies on targeted therapies and immunotherapies so far, single biomarkers and drug targets can be useful clinically; however, they must be used in the right context and with the right strategy in order to be effective and prevent adverse reactions [42]. The importance of MCU and its regulators in cancer is increasingly supported by multiple scientific studies and models; nevertheless, its function and role in pathobiological conditions are yet to be fully understood and reconciled across experimental conditions. To date, not all studies comprehensively examined MCU effects on proliferation, migration, invasion, adhesion, starvation, or treatment with various drugs, or examined Ca^{2+} and redox-dependent processes, so work is still needed to predict if targeting the MCU complex will be advantageous or not (Fig. 1). Additionally, many studies still feature only a handful of cell lines that do not fully recapitulate disease heterogeneity or function in vivo. This does not mean that the only path forward is to dissect the role of the MCU complex and each regulator in each cancer type, stage, environment, and mutational background before there are clinical insights and applications. Instead, there is a possibility to build on the knowledge gained from the MCU complex so far and focus future studies on research areas that show a more universal response. For example, as we have seen for breast cancer, aberrant expression of MCU and its key regulators is an indicator of poor prognosis; if combined with HIF1α- or p53-related information, results could be even more robust and predictive. Targeting MCU could also potentiate current therapeutic strategies to prevent resistance and this is a research area that still needs attention. The recent discovery of MCU's structural makeup, combined with further chemical engineering of known, but also of new specific compounds targeting the MCU complex, should help in such future endeavors and will hopefully pave the way for exploiting the mitochondrial Ca^{2+} signaling machinery in treating cancer.

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