Review

The Mitochondrial Calcium Uniporter (MCU): Molecular Identity and Role in Human Diseases

Donato D'Angelo and Rosario Rizzuto *

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The Mitochondrial Calcium Uniporter (MCU): Molecular Identity and Role in Human Diseases

Donato D'Angelo [1] and Rosario Rizzuto [2,*]

[1] Department of Biomedical Sciences, University of Padua, 35131 Padua, Italy; donato.dangelo.1@phd.unipd.it
[2] Department of Biomedical Sciences, University of Padua, 35131 Padua, Italy and National Center on Gene Therapy and RNA-based Drugs; rosario.rizzuto@unipd.it
* Correspondence: rosario.rizzuto@unipd.it

Abstract: Calcium (Ca^{2+}) ions act as second messenger, regulating several cell functions. Mitochondria are critical organelles for the regulation of intracellular Ca^{2+}. Mitochondrial calcium ($mtCa^{2+}$) uptake is ensured by the presence in the inner mitochondrial membrane (IMM) of the mitochondrial calcium uniporter (MCU) complex, a macromolecular structure composed of pore-forming and regulatory subunits. $MtCa^{2+}$ uptake plays a crucial role in the regulation of oxidative metabolism and cell death. Many evidences demonstrate that dysregulation of $mtCa^{2+}$ homeostasis can have serious pathological outcomes. In this review, we briefly discuss the molecular structure and the function of the MCU complex and then we focus our attention on human diseases in which a dysfunction in $mtCa^{2+}$ has been showed.

Keywords: MCU; mitochondrial Ca^{2+} signaling; cancer; cardiovascular diseases; metabolic diseases; skeletal muscle diseases; neurodegenerative disorders

1. MCU Complex Structure and Function

The MCU complex is composed of pore forming subunits, i.e. MCU and its dominant-negative isoform, MCUb, the essential MCU regulator (EMRE), which allows interaction with the MICU mitochondrial calcium uptake regulatory subunits (MICU1, MICU2 and MICU3), and possibly other modulators, such as the mitochondrial calcium uniporter regulator 1 (MCUR1) (Figure 1).

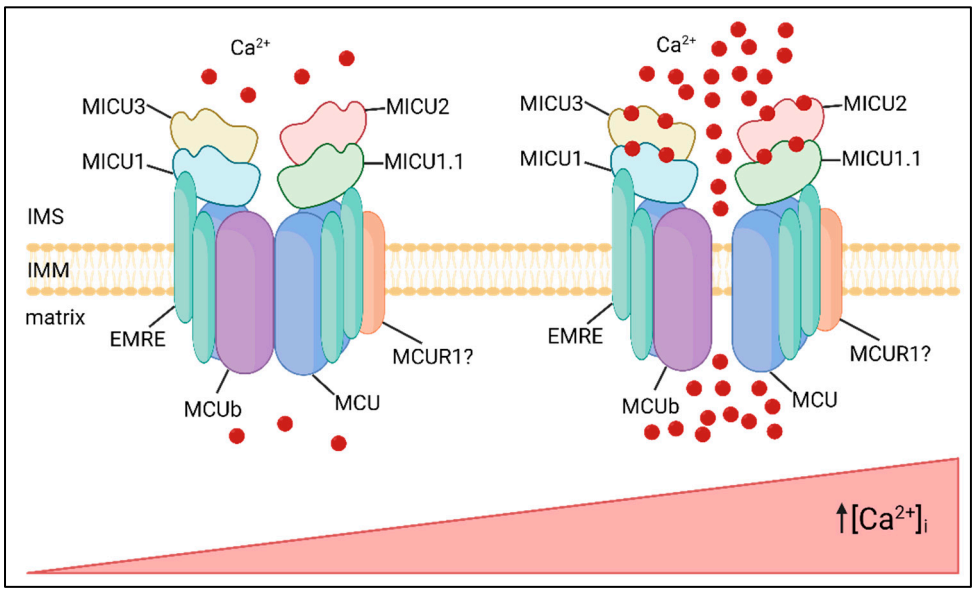

Figure 1. MCU complex composition and activity. The MCU complex localize in the IMM and is composed of pore-forming subunits, MCU and MCUb, regulatory subunits, MICUs family, and the

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structural protein EMRE. The MICUs proteins sense increases in $[Ca^{2+}]_i$ via EF-hand domains, undergoing conformational changes and allowing Ca^{2+} entry into the mitochondrial matrix.

The molecular identity of MCU was discovered in 2011 by two different studies. MCU is a 40 KDa protein, highly conserved and ubiquitously expressed, located in the IMM. These reports show that the downregulation of MCU strongly reduces $mtCa^{2+}$ uptake in cells after Ca^{2+} release from the ER after treatment with IP3-generating agonist. Notably, these changes occur without impinging on mitochondrial morphology and membrane potential. Coherently, MCU overexpression strongly enhances $mtCa^{2+}$ uptake after agonist-induced stimulation [1,2]. Structure analyses revealed that both the N- and C-termini of the protein face the mitochondrial matrix and that MCU contains two transmembrane domains linked by a highly conserved loop located in the intermembrane space, containing a "DIME" motif with negatively charged amino acids critical for Ca^{2+} permeation [3].

Raffaello et al., in 2013 described an alternative isoform of MCU, MCUb, a 33 KDa protein which shares high structural similarity with MCU. However, critical amino acids substitutions in the DIME motif explains why it exerts a dominant-negative effect, reducing the $[Ca^{2+}]$mt rise evoked by agonist stimulation. The opposite effect on $mtCa^{2+}$ uptake was obtained by MCUb silencing, confirming its role as a negative regulator of the channel [4]. The MCU/MCUb ratio varies greatly between different mammalian tissues, and this impinges on the intrinsic capacity of mitochondria of a tissue to rapidly accumulate Ca^{2+}. Not surprisingly, the heart, that undergoes repetitive Ca^{2+} spiking, at the risk of mitochondrial Ca^{2+} overload, displays a high MCUb/MCU ratio, while skeletal muscle ensures maximal and sustained metabolic upregulation in phasic responses though a very low expression of MCUb. In general, the great variability in expression and stoichiometry of MCU and MCUb accounts, at least in part, for the wide differences in the MCU currents measured in different mammalian tissues [5].

The Essential MCU Regulator (EMRE) is a 10 KDa metazoan-specific protein, identified by quantitative mass spectrometry of affinity-purified MCU complex components [6]. It is inserted in the IMM by a single transmembrane domain and is required for the interaction between MCU and MICU1, acting as a bridge between the pore-forming and the regulatory subunits of the complex. Experiments performed on EMRE knockout cells clearly demonstrate that $mtCa^{2+}$ uptake is impaired, phenocopying the effect of MCU silencing. This evidence enlightens the critical role of EMRE as an essential component of the MCU complex, required for its activity [6]. Coherently, reconstitution of the human MCU protein alone in yeast cells is not sufficient for uniporter activity, because the MCU channel is active only if MCU and EMRE are co-expressed [7]. It is not surprising that the proteolytic regulation of EMRE is a finely-tuned process, essential for the formation of a functional MCU complex [8].

MICU1 was the first member of the MCU complex to be identified in 2010. It is a 54 KDa protein located in the IMS where it regulates the activity of the MCU channel in Ca^{2+}-dependent way [9]. Indeed, it contains two EF-hand Ca^{2+} binding domains at its N-terminal sequence. This regulation ensured by MICU1 explains the sigmoidal response to $cytCa^{2+}$ levels of $mtCa^{2+}$ uptake. On one hand, at low $cytCa^{2+}$ concentrations, MICU1 keeps the channels closed and $mtCa^{2+}$ uptake is negligible, thus avoiding a constant entry of Ca^{2+} into the mitochondrial matrix. On the other hand, when $cytCa^{2+}$ concentration increases, MICU1 acts as cooperative activator of MCU, leading to an exponential increase of $mtCa^{2+}$ uptake [10]. An alternative splicing isoform of MICU1, MICU1.1, characterized by the addiction of a short exon (4 amino acids), was shown to be expressed predominantly in skeletal muscle, and at a lower level in the brain. Similar to MICU1, MICU1.1 act as a positive modulator of the channel, leading to even higher increases of $mtCa^{2+}$ uptake after stimulation. Interestingly, MICU1.1 when co-expressed with MICU2 does not show any reduction of $[Ca^{2+}]$ peaks, implying that the MICU1.1/MICU2 heterodimer is less sensitive to negative regulation of MICU2 (see below) [11].

Two paralogs of MICU1 residing in the IMS contributing to the regulation of $mtCa^{2+}$ handling: MICU2 and MICU3 [12]. MICU2 has an expression pattern similar to MICU1, it contains two EF-hand domains and interacts with MICU1 and MCU, forming obligate heterodimers with MICU1 [13]. Interestingly, MICU2 protein stability seems to be dependent on the presence of MICU1. Indeed, MICU1 silencing leads to the loss also of MICU2 [12]. Patron et al., proposed an inhibitory effect of

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MICU2 on MCU activity at low cytCa^{2+} concentration [13], while Kamer et al., proposed that both MICU1 and MICU2 act as gatekeeping of the channel [14]. Notably, another study indicates that MICU2 can regulate the Ca^{2+} threshold of MCU activation [15].

MICU3 is another MICU1 paralog mainly expressed in the nervous system, and to a lower extent in skeletal muscle. Similarly to MICU1 and MICU2, also MICU3 contains two EF-hand domains. Patron et al. demonstrated that MICU3 forms heterodimers exclusively with MICU1, and not with MICU2. Notably, MICU3 has a reduced gatekeeping activity compared to MICU1, mediating more rapid responses to elevated cytCa^{2+} levels. Thus, MICU3 is proposed to be a positive modulator of MCU channel ensuring mtCa^{2+} uptake in response to fast cytCa^{2+} rises, typical of neuronal stimulation [16].

MCUR1 is a 35KDa protein located in the IMM that interacts with MCU [17]. Its downregulation leads to a decrease in mtCa^{2+} uptake and ATP production [18]. In contrast to this view, Paupe et al., showed that MCUR1 is not a regulator of the MCU complex, but it is a cytochrome c oxidase assembly factor [19]. Thus, the precise role of MCUR1 related to mtCa^{2+} homeostasis still remains to be fully elucidated.

2. Cardiovascular Diseases

The dysregulation of mitochondrial calcium (mtCa^{2+}) homeostasis occurring in acute myocardial ischaemia reperfusion (I/R) injury points to a critical role of MCU in cardiovascular diseases. Upon reperfusion, mtCa^{2+} overload leads to excessive reactive oxygen species (ROS) production, promoting the opening of the mitochondrial permeability transition pore (PTP), which triggers cardiomyocyte death [20]. Thus, MCU was conceptually expected to be involved in ischemia-dependent sensitization of cardiac muscle to reperfusion damage.

The analysis of hearts from MCU knockout mice showed not only unaffected basal cardiac parameters, but, surprisingly, were not protected from ischemia/reperfusion damage, as expected of a role of mitochondrial Ca^{2+} loading upstream of permeability transition pore (PTP) opening. In addition, the hearts of $MCU^{-/-}$ mice were not protected by treatment with the pore desensitizer cyclosporin A (CsA) [21]. The same lack of protection was also observed in a transgenic mouse model in which MCU downregulation was achieved by the overexpression of a dominant-negative form of MCU (DN-MCU) [22]. Overall, these results suggest that constitutive ablation of MCU (and hence loss of mtCa^{2+} signals) from the embryonic phase could lead to mitochondrial compensatory mechanisms (e.g. in sensitivity to Ca^{2+} or modulators of PTP or prevalence of other cell death pathways).

To overcome this problem, a mouse model with acute deletion of MCU in adult cardiomyocytes was generated [23,24]. In contrast to constitutive MCU deletion, the conditional knockout model showed protection from I/R injury and cell death, thus confirming the view of MCU-dependent dysregulated Ca^{2+} signals upstream of PTP opening and myocardial cell death.

Interestingly, MCUb and mNCLX gene expression increases after ischemic damage to the heart, and their overexpression, that leads to a decrease in mtCa^{2+} by limiting the uptake or enhancing the efflux respectively, is protective against I/R injury [25,26].

In this contest the role of MICU1 is still controversial. Indeed, while on one hand it is protective in early stages after reperfusion since its knockdown worsens the I/R damage, on the other hand MICU1 is also found to be upregulated during the late stages after reperfusion. However, the mechanism behind this increase is still not clear [25,27].

Together, these findings highlight the relevance of MCU modulation as potential therapeutic approach in the treatment of cardiovascular diseases. However, further studies are needed to translate these findings in a clinical approach.

3. Metabolic Diseases

Normal blood glucose concentrations are ensured by glucose-induced insulin secretion from pancreatic β-cells [28]. In this process mitochondrial oxidative metabolism plays a key role. Indeed, an increase in cytosolic ATP level [29] results in the closure of ATP-sensitive K^+ channels (K_{ATP}) [30],

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leading to plasma membrane depolarization. This in turn promotes the opening of voltage-gated Ca^{2+} channels, allowing Ca^{2+} entry into the cell, ultimately leading to insulin release [31].

Considering the critical role of mitochondrial oxidative metabolism in glucose-induced insulin secretion, a properly functional MCU complex is required in pancreatic β-cells for the correct functioning of this process [32]. ATP rise upon glucose stimulation is impaired when MCU is downregulated [33], resulting in a decrease in insulin secretion [34]. Interestingly, not only the pore-forming subunit MCU, but also the regulatory subunit MICU1 is important for insulin secretion. Similar to MCU silencing, MICU1 downregulation leads to a decrease in ATP levels and glucose-induced insulin secretion in pancreatic β-cells [34]. Surprisingly, the strongest reduction in insulin secretion in β-cells is observed when the regulatory subunit MICU2 is downregulated [35]. Although the precise mechanism by which different subunits of the MCU complex influence insulin release still needs to be fully elucidated, the critical role of the MCU complex in the secretory function of pancreatic β-cells is undoubted.

MCU complex components are upregulated in insulin-resistant adipocytes and in mouse and human visceral adipose tissue (VAT) in conditions of obesity and diabetes. Interestingly, normal levels of MCU expression are restored in VAT of patients after bariatric surgery-induced weight loss. As for insulin secretion in pancreatic β-cells, also in this scenario not all the MCU complex components behave similarly, but a critical role emerges for MICU1, the only MCU complex component strongly upregulated during the transition from obesity to diabetes [36]. These data suggest a key role of mitochondrial Ca^{2+} dysregulation in obesity and diabetes, highlighting the relevance of MCU as a putative therapeutic target for the treatment of these metabolic diseases.

4. Cancer

A large number of studies have been published in the last decade associating MCU with development and progression of different tumors. This is not surprising, given the multifarious role of $mtCa^{2+}$ on key aspects of carcinogenesis. On the one hand, oncogenes of the Bcl-2 family have been shown to reduce $mtCa^{2+}$ loading by reducing ER Ca^{2+} levels and/or desensitizing the inositol 1,4,5 phosphate receptor (IP3R), thereby impairing apoptosis [37–40]. On the other hand, metastatic tumors have been shown to upregulate MCU, and the consequent increase of matrix Ca^{2+} favors motility and invasiveness, via a ROS/HIF-1α mitochondria-to-nucleus signaling pathway [41,42] (Figure 2). In the large number of reports highlighting a role for MCU is cancer, heterogeneity is thus the key word, not only between different cancer types, but also between different cell lines of the same cancer.

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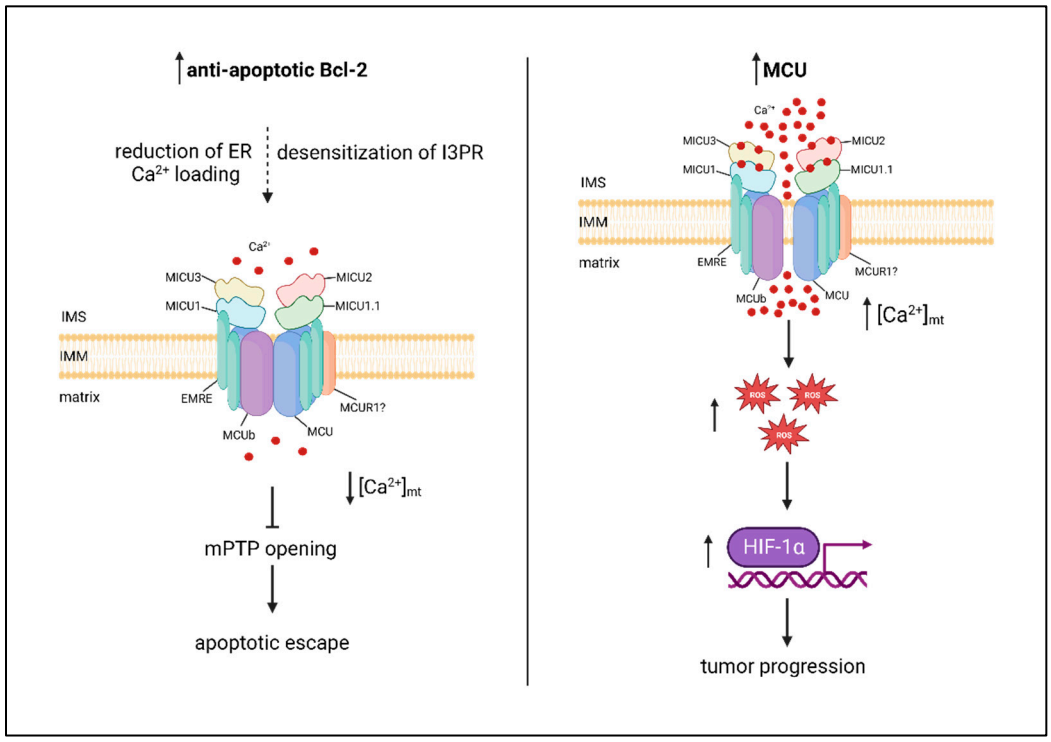

Figure 2. MtCa²⁺ dysfunctions in cancer. Left panel: anti-apoptotic Bcl-2 proteins reduce mtCa²⁺ loading, by reducing ER Ca²⁺ loading and/or desensitizing IP3R, impairing apoptosis. Right panel: MCU is upregulated in metastatic tumors, and the consequent increase in mtCa²⁺ uptake promotes tumor progression via ROS/HIF-1α mitochondria-to-nucleus signaling pathway.

4.1. Breast Cancer

The majority of studies about MCU and cancer are related to breast cancer. The picture that emerges from these studies, however, is quite complex, with marked differences in the various cell models [43]. One of the most aggressive breast tumor subtypes is the triple-negative breast cancer (TNBC). Tosatto et al., demonstrated that in TNBC the expression of MCU correlates with tumor size and lymph node infiltration, suggesting a potential role of MCU in tumor growth and metastatic potential. Accordingly, the authors showed that in xenografts models of TNBC, MCU downregulation reduced tumor size and cell motility as well as metastatic infiltrations. Finally, a positive correlation of MCU levels in TNBC with mROS and HIF-1α signaling was revealed [41]. Recently, a novel mechanism was proposed to explain the link between MCU and TNBC progression. Filadi et al., found that spontaneous and sustained inositol 1,4,5-trisphosphate (IP3)-dependent Ca²⁺ oscillations occurs in TNBC cells and they are associated with the regulation of fatty acid (FA) metabolism. The modulation of mitochondrial FA metabolism mediated by Ca²⁺ concentration has a deeply effect on TNBC cell migration, although further studies will be needed to get full mechanistic insight [44]. However, the critical role of MCU regulation of cell metabolism in supporting tumor growth and proliferation was recently further confirmed by Fernandez Garcia et al. [45].

The role of MCU in breast cancer was also studied in another widely used breast carcinoma cell line, MDA-MB-231. Curry at al., showed that MCU downregulation in this model did not cause changes in proliferation or viability. Interestingly, distinct effects of MCU downregulation were observed on cell death pathways. The authors showed that MCU silencing increased the cytotoxicity in MDA-MB-231 cells only in a caspase-independent way. Indeed, cell death was increased only when MCU silencing was accompanied by a treatment with ionomycin, while no effects were observed in combination with the Bcl-2 inhibitor ABT-263, initiator of caspase-dependent cell death [46]. In this case, similarly to data obtained by the same authors in other cancers, the authors postulate that impaired mtCa2+ uptake reduces Ca2+ buffering, and thus favors Ca²⁺-dependent apoptotic pathways.

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A way to induce cell-death through MCU modulation was also investigated by De Mario et al., in a study that identified positive and negative MCU modulators. Among the negative modulators, benzethonium emerged as an effective compound. In MDA-MB-231 cells, benzethonium, by negatively modulating MCU, delays tumor cell growth and migration [47]. Conversely, another recent study showed that MCU upregulation can be critical for MDA-MB-231 cells survival. Xue et al. showed that MCU is required for the pro-apoptotic effect of RY10-4, a protoapigenone analog, on MDA-MB-231 cells. Treatment with this compound causes a strong MCU upregulation which results in $mtCa^{2+}$ overload and finally apoptosis [48].

Interestingly, similarly to TNBC cells, also in MDA-MB-231 cells MCU levels correlate with metastasis and invasion and this was correlated with an effect on SOCE-dependent breast cancer cell migration, since MCU silencing abolished thapsigargin-induced SOCE activity [49]. Hall et al., further confirmed that poorer disease outcome was associated with MCU overexpression. Interestingly, the opposite effect was observed with MICU1, since its downregulation was associated with a negative disease outcome. Surprisingly, contrary to what was observe in TNBC cells, downregulation of MCU did not affect ROS production and that MCU was dispensable for clonogenic cell survival of MDA-MB-231 cells [50]. Finally, Yu et al., demonstrated that MCU overexpression in MC7F, a human breast carcinoma line, increase the invasiveness and metastatic potential of these cells [51].

4.2. Pancreatic Cancer

In hepatocellular carcinoma cells (HCC) cells, HINT2 was shown to promote cell death via apoptosis, however the molecular mechanism remained elusive [52]. In pancreatic cancer, HINT2 also promotes cell death, and Chen et al., showed that treatment with ruthenium red, an inhibitor of MCU, reduced HINT2-dependent induced apoptosis. Interestingly, overexpression of HINT2 increases the expression of EMRE and decreases the expression of MICU1 and MICU2. The authors hypothesized a possible mechanism in which a constitutively active channel, lacking the regulation of the MICUs subunits, could account for $mtCa^{2+}$ overload and consequent cell death [53]. In line with this study, Xie et al., showed that overexpression of EMRE positively correlates with pancreatic ductal adenocarcinoma (PDAC) prognosis [54]. Recently, elevated $mtCa^{2+}$ uptake was associated to metastasis formation in PDAC. The effect of MCU in PDCA metastasis is through the activation of the KEap-Nrf2 antioxidant program [55]. Overall, these studies suggest that MCU plays a role also in the pathogenesis of pancreatic cancer.

4.3. Colon Cancer

Marchi et al., identified miRNA-25, a cancer-related MCU-targeting microRNA. Experiments performed on HeLa cells, showed that miRNA-25 overexpression decreases $mtCa^{2+}$ uptake, without affecting cytosolic Ca^{2+} levels. The expression of this miRNA is upregulated in human colon cancers, where MCU expression is accordingly downregulated, and this correlates with apoptotic death resistance [56]. However, recently Yu et al., proposed that the lncRNA CERS6 antisense RNA 1 (CERS6-AS1) promotes colon cancer progression via upregulation of MCU [57]. Recently, another miRNA was found to target MCU in colorectal cancer (CRC), miR-138-5p. In CRC, miR-138-5p is downregulated, increasing MCU expression [58]. A further study by Zeng et al., showed that $mtCa^{2+}$ uptake promotes colorectal cancer progression, through the interaction between RIPK1, a signaling molecule essential for cell survival, and MCU [59]. Although further studies are needed to clarify the role of $mtCa^{2+}$ in colon cancer progression, these studies underly the importance of miRNA and lncRNA, instead of MCU directly, as possible target to modulate MCU activity in pathological contests.

4.4. Hepatocellular Carcinoma

Ren et al., demonstrated that in HCC cells the expression of MCU is enhanced, MICU1 is downregulated, while MICU2, MCUb and EMRE expression level is unaffected. Moreover, MCU

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upregulation is associated with poor survival and metastasis in HCC patients. The authors showed that the strong increase in $mtCa^{2+}$ uptake promotes ROS production by downregulating NAD+, sirtuin3 (SIRT3) and superoxide dismutase 2 (SOD2) activity. High ROS levels, in turn, stimulates metalloproteinase-2 activity increasing cell motility [60]. In a second publication by the same group it was showed that also the regulator of MCU complex, MCUR1, was enhanced in HCC cells. The consequent increase in $mtCa^{2+}$ uptake lead to increase ROS production and ROS-dependent p53 degradation, promoting cancer cell survival [61]. Another study investigating the expression profile of long noncoding RNA in sub-lethal heat-treated HCC cells, showed a downregulation of MCU. This model characterizes a transition zone of radiofrequency ablation (RFA), a treatment insufficient to kill all tumor cells, leading to residual cancer occurrence [62]. Further studies are needed to understand the contribution of MCU in this model.

4.5. Other Cancer Types

An emerging role of MCU in many other cancer types is emerging in the recent years.

A recent work by Stejerean-Todoran et al., enlightened the role of the MCU complex in melanoma, showing that silencing of MCU suppresses melanoma cell growth, but it promotes cell migration and invasion reducing the sensitivity to immunotherapies [42].

High levels of MCU expression were also found in ovarian cancer, and its silencing reduces ovarian cancer cells proliferation and migration. This was correlated to a decreased ROS production [63]. Similarly, reduction of MCU expression in renal cell carcinoma, through overexpression of EFHD1, a negative regulator of MCU activity, leads to reduction in cell migration and metastatic potential [64].

5. Skeletal Muscle Diseases

The molecular identification of MCU was followed by intensive studies on skeletal muscle aimed at characterizing the role of $mtCa^{2+}$ homeostasis in this tissue characterized with a specific physiology and Ca2+ signaling repertoire [65]. The study of the first global MCU knockout mouse model exhibited the most prominent alterations in the skeletal muscle. As expected, in this model both resting $mtCa^{2+}$ concentrations and stimulated $mtCa^{2+}$ uptake were reduced. These alterations caused an impairment in mitochondrial oxidative metabolism with an increase in the phosphorylation level of pyruvate dehydrogenase, leading to a reduction in TCA cycle activity. The defective mitochondrial energetic control is responsible for the reduction in exercise performance and muscle force [21]. Mammucari et al., studied the role of MCU in adult skeletal muscle, avoiding compensatory effects that can be present in the global knockout model. MCU expression was modulated through silencing and overexpression in vivo: overexpression of MCU caused muscle hypertrophy, while the silencing of MCU led to muscle atrophy. Interestingly, the control of muscle size and trophism by MCU, observed in both developing and adult muscles, did not depend on the effect on aerobic metabolism, but on the regulation of two major pathways of skeletal muscle hypertrophy, IGF1-Akt and PGC-1α4 [66]. To further characterize the role of MCU in skeletal muscle physiology, Gherardi et al., generated a skeletal muscle-specific MCU knockout, characterized by myofiber-specific impairment of $mtCa^{2+}$ uptake. This triggered a decrease in muscle exercise performance and force, and a fiber-type switch, from slow to fast MHC expression. Notably, loss of MCU rewired skeletal muscle metabolism toward fatty acid oxidation [67] (Figure 3).

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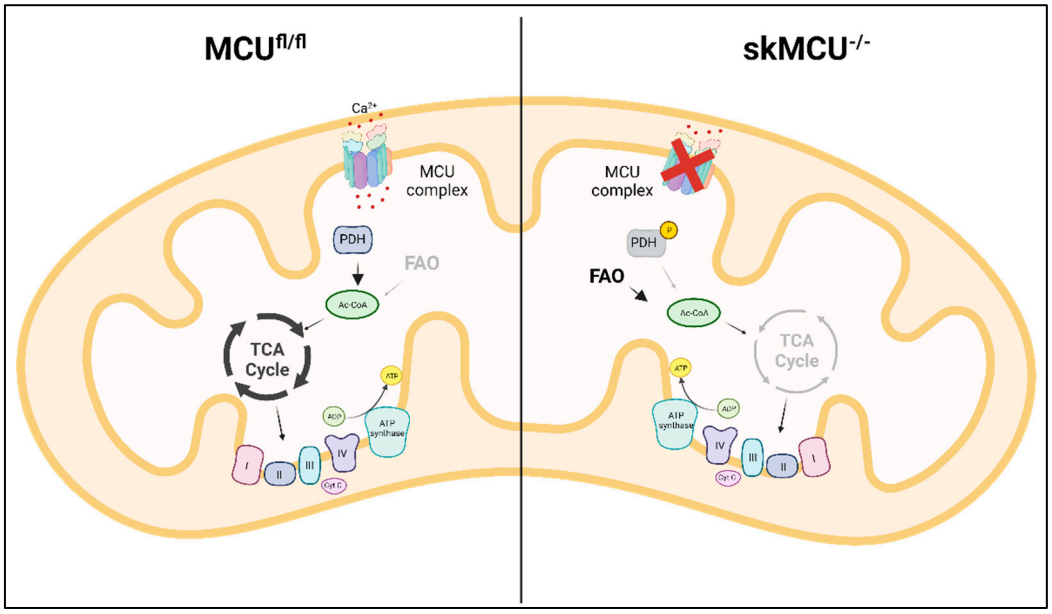

Figure 3. MCU deletion in skeletal muscle rewires metabolism towards FA utilization. In MCU$^{-/-}$ muscles the loss of mtCa^{2+} uptake leads to PDH inactivity, impairment in pyruvate oxidation and decrease in TCA cycle activity. The impairment in glucose oxidation is partially compensated by an increase in FA oxidation.

Interestingly, another MCU complex component, MCUb, the dominant-negative form of MCU, plays a key role during skeletal muscle regeneration, by modulating macrophage-driven stimulation and differentiation of satellite cells after muscle damage. In particular, MCUb was shown to drive macrophage polarization from the pro-inflammatory phenotype to the anti-inflammatory phenotype, with secretion of cytokines that promote satellite cells differentiation and fusion [68].

The role of the MCU complex in skeletal muscle physiology is critical, and mutations in MICU1 gene were reported in human patients with a disease phenotype characterized by learning difficulties, a progressive extrapyramidal movement disorder and learning difficulties. Clinically, the disease was characterized by early onset proximal muscle weakness, intellectual impairment and elevated levels of serum creatine kinases. At the genetic level, different loss-of-function mutations were found, resulting in the loss of MICU1 protein. This leads to in increased mtCa^{2+} load, increasing sensitivity to cell death stimuli but also resulting in lower cytoplasmic Ca^{2+} level, potentially impinging on muscle contraction and synaptic transmission [69]. To further understand the mechanisms behind this neuromuscular disease, Debattisti et al., characterized patient cells and skeletal muscle-specific MICU1 knockout mice. Lack of MICU1 was associated with a low threshold for MCU-mediated Ca^{2+} uptake. Notably, MICU1 loss causes muscle atrophy and a decrease in force. The alterations in mtCa^{2+} uptake during sarcolemma injury, leads to an ineffective muscle repair [70]. Recently, the potential other side of the coin, the neural pathogenesis, was characterized by Singh et al. They generated a neuron-specific MICU1-KO mouse model showing progressive motor and cognitive degeneration. MICU1-KO neurons are more susceptible to mtCa^{2+} overload and cell death, and this is reverted by the inhibition of the mPTP [71].

MICU1 was shown to be critical also in another skeletal muscle disorder, the Barth syndrome, characterized by cardiolipin deficiency. Ghosh et al., utilized several Barth syndrome models including yeast, mouse model, and patient cells, and showed that cardiolipin is required for the stability of MICU1, which is reduced in Barth syndrome patient-derived cells, together with MCU and MICU2. The reduction in mtCa^{2+} uptake results in reduced mitochondrial respiration [72].

Finally, mtCa^{2+} uptake was found to be critical in embryonal rhabdomyosarcoma (ERMS). Indeed, MCU expression is upregulated in ERMS and its downregulation causes mROS level decrease, and an increased propensity to differentiate, inhibiting the oncogenic phenotype [73].

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6. Neurodegenerative Diseases

Neurodegenerative diseases comprise a large group of heterogeneous disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis, that are all characterized by selective cell death of neuronal subtypes. A common feature of these disorders is $mtCa^{2+}$ overload, that can trigger the opening of mPTP, leading to cell death [74].

6.1. Alzheimer's Disease

The pathogenic mechanisms of Alzheimer's disease (AD) are still poorly characterized, but a hallmark is the aberrant processing of the amyloid precursor protein (APP) mediated by γ-secretase. The catalytic components of γ-secretase, presenilin-1 and -2 are enriched in the mitochondria-associate ER membranes (MAMs) in cell models of AD [75]. Zampese et al., showed that overexpression and the silencing of presenilin-2 modulate the transfer of Ca^{2+} between ER and mitochondria. In particular, overexpression of presenilin-2 mutants found in familial AD increased the physical interaction between ER and mitochondria, increasing $mtCa^{2+}$ entry [76]. Cheung et al., showed that familial AD presenilin-1 and -2 mutants interact with 1,4,5-trisphosphate receptor (InsP3R) to promote Ca^{2+} release from ER [77]. Importantly, this leads to $mtCa^{2+}$ overload and an increase in the open probability of mPTP [78]. Notably, the pathogenesis of familial AD was ameliorated in mouse model with the suppression of InsP3R-mediated Ca^{2+} signaling [79]. Interestingly, amyloid-beta and prion peptides can also induce the release of Ca^{2+} from the ER [80]. Reduction of NCLX activity, another way to increase the susceptibility to Ca^{2+}-induced cell death, was found in AD neurons, supporting the notion of $mtCa^{2+}$ overload as crucial in AD progression [81].

6.2. Parkinson's Disease

Parkinson's disease (PD) is a neurological disorder associated with the loss of dopaminergic neurons in the substantia nigra, characterized by typical motor symptoms including tremors and muscle rigidity [82]. Models of PD caused by alpha-synuclein overexpression show excessive $mtCa^{2+}$ uptake, enhancing ROS production and impairing oxidative metabolism [83]. Mutations in PINK1 are also causative of PD, and Gandhi et al., investigated the alterations in $mtCa^{2+}$ homeostasis in PINK1-deficient neurons. PINK1 deficiency causes $mtCa^{2+}$ overload, increasing ROS production and impairing respiration, resulting in neuronal cell death [84]. Genetic and pharmacological inactivation of MCU in a pink1-mutant zebrafish prevents dopaminergic neuronal cell death via rescue of mitochondrial respiration [85]. Interestingly, also mutations in the E3 ubiquitin-ligase Parkin cause familial PD, and Matteucci et al., showed that Parkin is required for the proteasome-mediated degradation of MICU1. Probably in indirect way, also MICU2 stability was affected upon Parkin overexpression. This study suggests that Parkin loss could contribute to the impairment in $mtCa^{2+}$ handling through its regulation of MICUs proteins [86]. Finally, mutations in leucin-rich repeat kinase 2 (LRRK2), a common genetic cause of PD, increased the expression of MCU and MICU2, enhancing $mtCa^{2+}$ uptake in cortical neurons and familial PD cells [87].

6.3. Huntington's Disease

Huntington's disease (HD) is a progressive neurodegenerative disorder in which the huntingtin gene is expanded by CAG triplet repeat leading to a N-terminal polyglutamine strand of variable length [88]. Panov et al., showed a reduction in $mtCa^{2+}$ uptake in HD cells from patients together with a reduction in membrane potential [89]. However, the susceptibility to mPTP opening is increased in mitochondria isolated from HD cells [90]. Another study conducted on striatal neurons of HD showed that mitochondria were unable to handle large Ca^{2+} loads, maybe for the increasing sensitivity to mPTP, which reduces membrane potential leading to Ca^{2+} release [91]. Further studies are needed to elucidate the contribution of $mtCa^{2+}$ in the generation and progression of this disease.

6.4. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal motoneuron disease characterized by muscle weakness, atrophy and eventually paralysis that leads to death [92]. The role of $mtCa^{2+}$ in ALS changes during the progression of the disease. Indeed, in cultured embryonic motor neurons from ALS mouse model, MCU is upregulated and its pharmacological inhibition is protective against excitotoxicity. Instead, MCU expression is reduced in motor neurons from symptomatic ALS mouse model [93]. However, another study showed that in motor neurons of ALS end stage, MCU and MICU1 were upregulated [94]. Thus, a different MCU modulation can be protective depending on the distinct phase of the disease.

7. Conclusions and Future Perspectives

In summary, since the molecular identification of MCU, research on this essential signaling component of mitochondria has literally boomed, with several hundreds of papers providing a deeper insight into the association with other proteins, the regulation of its activity and its role in the physiology of a broad variety of tissues. It has become clear that the essential constituent of the pore region, MCU itself, is part of a larger complex, and that the molecular composition of the latter (i.e. the presence of the dominant-negative MCUb pore subunit, or the identity of the associated regulatory elements) provides wide flexibility to the molecular machinery of a specific cell type, in agreement with the physiological properties of the cell. It is also becoming increasing clear that alterations of this finely tuned process have effects on events as diverse as metabolism, cell death and inflammation. In this review, we have summarized the increasing evidence associating dysregulation of MCU with the pathogenesis of diseases of high prevalence and social impact. While mechanistic insight in most cases still needs to be fully elucidated, these data highlight the mitochondrial calcium signaling machinery as a promising pharmacological target and suggest that the clarification of the role of the individual molecular components may lead to new drugs of high precision in these diseases.

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