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The Role of MCU Expression Regulation in Pathogenesis

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1. Abstract

Mitochondrial calcium uniporter is an intensively investigated calcium channel, its molecule components, structure features and gene encoding have been explored for a long time. Through the researches, the further findings illustrate that mitochondrial calcium unidirectional transporter (MCU) is a macromolecular complex related to regulate the calcium of Intracellular and extracellular. BasedΩ on our current understanding, MCU is crucial for maintaining the cytosolic Ca^{2+} homeostasis by modulating mitochondrial Ca^{2+} uptake. The dysfunction of MCU is confirmed to cause the disorder of intracellular metabolic pattern and cell death. Overexpressing MCU promotes the development of several types of cancer and also aggravates I/R injury, neuron apoptosis with mitochondrial Ca^{2+} overloaded. Besides, muscle atrophy and insulin-assistant adipocyte are also found to be relevant to MCU. In this review, we will summarize the MCU architecture, its regulation subunits, mechanism of MCU in pathogenesis and its expanding roles in osteolysis to provide a new possible target to reverse concerned diseases.

2. Introduction

Anterior researches have revealed that mitochondria regulates intracellular and extracellular calcium concentration and signaling, involved in a series of physiological and pathological processes including energy metabolism, signaling regulation, smooth-muscle contractility, cell proliferation, cell death, and so forth. Calcium's permeation into cellular is driven by the electrochemical gradient between the external space and matrix space [1]. Mitochondrial calcium uptake is mediated by a highly selective calcium channel which is localized to the inner mitochondrial membrane (IMM) called the mitochondrial calcium uniporter (MCU). The calcium has an extremely high affinity with the MCU, which substantially exerts the property of MCU [2]. Nevertheless, the precise and specific mechanism underlying MCU's function still remains elusive. There have been lots of studies that attempt to illustrate the exact structure and function of mitochondrial calcium uniporters. As the experiments progressing, it has been found that the uniporter is a complicated protein complex in humans, which consists of four essential elements: MCU (located at the mitochondrial inner transmembrane for ion conducting and being inhibited by Ru red or Ru360 [3]), MICU1 and MICU2 (two vital regulators that local-

ized to the intermembrane space as gatekeeper [4,5]), EMRE (an essential membrane crossing subunit prompting the entry of calcium [4]). As is well known that Ca^{2+} participates in various signaling pathway inducing multiple cellular processes. Nevertheless, the attributions of calcium uptake by mitochondria are concluded to three main aspects: 1) maintenance of cellular metabolic homeostasis between the cytosol and the mitochondria, 2) mediation of cCa^{2+} dynamics, 3) modulation in various cell death pathways of apoptosis and necrosis [6-8]. The Ca^{2+} enters into mitochondrial matrix under the large gradients across membrane to take part in the tricarboxylic acid cycle(TCA) through dehydrogenase to regulate the generation of ATP which is crucial to the responses required for energy like muscle contraction, exocytosis, biosynthesis, and neuronal signaling [6]. And Mitochondria has engaged in attenuating the acceleration of cytosolic calcium through the transportation of calcium when a transient influx of Ca^{2+} from intracellular stores and extracellular sources. Moreover, it is also vital for function of the current in the condition of weak intracellular Ca^{2+} buffering [7]. Cells killing is proceeded by constantly increasing mitochondrial free Ca^{2+}, resulting in the overloading of Ca^{2+} that ultimately attributes to intervention of ATP generation and appearance of mROS [8]. Taken together, MCU functioning as a calcium unidirectional transporter has been demonstrated to affect multiple cell types activities especially pathological processes. Plenty of experiments have been performed to manifest that MCU modulates the development of various cancers [9], I/R injury [10], neuron apoptosis [11], muscle atrophy [12], abnormal adipocyte metabolism [13] via calcium transition. Here we are about to analyze the architecture and regulation factor of MCU and reveal its vital role in pathogenesis.

2.1. The Structure of Mitochondrial Calcium Uniporter

MCU is defined to be an inward rectifying current, universally expressed in all eukaryotes except in yeast which have no homologs of the uniporter components to exhibit uniporter property. Because of this characteristics yeast becomes an excellent heterologous expression system by reconstruction of MCU. A mCa^{2+} uptake response has been evoked after reconstruction, suggesting that MCU is the pore-forming subunit of the uniporter complex [14]. Electrophysiological studies have demonstrated that MCU as an ion channel shows an essential selectivity and high affinity of Ca^{2+}, but it is sensitive to Ruthenium Red (RR) and its analog Ru360 [2]. The computational analysis provides a hypothesis of ion channel topologies of MCU pore-forming domain which includes four identical subunits composed of two transmembrane helices (TM1 and TM2) that separated by a conserved linker. The linker, facing the intermembrane space, is a short stretch of amino acid and contains a motif called “DIME(Asp-Ile-Met-Glu)”. Besides, the structure of MCU comprise two coiled-coils and N-terminal domain [3,15]. In recent years, researchers have applied Cryo-EM to further study the precise architecture. The overall structure is in accordance with the hypothesis as we predicted previously. MCU shows exactly a tetrameric architecture by means of EM under both high and low concentration of calcium. TM1 and CC1 form a long and continuous helix at the periphery of the channel, while the TM2 helices line the central symmetry axis. There is a short helix that is defined as junctional helix (JH), situated almost perpendicular to TM1. JH forms a junction between TM2 and CC2. In the CCD, CC1 and CC2 form a dimeric coiled-coil, resulting in four dimeric coiled-coils within the tetramer. Follow the coiled-coils domain, the NTD, comprising six β-strands (β1 to β6) and two α-helices (α1 and α2) that form the central core with two highly conserved leucine rich loops [16], is directly connected to CC1 [17]. The structure of NTD forms MCU oligomers and the deletion of NTD shows a rapid decline of mCa^{2+} with complete cCa^{2+} dynamics [16]. A residue of NTD called MCU-regulating acidic patch (MRAP) binds divalent cations to autoregulated Ca^{2+} uptake by matrix Ca^{2+} and Mg^{2+} occupying the MRAP domain just like feedback mechanism [18]. The strictly conserved sequence motif existing in all MCU homologs has been demonstrated to shape the selectivity filter of Ca^{2+}. This sequence motif is located in the N-terminal region of TM2, with the carboxylate side chains of conserved acidic residues Asp and Glu from each protomer pointed into the central symmetry axis, respectively forming two acidic rings along the channel pore. Asp is located at the intermembrane end of TM2, its carboxylate ring could co-ordinate a hydrated Ca^{2+}, while the diameter of Glu carboxylate ring is too small for hydrated Ca^{2+} to go through, suggesting that only dehydrated Ca^{2+} can be allowed to pass through [17, 19, 20]. Consequently, we have enough reason to deduce that Glu carboxylate ring is unsubstitutable to the selective permeation.

2.2. The Regulation Subunits of MCU

It has been generally accepted the main regulation subunits centered around the Ca^{2+} channel protein is MICU1, MICU2, MCUR1, MCUB and EMRE. MICU1, co-occurrence with MCU in mammalian but absent from most fungi, is a peripheral membrane protein with two EF-hand motifs and functions as calcium-sensing regulators [21]. MICU1 interacts with MCU through the D-ring of DIME motif to constitute the conserved unit of a eukaryotic uniporter. It is not until cCa^{2+} rises above 3 μM that the gate-keeper MICU1 will activate MCU channel through dissociating from MCU, thus making it in open confirmation [22]. To prove it, genetic deletion of MICU1 was performed and the results is complemented to assumption that the mCa^{2+} increased even at low cCa^{2+} and simultaneously caused mCa^{2+} overload [23]. Moreover, when MICU1 was knockdown, the sensitivity of Ru Red/Ru 360 to MCU has increased while calcium uptake has remarkably decreased, proving that MICU1 probably competes with Ru Red/Ru 360 by the site of pore-forming domain [5, 24].

MICU2 is a paralog of MICU1 sharing the similar function as a gatekeeper which assist MCU to take up calcium from cytoplasm. Silencing MICU2 by RNAi reveals a reduced rate of mCa^{2+} uptake which signifies that MICU2 is dispensable to mCa^{2+} uptake [25]. Importantly, utilizing transcription activator-like effector nuclease (TALEN) technology to knock out MICU1 and MICU2 in HEK293 T cells displayed a phenomenon that there was a steep Ca^{2+} uptake by MICU1 KO mitochondria due to the loss of gate-keeping. MICU2 KO mitochondria exhibited a decreased rate of Ca^{2+} influx [26]. It follows that MICU2 possibly has a positive effect on co-operating with MICU1 to achieve the function of gatekeeping. Except the MICU1, hetero-oligomers of MICU1 and MICU2 also interact with MCU to control the gating and cooperative activation of the uniporter. Whereas, the respective function and mechanism of each subunits in mediating the activity of uniporter is complicated in mammalian system because of the differences ranging from the degree of gene silencing, tissue-specific protein composition [27, 28] from stoichiometry, and compensatory remodeling of the channel [23, 29].

By contrast, MCU regulator 1 (MCUR1), a positive regulator of the channel, exerted blunt MCU current when the transmembrane voltage was clamped, indicating that MCUR1 act directly on MCU to promote Ca^{2+} entry into the mitochondria instead of the reduced electrochemical driving force [30]. Another laboratory finding shows that MCUR1 is an assembly factor of complex IV cytochrome oxidase leading to impaired oxidation phosphorylation in the absence of MCUR1 and certainly declined driving force so that calcium uptake is decreased [31].

MCU proteome analysis reveals that EMRE is a 10-kilodalton protein with EF hand domain whose homologs is found only in metazoans and is ubiquitously expressed in all mammalian's mitochondria. The experiments have exhibited that the knockout of EMRE specifically declines the uptake of calcium while the appearance and proliferation of cells is not affected. Moreover, abundance of other uniplex proteins and their mitochondrial localization also remain still. What has been changed is the association of MCU with MICU1 and MICU2. Hence, we can link the function of EMRE and MICU to calcium-sensing activity [4]. Moreover, it has been proposed that EMRE senses the matrix calcium concentration with the acidic patch at its carboxy terminal to regulate the MCU activity [32]. However, in Tsai et al study, MICU1's interaction with MCU in the absence of EMRE was also displayed by co immunoprecipitation [33].

Recently, MCUB, a paralog of pore forming subunit-MCU, has been investigated whether it has a prominent influence in Ca^{2+} permeation. MCUB is conserved across all vertebrates and absent in species of plants, kinetoplastids, Nematoda, and Arthropoda, where MCU is present [15]. The results of present experiments suggest that MCUB is an important continuance of uniplex and have a negative effect on Ca^{2+} uptake. It has been studied that MCUB dislocates the position of MCU in order to disconnect MCU with MICU1 and MICU2 to alter channel gating. Meanwhile, the presence of MCU and the expression of channel regulators MICU1 and MICU2 rapidly increase after MCUB depletion [34].

2.3. The Modulation in MCU Expression

As the experiments proved, cytosolic Ca^{2+} (cCa^{2+}) signaling is influenced by both high and low cytosolic calcium concentration [24, 34]. MCU, as a core ion channel transmitting Ca^{2+} maybe an effective target to interrupt Ca^{2+} homeostasis and subsequently get involved in various pathological processes. In line with it, regulating the proceeds associated with MCU protein generation including transcription, post transcriptional modification, translation, post translational modification can remarkably influence the expression level of protein to change the interplay between cytosolic Ca^{2+} and mitochondrial Ca^{2+} (mCa^{2+}). The Ca^{2+} regulated transcription factor CREB (cyclic adenosine monophosphate response element) is the binding of MCU promoter. When cCa^{2+} decreases, the phosphorylation of CREB will instantly be initiated to alter the transcription followed the downregulation of MCU abundance and the diminishing of mCa^{2+} [35]. In the post-transcription level, microRNAs (miRNAs) are non-coding nucleotides and can modulate the gene expression by combining with the specific mRNA to degradate target mRNA or restrain translation [36]. There have been valid findings to link miRNA-associated MCU expression with cell survival. The downregulation of MCU RNA and protein by miR-25 is showed to be able to protect the tumor cells from cell death in colon cancer [37]. Furthermore, later studies have utilized anti-miR25 and anti-miR138 applying to pulmonary arterial hypertension (PAH) patients whose MCU expression is down-regulation, and the final conclusion has signified a predictable over-expression of MCU protein [38]. Post translational modifications (PTM) are composed of two predominant forms, namely oxidation and phosphorylation [39]. Previous studies confirmed the findings about PTM of MCU. In the N-terminal domain of MCU there are conserved cysteins, which carried on S-glutathionylation under oxidative stress to reconstruct NTD confirmation that accelerates the sustained activity of MCU and high rate of calcium uptake. In particular, the biochemical gel shift assay finds the evidence that MCU is the only element sensing the mROS. When the inflammation and oxidative stress raise the number of mROS, modification of channel by mROS will bring about mCa^{2+} overload-mediated cell death [18]. In addition, phosphorylation is also proposed to undergo in MCU PTM at the site S57 and S92 of NTD in the presence of Calmodulin kinase II (CaMKII). The implication of further studies reveals facilitated MCU current compared to the control which inhibited the expression of CaMKII [16]. These proceedings highlight the significance of regulating MCU expression and put forward a novel route to interpose cell killing and metabolic

homeostasis.

2.4. The Pathological Processes That MCU Involved In

The intracellular calcium acting as the second messenger is ubiquitously participating in all kinds of cells biological events. Because of the importance of Ca^{2+} in signaling pathway, cCa^{2+} dynamics has been strictly controlled. Dysregulating calcium signaling commonly leads to abnormal cell activities and causing disease. A significant quantity of data indicates that a small amount of calcium entering into mitochondria is beneficial to metabolic homeostasis while a large amount of calcium is considered to arouse cell death. This phenomenon is based on MCU-dependent calcium overloading and the opening of mitochondria permeability transition pore (mPTP) [40]. On one hand, calcium overloading gives rise to disorder of oxidative phosphorylation, thus leading to excessive production of mROS that initiates cell killing. On the other hand, mPTP abruptly allows all solutes of molecular weight up to about 1500 Da to permeate into mitochondria matrix which results in mitochondrial depolarization, uncoupled oxidative phosphorylation and mitochondrial swelling that eventually provokes cell apoptosis and autophagy [41-43]. Additionally, calcium is reported to change the pattern of cellular energy metabolism from glycolysis to fatty acid oxidation, which is commonly presumed to affect cellular function even pathogenesis [13]. Mitochondria has consistently played an irreplaceable role in maintaining the Ca^{2+} homeostasis. With the understanding of MCU increasing steeply, a spectrum of experiments has been performed to reveal the effects of MCU in a myriad of pathological proceeds. Whereas, depending on the different types of cell, the abnormal expression of MCU has completely distinct impact [9].

3. The Role of MCU in Cancer

Evidence that abnormal expression levels or function of MCU complex that has an affinity association with different cancer-related phenotypes, such as prostate cancer, hepatocarcinoma, breast carcinoma, colorectal cancer, and pancreatic cancer [9]. For instance, Yang et al. [44] have reported that MCU is instrumental for the growth of colorectal cancer (CRC). RT-qPCR and western blotting analysis have showed the expression level of both MCU protein and mRNA has markedly upregulated the majority of malignant colorectal tissue compared to adjacent normal tissue and immunohistochemical and kaplan-Meier analysis also validated the results. As a consequence, mitochondrial Ca^{2+} uptake is induced to be augmented without calcium overloading. And the increased mCa^{2+} promotes mitochondrial biogenesis including relative mitochondria component, ATP production-associated protein, mtDNA copy number and so on by enhancing dephosphorylation of TFAM, which is the key factor of mitochondrial biogenesis [45-47].

ROS/NF-κB signaling is well demonstrated to be crucial role in plenty of types of cancer [48, 49]. Overexpression of MCU facilitates protein expression of phosphorylated p65 which is the principle protein in ROS/NF-κB signaling. More importantly, the studies implicate that treatment with H_2O_2 and ROS scavenger respectively reversed the effects of MCU knockdown and MCU overexpression in vivo. It is quite easy to summarize that MCU-mediated calcium uptake stimulates mitochondria biogenesis to expedite CRC growth via ROS/NF-κB signaling.

Triple-negative breast cancer (TNBC), the most aggressive breast carcinoma type, is also influenced by MCU in tumor size, lung metastasis, lymph node infiltration. In Tosatto et al. study [50], the abundance of MCU protein has been persistently increasing while MCUB, the channel isoform, decreases with tumor progressing, that is in line with the uplift of mitochondrial Ca^{2+} uptake. These data show the relationship between TNBC and MCU. In order to further explore the mechanism of MCU in tumor process, MCU deletion of MDA-MB-231 cells model has been created by CRISPR/Cas9 Nuclease RNA-guided genome editing technology. The authors have observed that MCU-silencing obviously hampered tumor growth but not viability, meanwhile lymph node infiltration and lung metastasis have all been impaired. Notably, the protein and mRNA levels of human hypoxia-inducible factor 1α(HIF-1α), main regulator of cell transformation and cancer development [51], simultaneously declined. Later the rescue experiment was carried out by reconstructing HIF-1α in MCU depletion cells. And the MCU-silence mediated tumor migration was reversed by overexpression of HIF-1α, indicating that HIF-1α is the downstream factor of MCU in TNBC progression. Similarly, in hepatocellular carcinoma (HCC) cells, it has also been found the up-expression of MCU and furthermore the down-expression of MICU1 leading to the increased basal mCa^{2+} compared with the control cells. As is well known that electron transport chain will substantially be strengthened with more calcium entering into mitochondria and thus generating additional mROS. In particular, mCa^{2+} uptake increases NAD+ conversion into NADH and downregulates deacetylase activity of sirtuin 3 (SIRT3) making SOD2 inactivate which subsequently lose the capacity of eliminating mROS. As a result, HCC proliferation, cell migration and invasion have been enhanced via ROS-activated c-Jun N-terminal kinase (JNK) pathway with high level expression of relative protein [52]. Beyond these, Marchi et al. [53] have established the link of high mCa^{2+} related prostate cancer with inhibition of mitochondrial permeability transition pore (mPTP) while Arvizo et al. [54] have probed novel roles of MCU in ovarian cancer. As the pivotal function of MCU in cancer development, it can provide an evolutionary therapeutic target to reverse the pathological progress.

4. The Role of MCU in Cardiopulmonary System

In the physiological condition, mCa^{2+} is coupled with cardiac excitation-contraction process [55] and contributes to supply the energy for cardiomyocytes via generation of ATP [56, 57]. However,

Mitochondrial Ca^{2+} overload triggers mitochondrial dysfunction and cell death instead of enhancing energy production by means of opening mPTP causing permeabilization of inner mitochondrial membrane and collapse of electrochemical Potential grad [58]. Owing to the discovery of Ru red and its derivative Ru360 effectively inhibited MCU, numerous cell death models, such as cardiac ischemia-reperfusion (I/R) injury [10] and neuronal excitotoxicity [59] have validated the evidence of its negative effect In the I/R injury mouse model, the abundance of MCU protein has suggested to be increased following high concentration of mCa^{2+}. MCU-mPTP axis mainly regulates the cell death pathway and is abolished by acute MCU ablation, accompanied by greatly alleviated cardiomyocyte death in vivo. Besides, cardiomyocyte contractile capability in response to acute adrenalin stimulation and mitochondrial dehydrogenases activation was discovered to be attenuated in MCU knockout mice which confirms the assumption that MCU is required to work during acute stress while it is dispensable for homeostatic cardiac function [58, 60]. To prove it, there have been diverse conjecture report that the rest/ Basel mCa^{2+} showed no difference in MCU-deletion cardiomyocyte versus controls. In contrast, the mitochondrial Ca^{2+} uptake displayed a rapid attenuation with MCU depletion under acute Ca^{2+} pulse. Likewise, at rest state, mitochondria ATP generation, biogenesis rate, ATP generation, and mROS production were similar in MCU-KO and control group with unaltered basal Ca^{2+} levels while treatment with exogenous Ca^{2+} fiercely increased ATP generation in cells which possess intact MCU. These effects were expected to be blocked by MCU knockout or MCU inhibitor [58]. Wu et al. [61] also observed that the physiological fight or flight heart rate cannot be accelerated but the resting heart rates was unchanged in dominant-negative mice model. These results consistently disclose that MCU plays a vital role in short-term acute stimulation and its effects can be compensated by other calcium pathway in cardiomyocyte system. In the course of ischemia-reperfusion, the imbalance between mitochondria fission and fusion/ mitophagy intervenes the dynamic homeostasis of mitochondria function, resulting in a series of apoptosis cascading effect [62]. Calpains, belonging to calcium-dependent thiol-protease, are activated during (I/R) injury by calcium overload to phosphorylates the dynamin-related protein 1 (Drp1), evoking mitochondrial fission and fragmentation [63]. The investigation has confirmed that MCU inhibition evidently alleviates Drp1 accumulation and enhances mitochondrial LC3II expression, which respectively indicates the occurrence of fission and mitophagy. Additionally, atrophy type 1 (OPA1) expression, which is supposed to dominate mitochondrial fusion and is suppressed in (I/R) injury models, has been recovered when calpain or MCU is repressed. Nevertheless, the downregulation of OPA1 by siRNA transfection surprisingly abrogates the protective effects of calpains suppression in mitochondrial fission/fusion and mitophagy, manifesting that OPA1 mediate MCU-calpains induced mitochondria dysfunction to shrink myocardial infarction size and level of apoptosis [64].

Apart from the property of MCU in cell killing, it also matters in metabolism regulation mediating pathogenesis. There have been findings which revealed that MCU induced pulmonary fibrosis process via metabolic reprogramming. In the study, they found PGC-1α, which increases the enzymatic capacity for fatty acid oxidation (FAO) and abolishes the glycolysis, was augmented in macrophages from idiopathic pulmonary fibrosis (IPF)subjects, whereas the effects of PGC-1α were diminished in dominant-negative MCU model. Since ATF2 is well acknowledged to bind to the CRE domain in PGC-1α promoter under phosphorylation by p38 MAPK to improve the expression of PGC-1α [65-67]. Interestingly, all data has suggested the activation of p38 MAPK was regulated by MCU, which is simultaneously dependent on mROS.

5. The Role of MCU in Neural System

Owing to the key role of MCU in cell death via calcium overload, it causes mitochondrial permeability transition pore (mPTP) activated and thus interrupt energetic metabolism and mitochondrial swelling. Researchers have found that MCU involves in neuron apoptosis, which triggers various neural system diseases. In a rat cerebral ischemic stroke injury model, proline-rich tyrosine kinase 2 (Pyk2) was observed to be activated, responsible for dysfunction of mitochondria and calcium overload, which eventually evoke neuronal apoptosis and brain damage. More importantly, with Pyk2/ MCU pathway inhibited by a Pyk2 inhibitor, mitochondrial Ca^{2+} overload, proapoptotic protein release and even cell death has been obviously prevented [68]. In Matthew et al. study, they have utilized Tamoxifen to induce MCU-knockdown Thy1-expressing neurons model and highly-efficacious protectants of neuron for acute hypoxic/ ischemic brain damage [11]. In addition, Veronica et al. also investigated that mitochondrial Ca^{2+} overload is sufficient to decide neural cell fate by over-expressing MCU both in exosomatic model of mouse primary cortical neurons and endosomatic model of injecting MCU-coding adenoviral particles into mouse brain cortex [69]. Given all that, enlightened by the examination of MCU in ischemia-reperfusion injury of cardiomyocytes, MCU-medicated neuron apoptosis and necrosis which crucially account for neurodegenerative and neural ischemic disease have been universally probed to seek for a novel therapeutic target. Maria et al. [70] and Smijin K et.al [71] have respectively reported the relevance of hereditary spastic paraplegia (HSP7) and Parkinson's disease (PD) to MCU function. As is well known, m-AAA plays an important role in regulating the function of mitochondria. The protein synthesis and respiration in mitochondria will be damaged after inhibiting m-AAA [72]. Especially, m-AAA depletion impairs especially MCU assembly leading to degradation of EMRE subunit which induces massive entry of mCa^{2+} in Purkinje cells

and finally evokes HSP7. And in the pink1-/- zebrafish model of PD, DA neurons with hybridization and immunohistochemical labels have examined to be rescued after deletion of MCU.

6. The Role of MCU in Skeletal Cell

It has been extensively explored in skeletal muscle about energy utilization under both rest and stress state. It has been widely believed that the increased demand of ATP is achieved by entry of mitochondrial calcium. cCa^{2+} transients stimulates a rapid increase of calcium in matrix to promote the activation of Ca^{2+}-sensitive dehydrogenases of the Krebs cycle [73, 74]. The study designed an array of function tests under increased skeletal muscle workload and they revealed the impairment ATP supplement in MCU knockout mice after assessing the skeletal muscle peak performance [75]. Furthermore, skeletal muscle atrophy has been examined to be related to mitochondria calcium homeostasis by silencing and overexpressing MCU. And the findings indicating the trophic effects of MCU-dependent mitochondrial Ca^{2+} uptake on muscles [12]. PGC-1α4, a novel isoform of the mitochondria-related PGC-1α family and IGF1-Akt/ PKB are widely defined to contribute to muscle hypertrophy signaling pathway, which has been largely eliminated with MCU-silence [76]. On the whole, MCU function may represent a viable target in Sarcopenia.

7. The Role of MCU in Adipose Tissue

Several previous studies have reported the enhanced sensitivity of insulin in adipocyte under decreased intracellular calcium concentration [77]. Given the crucial modulation function of calcium, with ER-mitochondria contacting, ER calcium stores can be rapidly transferred to the mitochondria upon demand via the MCU transmission [78].

In insulin-resistant adipocytes, MCU and its relevant components have been observed to be upregulated together with increased mCa^{2+} uptake. The experiments have performed on human IR adipocytes both in vivo and vitro which signified slightly different outcomes. The expression of MCU and MICU1 increased while the other components such as MICU2, were unaltered in vitro situation. In contrast, MCU, MICU1 and MICU2 in vivo experiments all increased but not MCUB, which is so low that it is unable to be accurately measured. Intriguingly, the authors have also noticed that IR-induced mROS and total ROS were diminished by over-expression of MCUB via adenoviral infection (ad-MCUB) which implies protective effects of MCUB against oxidation stress. For this reason, the study further explored the relationship between inflammatory factors and MCU. The data showed that ad-MICUB caused a decreased release of TNF-α and IL-6 indicating that impressively declining MCU-induced calcium uptake remarkably influences the inflammatory cytokine release [13]. These results display a possible role of MCU in participating cell metabolism in adipocyte However, the accurate mechanism is remained to be fully understood.

8. Perspective

With the findings aiming at studying molecular identity, stoichiometry and its regulatory proteins of MCU, precise construction and function of it have been demonstrated. Lots of physiological and pathological processes still remain elusive. Previous studies have already proved the main function of MCU as a unidirectional pore transmitting calcium into matrix. MCU-dependent calcium uptake involved in diversities of intracellular activities. This review emphasizes the relevance of MCU in pathogenesis within different cell types and tissues. The researches in metabolism and cell death induced by mCa^{2+} emerge evolutionary therapeutic target in cancer, I/R injury, neuron apoptosis, muscle atrophy and FAO.

As a result, we may propose an assumption that MCU is also involved osteolysis. It is universally acknowledged that osteolysis involves immune response and osteoclastogenesis (Figure1). Inflammatory cells secret diversities of mediators including nuclear factor-κB (NF-κB), TNF-α, IL-1, IL-1β and IL-6 [79-83]. Importantly, these inflammatory factors not only directly participate in the process of inflammation response, but also in turn can stimulate RANKL expression, augment RANK-induced osteoclastogenesis or inhibit osteoclast apoptosis [84-86]. RANK-induced bone marrow macrophages differentiation evoking osteolysis has commonly been examined. RANKL, which is mostly expressed in osteoblast and stromal cell, combined with RANK, a receptor that exists in osteoclast precursor, to deliver intracellular signaling. Calcium is released from intracellular stores by activated phospholipase C which is a downstream effector of G-protein-coupled receptor, and subsequently triggers calcium-induced calcium release. Calcium as a second messenger activates extracellular signal-regulated kinase (ERK), Akt, p38 and MAPK in osteoclasts precursor [87]. As a consequence, a cascade of transcription factors including NF-κB, AP-1 (c-Fos/c-Jun), and NFATc1 have been activated to upregulate the expression of osteoclastic genes leading to bone absorption [88, 89]. Another important signaling pathway, the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN) and its targets, the NFAT family of transcription factors, have equally been positioned as a master effector of osteoclastogenesis [90]. Moreover, there have been existed outcomes which indicates that alterations in mCa^{2+} uptake substantially decreased the cCa^{2+} transient amplitude, which results in impaired cCa^{2+} signaling [34].

Therefore, we can conclude that Ca^{2+} evidently plays a pivotal role in osteoclastogenesis signaling pathway. If the intracellular calcium homeostasis is destroyed by outside intervention, calcium-dependent osteolysis probably can be attenuated or even eliminated. A universally known inhibitor of MCU is Ruthenium Red or Ru 360, which can bind to the selectivity filtration to directly destroy the function of MCU. More importantly, with further research the investigators can weaken the abundance of MCU expression in

Nucleic acid and protein level to impact the whole cell fate and ultimately present the macroscopic effect. In a word, with early detection and multi-routes therapy targets, osteolysis can be prevented or even reversed.

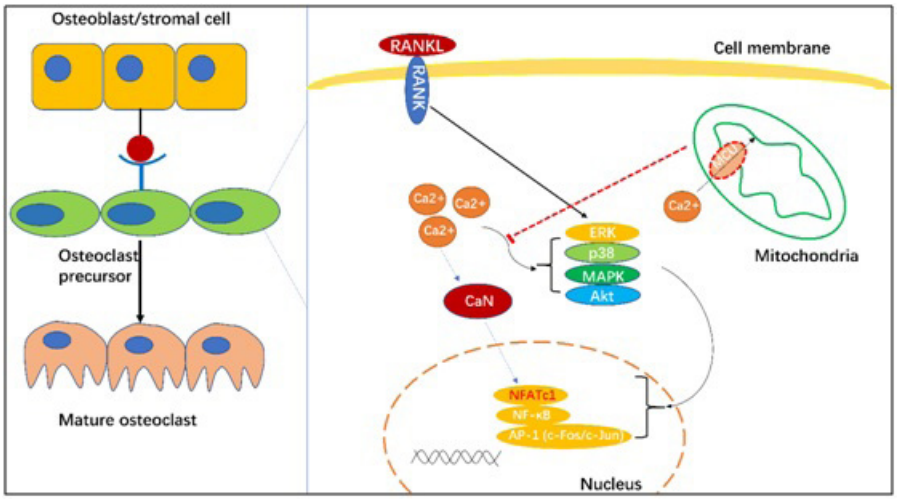

Figure 1: A graphical abstract of mechanism related with RANK-induced osteolysis involved in MCU. RANKL, which is mostly expressed in osteoblast and stromal cell, combined with RANK, a receptor that exists in osteoclast precursor, to deliver intracellular signaling. Calcium as a second messenger activates extracellular signal-regulated kinase (ERK), Akt, p38 and MAPK in osteoclasts precursor, triggering a cascade of transcription factors including NF-κB, AP-1 (c-Fos/c-Jun), and NFATc1 activated to upregulate the expression of osteoclastic genes leading to the generation of mature osteoclast. This process is possibly suppressed by MCU-mediated calcium uptake which impaired cytosolic calcium signaling pathway.

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