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REVIEW ARTICLE

Progress in understanding mitochondrial calcium uniporter complex-mediated calcium signalling: A potential target for cancer treatment

Chaochu Cui[1,2,3] | Jianbo Yang[1] | Liwu Fu[3] | Mingyong Wang[4] | Xianwei Wang[1]

[1] Henan Key Laboratory of Medical Tissue Regeneration, College of Basic Medical Sciences, Xinxiang Medical University, Xinxiang, China

[2] Henan Key Laboratory of Neurorestoratology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, China

[3] State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

[4] School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, China

Correspondence
Xianwei Wang, Henan Key Laboratory of Medical Tissue Regeneration, College of Basic Medical Sciences, Xinxiang Medical University, Xinxiang, Henan, China.
Email: wangxianwei1116@126.com

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Due to its Ca^{2+} buffering capacity, the mitochondrion is one of the most important intracellular organelles in regulating Ca^{2+} dynamic oscillation. Mitochondrial calcium uniporter (MCU) is the primary mediator of Ca^{2+} influx into mitochondria, manipulating cell energy metabolism, ROS production, and programmed cell death, all of which are critical for carcinogenesis. The understanding of the uniporter complex was significantly boosted by recent groundbreaking discoveries that identified the uniporter pore-forming subunit MCU and its regulatory molecules, including MCU-dominant negative β subunit (MCUb), essential MCU regulator (EMRE), MCU regulator 1 (MCUR1), mitochondrial calcium uptake (MICU) 1, MICU2, and MICU3. These provide the means and molecular platform to investigate MCU complex (uniplex)-mediated impaired Ca^{2+} signalling in physiology and pathology. This review aims to summarize the progress of the understanding regulatory mechanisms of uniplex, roles of uniplex-mediated Ca^{2+} signalling in cancer, and potential pharmacological inhibitors of MCU.

1 | INTRODUCTION

Mitochondria play critical roles in cell physiology, including bioenergetics, cell cycle, autophagy, and apoptosis (Jouaville, Pinton, Bastianutto, Rutter, & Rizzuto, 1999; Kroemer & Reed, 2000; Strzyz, 2018; Westermann, 2010). The second messenger, Ca^{2+}, is essential for carcinogenesis and tumour development and, therefore, is a promising target for cancer treatment (Cui et al., 2018; Cui, Merritt, Fu, & Pan, 2017; Monteith, McAndrew, Faddy, & Roberts-Thomson, 2007). Since it was discovered that mitochondria can take in and store large

Abbreviations: $[Ca^{2+}]_c$, cytoplasm Ca^{2+}; $[Ca^{2+}]_m$, mitochondrial matrix Ca^{2+}; ΔΨ(m), mitochondrial transmembrane potential; $^{+}$AuNPs, positively charged gold nanoparticles; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cl-CD95L, cleaved CD95L; COXs, cytochrome c oxidases; CytC, cytochrome c; EMRE, essential MCU regulator; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; HCX, H^{+}/Ca^{2+} exchanger; HINT2, histidine triad nucleotide-binding 2; hMCU, human MCU; IMM, inner mitochondrial membrane; IMS, intermembrane space; IP_3R, inositol trisphosphate receptor; MCU, mitochondrial calcium uniporter; MCUb, MCU-dominant negative beta subunit; MCUR1, MCU regulator 1; MICU, mitochondrial calcium uptake; mROS, mitochondrial ROS; Mfn2, mitofusin-2; mPTP, mitochondrial permeability transition pore; NCX, Na^{+}/Ca^{2+} exchanger; PM, plasma membrane; PRMT1, protein arginine methyl transferase 1; Pyk2, proline-rich TK 2; RIPK1, receptor-interacting protein kinase 1; RuR, ruthenium red; TCA, tricarboxylic acid; TMD, transmembrane domain; TNBCs, triple-negative breast cancers

amounts of Ca^{2+}, it has been well established that most mitochondrial functions are dependent on Ca^{2+} (Deluca & Engstrom, 1961; Drago, Pizzo, & Pozzan, 2011; Hajnoczky et al., 2006; Jouaville et al., 1999; Zeng et al., 2018). Together with the sarcoplasmic/endoplasmic reticulum (SR/ER) and Ca^{2+} channels/pumps located in plasma membrane (PM), mitochondria are vital for the fine tuning of Ca^{2+} oscillation signalling (Figure 1a). Upon Ca^{2+} influx from extra cytoplasm or its release from the SR/ER, mitochondria translocate to the areas close to either the PM or SR/ER. Through this process, mitochondria regulate the activities of corresponding Ca^{2+} channels, and either propagate Ca^{2+} oscillation-encoded signals or restrict Ca^{2+} signalling to specific cellular domains (Clapham, 2007; Glitsch, Bakowski, & Parekh, 2002; Hoth, Button, & Lewis, 2000; Rizzuto et al., 1998; Rizzuto, Brini, Murgia, & Pozzan, 1993; Rizzuto, De Stefani, Raffaello, & Mammucari, 2012; Szabadkai & Duchen, 2008).

There is a growing body of evidence suggesting that the mitochondrial calcium uniporter (MCU) is the primary transporter to mediate Ca^{2+} influx into mitochondria (Figure 1a; De Stefani, Raffaello, Teardo, Szabo, & Rizzuto, 2011; Gunter, Buntinas, Sparagna, Eliseev, & Gunter, 2000). The MCU resides in the inner mitochondrial membrane (IMM) and is a highly selective Ca^{2+} uniporter (Baughman et al., 2011; Chaudhuri, Sancak, Mootha, & Clapham, 2013; Kirichok, Krapivinsky, & Clapham, 2004). Under resting conditions, mitochondrial matrix Ca^{2+} ($[Ca^{2+}]_m$) level is similar to that of cytoplasm (~100 nM), and the MCU is closed, despite the driving force of the mitochondrial transmembrane potential ($\Delta\Psi(m)$) produced by the respiratory chain. However, once cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$) is rapidly elevated (>300–500 nM), the MCU will be activated promptly and transport Ca^{2+} into mitochondria, even against the Ca^{2+} concentration gradient (Chaudhuri et al., 2013; Kamer, Grabarek, & Mootha, 2017; Marchi & Pinton, 2014). The MCU is universally expressed in different organs and tissues, but its tissue-specific activities are various (Fieni, Lee, Jan, & Kirichok, 2012). The MCU and its regulatory molecules, including the MCU-dominant negative beta subunit (MCUb), essential MCU regulator (EMRE), MCU regulator 1 (MCUR1), mitochondrial calcium uptake (MICU) 1, MICU2, and MICU3, form a large complex to

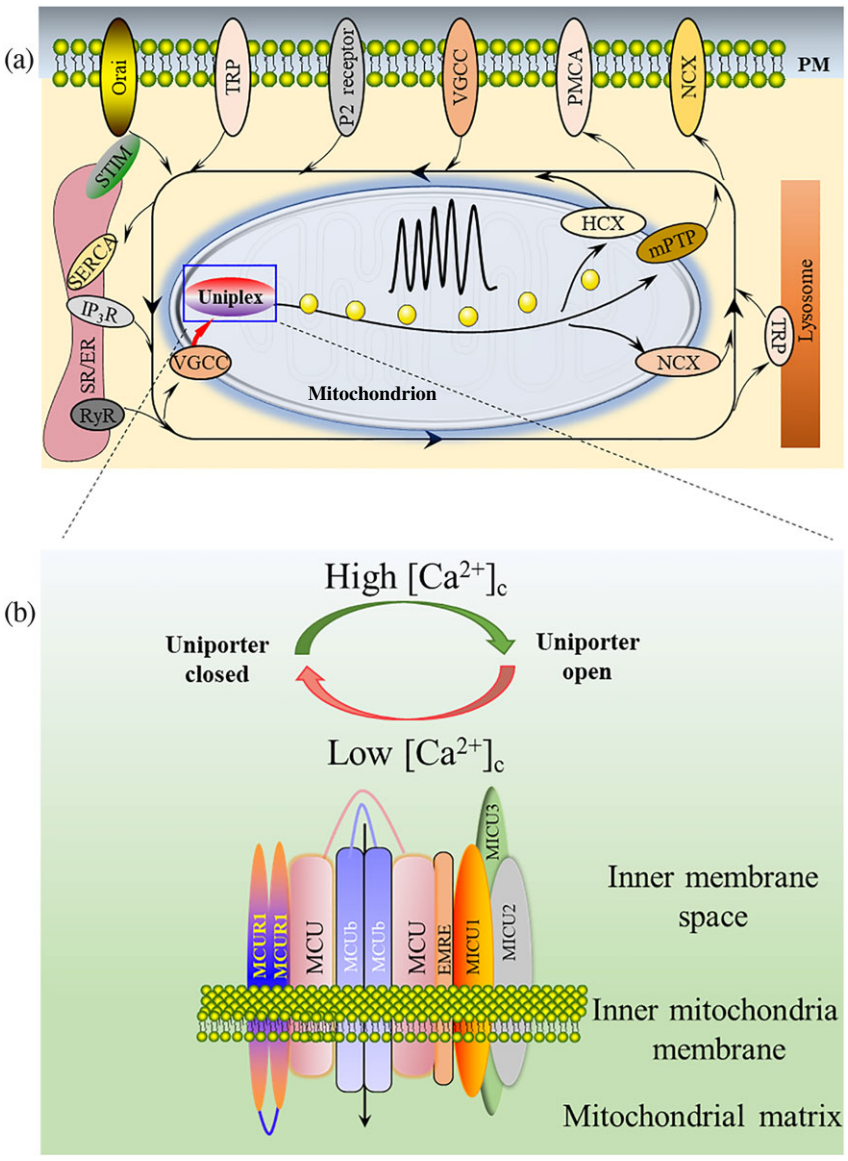


FIGURE 1 Uniplex components and uniplex-mediated mitochondrial Ca^{2+} uptake in the global Ca^{2+} dynamic. (a) The dynamics of the intracellular Ca^{2+} oscillation are finely regulated by the mitochondria, SR/ER, lysosome, and channels located on the PM. When Ca^{2+} is transported from the extracellular space or released from intracellular Ca^{2+} stores, mitochondria will move close to the corresponding channel to take up the elevated $[Ca^{2+}]_c$, thus propagate or restrict Ca^{2+} signals to specific cellular domains. Ca^{2+} influx into mitochondria is mainly mediated by the uniplex. (b) Uniplex is composed of the pore-forming subunit MCU, MCUb, EMRE, MCUR1, MICU1, MICU2, and MICU3. All these molecules localize at the inner mitochondrial membrane. MICU family protein or EMRE molecule contains only single TMD. Both MCU and MCUb contain two TMDs, while both N- and C-terminals face the mitochondrial matrix. With two TMDs, N- and C-terminals of MCUR1 face the mitochondria inner membrane space

manipulate its activities (Lambert, Luongo, Shah, & Elrod, 2016; Mallilankaraman, Cardenas et al., 2012; Patron, Granatiero, Espino, Rizzuto, & De Stefani, 2019; Perocchi et al., 2010; Plovanich et al., 2013; Sancak et al., 2013).

A respiratory chain-generated proton gradient across the IMM is required for mitochondrial Ca^{2+} uptake. The primary functions of $[Ca^{2+}]_m$ include modulation of tricarboxylic acid (TCA) cycle and oxidative phosphorylation via stimulating Ca^{2+}-sensitive dehydrogenases, resulting in increased **NADH** oxidase activity, **ATP**, and ROS production. Thus, it forms a positive feedback regulatory loop for the calcium influx into mitochondria induced by the respiratory chain-IMM proton gradient (Cárdenas et al., 2010; Jouaville et al., 1999; Nicholls, 2005; Rizzuto et al., 2012). Silencing the MCU or MICU1 attenuates Ca^{2+}-dependent activation of the TCA cycle and NAD(P)H oxidase (Perocchi et al., 2010; Tosatto et al., 2016). $[Ca^{2+}]_m$ signalling goes far beyond the general stimulation of cellular energetics (Figure 2). An elevated $[Ca^{2+}]_m$ increases mitochondrial ROS (mROS) production, which promotes the oxidation of membrane phospholipids and proteins. Ca^{2+} binding to the β subunit of **F-type ATP synthase** activates the mitochondrial permeability transition pore (mPTP), leading to a reduction in the ΔΨ(m) and the release of pro-apoptotic factors such as cytochrome c (CytC) into the cytoplasm, which finally activate the mitochondria-dependent apoptotic signalling pathway. $[Ca^{2+}]_m$-activated **calpain** can also directly stimulate **caspases**, thus promoting apoptosis independent of CytC release (C. Giorgi et al., 2012; V. Giorgio et al., 2017; Kim et al., 2013; Mattson & Chan, 2003; Schinzel et al., 2005). Besides apoptosis, it is well accepted that $[Ca^{2+}]_m$ also regulates necrosis, autophagy, and necroptosis (Gomez-Suaga, Paillusson, & Miller, 2017; Rasola & Bernardi, 2011).

It is well known that mitochondria are critical for tumour initiation and development, while the reprogramming of mitochondrial metabolism is currently considered to be an emerging hallmark of cancer (Boroughs & Deberardinis, 2015; Hanahan & Weinberg, 2011; Wallace, 2012; Weinberg et al., 2010; Weinberg & Chandel, 2015). Ca^{2+}-dependent ROS production and Ca^{2+} itself are versatile and are the main effectors of mitochondria-mediated cancer signalling (Figure 2). A reduction in $[Ca^{2+}]_m$ in cancer inhibits mitochondrial apoptotic signals and thus favours cancer cells resistant to apoptotic stimuli. The mROS are commonly deregulated in cancers and are viewed as vital molecular effectors for cancer progression (Klimova & Chandel, 2008; Porporato, Filigheddu, Pedro, Kroemer, & Galluzzi, 2017; Ren et al., 2017; Tosatto et al., 2016). Therefore, uniplex-mediated mitochondrial Ca^{2+} signalling is a promising to target for cancer chemotherapy.

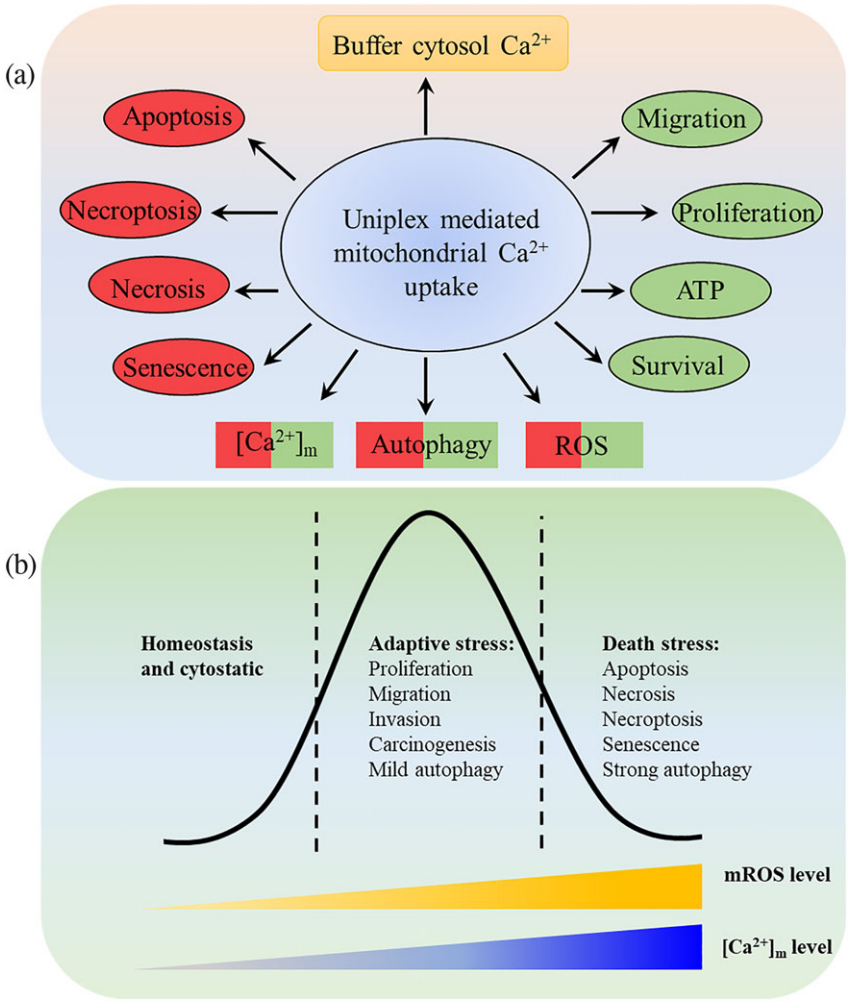


FIGURE 2 Functions of uniplex-mediated mitochondrial Ca^{2+} uptake. (a) Effect of uniplex-mediated Ca^{2+} signalling. It buffers the elevated $[Ca^{2+}]_c$, thus transduces or restricts calcium oscillation coded signalling. $[Ca^{2+}]_m$ binding to specific proteins directly induces biochemical events, including cell migration, proliferation, survival, ATP production, autophagy, ROS, apoptosis, necrosis, necroptosis, and senescence. Autophagy, $[Ca^{2+}]_m$, and ROS are multifunctional events or factors. (b) Dual roles of $[Ca^{2+}]_m$ and mROS. $[Ca^{2+}]_m$ and mROS are required for normal cell functions and signalling. Under moderate stress, the increased Ca^{2+} and ROS could enhance the corresponding signalling pathway for adapting to the elevated requirement of ATP, proliferation, and autophagy. Once this promoted adaptive stress is out of control, it may lead to tumourigenesis and cell invasion. However, once $[Ca^{2+}]_m$ and mROS accumulation exceed the threshold, death-related events, such as apoptosis and strong autophagy, will be induced. Manipulation of these processes can regulate the fate

2 | UNIPLEX COMPONENTS AND REGULATORY MECHANISMS

2.1 | Mitochondrial calcium uniporter

In 2011, by using integrative genomics, Baughman et al. first reported a novel gene CCDC109A that encodes the pore-forming subunit of uniplex (Baughman et al., 2011; De Stefani et al., 2011). The ubiquitously expressed MCU is a ~35 kDa protein containing two transmembrane domains (TMDs) that are linked by a highly conserved sequence (Figure 1b). This conserved sequence faces the intermembrane space (IMS), while both the N- and C-terminal regions of MCU protrude into the mitochondria matrix (Baughman et al., 2011). The channel formed by MCU is sensitive to **ruthenium red** (RuR), RU360, and lanthanides (Cao, Wang, Cui, Su, & Chou, 2017; Crompton, Heid, Baschera, & Carafoli, 1979; De Stefani et al., 2011). The high affinity of MCU binding to Ca^{2+} (K_D < 2 nM) relies on its WDXXEP motif located between TM1 and TM2 (X denotes hydrophobic amino acids, WDIMEP in humans). This motif forms the narrowest region of the pore and is the only region lined by acidic amino acids. The Asp residues of these motifs form a mouth with a radius of ~2.2 Å on the side of the pore-facing IMS, while Glu residues form a second ring with a radius of <1 Å (Baradaran, Wang, Siliciano, & Long, 2018; Kirichok et al., 2004; Yoo et al., 2018). The first X residue in the DXXE motif (Ile^{262} in human MCU, $hMCU^{I262}$) is insensitive to mutation, since both the $hMCU^{I262V}$ and $hMCU^{I262A}$ have full activity. The second X residue (Met^{263} in hMCU) is sensitive to mutation, since $hMCU^{M263A}$ displays reduced activity compared to the wild type. The various effects of the mutant X residues on MCU activities are probably due to the first X residue that has no protein contacts in the structure, while the second X residue can interact with TM1 (Baradaran et al., 2018). The replacement of the DXXE motif with QXXQ in mouse MCU abolishes MCU activity in lipid bilayer membranes. In HeLa cells, the overexpressed $hMCU^{QIMQ}$ acts as a dominant negative regulator, suggesting that the active MCU channel works as an oligomer (Baradaran et al., 2018; De Stefani et al., 2011; Yoo et al., 2018). The $hMCU^{S259A}$ retains Ca^{2+} transport ability but has diminished sensitivity to RuR (Chaudhuri et al., 2013).

MCU activities are also affected by phosphorylation. Under **phenylephrine** treatment, the **proline-rich TK 2 (Pyk2)** will translocate to mitochondria and subsequently binds to and phosphorylates MCU in an mROS-dependent manner. This promotes MCU oligomerization and enhances mitochondrial Ca^{2+} uptake, leading to MCU-dependent mROS generation and forms a positive feedback loop between ROS accumulation and MCU activation. The MCU-mediated mitochondrial Ca^{2+} uptake enhanced by Pyk2 also results in $[Ca^{2+}]_m$ overload and cell apoptosis. The phosphorylation site of hMCU by Pyk2 is most likely Y289; this has not yet been proved. Other mechanisms have also been proposed for the phosphorylation of MCU, and Pyk2 may also phosphorylate other uniplex components (O-Uchi et al., 2014). The multifunctional Ca^{2+}/**calmodulin-dependent protein kinase II (CaMKII)** has also been demonstrated to interact with MCU and increase MCU activities. The CaMKII-dependent MCU activities could be significantly reduced by Ru360 or S57A/S92A mutation in MCU (Joiner et al., 2012). The $hMCU^{C97}$ is sensitive to ROS and will undergo *S*-glutathionylation, resulting in the formation of a MCU higher order oligomer and an elevated $[Ca^{2+}]_m$ uptake rate (Dong et al., 2017). The N-terminal matrix domain of hMCU contains a β-grasp-like fold with a cluster constituted by negatively charged residues. These residues bind to Ca^{2+} or Mg^{2+} and destabilize and shift the self-association equilibrium of the domain towards a monomer, which markedly decreases MCU activity (Lee et al., 2016).

2.2 | MCU-dominant negative beta subunit

MCUb protein shares 50% sequence similarity with MCU and also has two highly conserved putative TMDs. It contains several amino acid substitutions (key replacements of R252W and E257V in human) in the critical pore-forming region. MCUb directly interacts with MCU (Figure 1b) and exerts a dominant negative effect on MCU (Raffaello et al., 2013; Tomar et al., 2016). Once MCUb presents in uniplex, MCU activity will be reduced (Figure 3a). The silencing of MCUb markedly increases histamine-induced mitochondrial Ca^{2+} uptake, without affecting the protein expression of MCU. Unlike MCU, MCUb is expressed only in vertebrates (Raffaello et al., 2013). Even in vertebrates, the ratio of MCU/MCUb is highly variable (from 3:1 to 40:1), indicating that the functions of uniplex-mediated Ca^{2+} signals are tissue-specific (Raffaello et al., 2013; Tomar et al., 2016).

2.3 | MCU regulator 1

MCUR1 (CCDC90A), a ~40 kDa protein with two TMDs localized to IMM (Figure 1b), has been identified as another regulatory molecule of MCU. It has been predicted that both N- and C-terminal regions of MCUR1 face the cytosolic side, exposed to the IMS (Mallilankaraman, Cardenas, et al., 2012). At first, it was proposed that the ubiquitously expressed MCUR1 is required for MCU activity and physically interacts with MCU (Figures 1b and 3). Although MCUR1 knockdown does not affect the expression and localization of MCU, it reduces the basal $[Ca^{2+}]_m$ level and attenuates mitochondrial Ca^{2+} uptake (Mallilankaraman, Cardenas, et al., 2012; Tomar et al., 2016). Ablation of MCUR1 can disrupt oxidative phosphorylation, decrease cellular ATP, and activate **AMP kinase**-dependent pro-survival autophagy. When exogenous MCUR1 is increased, the enhanced mitochondrial Ca^{2+} uptake can be inhibited by Ru360. Thus, MCUR1 is required for mitochondrial Ca^{2+} uptake and is a critical component of the uniplex (Mallilankaraman, Cardenas, et al., 2012).

However, in 2015, Paupe et al. reported that a fraction of MCUR1 consistently locates outside the IMM, indicating a potential unknown function of MCUR1. They proposed that MCUR1 primarily functions as a CytC oxidases (COXs) assembly factor but not a regulator of MCU. Inhibition of MCUR1 specifically results in COXs assembly defect. This leads to an impaired respiratory chain and reduced ΔΨ(m), which are the real reasons that MCUR1 affects mitochondrial Ca^{2+} uptake (Paupe, Prudent, Dassa, Rendon, & Shoubridge, 2015).

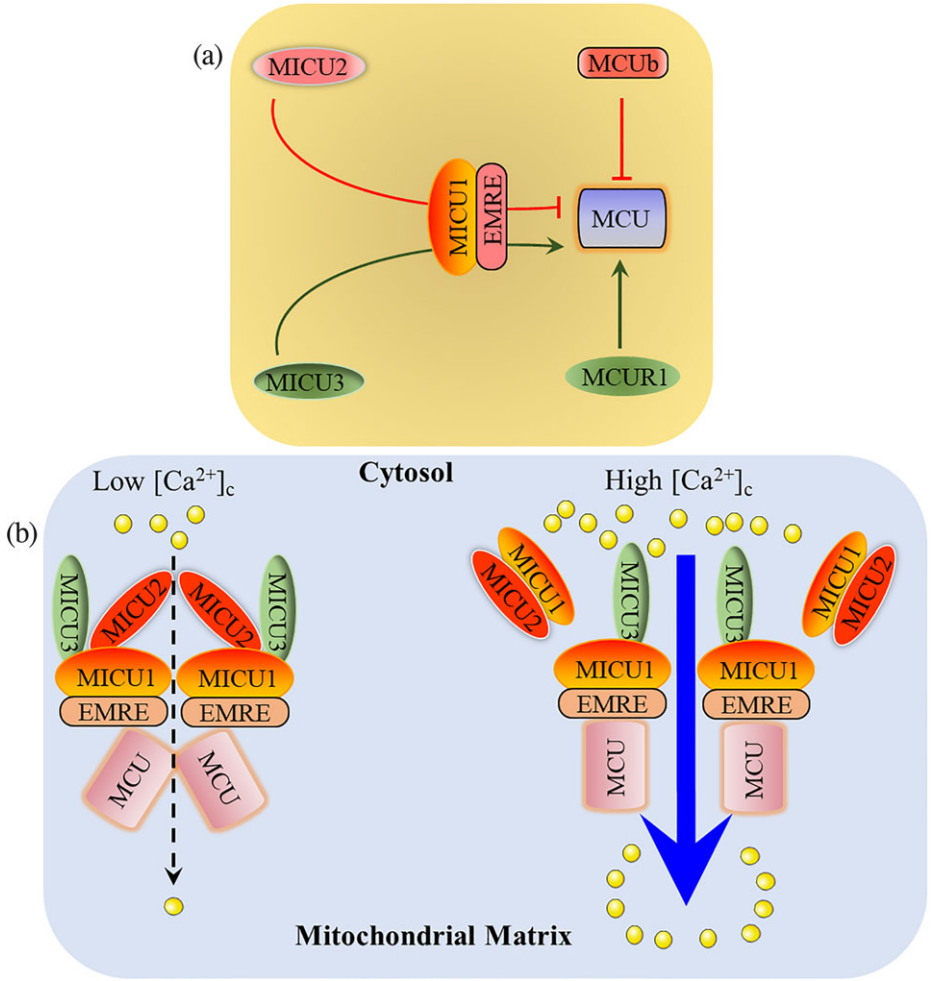


FIGURE 3 Regulatory mechanisms of uniplex. (a) MCUR1 and MCUb directly bind to MCU, which exerts a positive and dominant negative effect on MCU, respectively. MICU2 is a gatekeeper and MICU3 is an enhancer of MCU, both of which require MICU1-EMRE heterodimer as a bridge. (b) EMRE directly binds to MICU1 and MCU. At low $[Ca^{2+}]_c$ levels, both MICU2 and MICU3 bind to MICU1, and MICU2 inhibits MCU activation. When $[Ca^{2+}]_c$ increases and exceeds the Ca^{2+} threshold set by MICU2, the MICU1–MICU2 dimer will dissociate from the uniplex, and MICU2 will lose its inhibitory function; MICU3 enhances MCU activity to transfer Ca^{2+} from the cytosol into the mitochondrial matrix

Interestingly, not long after these findings, using immune-affinity binding assays and bimolecular fluorescence complementation, Tomar et al. demonstrated that MCUR1 could directly bind to MCU and EMRE (Tomar et al., 2016). It has been clearly proven that MCUR1 and EMRE can co-localize in the IMM, and a deficiency in MCUR1 considerably decreases the molecular size of the uniplex. The highly conserved regions of the coiled-coil domain in both the MCU and MCUR1 are required for the formation of their heterooligomeric complex. MCUR1 functions as a scaffold factor for the uniplex assembly (Tomar et al., 2016). Deletion of either MCU or MCUR1 does not affect ΔΨ(m) in cardiomyocytes, endothelial cells, and fibroblasts, which is contrary to Paupe's conclusion that MCUR1 is a COX assembly factor that affects the ΔΨ(m) (Paupe et al., 2015; Tomar et al., 2016; Vais et al., 2015). It has also been reported that MCUR1 is not essential for mitochondrial Ca^{2+} uptake and only serves as a regulator to set the Ca^{2+} threshold for the mitochondrial permeability transition. Inhibition of MCUR1 expression increases the Ca^{2+} threshold for inducing mPTP transition, thus decreasing mitochondrial cell death induced by Ca^{2+} overload (Chaudhuri, Artiga, Abiria, & Clapham, 2016). Therefore, the current findings regarding the actions of MCUR1 on MCU are still controversial and need to be further elucidated.

2.4 | MICU family proteins

MICU1 was identified by scientists 1 year preceding MCU. MICU1 is a ~50 kDa protein localized at the IMM and characterized by a single putative TMD (Figure 1b), which makes it unlikely that MICU1 functions as a Ca^{2+} channel. Initially, it was reported that silencing MICU1 neither disrupts mitochondrial respiration nor affects ΔΨ(m), whereas it abolishes mitochondrial Ca^{2+} uptake. Two canonical EF-hands of MICU1 are required for MCU-mediated Ca^{2+} influx into mitochondria (Perocchi et al., 2010). Based on the characteristic of the EF-hand-sensitive Ca^{2+} concentration, it was later proposed that MICU1 sets the Ca^{2+} threshold for MCU activation, which

means MICU1 works as a vital gatekeeper of MCU and inhibits MCU activity to maintain a normal $[Ca^{2+}]_m$ level under resting conditions.

Knockdown of MICU1 leads to a constitutive MCU-mediated Ca^{2+} transfer into mitochondria, inducing the accumulation of mROS and sensitizing cells to apoptotic stimulation (Mallilankaraman, Doonan, et al., 2012). MICU1 also imparts MCU selectivity to discriminate between Ca^{2+} and Mn^{2+}. Although both Mn^{2+} and Ca^{2+} can bind to MICU1, only Ca^{2+} is capable of inducing a conformation change in MICU1 and activating MCU, further supporting the negative function of MICU1 on MCU (Kamer et al., 2018).

Scientists further showed that MICU1 enhances MCU activity under the elevated $[Ca^{2+}]_c$ condition. A lack of MICU1 not only induces a continuous elevation of $[Ca^{2+}]_m$ under resting conditions but also reduces MCU-mediated Ca^{2+} influx into mitochondria while fast $[Ca^{2+}]_c$ increases (de la Fuente, Matesanz-Isabel, Fonteriz, Montero, & Alvarez, 2014; Mallilankaraman, Doonan, et al., 2012). In the planar lipid membrane, MICU1 exerts a stimulatory effect on MCU activity even at low concentrations of Ca^{2+}or in the absence of Ca^{2+}. However, in intact cells, MICU1 exerts an inhibitory effect on MCU activity under lower $[Ca^{2+}]_c$ levels (Mallilankaraman, Doonan, et al., 2012). These interesting results suggest that MICU1 itself is not enough as a gatekeeper, and other molecules are required for maintaining its actions. Now it has been demonstrated that the action of MICU1 as a gatekeeper or enhancer of MCU depends on MICU2 and MICU3, respectively (Figure 3).

By using bioinformatics, Plovanich et al. identified MICU2 (EFHA1) and MICU3 (EFHA2) as paralogous genes to MICU1, which also have mitochondrial localization targeting sequences at their N-terminals. MICU2 has 41% sequence similarity to MICU1, while MICU3 has 34% sequence similarity to MICU1 and 47% sequence similarity to MICU2 (Csordas et al., 2013; Patron et al., 2019; Plovanich et al., 2013). The expression patterns of the MICU family proteins are different. MICU1 is broadly expressed in most of the tissues; MICU2 is expressed primarily in visceral organs, whereas MICU3 is mostly in skeletal muscles and the CNS (Pagliarini et al., 2008; Plovanich et al., 2013). This suggests that the MCU-mediated Ca^{2+} currents regulated by MICU molecules are tissue- or cell-specific.

MICU2 interacts with MICU1 to increase the $[Ca^{2+}]_c$ threshold for MCU activation; thus, MICU2 has the ability to inhibit MCU activity under low $[Ca^{2+}]_c$ conditions (Payne, Hoff, Roskowski, & Foskett, 2017). It has been reported that MICU2 rather than MICU1 might act as the gatekeeper of MCU (Figure 3). MICU2 has a conserved domain architecture like MICU1, and is found to reside within mitochondria with high-content microscopy and MitoCarta (an inventory of human and mouse genes encoding proteins with strong support of mitochondrial localization). MICU3 also has the targeting sequence to support its mitochondrial localization, and has been shown to localize in mitochondria (Patron et al., 2019; Plovanich et al., 2013), although it does not achieve the score needed to be listed in MitoCarta (Patron et al., 2019; Plovanich et al., 2013).

Once MICU1 matures and localizes to the IMM, it will bind to MCU, this enables MICU1 to bind to Mia40. Following MICU1 interacting with oxidoreductase Mia40, MICU1 and MICU2 form a heterodimer (Figure 3b) by a disulfide bond. The binding of MICU1 to MCU only occurs under low Ca^{2+} or no Ca^{2+} conditions. High Ca^{2+} levels will result in the dissociation of the MICU1–MICU2 dimer from the uniplex. However, this hypothesis is still controversial because MICU1 has been shown to cooperatively activate MCU at high Ca^{2+} concentrations (Csordas et al., 2013; Petrungaro et al., 2015). This may result from the remnant MICU1 proteins in the uniplex binding to MICU3 (Figure 3b). The dimerization of MICU1 and MICU2 would stabilize both proteins, without affecting their mRNA level (Patron et al., 2014; Petrungaro et al., 2015; Plovanich et al., 2013). MCU was reported to physically interact with and stabilize MICU1 and MICU2. A later study demonstrated that this interaction is mediated by EMRE (Figures 1b and Figure 3; Plovanich et al., 2013; Sancak et al., 2013).

The overexpression of MICU3 causes a 10-fold increase in $[Ca^{2+}]_m$ transients. However, MICU1 overexpression only induces a threefold increase. $MICU3^{EFmut}$ still retains a certain ability to enhance MCU, but its efficiency is less than the wild type. The silencing of MICU1 also abolishes the cooperative function of MICU3 on MCU, indicating that MICU1 is a common platform for both MICU2 and MICU3. MICU3 also binds to MICU1 via a disulfide bond, in which C465 of MICU1 and C515 of MICU3 are required (Patron et al., 2019). The **protein arginine methyl transferase 1 (PRMT1)** is able to methylate R455 of hMICU1, thus reduces MICU1 sensitivity to Ca^{2+} and increases the threshold of $[Ca^{2+}]_c$ for activation of MCU. However, methylation of MICU1 by PRMT1 enables MICU1 to bind to **uncoupling protein** 2 (UCP2), which rescues the decreased MCU activity (Madreiter-Sokolowski et al., 2016). Whether MICU2 can be methylated by PRMT1 is not yet known.

MICU1, MICU2, and MICU3 exert various effects on MCU, although they belong to the same family (Figure 3). MICU2 is a gatekeeper of MCU, while MICU3 mainly functions as an enhancer of MCU activation. The diverse functions of the MICU family proteins maintain normal $[Ca^{2+}]_m$ levels under the resting conditions and enable prompt activation of MCU to mediate rapid mitochondrial Ca^{2+} uptake.

2.5 | Essential MCU regulator

Sancak et al. identified another regulatory protein of MCU, called essential MCU regulator (EMRE, previously known as C22orf32) in 2013 (Sancak et al., 2013). EMRE is a ~10 kDa metazoan-specific protein with a single TMD, located at IMM (Figure 1b). By use of a protease sensitivity biochemistry experiment, it was shown that the C-terminal of EMRE faces the mitochondrial matrix and contains a motif similar to the Ca^{2+} binding "Ca^{2+} bowl" and "Ca^{2+} clasp" to sense Ca^{2+}. However, due to the small size of EMRE's extra-membrane regions, it is not suitable for standard protease digestion assays. Tsai's group used directed mass-tagging and MCU-EMRE fusion construction demonstrated that EMRE exposes its N-terminal region to the matrix and C-terminal to IMS (Tsai et al., 2016).

Overexpression of MCU in cells lacking EMRE fails to restore MCU activity. Interestingly, depletion of MCU can decrease EMRE abundance without affecting its mRNA level, indicating that MCU is able to stabilize EMRE protein post-transcriptionally. Depletion of EMRE considerably impairs MCU-mediated mitochondrial Ca^{2+} uptake, despite the normal expression and oligomerization of MCU (Sancak et al., 2013). The interaction of MCU and EMRE is mediated by the transmembrane helices from both proteins. Loss of EMRE or MICU1 results in a similar decrease in molecular size of the uniplex to ~300 kDa, suggesting that EMRE may serve as a bridge between MCU and MICU1 (Figures 1b and 3), which has been demonstrated by an immunoprecipitation assay (Sancak et al., 2013). When EMRE is depleted, the interaction between MICU1 and MICU2 does not change, and MCU oligomerization and interaction with MCUb are also normal, whereas the association of MCU with the MICU1–MICU2 dimer is significantly decreased (Sancak et al., 2013).

3 | REPROGRAMMING OF UNIPLEX-MEDIATED CALCIUM SIGNALLING IN CANCER

Metabolic reprogramming is essential for the increased activity of energetic and biosynthetic pathways in cancer. The TCA cycle-dependent production of ATP and metabolic intermediates are required for the synthesis of fatty acids and nucleotides (Boroughs & Deberardinis, 2015). $[Ca^{2+}]_m$ overload is commonly considered as a trigger for the initiation of cell death. Reduced mitochondrial Ca^{2+} uptake has been demonstrated to reduce O_2 consumption and ATP level, thus activate AMP-activated protein kinase-dependent pro-survival macro-autophagy (Cárdenas et al., 2010). Down-regulation or inhibition of MCU increases proliferation and renders cells resistant to apoptosis by diminishing Ca^{2+} entry into mitochondria. Oncogenes or tumour suppressors can promote cell survival or induce death by modifying uniplex activity (Garcia-Prieto et al., 2013; Marchi et al., 2013). Here, we summarize the data available on the deregulation of uniplex-mediated Ca^{2+} signalling in cancer (Table 1).

3.1 | Pancreatic cancer

The histidine triad nucleotide-binding 2 (HINT2) protein is an adenosine phosphoramidase localized in mitochondria. Decrease of HINT2 expression is closely associated with metastasis of pancreatic cancer lymph nodes, pathological grade, and poor prognosis. Forced overexpression of HINT2 in pancreatic cancer cells results in almost threefold elevation of $[Ca^{2+}]_m$ and induction of apoptosis. These processes can be prevented by RuR, suggesting that the uniplex is involved. Coupled with the elevated expression of exogenous HINT2 in pancreatic cancer cells, MICU1 and MICU2 proteins are decreased, whereas EMRE is increased (Chen et al., 2017). However, the expression patterns and the roles of MICU1, MICU2, EMRE, and other

TABLE 1 Alterations of uniplex in cancer

Cancer type		$[Ca^{2+}]_m$ alteration	Uniplex alteration	Upstream modulator and alteration	Reference
Pancreatic cancer		Decreased	MICU1 and MICU2 increased; EMRE decreased	HINT2, decreased	Chen et al. (2017)
Breast cancer		Increased	MCU increased and MCUb decreased	miR-340, decreased	Curry, Peters, Kenny, Roberts-Thomson, and Monteith (2013), Fouque et al. (2016), Tang et al. (2015), Tosatto et al. (2016), and Yu et al. (2017)
Colon cancer		Decreased	MCU decreased	MiR-25 Increased	Marchi et al. (2013)
Prostate cancer		Decreased	MCU decreased	miR-25, increased	Marchi et al. (2013)
Ovarian cancer		—	MICU1 mRNA increased	Unknown	Sancak et al. (2013)
HCC		Increased	MCU, MCUR1, and MICU2 increased; alteration of MICU1 was controversial	Mfn2, decreased	Ren et al. (2017, 2018) and Wang et al. (2015)
Multiple myeloma	Bortezomib-sensitive cells	Resting: increased, compared with resistant cells; after bortezomib treatment: increased ~10 fold	Increased by bortezomib treatment	—	Song et al. (2013)
	Bortezomib-resistant cells	Resting: Decreased, compared with sensitive cells; after bortezomib treatment: increased ~2 fold	No change with bortezomib treatment	—	

uniplex components in pancreatic cancer, as well as the mechanisms responsible for the effects of HINT2 on MICU1, MICU2, and EMRE remain unclear.

3.2 | Cervical carcinoma

Currently, there have been a few reports on the role of the uniplex in the pathobiology of cervical cancer, primarily obtained using HeLa cells. Knockdown of MICU1 in HeLa cells results in an elevated $[Ca^{2+}]_m$, while ΔΨ(m) and the histamine-induced mitochondrial Ca^{2+} uptake seem to be unaffected. Although MICU1 knockdown does not affect the proliferation of HeLa cells, it considerably enhances ceramide-induced cell death, which is attributed to the $[Ca^{2+}]_m$-dependent chronic elevation of mROS (Csordas et al., 2013; Mallilankaraman, Doonan, et al., 2012). After silencing MCU, most of the mitochondrial parameters, such as morphology and ΔΨ(m), remain unchanged. Ablation of EMRE or MCU causes a decrease in Ca^{2+} influx into mitochondria, while cell proliferation is not affected (Sancak et al., 2013).

3.3 | Ovarian cancer

Ca^{2+}-dependent permeabilization of the outer mitochondrial membrane is a key event in the mitochondrial intrinsic apoptotic pathway (Deniaud et al., 2008). MICU1 mRNA level is increased in ovarian cancer cells, per mitochondrion and per cell (Table 1). Compared to normal cells, ovarian cancer cells are highly resistant to cytotoxicity induced by positively charged gold nanoparticles ($^{+}$AuNPs). $^{+}$AuNPs can substantially increase $[Ca^{2+}]_c$ levels. A2780 and OV202 ovarian cancer cells display at least a threefold higher expression of MICU1 than normal ovarian surface epithelial cells. Ovarian cancer cells exposed to $^{+}$AuNPs display a rapid increase in $[Ca^{2+}]_c$ and a chronic sustained increase in MCU-mediated mitochondrial Ca^{2+} uptake. Silencing MICU1 promotes $^{+}$AuNPs-induced mitochondrial membrane depolarization and mitochondrial-dependent apoptosis in ovarian cancer cells, coupled with reduced mitochondrial Ca^{2+} uptake. These results suggest that an up-regulation of MICU1 can enhance mitochondrial ability to buffer $[Ca^{2+}]_c$ elevations induced by $^{+}$AuNPs, thus prevent cells from $[Ca^{2+}]_c$-dependent apoptosis (Arvizo et al., 2013).

3.4 | Multiple myeloma

Multiple myeloma is a haematological malignancy with a high risk of relapse and death. The proteasome inhibitor **bortezomib** is a first-line chemotherapeutic agent for clinical treatment of multiple myeloma by inducing apoptosis. Intrinsic and acquired drug resistances are obstacles encountered when bortezomib is applied for treating multiple myeloma patients. Bortezomib treatment induces ER Ca^{2+} release, activation of a store-operated ion, **Orai channel**, and mitochondrial Ca^{2+} uptake. $[Ca^{2+}]_m$ deregulation induced by bortezomib treatment precedes caspase activation, which can be prevented by RuR and Ru360 (Rizzuto et al., 2012). Under bortezomib treatment, MCU level increases almost 15- to 20-fold in the cells sensitive to bortezomib, while few changes occur in the cells resistant to bortezomib, although the resistant cells have higher MCU expression levels under the resting conditions (Table 1) (Song et al., 2013). When the transfer of Ca^{2+} from ER to mitochondria is inhibited, AMPK -dependent autophagy will be activated, and the growth of xenografted melanoma cells will be reduced by nearly 60–70% (Boroughs & Deberardinis, 2015). This indicates that the uniplex is essential for bortezomib-induced apoptosis. It may be a promising strategy to combine uniplex-targeted agents with bortezomib to overcome chemotherapy resistance in multiple myeloma treatment.

3.5 | Breast cancer

Analysis of the Oncomine cancer microarray database and qPCR results from clinical breast cancer samples reveals that breast cancer cells display high levels of MCU mRNA (Curry et al., 2013; Tang et al., 2015). Analysis of the Cancer Genome Atlas breast cancer dataset shows that the increased MCU and reduced MCUb mRNA, but not MICU molecules and EMRE, are positively correlated with the growth of triple-negative breast cancers (TNBCs) and lymph node metastasis (Table 1; Tosatto et al., 2016). Orais have been demonstrated to mediate migration and metastasis of breast tumour cells (Yang, Zhang, & Huang, 2009). RNAi or pharmacological inhibition of MCU activity significantly reduces Orais' activity, migration, and viability of breast cancer cells in vitro. $MCU^{-/-}$ basal-like MDA-MB-231 breast cancer cells consistently display significantly reduced lymph node infiltration and lung metastasis in vivo. Knockdown of MCU decreases NADH and ROS levels while increasing the **NADPH**/NADH ratio. Transcriptions of HIF-1α and its target genes are significantly reduced by MCU knockdown (Tang & Wang et al., 2015; Tosatto et al., 2016). Knockdown of MCU in MDA-MB-231 cancer cells does not affect proliferation and $[Ca^{2+}]_c$ levels but markedly enhances the Ca^{2+} ionophore ionomycin-induced cell death that is independent of caspase. This results from alterations in mitochondria-associated membrane Ca^{2+} signals and the elimination of the $[Ca^{2+}]_m$-induced autophagic survival pathway (Curry et al., 2013).

Fouqué et al. demonstrated that the cleaved **CD95L** (cl-CD95L)-induced Ca^{2+} transfer from ER to mitochondria in TNBC cells is dependent on a **Bcl-xL**/VDAC1/MCU complex. Overexpression of cl-CD95L results in poor prognosis and a higher metastatic ability of TNBCs. Treating cells with Ru360 inhibits CD95-mediated mitochondrial Ca^{2+} influx and cell migration (Fouque et al., 2016). Compared to normal tumour-adjacent tissues and ductal carcinoma tissues, the breast cancers with distant metastases and/or lymph node infiltration display an increased expression of MCU protein. Also, the expression of MCU protein in the highly migratory and invasive ZR-75-30 and MDA-MB-231 breast cancer cells is higher than that in BT-474 and MCF-7 cells with poorer invasive ability (Table 1). Exogenous MCU markedly increases the migratory and invasive ability of MCF-7 cells, which are sensitive to the inhibitory action of Ru360. It is known that the Warburg effect contributes to cell metabolism and motility, while an up-regulation of MCU enhances the Warburg effect in breast cancer

cells. Yu et al. demonstrated that miR-340 directly interacts with MCU mRNA and inhibits MCU-mediated mitochondrial Ca^{2+} influx, the Warburg effect, and breast cancer cells motility (Table 1). Nude mice injected with MCF-7 cells with overexpressed MCU or down-regulated miR-340 developed more metastatic lesions in their lungs (Yu et al., 2017).

3.6 | Prostate cancer and colon cancer

Prostate cancer is one of the widely studied cancers that commonly exhibit impaired Ca^{2+} signals (Prevarskaya, Skryma, Bidaux, Flourakis, & Shuba, 2007; Thebault et al., 2006; Vanden Abeele et al., 2002). It has been demonstrated that the **Na^+/Ca^{2+} exchanger (NCX)**-specific inhibitor, CGP-37157, markedly enhances **TNF-related apoptosis-inducing ligand** (TRAIL)-induced apoptosis in prostate cancer cells by causing $[Ca^{2+}]_m$ overload. Although the MCU inhibitor, Ru360, failed to attenuate TRAIL-induced cell death, MCU agonists are showing promise in enhancing this action, which is still worthy of further study (Kaddour-Djebbar et al., 2006). MCU is down-regulated in prostate cancer and colon cancer (Table 1; Marchi et al., 2013). MiR-25 with 22 nucleotides is highly expressed in these cancers. MiR-25 has the potential to decrease mitochondrial Ca^{2+} uptake by selectively inhibiting MCU expression, resulting in resistance of cancer cells to apoptotic challenges. Anti-miR-25 in PC-3 prostate cancer cells and HCT116 colon cancer cells can increase the $[Ca^{2+}]_m$ level and re-sensitize cells to apoptosis. MiR-92a, miR-363, and miR-1 exert a similar effect on MCU expression and Ca^{2+} signalling (Marchi et al., 2013; Marchi & Pinton, 2013; Zaglia et al., 2017). These results reveal how cancer cell survival is favoured by the down-regulation of MCU and suggest that activation of MCU is a potential target for cancer therapy.

Receptor-interacting protein kinase 1 (RIPK1) plays a central role in cancers via apoptosis and necroptosis. It has been shown that expression of RIPK1 positively correlates with 3 years mortality in colorectal cancer patients. Zeng et al. demonstrated that MICU1 is decreased, and MCU is significantly up-regulated in colorectal cancer tissues (Table 1), and these changes correlate with RIPK1 levels. Co-immunoprecipitation results further revealed a physical interaction between RIPK1 and MCU, which was enhanced in colorectal cancer. Silencing RIPK1 decreased MCU protein levels, whereas an overexpression of RIPK1 increased $[Ca^{2+}]_m$ level in an MCU-dependent manner thus modulating colorectal cancer energy metabolism (Zeng et al., 2018).

3.7 | Hepatocellular carcinoma

Based on the bioinformatic analysis of public microarray mRNA expression data sets, MCU is found to be increased and MICU1 decreased at both mRNA and protein levels in hepatocellular carcinoma (HCC) tissues. Furthermore, MCU is markedly higher and MICU1 is lower in the metastatic HCC tissues than in the primary HCC tissues (Table 1). The expression of these two deregulated molecules is closely correlated with the risk of recurrence and death in HCC patients. Highly metastatic HCC cells display higher basal levels of $[Ca^{2+}]_m$ than HCC cells with low metastatic ability or normal hepatocyte HL-7702 cells. Ca^{2+} influx to mitochondria is reduced by MCU knockdown or MICU1 overexpression, which is dissimilar to effects observed in ovarian cancer, suggesting that MCU-mediated Ca^{2+} signalling may be tissue-specific (Arvizo et al., 2013; Ren et al., 2017). With pro-apoptotic and anti-proliferative effects, mitofusin-2 (Mfn2) plays a critical role in mitochondrial fusion and contributes to the maintenance of mitochondrial function. Expression of Mfn2 is reduced in HCC and correlates with poor prognosis. When exogenous Mfn2 is introduced into HCC HepG2 cells, $[Ca^{2+}]_m$ levels and apoptotic cells will be increased, which can be inhibited by Ru360. The introduced Mfn2 can reduce the originally up-regulated MICU1 and MICU2 proteins in HCC (Wang et al., 2015). How Mfn2 affects MICU1 and MICU2 expression has not yet been elucidated; thus, the lack of conformity of MICU1 expression in HCC is still in need of a careful validation. Silencing of MCU increases the ratio of **NAD^+**/NADH both in the cellular and mitochondrial compartments, the deacetylase activity of **SIRT3**, and enzymatic activity of SOD2, while decreasing ATP production and the acetylated SOD2 (Ac-SOD2) level. By inhibiting the NAD^+/**SIRT**3/SOD2 signalling pathway, MCU-mediated mitochondrial Ca^{2+} uptake promotes mROS accumulation and subsequently activates ROS/**JNK** signalling to facilitate HCC metastasis in vitro and in vivo (Ren et al., 2017).

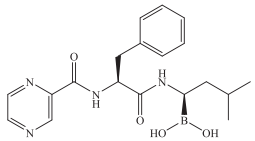
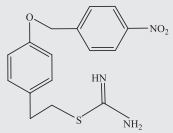
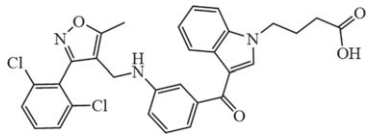
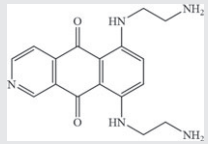
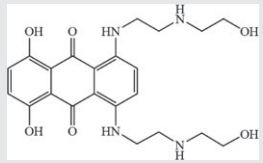
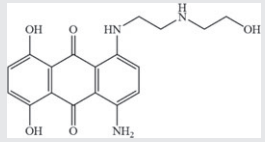
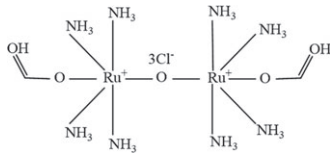
Expression of MCUR1 is also increased in HCC (Table 1), which is related to poorer overall survival and recurrence-free survival. Knockdown of MCUR1 arrests cells in G1 phase, decreases HCC cell growth and colony-formation, while increasing cell apoptosis. This leads to markedly reduced mitochondrial Ca^{2+} uptake, basal $[Ca^{2+}]_m$, and mROS production. As a well-known tumour suppressor, p53 displays a negative correlation with MCUR1 levels. Elimination of ROS inhibits MCUR1-dependent activation of the Akt/MDM2 pathway, while it increases p53 expression (Ren et al., 2018). The controversial observations regarding the levels and functions of $[Ca^{2+}]_m$ in HCC cells probably result from the dual roles of both $[Ca^{2+}]_m$ and mROS (Figure 2), highlighting the complexity of $[Ca^{2+}]_m$ signalling.

Increasing evidence indicates that the uniplex manipulates proliferative, survival and metastatic signals, and death-related pathways in cancer. Uniplex can serve as a promising target for cancer therapy. Since the uniplex is the primary channel mediating Ca^{2+} entrance into mitochondria, the altered Ca^{2+} current will be easily remolded by potential uniplex-modulatory compounds and result in non-resistant drugs. The limitations are that regulatory mechanisms of uniplex are still not completely clear and even remain controversial. Alterations of the MCU are tissue- and tumour-specific. Currently, only MCU inhibitors are available but not agonists.

4 | PHARMACOLOGICAL INHIBITORS OF THE MCU

Previously, the uniplex modulators were limited to lanthanides and ruthenium-related compounds, which had been shown to inhibit the

TABLE 2 Pharmacological inhibitors of uniplex

Compound		Molecular formula	Skeletal formula	Formula weight	Target	IC_{50}	Reference
Bortezomib		$C_{19}H_{25}BN_4O_4$		384.24	–	–	Landowski, Megli, Nullmeyer, Lynch, and Dorr (2005)
KB-R7943		$C_{16}H_{17}N_3O_3S.CH_3SO_3H$		427.49	–	~5.5 μM	Santo-Domingo et al. (2007)
DS16570511		$C_{30}H_{25}Cl_2N_3O_4$		562.44	–	~7 μM	Kon et al. (2017
Mitoxantrone analogues	Pixantrone	$C_{17}H_{19}N_5O_2$		325.37	Asp residue in DXXE motif of MCU	~15 μM	Arduino et al. (2017)
	Mitoxantrone	$C_{22}H_{28}N_4O_6$		444.48		~8.3 μM	
	Mitoxantrone impurity A	$C_{18}H_{19}N_3O_5$		357.36		~36 μM	
Ruthenium red analogues	Ru360	$C_2H_{26}N_8O_5Ru_2Cl_3$		550.78	Asp residue in DXXE motif of MCU	2 nM	Fouque et al. (2016) and Yu et al. (2017)

(Continues)

TABLE 2 (Continued)

Compound		Molecular formula	Skeletal formula	Formula weight	Target	IC_{50}	Reference
	Ruthenium red	$H_{42}N_{14}O_2Ru_3Cl_6$		786.35	DXXE motif of MCU	9 nM	Fouque et al. (2016) and Yu et al. (2017)
Lanthanides	La^{3+}, Gd^{3+}, and Pr^{3+}	–	–	–	–	–	Crompton et al. (1979)
Tetracycline analogues	Minocycline	$C_{23}H_{27}N_3O_7$		457.48	–	–	Schwartz et al. (2013)
	Doxycycline	$C_{22}H_{24}N_2O_8$		444.43	–	–	Schwartz et al. (2013)

The molecular structures were generated from ChemDraw15.0 programme.

development of various cancers (Anghileri, 1975; Cao et al., 2017; Kirichok et al., 2004; Moore, 1971). Ru360 and RuR (Table 2) have been shown to directly bind to the solvent-accessible Asp ring formed by the DXXE motif in MCU (Cao et al., 2017). The tetracycline-derived minocycline and doxycycline (Table 2), but not tetracycline itself and other tetracycline-derived compounds, exert inhibitory effects on MCU. They can suppress mitochondrial Ca^{2+} uptake, $[Ca^{2+}]_m$ overload-induced mPTP open and cell death, similarly to that of Ru360 (Schwartz et al., 2013). The promising anti-cancer potential of minocycline and **doxycycline** has been shown in numerous studies on a variety of cancers (Ali, Alfarouk, Reshkin, & Ibrahim, 2017; Garrido-Mesa, Zarzuelo, & Gálvez, 2013). Whether the inhibitory functions of these two compounds on MCU are related to their anti-cancer effect and the underlying mechanisms of these compounds inhibiting MCU are still in need of further investigation.

The meaningful molecular discoveries of uniplex components will definitely provide the platform and possibility for developing specific inhibitors or agonists for cancer chemotherapy. By using high-throughput screening, Kon et al. identified DS16570511 (Table 2) as a cell-permeable inhibitor of MCU. Without disruption of the ΔΨ(m), DS16570511 can inhibit serum-induced mitochondrial Ca^{2+} influx in HEK293A cells with an $IC_{50} \approx 7$ μM, but the underlying mechanisms are still unclear (Kon et al., 2017). The thiourea derivative, **KB-R7943** (Table 2), was originally developed as a selective inhibitor of the NCX located at PM. KB-R7943 has also been shown to be a potent inhibitor of MCU without modifying ΔΨ(m) in cervical carcinoma HeLa cells (Santo-Domingo et al., 2007). By inhibiting MCU, KB-R7943 further reduces the fast initial ER Ca^{2+} release via Ca^{2+}-inhibition feedback on the **inositol trisphosphate receptor** (IP_3R). As it has no detectable PM NCX activity, KB-R7943 shows promise as a specific MCU inhibitor in treatment of these cancers (Furman, Cook, Kasir, & Rahamimoff, 1993; Iwamoto, Watano, & Shigekawa, 1996; Santo-Domingo et al., 2007). But Brustovetsky et al. reported that KB-R7943 did not directly affect MCU in their research. They postulated that KB-R7943 reduced the activity of complex I in the respiratory chain and the energy required for Ca^{2+} transport, which are responsible for the decreased mitochondrial Ca^{2+} uptake (Brustovetsky et al., 2011; Clerc & Polster, 2012). Currently, the findings on the effect of KB-R7943 on MCU-mediated mitochondrial Ca^{2+} uptake are contraversial and in need of further studies.

There are challenges associated with high-throughput screening to develop MCU modulators. As an intracellular protein complex, uniplex's activities are dependent on the alterations of $[Ca^{2+}]_c$ and its driving force is dependent on the respiratory chain. These features of uniplex make the traditional high-throughput screening prone to getting false-positive hits, such as modulators of the respiratory chain, TCA cycle, or other molecules involved in intracellular Ca^{2+}-signalling networks. Many false-positive hits could be eliminated by introducing hMCU and EMRE into yeast *Saccharomyces cerevisiae*, which is deficient in endogenous MCU and with delicately designed bioenergetic characteristics. Utilizing this system, Arduino et al. identified **mitoxantrone** as a selective and specific inhibitor of MCU (Arduino et al., 2017). In traditional cancer chemotherapeutics,

mitoxantrone, is a well-known agent with DNA intercalating activity to inhibit **DNA topoisomerase** II and cell proliferation. Mitoxantrone has been widely applied in the clinic for several cancers, such as non-Hodgkin's lymphomas and acute myeloid leukaemia. Mitoxantrone has a concentration-dependent inhibitory effect on MCU. In HEK293 cells, 10 μM mitoxantrone significantly inhibits (~85%) MCU-mediated Ca^{2+} currents via its charged side arms binding to acidic residues in the selectivity filter of MCU (Arduino et al., 2017).

There is no difference in reduction of viability in MDA-MB-231 breast cancer cell and PLB-985 peripheral blood acute myeloid leukaemia cell, regardless of MCU expression level. Certain MCU-dependent pro-oncogenic events are thought to be more sensitive to mitoxantrone, such as the increased metastatic abilities of MCF-7 cells and HCC cells, as well as the MCU-dependent chemotherapy resistance in multiple myeloma cells (Arduino et al., 2017; Ren et al., 2017; Song et al., 2013; Yu et al., 2017). If this hypothesis is true, as double-targeting agents, mitoxantrone and its effective analogues should be more potent in treating specific cancers.

Recently, the cryo-EM/crystal structures of full length MCU were revealed by several groups. By using an N-terminal domain (NTD) dimer-of-dimers assembly manner, which is similar to that of **ionotropic glutamate receptors**, MCU forms the channel. The interactions between the Tyr residue (Y268 in hMCU) with nearby transmembrane helices facilitate a rotamer switch of one pair of tyrosines to control Ca^{2+} flow through the pore. This is probably how the phosphorylation of the NTD or divalent cation binding to the NTD manipulate MCU function. In the selectivity filter, unlike the large superfamily of cation channels that share loop-like secondary structures, it is formed by α-helices that coordinate Ca^{2+} with rings of acidic amino acids in MCU. They also indicate potential sites of channel regulation by other molecules within the mitochondrial matrix, besides the above listed MCU accessory proteins located at the IMM (Baradaran et al., 2018; Fan et al., 2018; Yoo et al., 2018). In *Metarhizium acridum* MCU, a conserved interaction network among Trp^{332}, Glu^{336}, and Pro^{337} stabilizes closely spaced carboxylate groups of the Glu^{336} ring, which might contribute to the high-affinity binding and high selectivity of Ca^{2+} (Fan et al., 2018). Although higher resolution is needed for further validation of the structure and functional mechanism of MCU, this approach will definitely accelerate the development of structure-based computer-aided drug design, such as the identification of novel molecules, hit-to-lead optimization of affinity, and selectivity.

5 | CONCLUDING REMARKS

The discoveries of MCU and its regulatory molecules open a new era in study of the uniplex-mediated mitochondrial Ca^{2+} homeostasis in cancer. It is now possible to search the specific modulators of MCU based on molecular structure and to create transgenic animals by genetic tools. Studies have revealed that the regulation of MCU-dependent mitochondrial Ca^{2+} homeostasis may be context- and tissue-specific. Some questions regarding the regulatory mechanisms still remain to be answered. For example, why uniplex needs dual regulators (MCUb and MICU2) as negative regulators or gatekeeper proteins? The post-translational regulation mechanisms of MCU, especially its regulatory proteins, are still mysterious. Currently, investigations into the alterations of uniplex-mediated $[Ca^{2+}]_m$ signalling in cancer are still at the nascent stage, and more studies are urgently needed. The range of candidate agents available for targeting the uniplex for cancer chemotherapy is still limited, especially with regard to MCU agonists and modulators of MCU regulatory proteins. Once these problems are solved, they will definitely shed light on the contribution of uniplex-mediated Ca^{2+} signalling in carcinogenesis and improve the clinical chemotherapeutic outcomes in cancer patients.

5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander, Fabbro et al., 2017; Alexander, Kelly, Marrion, Peters, Faccenda, Harding, Pawson, Sharman, Southan, Buneman et al., 2017; Alexander, Kelly, Marrion, Peters, Faccenda, Harding, Pawson, Sharman, Southan, Davies et al., 2017a,b; Alexander, Peters et al., 2017).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Chaochu Cui https://orcid.org/0000-0003-0907-9766
Liwu Fu https://orcid.org/0000-0003-2834-2961

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