Emergence of repurposed drugs as modulators of MCU channel for clinical therapeutics

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Abstract:

In metazoans, the compartmentalization of cellular Ca^{2+} is essential for its messenger activity to control signal transduction, bioenergetics, and cell death. Upon cellular activation via numerous ligands, hormones, mechanical forces and small molecule drugs elicit cytosolic Ca^{2+} dynamics that is rapidly cleared by multiple modalities including mitochondrial highly selective Ca^{2+} ($_mCa^{2+}$) uptake channel, mitochondrial calcium uniporter (MCU) complex. Recently, De Mario et al., conducted small molecule screen to identify MCU modulators that control mitochondrial Ca^{2+} uptake as a proof-of-concept.

Mitochondrial Ca^{2+} uptake is mediated by the highly selective channel, the mitochondrial calcium uniporter (MCU) (Baughman et al., 2011; De Stefani et al., 2011; Kirichok et al., 2004; Nemani et al., 2018) and occurs in response to various physiological stimuli, which are often triggered by the release of Ca^{2+} from intracellular stores within the endoplasmic reticulum.. The core components of the MCU complex include pore-forming subunits (i.e., MCU, and Essential MCU Regulator [EMRE]) and regulatory proteins (i.e., MCUb, MCUR1, MICU1, MICU2, MICU3, LETM1, and SLC25A23) (Alevriadou et al., 2021) Several studies have elucidated the structure of the MCU alone (Baradaran et al., 2018; Fan et al., 2018; Nguyen et al., 2018; Yoo et al., 2018) and in combination with EMRE (Fan et al., 2020; Wang et al., 2019), revealing this channel to be tetrameric and the stoichiometry of MCU subunits to EMRE as one to one. Genetic variants of the MCU complex components have been linked to the development of several diseases, suggesting that this channel plays an important role organismal physiology. For example, MCU overexpression is associated with the progression of lung (Tosatto et al., 2016), gastric (Wang et al., 2001), and liver cancers (Li et al., 2020). Furthermore, the MCU positively regulates myofiber size, and a skeletal muscle-specific MCU deletion inhibits myofiber mitochondrial Ca^{2+} uptake, resulting in impaired muscle force and exercise performance (Gherardi et al., 2019).

Mutations in the regulatory component MICU1 have been reported in patients affected by proximal myopathy, learning difficulties, and extrapyramidal movement disorder (Logan et al., 2014). Furthermore, MICU1 was downregulated in db/db mouse hearts, which contributes to myocardial apoptosis in diabetes (Ji et al., 2017). As observed with MICU1, a homozygous truncating mutation in MICU2 lead to severe neurodevelopmental disorder

affecting consanguineous patients (Shamseldin et al., 2017). Additionally, silencing of MICU2 was recently been linked to impaired pancreatic beta-cell function (Vishnu et al., 2021). Taken together, these results paint a compellingly picture regarding the physiological importance of the MCU complex in maintaining normal cellular function.

Considering the fundamental importance of mitochondrial Ca^{2+} uptake for organ physiology and the pathological consequences of its dysregulation (Mammucari et al., 2018), the development of small-molecule modulators of the MCU are valuable tools for understanding these processes (Woods and Wilson, 2020). Ruthenium-based MCU inhibitors such as Ru360 (Arduino et al., 2017) and its more cell-permeable and stable analogue Ru265 (Woods et al., 2019) with inhibitory activities in the low micromolar to nanomolar range, have recently been discovered. Although these compounds are generally potent and effective, some ruthenium compounds (PMID: 7543979) have been associated with neurotoxic effects. Therefore, ongoing research has sought new MCU-modulators that have established biological applications. As a step towards this direction, a high throughput screen (HTS) for chemical compounds was carried out by expressing mitochondrially targeted aequorin, which upon stimulation with inositol tris phosphate (IP_3)-generating agonist gives a high fluorescence emission in response to mitochondrial Ca^{2+} entry. Using this assay, two compounds called MCU-i4 and MCU-i11 were identified to be MCU inhibitors with μM activity. Further research on these compounds revealed them to specifically bind to a cleft in MICU1, resulting in mitochondrial Ca^{2+} uptake inhibition (Di Marco et al., 2020). With respect to compounds that increase, rather than inhibit, the activity of the MCU, the p38 mitogen-activated protein kinase inhibitor SB202190 was found to modulate mitochondrial Ca^{2+} uptake in this manner with a

mechanism that is independent of p38 activity (Montero et al., 2002). In addition, several natural plant flavonoids have been shown to increase MCU activity via mechanisms that are distinct from those that mediate their antioxidant activity (Montero et al., 2004). In particular, 4,4′,4″-(4-propyl-[1 h]-pyrazole-1,3,5-triyl) trisphenol (PPT), diethylstilbestrol, and 17-β-estradiol activate mitochondrial Ca^{2+} uptake, whereas tamoxifen and 4-hydroxy-tamoxifen inhibits MCU activity (Lobaton et al., 2005). Although several strategies have been employed to identify the selective modulators of MCU, newer approaches are forthcoming.

In this work De Mario et. al utilized both mitochondria matrix-targeted (mitAEQ) and cytoplasm-targeted (cytAEQ) versions of the Ca^{2+}-responsive recombinant protein aequorin to screen a library of small molecules comprising 1,600 US Food and Drug Administration (FDA)-approved drugs for their ability to act on mitochondrial Ca^{2+} uptake without affecting cytosolic Ca^{2+} transients (De Mario et al., 2021). They used inositol 1,4,5-trisphosphate (IP_3)-generating agonists to release Ca^{2+} from ER stores and measured the subsequent elevation in mitochondrial and $[Ca^{2+}]_c$ upon incubation with a panel of compounds. False-positive hits, defined as compounds that dissipated the $\Delta\Psi_m$ or failed to alter mitochondrial Ca^{2+} uptake speed in permeabilized cells, were removed from the screen. From this assay, the authors successfully identified amorolfine and benzethonium as an activator and inhibitor of mitochondrial Ca^{2+} uptake, respectively. Amorolfine, a morpholine antifungal drug, triggers mitochondrial Ca^{2+} uptake in both intact and permeabilized cell systems with an EC_{50} value of 86.88 μM. Furthermore, amorolfine increased myotube size in vitro in an MCU-dependent manner and induced muscle hypertrophy in vivo, consistent with prior studies

that have correlated MCU overexpression with these phenomenon (Mammucari et al., 2015). Taken together, amorolfine is a genuine, selective MCU channel activator. In future studies, it will be valuable the binding site of this molecule within the MCU complex to further understand its mechanism of action and aid in the design of new, more potent synthetic analogs.

Benzethonium is a synthetic quaternary ammonium salt with antiseptic properties. The inhibitory effects of benzethonium on mitochondrial Ca^{2+} uptake in this study were verified in MDA-MB-231 cells, a triple-negative breast cancer cell line in which. Consistent with prior studies that showed MCU silencing to diminish both migration and growth of this breast cancer cell line (Tosatto et al., 2016), this compound induced a similar phenotypic response, indicating that it effectively inhibits the MCU in vitro. In addition, consistent with the role of mitochondrial Ca^{2+} uptake in cell death (Dong et al., 2017; Mallilankaraman et al., 2012), benzethonium protected cells from pro-apoptotic stimuli, like ceramide. Moreover, benzethonium significantly reduced histamine-induced mitochondrial Ca^{2+} uptake with EC50 of 21.55 μM in cells pre-treated for 1 h with the compound. Furthermore, it reduced basal, ATP-linked, and maximal respiration when administered at a concentration of 1 μM. However, a point of concern regarding its activity was its limited cellular permeability, as it was required to incubate the cells for 1 h with this compound to observe a substantial MCU channel inhibition. Overall, this study utilized a new approach to identify FDA-approved drugs as modulators of MCU channel activity, which could be leveraged for therapeutic applications in the future.

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Figure legend:

Figure 1: Targeted compound library screen identifies MCU channel modulators: De Mario et al., utilized FDA-approved drug library to identify the MCU channel modulators. Amorolfine and Benzethonium emerged as a positive and negative regulator of the channel activity.

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