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Commentary

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

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Mitochondrial Ca^{2+} uptake is mediated by the highly selective channel the mitochondrial calcium uniporter (MCU) [1-4] and occurs in response to various physiological stimuli, which are often triggered by the release of Ca^{2+} from 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). Several studies have elucidated the structure of the MCU alone and in combination with EMRE [4], revealing a tetrameric structure with 1:1 stoichiometry with EMRE. Genetic variants of the MCU complex components have been linked to the development of several diseases, suggesting that this channel plays an important role in organismal physiology. For example, MCU overexpression is associated with the progression of lung, gastric, and liver cancers. 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.

Mutations in the regulatory component MICU1 have been reported in patients with proximal myopathy, learning difficulties, and extrapyramidal movement disorder [5]. Furthermore, MICU1 was downregulated in db/db mouse hearts, which contributes to myocardial apoptosis in diabetes. A homozygous truncating mutation in MICU2 leads to severe neurodevelopmental disorder affecting consanguineous patients. Additionally, silencing of MICU2 was recently been linked to impaired pancreatic beta-cell function. Together, these finding 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, the development of small-molecule modulators of the MCU are valuable tools for understanding these processes [6]. Ruthenium-based MCU inhibitor such as Ru360 [7] and its more cell-permeable and stable analogue Ru265 [8] with inhibition in the low micromolar to nanomolar range, have recently been discovered. Although these compounds are generally potent and effective, some ruthenium compounds 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) of 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 potency. These compounds specifically bind to a cleft in MICU1, resulting in mitochondrial Ca^{2+} uptake inhibition [9]. 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 with a mechanism that is independent of p38 activity. 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. 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 inhibit MCU activity. Although several strategies have been employed to identify 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 1600 US Food and Drug Administration (FDA)-approved drugs for their ability to act on mitochondrial Ca^{2+} uptake without affecting cytosolic Ca^{2+} transients [10]. 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 mitochondrial membrane potential ($\Delta\Psi_m$) or failed to alter mitochondrial Ca^{2+} uptake speed in

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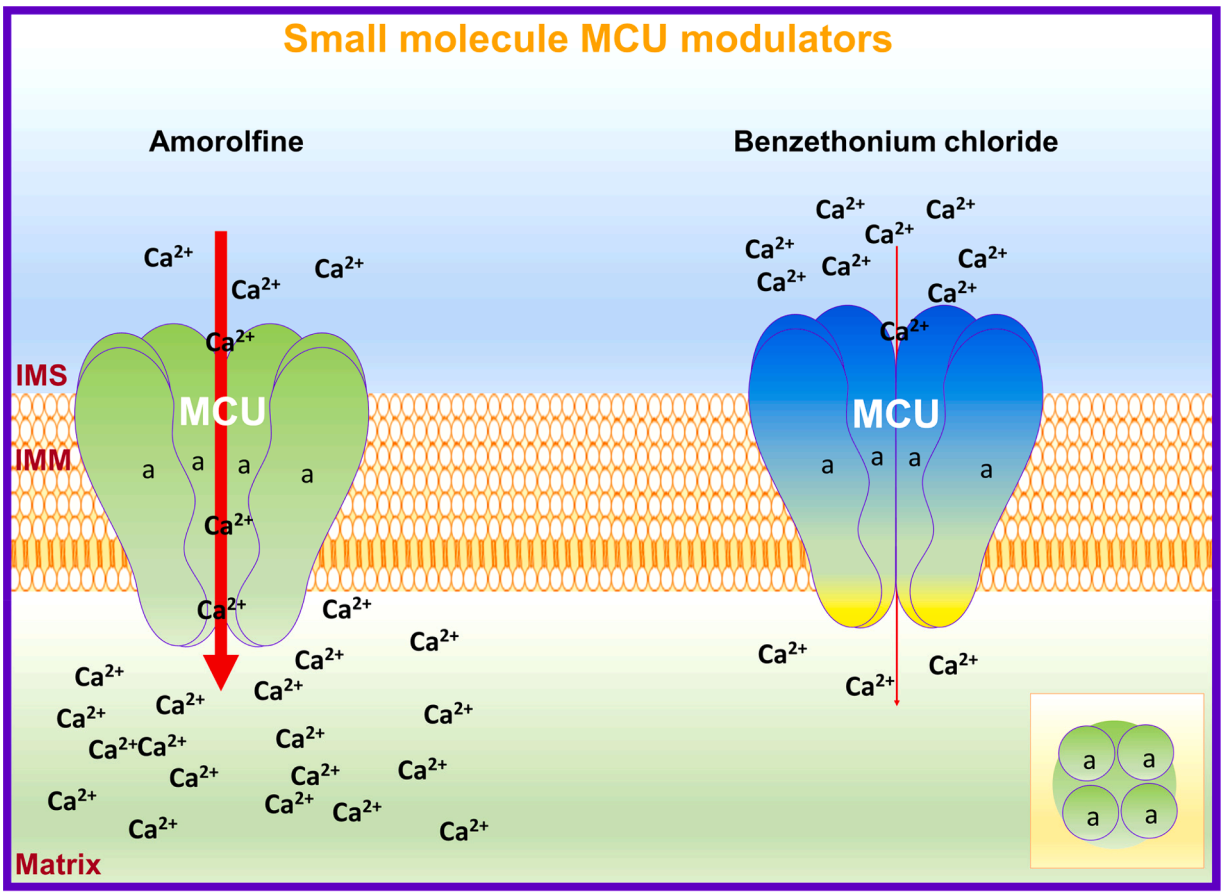

Fig. 1. Targeted compound library screen identifies MCU channel modulators: De Mario et al., utilized FDA-approved drug library to identify the MCU channel modulators. Amorolfine and Benzethonium emerged as a positive and negative regulator of the channel activity.

permeabilized cells, were removed from the screen. With 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. Taken together, amorolfine is a genuine, selective MCU channel activator. In future studies, it will be valuable to determine 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. Fig. 1.

Benzethonium is a synthetic quaternary ammonium salt with antiseptic properties. The inhibitory effects of benzethonium on mitochondrial Ca^{2+} uptake was verified in MDA-MB-231 cells, a triple-negative breast cancer cell line. Consistent with prior studies that showed MCU silencing to diminish both migration and growth of this breast cancer cell line, 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, 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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