Cell Calcium 87 (2020) 102193

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Nuclear-encoded NCX3 and AKAP121: Two novel modulators of mitochondrial calcium efflux in normoxic and hypoxic neurons

Maria Josè Sisalli[a], Antonio Feliciello[b], Salvatore Della Notte[a], Rossana Di Martino[a], Domenica Borzacchiello[b], Lucio Annunziato[c], Antonella Scorziello[a,*]

[a] Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples, Italy
[b] Department of Molecular Medicine and Medical Biotechnology, Federico II University of Naples, Italy
[c] IRCCS SDN Naples, Italy

ARTICLE INFO

Keywords:
mNCX3
AKAP121
Siah2
Fission
Fusion
Hypoxia
Mitochondria calcium handling

ABSTRACT

Mitochondria are highly dynamic organelles extremely important for cell survival. Their structure resembles that of prokaryotic cells since they are composed with two membranes, the inner (IMM) and the outer mitochondrial membrane (OMM) delimitating the intermembrane space (IMS) and the matrix which contains mitochondrial DNA (mtDNA). This structure is strictly related to mitochondrial function since they produce the most of the cellular ATP through the oxidative phosphorylation which generate the electrochemical gradient at the two sides of the inner mitochondrial membrane an essential requirement for mitochondrial function. Cells of highly metabolic demand like those composing muscle, liver and brain, are particularly dependent on mitochondria for their activities. Mitochondria undergo to continual changes in morphology since, they fuse and divide, branch and fragment, swell and extend. Importantly, they move throughout the cell to deliver ATP and other metabolites where they are mostly required. Along with the capability to control energy metabolism, mitochondria play a critical role in the regulation of many physiological processes such as programmed cell death, autophagy, redox signalling, and stem cells reprogramming. All these phenomena are regulated by Ca^{2+} ions within this organelle. This review will discuss the molecular mechanisms regulating mitochondrial calcium cycling in physiological and pathological conditions with particular regard to their impact on mitochondrial dynamics and function during ischemia. Particular emphasis will be devoted to the role played by NCX3 and AKAP121 as new molecular targets for mitochondrial function and dysfunction.

1. Introduction

Mitochondria are intracellular organelles which play a key role for cell survival. Their structure resembles that of prokaryotic cells since they are delimitated by a double membrane, the inner (IMM) and the outer mitochondrial membrane (OMM) that on their own are able to outline two aqueous compartments, the intermembrane space (IMS) [1,2] and the matrix which contains mitochondrial DNA (mtDNA) as depicted in Fig. 1. This structure is strictly related to mitochondrial function. Indeed, the OMM oriented towards the cytosol is important to allow mitochondrial communication with the cytosol, and the IMM protruding into the mitochondrial matrix which is organized in cristae and contains all the component of oxidative phosphorylation system, including the respiratory complexes I to IV and the F1F0-ATP synthase [3]. The matrix represents the place in which metabolic activity takes place (Fig. 1). Indeed, mitochondria are the primary generators of cellular energy since they efficiently produce more than 90 % of cellular ATP through oxidative phosphorylation, a chemiosmotic process in which the high energy of electrons are funnelled from NADH to oxygen through the respiratory chain. The energy consumed in this process allows the H^+ pumping from mitochondrial matrix to the intermembrane space in order to generate the electrochemical gradient at the two sides of the inner mitochondrial membrane. This contribute to generate the mitochondrial membrane potential ($\Delta\Psi_m$), that is essential for mitochondrial function [4–6] since it is fundamental for the efficient production of ATP and requires the coordinated activity of several enzyme complexes (Fig. 1). Cells with a high metabolic demand as those composing muscle, liver and brain, are therefore dependent for their activities on mitochondrial efficiency. More in details, mitochondrial distribution within the cells is mainly dictated by the ATP consumption

* Corresponding author at: Division of Pharmacology, Department of Neuroscience, School of Medicine, Federico II University of Naples, Via Sergio Pansini 5, 80131, Naples, Italy.
E-mail address: scorziel@unina.it (A. Scorziello).

https://doi.org/10.1016/j.ceca.2020.102193
Received 19 February 2020; Received in revised form 2 March 2020; Accepted 4 March 2020
Available online 06 March 2020

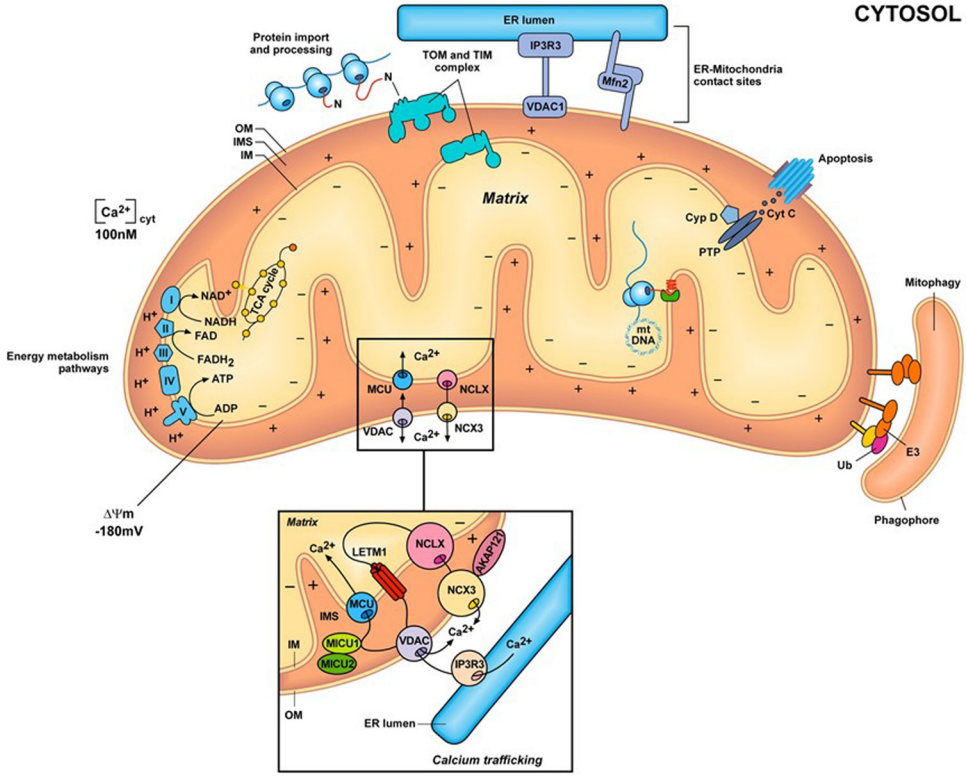

Fig. 1. Mitochondrial structure and functions. Mitochondria consist of four compartments: outer membrane (OM), intermembrane space (IMS), inner membrane (IM) and matrix. Mitochondria coordinate many essential metabolic processes and contribute to different cellular pathways. Among them: energy metabolism with respiration and synthesis of ATP; maintenance of mitochondrial electrochemical gradient; expression of the mitochondrial genome; protein import and signaling; endoplasmic reticulum interaction and cytosolic calcium homeostasis, quality control and degradation processes including mitophagy and apoptosis.
Inset: molecular pathways involved in the regulation of mitochondrial calcium cycling.
E3, ubiquitin-protein ligase; ER, endoplasmic reticulum; mtDNA, mitochondrial DNA; TCA, tricarboxylic acid; Ub, ubiquitin.

as it happens in neurons at synaptic level or in correspondence of Ranvier's nodes [7–10]. Along with the ability to control cellular energy metabolism, mitochondria take part to numerous other physiological processes such as apoptosis, autophagy, redox signalling, and stem cells reprogramming [11–14]. All these phenomena are dependent by the amount of Ca^{2+} ions contained into the organelles (Fig. 1) [15–17]. In this review will be discussed the molecular mechanisms regulating mitochondrial calcium cycling in physiological and pathological conditions with particular regard to their impact on mitochondrial dynamics and function during ischemia. Particular emphasis will be devoted to the role played by the nuclear encoded NCX3/AKAP121 mitochondrial complex as new molecular target for mitochondrial function and dysfunction.

2. Molecular mechanisms regulating mitochondrial calcium homeostasis

Mitochondria represent one of the major cellular compartment involved in the regulation of calcium homeostasis thank to the coordinated action of different release/uptake systems localized on the IMM, thus playing a critical role in the control of cytosolic Ca^{2+} signals and Ca^{2+}-dependent proteins' activity like kinases, phosphatases, proteases, transcription factors and ion channels. Moreover, Ca^{2+} ion represents an important intracellular messenger responsible for many physiological and pathophysiological processes [18]. On the other hand, the maintaining of Ca^{2+} concentration within a range is an important requirement ensuring mitochondrial function. In fact, mitochondrial dehydrogenases are dependent on calcium for their activities, and they are mainly involved in the regulation of oxidative phosphorylation and ATP synthesis [19]. These mitochondrial Ca^{2+}-mediated processes occur within milliseconds and depend on the spatiotemporal distribution of $[Ca^{2+}]_I$, the so called high-Ca^{2+}-contained-microdomains, identified near the plasma membrane, in proximity of Ca^{2+} channels, and close to the endoplasmic reticulum (ER), two cellular regions where mitochondria are more localized [20–22].

The driving forces for mitochondrial calcium uptake are the electrochemical gradient, generated at the level of IMM, and a relatively low $[Ca^{2+}]_m$. However, $[Ca^{2+}]_m$ is finely regulated by the coordinated activity of many transporters and channels distributed along the IMM and the OMM. Calcium enters into mitochondria through the mitochondrial Ca^{2+} uniporter (MCU) which mediates the Ca^{2+} uptake across the IMM into the matrix in an energy-dependent manner [23,24], and is extruded from the mitochondria by two different mechanisms one Na^+-dependent (NCE) and another one Na^+-independent (NICE) [25–28], both mechanisms endowed with proper activation and calcium binding capabilities [29–31]. The Na^+-independent Ca^{2+} efflux, which exchanges Ca^{2+} with H^+, is the main mitochondrial Ca^{2+} efflux system in non-excitable cells [6]. This transporter saturates at low calcium loads with an extremely slow kinetic [32]. These features of the mitochondrial Ca^{2+} machinery, high Vmax uptake transport systems coupled to slow and easily saturable efflux system, increase the risk of mitochondrial Ca^{2+} overload [33]. Another actor in mitochondrial calcium handling is the voltage-dependent anion channel (VDAC) which is localized on the OMM and contributes to Ca^{2+} flux within the intermembrane space to promote the activity of the specific transport systems of the IMM [34]. Moreover, VDAC is a component of the mitochondrial permeability transition pore (PTP) which concurs in mitochondrial Ca^{2+} efflux [32,34].

During the last years, extensive efforts have been devoted to clarify the identity of the systems regulating mitochondrial calcium efflux through the Na^+-dependent pathways. The Li+-dependent Na^+/Ca^{2+} exchanger (NCLX), a member of the cation/Ca^{2+}- exchanger (CCX) superfamily localized on the IMM, mediates sodium- and lithium-dependent calcium efflux from the mitochondrial matrix to the intermembrane space [27] (Fig. 1, inset). Nevertheless, the mechanisms regulating the expression, transport, tissue distribution of NCLX, as well as its contribution to mitochondrial activity in pathological conditions were only recently addressed [35,36]. On the other hand, Duchen's group demonstrated that the OMM is not so permeable to calcium fluxes as it was previously thought, since this outer mitochondrial membrane plays an active role in directing mitochondrial function and Ca^{2+} cycling [37]. This emerging concept let to hypothesize that the

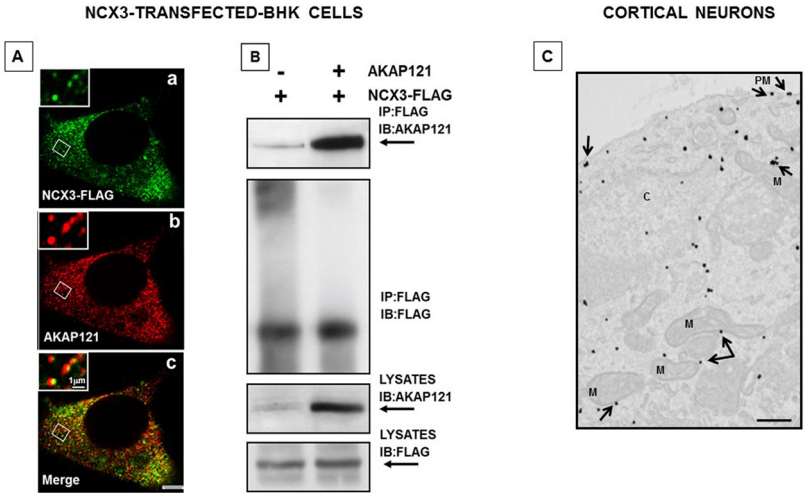

Fig. 2. Mitochondrial localization of NCX3 and AKAP121 in NCX3-stably transfected BHK cells and in cortical neurons. (A-B) NCX3 and AKAP121 colocalization and interaction in BHK cells. Scale bar: 5μm; (C) NCX3 localization on the OMM in primary cortical neurons. Scale bar: 200 nm.

mechanisms regulating mitochondrial calcium homeostasis are more complex and dynamic. Recent findings provided evidence that NCX3 isoform, belonging to the NCX family, coded by nuclear DNA, as the majority of mitochondrial proteins [38], is present on the OMM of neurons (Fig. 2C) and contributes to mitochondrial calcium efflux [28]. In particular, NCX3 plays this role due to the physical interaction with AKAP121 (Fig. 2A), a protein that anchors protein kinase A (PKA) to the OMM, and it controls mitochondrial metabolism and neuronal survival [39]. This interaction between the nuclear encoded NCX3 and AKAP121 occurs on the OMM in a PKA-dependent manner and is crucial for improving neuronal survival since it allows mitochondrial Ca^{2+} extrusion in physiological and in pathophysiological conditions such as hypoxia [28].

Different families of mitochondrial AKAPs have been identified [39,40]. Mitochondrial AKAP-PKA complex controls the activation and integration of distinct signaling events at mitochondrial level, supporting oxidative phosphorylation, metabolism, calcium homeostasis, survival and global protein synthesis [39,41]. AKAPs, thus, represent an important signaling hub that optimally adapt mitochondrial functions to rapid changes of the extracellular microenvironment. It has been demonstrated that AKAP121 promotes the mitochondrial respiratory activity, induces mitochondrial membrane hyperpolarization and improves the ATP synthesis in a PKA dependent manner [42]. Interestingly, in conditions of low oxygen availability, the cells promotes AKAP121 degradation in order to preserve mitochondrial activity [39].

3. Calcium-dependent mechanisms regulating mitochondrial dynamics

It is now largely proved that mitochondria are highly dynamic organelles that continually undergo to changes in their morphology known as mitochondrial dynamics. Indeed, they are able to fuse and fragment, to cluster or to stay as individual entities and to move throughout the cell in anterograde and in retrograde way to provide ATP and other metabolites where they are mostly required. These events are particularly evident in neurons, at presynaptic and at postsynaptic terminals, where mitochondria are more concentrated and where the energetic demand is particularly high [43]. Moreover, mitochondria contribute to synaptic plasticity thank to their ability to maintain calcium homeostasis in the synaptic microenvironment. The changes in mitochondrial morphology are also important to preserve mitochondrial function since they allow the mixing and the exchange of small molecules, proteins and mtDNA among the organelles [44]. Finally, fusion and fission are crucial for the mitochondrial quality control, since these processes regulate not only the overall morphology of the mitochondrial population, but also their proper functions including oxidative phosphorylation, membrane potential, and DNA replication/repair [45]. Fusion is regulated by three proteins: the two mitofusins, Mfn1 and Mfn2, and OPA1. Mfn1 and Mfn2 are GTPases localized on the OMM [46–48], whereas OPA1 is a dynamin-related GTPase localized at the IMS very close to the IMM [49–51]. Fission consists in division of one mitochondrion into two daughter mitochondria, and is essential for organelle biogenesis and inheritance. The deregulation of this phenomenon brings out to the formation of dysfunctional organelles with an unequally mtDNA content, an altered energy production, and a larger capacity to produce reactive oxygen species which in turn make the cells more prone to apoptosis [51]. Therefore, fission is extremely relevant to remove damaged mitochondria through a process known as mitophagy [52,53]. Two are the proteins regulating mitochondrial fission: Fis1 and Drp1 [54]. Fis1 is uniformly distributed in mitochondria at the level of the OMM, whereas Drp1, a dynamin-related GTPase, is a cytosolic protein which oligomerizes into puncta on the OMM, depending on cellular stimuli, to activate mitochondrial fission. Blocking the activity of these proteins leads to the elongation and interconnectivity of mitochondria [54–57]. It is interesting to underline that Drp1 activity is inhibited by PKA activation [58] whereas, it is stimulated by the calcium-activated phosphatase calcineurin [58,59]. Studies performed in a cardiac cell model of ischemia demonstrated that mitochondria undergo to fragmentation through Drp1 activation [60], thus indicating the importance of Drp1 in the pathogenesis of cerebral ischemia/riperfusion.

Interestingly, we have recently demonstrated that the nuclear encoded NCX3/AKAP121 mitochondrial complex on the OMM regulates mitochondrial dynamics by controlling mitochondrial shape and mitochondrial oxidative metabolism in neurons exposed to hypoxia and hypoxia followed by reoxygenation [61].

4. Mitochondrial calcium handling affects mitochondrial function and dynamics in *in vitro* anoxia and *in vivo* ischemia

Mitochondria play a central role in ischemia-induced cell death either because they became unable to produce the adequate amount of ATP for neuronal function, or because they release factors leading to cell demise [62–68]. The cascade of events activated by ATP depletion during ischemia, causes neuronal membrane depolarization and glutamate release which provokes a massive increase in cytosolic Ca^{2+} with the consequent swelling of the cells [69–72]. Due to coordinated activity of specific channels and transporters on the OMM and IMM,

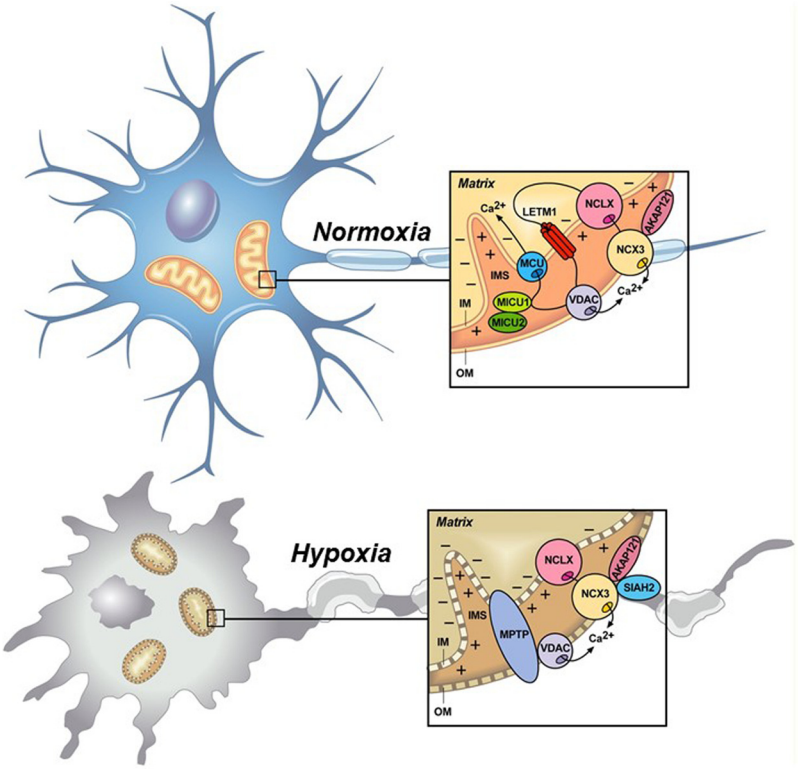

Fig. 3. NCX3 and AKP121 involvement in mitochondrial dynamics during hypoxia. (A) In normoxic conditions the nuclear encoded NCX3/AKAP121 mitochondrial complex on the outer mitochondrial membrane preserves mitochondrial function; (B) In hypoxic conditions, the binding of Siah2 to the nuclear encoded NCX3/AKAP121 mitochondrial complex on the outer mitochondrial membrane promotes its ubiquitin-mediated proteasome degradation impairing mitochondrial function and promoting mitochondrial fission.

mitochondria are able to face the increase in free cytosolic calcium by promoting mitochondrial Ca^{2+} uptake. The excessive increase in matrix [Ca^{2+}] alters the mitochondrial membrane potential and impairs mitochondrial capacity to produce ATP with consequent release of pro-apoptotic factors [73,74] (Fig. 1). On the other hand, calcium released from the endoplasmic reticulum also contributes to ischemia induced-cell damage [75]. Due to the close proximity between mitochondria and ER, the release of calcium ions through IP3 receptor further promotes the entry of calcium in adjacent mitochondria [76–78] (Fig. 1). These events, by exposing mitochondria to high Ca^{2+} concentrations for long period of time favours Ca^{2+} uptake by the MCU, and stimulate mitochondria calcium buffering activity [79]. It is clear that calcium accumulation by mitochondria induces the membrane permeability transition, *via* the calcium-activated pore (mPTP) in the inner membrane and impairs their ability to face cellular metabolism (Fig. 3).

The recent demonstration that new mitochondrial proteins have been identified as regulators of mitochondrial Ca^{2+} uptake and efflux provide a chance to deeply investigate the role of mitochondrial Ca^{2+} handling in the ischemic neuronal damage. This is supported by the finding that in neurons the nuclear encoded NCX3 is localized on the OMM (Fig. 2 C) and it is particular evident in those mitochondria distributed close to the plasmatic membrane [28], where energy is required to promote the activity of proteins involved in the regulation of ionic homeostasis [80,81].

In the light of these findings and of the results obtained by Palty et al., who demonstrated that NCLX, the component of the Na^+/Ca^{2+} exchanger superfamily in the IMM, has a role in removing Ca^{2+} ions from the mitochondria [27], it is reasonable to hypothesize that in anoxic conditions the Na^+/Ca^{2+} exchange from mitochondria requires two consecutive steps. The first one, mainly mediated by NCLX, which participate to the removal of Ca^{2+} from the matrix to the intermembrane space, and the second one, mainly operated by the nuclear encoded NCX3/AKAP121 mitochondrial complex, that promotes the Ca^{2+}removal from the intermembrane space to the cytosol (Fig. 1 inset). Consistent with the role played by the nuclear encoded NCX3/AKAP121 mitochondrial complex in the regulation of mitochondrial Ca^{2+} efflux on the OMM we demonstrated that this antiporter preserves mitochondrial function and contributes to cells survival in hypoxic conditions [28]. The relevance of the interaction between these two proteins, NCX3 and AKAP121 (Fig. 2 A–B), is extremely important when we look at mitochondrial function in cells exposed to hypoxic condition since, we reported that in the absence of AKAP121, the mNCX3-promoted Ca^{2+} efflux was reduced with consequent risk of increased cell death. Therefore, it is possible to speculate that, when activated on the OMM, the nuclear-encoded-NCX3/AKAP121 mitochondrial complex participates to mitochondrial Ca^{2+} handling, thus helping mitochondrial metabolism and, in turn, promoting cellular survival. This hypothesis is in accordance with data previously described by Secondo et al. in BHK cells stably transfected with NCX3 [82]. In these cells, NCX3 significantly contributes to $[Ca^{2+}]_i$ homeostasis during ischemia and preserves mitochondria preventing $\Delta\Psi_m$ collapse and, consequently, counteracts cell death [82]. Accordingly, the experiments performed in primary culture of cortical neurons under OGD and OGD/reoxygenation conditions emphasize the key role played by the nuclear encoded mNCX3/AKAP121 complex in the regulation of mitochondrial Ca^{2+} extrusion in pathological conditions. In fact, $[Ca^{2+}]_m$ significantly increased in neurons during OGD, when NCX3 is impaired [83], and decreased during OGD followed by reoxygenation, when NCX3 expression returned to the basal values [83]. Interestingly, the NCX3 knocking down affected mitochondrial [Ca^{2+}] under basal and OGD/reoxygenation conditions, since it was reduced, whereas no alteration in mitochondrial Ca^{2+} extrusion can be detected during OGD [28]. This is in line with data previously reported about the changes in the expression of NCX3 and AKAP121 detected during OGD and OGD followed by reoxygenation [39,83]. Moreover, these findings have relevant physiological and pathophysiological implications since, the crosstalk between energetic function and Ca^{2+} homeostasis strongly affects mitochondrial oxidative metabolism and promotes mitochondrial respiration [84–86]. However, when $[Ca^{2+}]_m$ rises over its set-point, the ability of mitochondria to produce ATP dramatically decreases leading to irreversible depolarization of IMM and consequent apoptosis [87,88]. Therefore, the nuclear encoded NCX3/AKAP121

mitochondrial complex for its ability to finely tune mitochondrial calcium handling from the OMM is extremely important for preserving mitochondrial function. The functional relevance of this finding resides in the demonstration that the hypoxia-induced E3–Ub ligase Siah2 was detected in association with the nuclear encoded mNCX3/AKAP121 mitochondrial complex on the OMM. More interestingly, this interaction regulates AKAP121 and the nuclear encoded mNCX3 expression and activity through the stimulation of their ubiquitin-dependent proteasome degradation which in turn leads to mitochondrial dysfunction during ischemia [39,61]. The proteolytic degradation of the nuclear encoded mNCX3/AKAP121 complex is also associated to an imbalance between mitochondrial fission and fusion events with a consequent increase in mitochondrial fragmentation [61]. It is worth to underline that, in cortical neurons obtained from siah2 KO mice, the exposure to OGD and OGD followed by reoxygenation does not impair the expression of the nuclear encoded NCX3/AKAP121 mitochondrial complex, and as a consequence, mitochondrial function is preserved, as well as the balance between fission and fusion that is restored [61]. These findings confirm the key role played by the nuclear encoded NCX3/AKAP121 mitochondrial complex in the regulation of mitochondrial shape and functional properties. In fact, Siah2 ablation by preventing NCX3 degradation elicited by OGD, was able to preserve the balance between fragmentation and fusion, and to counteract the increases in $[Ca^{2+}]_m$ and mitochondrial depolarization observed in mice during hypoxic/ischemic conditions [39,61]. On the other hands, that NCX3 exerts a beneficial role on mCa^{2+} homeostasis is also reinforced by the data obtained in neurons exposed to a sub-lethal ischemic insult, known as ischemic preconditioning, demonstrating that NCX3 expression and activity was increased. This effect is associated to a reduction of mitochondrial calcium levels, which in turn, renders the neurons more tolerant to the subsequent lethal ischemic insult. Interestingly, this effect is prevented by siNCX3 and CGP37157, a selective inhibitor of mitochondrial NCX [90], without affecting cytosolic calcium concentration [89].

5. Conclusions

Collectively, the data reported suggest new perspectives to explore the role of mitochondrial Ca^{2+} handling in neuronal death occurring in pathological conditions like cerebral ischemia. Indeed, targeting the proteins that play a key role in governing Ca^{2+} fluxes across the IMM and the OMM not only might help to understand whether the altered mitochondrial Ca^{2+} handling is causally related to ischemic neuronal death, but it might also contribute to enrich the repertoire of therapeutic tools currently employed to interfere with ischemic brain demise. In this scenario, a strategy aimed to reduce mitochondrial Ca^{2+} concentration during ischemia might be focused to stimulate mitochondrial efflux either at the level of the IMM or of the OMM. The identification of NCLX and of nuclear encoded NCX3/AKAP121 mitochondrial complex as proteins able to regulate mitochondrial Na^+/Ca^{2+} exchange might, therefore, result in the identification of new promising targets for developing therapeutic strategies aimed to counteract mitochondrial dysfunction during ischemia/reperfusion in the brain. In this regard, the demonstration that the SIAH2/NCX3/AKAP121 pathway regulates fission and fusion balance by controlling mitochondrial morphology and mitochondrial oxidative metabolism let to speculate that the inhibition of Siah2 might be explored as a new druggable target to selectively prevent mNCX3 degradation and mitochondrial damage in ischemic conditions.

CRediT authorship contribution statement

Maria Josè Sisalli: Data curation, Formal analysis, Investigation. **Antonio Feliciello:** Writing - review & editing. **Salvatore Della Notte:** Writing - review & editing. **Rossana Di Martino:** Writing - review & editing. **Domenica Borzacchiello:** . **Lucio Annunziato:** Writing - review & editing, Funding acquisition. **Antonella Scorziello:** Conceptualization, Writing - review & editing, Funding acquisition, Supervision.

Acknowledgements

This work was supported by: Programma Operativo Nazionale: PerMedNet, from MIUR; NANOmiRNICTUS: Project from Ministero dello Sviluppo Economico, MISE to L.A. (F/050139/01-02/X32); PRIN 2015 and 2017 MIUR to A.S.

References

[1] A.J. Anderson, T.D. Jackson, D.A. Stroud, D. Stojanovski, Mitochondria—hubs for regulating cellular biochemistry: emerging concepts and networks, Open Biol. 9 (8) (2019) 190126.
[2] M. Picard, T. Taivassalo, G. Gouspillou, R.T. Hepple, Mitochondria: isolation, structure and function, J. Physiol. 589 (2011) 4413–4421.
[3] P. Mitchell, J. Moyle, Chemiosmotic hypothesis of oxidative phosphorylation, Nature 213 (1967) 137–139.
[4] I. Martínez-Reyes, N.S. Chandel, Mitochondrial TCA cycle metabolites control physiology and disease, Nat. Commun. 11 (1) (2020) 102.
[5] N. Pfanner, B. Warscheid, N. Wiedemann, Mitochondrial proteins: from biogenesis to functional networks, Nat. Rev. Mol. Cell Biol. 20 (2019) 267–284.
[6] N.E. Saris, E. Carafoli, A historical review of cellular calcium handling, with emphasis on mitochondria, Biochemistry Mosc. 70 (2005) 187–194.
[7] C.H. Berthold, C. Fabricius, M. Rydmark, B. Andersén, Axoplasmic organelles at nodes of Ranvier. I. Occurrence and distribution in large myelinated spinal root axons of the adult cat, J. Neurocytol. 22 (1993) 925–940.
[8] E.A. Bristow, P.G. Griffiths, R.M. Andrews, M.A. Johnson, D.M. Turnbull, The distribution of mitochondrial activity in relation to optic nerve structure, Arch. Ophthalmol. 120 (2002) 791–796.
[9] G.H. Kageyama, M.T. Wong-Riley, Histochemical localization of cytochrome oxidase in the hippocampus: correlation with specific neuronal types and afferent pathways, Neuroscience 7 (1982) 2337–2361.
[10] K.C. Rowland, N.K. Irby, G.A. Spirou, Specialized synapse-associated structures within the calyx of held, J. Neurosci. 20 (2000) 9135–9144.
[11] K.J. Kamer, V.K. Mootha, The molecular era of the mitochondrial calcium uniporter, Nat. Rev. Mol. Cell Biol. 16 (2015) 545–553.
[12] V. Nikoletopoulou, M. Markaki, K. Palikaras, N. Tavernarakis, Crosstalk between apoptosis, necrosis and autophagy, Biochim. Biophys. Acta 1833 (2013) 3448–3459.
[13] A.S. Rambold, E.L. Pearce, Mitochondrial dynamics at the interface of immune cell metabolism and function, Trends Immunol. 39 (2018) 6–18.
[14] L. Tilokani, S. Nagashima, V. Paupe, J. Prudent, Mitochondrial dynamics: overview of molecular mechanisms, Essays Biochem. 62 (2018) 341–360.
[15] R. Rizzuto, D. De Stefani, A. Raffaello, C. Mammucari, Mitochondria as sensors and regulators of calcium signaling, Nat. Rev. Mol. Cell Biol. 13 (2012) 566–578.
[16] M. Giacomello, I. Drago, P. Pizzo, T. Pozzan, Mitochondrial Ca2+ as a key regulator of cell life and death, Cell Death Differ. 14 (2007) 1267–1274.
[17] G. Pallafacchina, S. Zanin, R. Rizzuto, Recent advances in the molecular mechanism of mitochondrial calcium uptake, eCollection (2018).
[18] E. Carafoli, R. Tiozzo, G. Lugli, F. Crovetti, C. Kratzing, The release of calcium from heart mitochondria by sodium, J. Mol. Cell. Cardiol. 6 (1974) 361–371.
[19] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[20] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11–21.
[21] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (2003) 517–529.
[22] M. Brini, R. Marsault, C. Bastianutto, J. Alvarez, T. Pozzan, R. Rizzuto, Transfected aequorin in the measurement of cytosolic Ca2+ concentration ([Ca2+]c). A critical evaluation, J. Biol. Chem. 270 (1995) 9896–9903.
[23] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R. Bao, L. Strittmatter, O. Goldberger, R.L. Bogorad, V. Koteliansky, V.K. Mootha, Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter, Nature 476 (2011) 341–345.
[24] D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340.
[25] E. Carafoli, R. Tiozzo, G. Lugli, F. Crovetti, C. Kratzing, The release of calcium from heart mitochondria by sodium, J. Mol. Cell. Cardiol. 6 (1974) 361–371.
[26] E. Carafoli, Historical review: mitochondria and calcium: ups and downs of an unusual relationship, Trends Biochem. Sci. 28 (2003) 175–181.
[27] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvili, I. Sekler, NCLX is an essential component of mitochondrial Na+/Ca2+ exchange, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 436–441.
[28] A. Scorziello, C. Savoia, M.J. Sisalli, A. Adornetto, A. Secondo, F. Boscia, A. Esposito, E.V. Polishchuk, R.S. Polishchuk, P. Molinaro, A. Carlucci, L. Lignitto, G. Di Renzo, A. Feliciello, L. Annunziato, J. Cell. Sci. 126 (2013) 5566–5577.
[29] E.J. Harris, Modulation of Ca2+ efflux from heart mitochondria, Biochem. J. 178

(1979) 673–680.
[30] A.L. Lehninger, A. Vercesi, E.A. Bababunmi, Regulation of Ca2+ release from mitochondria by the oxidation-reduction state of pyridine nucleotides, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 1690–1694.
[31] C. Ramachandran, F.L. Bygrave, Calcium ion cycling in rat liver mitochondria, Biochem. J. 174 (1978) 613–620.
[32] P. Bernardi, Mitochondrial transport of cations: channels, exchangers, and permeability transition, Physiol. Rev. 79 (1999) 1127–1155.
[33] C. Murgia, P. Giorgi, P. Pinton, R. Rizzuto, Controlling metabolism and cell death: at the heart of mitochondrial calcium signaling, J. Mol. Cell. Cardiol. 46 (2009) 781–788.
[34] M. Crompton, E. Barksby, N. Johnson, M. Capano, Mitochondrial intermembrane junctional complexes and their involvement in cell death, Biochimie 84 (2002) 143–152.
[35] M. Kostic, I. Sekler, Functional properties and mode of regulation of the mitochondrial Na +/Ca 2+ exchanger, NCLX, Semin. Cell Dev. Biol. 94 (2019) 59–65.
[36] P. Jadiya, D.W. Kolmetzky, D. Tomar, A. Di Meco, A.A. Lombardi, J.P. Lambert, T.S. Luongo, M.H. Ludtmann, D. Praticò, J.W. Elrod, Impaired mitochondrial calcium efflux contributes to disease progression in models of alzheimer's disease, Nat. Commun. 10 (2019) 3885–3898.
[37] G. Szabadkai, M.R. Duchen, Mitochondria: the hub of cellular Ca2+ signaling, Physiology 23 (2008) 84–94.
[38] D. Stojanovski, A.J. Johnston, I. Streimann, N.J. Hoogenraad, M.T. Ryan, Import of nuclear-encoded proteins into mitochondria, Exp. Physiol. 88 (2003) 57–64.
[39] A. Carlucci, A. Adornetto, A. Scorziello, D. Viggiano, M. Foca, O. Cuomo, L. Annunziato, M. Gottesman, A. Feliciello, Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischaemia, EMBO J. 27 (2008) 1073–1084.
[40] L. Rinaldi, R. Delle Donne, D. Borzacchiello, L. Insabato, A. Feliciello, The role of compartmentalized signaling pathways in the control of mitochondrial activities in cancer cells, Biochim. Biophys. Acta Rev. Cancer 1869 (2018) 293–302.
[41] R.A. Merrill, S. Strack, Mitochondria: a kinase anchoring protein 1, a signaling platform for mitochondrial form and function, Int. J. Biochem. Cell Biol. 48 (2014) 92–96.
[42] A. Livigni, A. Scorziello, A. Agnese, A. Adornetto, A. Carlucci, C. Garbi, I. Castaldo, L. Annunziato, E.V. Avvedimento, A. Feliciello, Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism, Mol. Biol. Cell 17 (2006) 263–271.
[43] M. Liesa, M. Palacín, A. Zorzano, Mitochondrial dynamics in mammalian health and disease, Physiol. Rev. 89 (2009) 799–845.
[44] H. Chen, D.C. Chan, Emerging functions of mammalian mitochondrial fusion and fission, Hum. Mol. Genet. 2 (2005) R283–289.
[45] L.D. Osellame, T.S. Blacker, M.R. Duchen, Cellular and molecular mechanisms of mitochondrial function, Best Pract. Res. Clin. Endocrinol. Metab. 26 (2012) 711–723.
[46] H. Chen, S.A. Detmer, A.J. Ewald, E.E. Griffin, S.E. Fraser, D.C. Chan, Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development, J. Cell Biol. 160 (2003) 189–200.
[47] A. Santel, M.T. Fuller, Control of mitochondrial morphology by a human mitofusin, J. Cell. Sci. 114 (2001) 867–874.
[48] T. Koshiba, S.A. Detmer, J.T. Kaiser, H. Chen, J.M. McCaffery, D.C. Chan, Structural basis of mitochondrial tethering by mitofusin complexes, Science 305 (2004) 858–862.
[49] S. Cipolat, O. Martins de Brito, B. Dal Zilio, L. Scorrano, OPA1 requires mitofusin 1 to promote mitochondrial fusion, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 15927–15932.
[50] L. Tilokani, S. Nagashima, V. Paupe, J. Prudent, Mitochondrial dynamics: overview of molecular mechanisms, Essays Biochem. 62 (2018) 341–360.
[51] M. Satoh, T. Hamamoto, N. Seo, Y. Kagawa, H. Endo, Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria, Biochem. Biophys. Res. Commun. 300 (2003) 482–493.
[52] P.A. ParoneA, S. Da Cruz, D. Tondera, Y. Mattenberger, D.I. JamesI, P. Maechler, F. Barja, J.C. Martinou, Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA, PLoS One 3 (2008) e3257.
[53] I. Kim, S. Rodriguez-Enriquez, J.J. Lemasters, Selective degradation of mitochondria by mitophagy, Archives of Biochem. and Biophysics 462 (2007) 245–253.
[54] Y.J. Lee, S.Y. Jeong, M. Karbowski, C.L. Smith, R.J. Youle, Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis, Mol. Biol. Cell 15 (2004) 5001–5011.
[55] R. Sugioka, S. Shimizu, Y. Tsujimoto, Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis, J. Biol. Chem. 279 (2004) 52726–52734.
[56] R.J. Youle, M. Karbowski, Mitochondrial fission in apoptosis, Nat. Rev. Mol. Cell Biol. 6 (2005) 657–663.
[57] E. Smirnova, L. Griparic, D.L. Shurland, A.M. van Der Bliek, Dynaminrelated protein drp1 is required for mitochondrial division in mammalian cells, Mol. Biol. Cell 12 (2001) 2245–2256.
[58] J.T. Cribbs, S. Strack, Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death, EMBO Rep. 8 (2007) 939–944.
[59] C.R. Chang, C. Blackstone, Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology, J. Biol. Chem. 282 (2007) 21583–21587.
[60] S.B. Ong, S. Subrayan, S.Y. Lim, D.M. Yellon, S.M. Davidson, D.J. Hausenloy, Inhibiting mitochondria fission protects the heart against ischemia/reperfusion injury, Circulation 121 (2010) 2012–2022.
[61] M.J. Sisalli, G. Ianniello, C. Savoia, O. Cuomo, L. Annunziato, A. Scorziello, Knocking-out the Siah2 E3 Ubiquitin Ligase Prevents Mitochondrial NCX3 Degradation, Regulates Mitochondrial Fission and Fusion, and Restores Mitochondrial Function in Hypoxic Neurons, in Press on Cell Communication and Signaling, (2020).
[62] R.L. Martin, H.G. Lloyd, A.I. Cowan, The early events of oxygen and glucose deprivation: setting the scene for neuronal death? Trends Neurosci. 17 (1994) 251–257.
[63] T. Rich, R.L. Allen, A.H. Wyllie, Defying death after DNA damage, Nature 407 (2000) 777–783.
[64] G. Kroemer, L. Galluzzi, C. Brenner, Mitochondrial membrane permeabilization in cell death, Physiol. Rev. 87 (2007) 99–163.
[65] M.O. Hengartner, The biochemistry of apoptosis, Nature 407 (2000) 770–776.
[66] R.J. Clem, E.H. Cheng, C.L. Karp, D.G. Kirsch, K. Ueno, A. Takahashi, M.B. KastanB, D.E. Griffin, W.C. Earnshaw, M.A. Veliuona, J.M. Hardwick, Modulation of cell death by Bcl-XL through caspase interaction, Proc. Nat. Acad. Sci. USA 95 (1998) 554–559.
[67] N. Fujita, A. Nagahashi, K. Nagashima, S. Rokudai, T. Tsuruo, Acceleration of apoptotic cell death after the cleavage of Bcl-XL protein by caspase-3-like proteases, Oncogene 17 (1998) 1295–1304.
[68] E.A. Jonas, D. Hoit, J.A. Hickman, T.A. Brandt, B.M. Polster, Y. Fannjiang, E. McCarthy, M.K. Montanez, J.M. Hardwick, L.K. Kaczmarek, Modulation of synaptic transmission by the BCL-2 family protein BCL-Xl, J. Neuroscience 23 (2003) 8423–8431.
[69] D.W. Choi, Calcium and excitotoxic neuronal injury, Annals N. Y. Acad. Sci. 747 (1994) 162–171.
[70] R. Sattler, M. Tymianski, Molecular mechanisms of calcium-dependent excitotoxicity, J. Mol. Med. 78 M (2000) 3–13.
[71] Y. Nishizawa, Glutamate release and neuronal damage in ischemia, Life Sci. 69 (2001) 369–381.
[72] H. Tanaka, H. Yokota, T. Jover, I. Cappuccio, A. Calderone, M. Simionescu, M.V. Bennett, R.S. Zukin, Ischemic preconditioning: neuronal survival in the face of caspase-3 activation, J. Neurosci. 11 (2004) 2750–2759.
[73] N.R. Sims, H. Muyderman, Mitochondria, oxidative metabolism and cell death in stroke, Biochim. Biophys. Acta 1 (2010) 80–91.
[74] A.A. Starkov, C. Chinopoulos, G. Fiskum, Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury, Cell Calcium 36 (2004) 257–264.
[75] W. Paschen, J. Doutheil, Disturbance of endoplasmic reticulum functions: a key mechanism underlying cell damage? Acta Neurochir. (Wien) 73 (1999) 1–5.
[76] G. Csordas, C. Renken, P. Varnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, G. Hajnoczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J. Cell Biol. 174 (2006) 915–921.
[77] G. Csordas, P. Varnai, T. Golenar, S. Roy, G. Purkins, T.G. Schneider, T. Balla, G. Hajnoczky, Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface, Mol. Cell 39 (2010) 121–132.
[78] M. Giacomello, I. Drago, M. Bortolozzi, M. Scorzeto, A. Gianelle, P. Pizzo, T. Pozzan, Ca2+ hot spots on the mitochondrial surface are generated by Ca2+ mobilization from stores, but not by activation of store-operated Ca2+ channels, Mol. Cell 38 (2010) 280–290.
[79] D.G. Nicholls, S. Chalmers, The integration of mitochondrial calcium transport and storage, J. Bioenerg. Biomembr. 36 (2004) 277–278.
[80] M.P. Blaustein, M. Juhaszova, V.A. Golovina, P.J. Church, E.F. Stanley, Na+/Ca2+ exchanger and PMCA localization in neurons and astrocytes: functional implications, Ann. NY Acad. Sci. 976 (2002) 356–366.
[81] J. Lytton, Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport, J. Biochem. 406 (2007) 365–382.
[82] A. Secondo, R.I. StaianoI, A. Scorziello, R. Sirabella, F. Boscia, A. Adornetto, V. Valsecchi, P. Molinaro, L.M. Canzoniero, G. Di Renzo, L. Annunziato, BHK cells transfected with NCX3 are more resistant to hypoxia followed by reoxygenation than those transfected with NCX1 and NCX2: possible relationship with mitochondrial membrane potential, Cell Calcium 42 (2007) 521–535.
[83] R. Sirabella, A. Secondo, A. Pannaccione, A. Scorziello, V. Valsecchi, A. Adornetto, L. Bilo, G. Di Renzo, L. Annunziato, Anoxia-induced NF-kappaB-dependent upregulation of NCX1 contributes to Ca2+ refilling into endoplasmic reticulum in cortical neurons, Stroke 40 (2009) 922–929.
[84] R.M. Denton, J.G. McCormack, On the role of the calcium transport cycle in heart and other mammalian mitochondria, FEBS Lett. 119 (1980) 1–8.
[85] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[86] R.M. Denton, Regulation of mitochondrial dehydrogenases by calcium ions, Biochim. Biophys. Acta 1787 (2009) 1309–1316.
[87] C. Krieger, M.R. Duchen, Mitochondria, Ca2+ and neurodegenerative disease, Eur. J. Pharmacol. 447 (2002) 177–188.
[88] S.Y. Jeong, D.W. Seol, The role of mitochondria in apoptosis, BMB Rep. 41 (2008) 11–22.
[89] M.J. Sisalli, A. Secondo, A. Esposito, V. Valsecchi, C. Savoia, G. Di Renzo, L. Annunziato, A. Scorziello, Endoplasmic reticulum refilling andmitochondrial calcium extrusion promoted in neurons by NCX1 and NCX3 in ischemic preconditioning are determinant for neuroprotection, Cell Death Differ. 7 (2008) 1142–1149.
[90] D.A. Cox, L. Conforti, N. Sperelakis, M.A. Matlib, Selectivity of inhibition of Na(+)-Ca2+ exchange of heart mitochondria by benzothiazepine CGP-37157, J. Cardiovasc. Pharmacol. 21 (1993) 595–599.