Cell Calcium 87 (2020) 102195

Contents lists available at ScienceDirect

Cell Calcium

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

Sumoylation of sodium/calcium exchanger in brain ischemia and ischemic preconditioning

Ornella Cuomo[a,*], Antonella Casamassa[a], Paola Brancaccio[a], Giusy Laudati[a], Valeria Valsecchi[a], Serenella Anzilotti[b], Antonio Vinciguerra[a], Giuseppe Pignataro[a], Lucio Annunziato[b]

[a] Division of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples Federico II, Naples, Italy
[b] IRCCS SDN, Naples, Italy

ARTICLE INFO

Keywords:
Na^+/Ca^{2+} exchanger
NCX
Cerebral ischemia
Ischemic preconditioning
SUMO

ABSTRACT

The small ubiquitin-like modifier (SUMO) conjugation (or SUMOylation) is a post-translational protein modification mechanism activated by different stress conditions that has been recently investigated in experimental models of cerebral ischemia. The expression of SUMOylation enzymes and substrates is not restricted to the nucleus, since they are present also in the cytoplasm and on plasma membrane and are involved in several physiological and pathological conditions.

In the last decades, convincing evidence have supported the idea that the increased levels of SUMOylated proteins may induce tolerance to ischemic stress. In particular, it has been established that protein SUMOylation may confer neuroprotection during ischemic preconditioning.

Considering the increasing evidence that SUMO can modify stability and expression of ion channels and transporters and the relevance of controlling ionic homeostasis in ischemic conditions, the present review will resume the main aspects of SUMO pathways related to the key molecules involved in maintenance of ionic homeostasis during cerebral ischemia and ischemic preconditioning, with a particular focus on the on Na^+/Ca^{2+} exchangers.

1. Introduction

Small Ubiquitin-like Modifier (SUMO) protein conjugation is an enzymatic process which shares several biochemical analogies with ubiquitination, although the two precesses are functionally distinct [1,2]. Indeed, while protein ubiquitination leads to target protein degradation, SUMO conjugation may induce protein-protein interaction and, may influence protein stability and activity [3,4]. So far, five SUMO isoforms have been identified, SUMO-1, SUMO-2, SUMO-3, SUMO-4 and SUMO-5. SUMO-1 has a 47 % homology to SUMO-2 and -3. On the other hand, SUMO-2 and SUMO-3 show a 95 % homology, act very similar, and are therefore often referred to as SUMO2/3. All SUMO isoforms are ubiquitously expressed and can be found in the Central Nervous System (CNS) of vertebrate animals. SUMO-4, an isoform not so much investigated so far, is mainly expressed in kidney and liver, but not in the brain [5,6]. Finally, it has been recently suggested the existence of SUMO-5. Indeed, Liang and colleagues demonstrated that the fifth SUMO isoform is involved in the regulation of promyelocytic leukaemia nuclear bodies [7]. SUMOylation is a reversible process where conjugation may be mediated by the ubiquitin-conjugating enzyme Ubc9 and de-conjugation is mediated by SUMO-specific proteases (SENPs). The balance between conjugation mediated by Ubc9 and de-conjugation mediated by SENPs may change the functional properties of proteins that are targets of SUMO [8].

Although SUMOylation was initially described as a post-translational modification of just nuclear proteins, more recently, convincing evidence showed that the expression of SUMOylation enzymes and substrates is not restricted to the nucleus, since they are present also in the cytoplasm and on plasma membrane and are involved in several physiological and pathological conditions. In the last decades, several studies highlighted the role of SUMOylation during cerebral ischemia and supported the idea that increased levels of SUMOylated proteins may induce tolerance to ischemic stress [9–11]. Recently, several evidence showed a neuroprotective role of SUMOylation during

Abbreviation: CNS, Central Nervous System; NCX, Na^+/Ca^{2+} exchanger; NCX3F, NCX3FLAG; NCX3FΔf, NCX3FLAG mutant lacking the f-loop; OGD, Oxygen and glucose deprivation; pMCAO, Permanent Middle Cerebral Artery Occlusion; SUMO, Small Ubiquitin-like Modifier; SUMO-KD, SUMO knock down; SENPs, SUMO-specific proteases; tMCAO, Transient Middle Cerebral Artery Occlusion

* Corresponding author at: Università degli Studi di Napoli Federico II Dipartimento di Neuroscienze e Scienze, Riproduttive ed Odontostomatologiche, Italy.
E-mail address: ornella.cuomo@unina.it (O. Cuomo).

https://doi.org/10.1016/j.ceca.2020.102195
Received 24 January 2020; Received in revised form 10 March 2020; Accepted 13 March 2020
Available online 16 March 2020

endogenous neuroprotective events like ischemic preconditioning [3,9].

The present review will provide an overview of SUMOylation process in the regulation of key molecules involved in the control of ionic homeostasis under brain ischemia and ischemic preconditioning. In particular, it will deeply focus on the role of sodium calcium exchangers SUMOylation.

2. Physiological functions of protein SUMOylation process

An increasing body of evidence suggests a key role of SUMOylation in several physiological processes. Although SUMOylated targets have been described in different cellular compartments including the nucleus, the cytoplasm, the mitochondria and the cell membrane [12], the vast majority of SUMO substrates are located in or near the nucleus. These proteins participate in complex cell pathways and regulate several cellular processes including gene expression, DNA damage repair, RNA processing, transcriptional regulation, protein localization, and protein-protein interaction [13,14]. Interestingly, a plethora of targets and pathways have been identified as substrates for SUMO modifications.

In addition to the well established role of SUMO signaling in the regulation of nuclear proteins, recent studies revealed the crucial function of SUMO modifications at the cytoplasmic side of membrane. Indeed, a wealth of studies supports a crucial role for SUMO modifications outside of the nucleus and several non-nuclear substrates have been identified by proteomic analyses [13].

In the CNS, SUMO activity plays a crucial role in maintaining several physiological functions. SUMOylation is required in neural cell differentiation and synaptogenesis [15] and dynamic SUMOylation can modulate neurotransmitter release [16] and synaptic transmission. Interestingly, SUMOylation of target proteins can influence axonal regeneration including growth cone orientation and synaptic plasticity [17]. In addition, SUMOylation exerts key regulatory roles in several metabolic processes [18,19] and, finally, SUMO pathways regulate resting potential, neuronal excitability and ion flow/distribution [13,20]. In this regard, SUMOylation is a pleiotropic regulator of expression and stability of membrane proteins involved in transport and homeostasis of ions, including receptor, ion channels and transporters, not only in physiological but also in pathological conditions such as brain ischemia [13].

3. SUMOylation process during brain ischemia

In the last decades, several studies have been investigating the role of SUMOylation during cerebral ischemia, and it has been established that both global and focal transient cerebral ischemia strongly induce an increase of the levels of SUMO-conjugated proteins in rodent brains after reperfusion [3,10,11,21,22].

The role of SUMOylation during ischemia has been assessed by genetically manipulating the SUMO pathway in order to increase the levels of SUMOylated proteins, overexpressing each SUMO isoforms or the conjugating enzyme Ubc [9,23] or with the attempt to suppress SUMOylation by microRNA or siRNA against SUMO isoforms [9,24,25]. More specifically, Yang et al. showed that an increase in protein SUMOylation by SUMO-2/3 was maximally induced in both the hippocampus and cerebral cortex of mice three hours after a 10 min global ischemia exposure [11]. The same authors also demonstrated that in a rat model of transient brain ischemia, SUMOylation decreased during transient middle cerebral artery occlusion (tMCAO) and increased during the reperfusion phase when ATP levels were restored [10]. In a similar stroke model, protein SUMOylation by SUMO-1 significantly increased after tMCAO in the ipsilateral temporoparietal cortex, whereas it remained unchanged in the striatum. The fact that this increase involved just the brain area less injured by the ischemic damage and not the striatum, supports the idea that SUMOylation represents a cell protective response to ischemic injury [3]. In this model, SUMO-1 silencing significantly exacerbated the ischemic damage mediated by tMCAO. Consistent with these data, also SUMO-2/3 conjugation resulted activated in neurons located in the ischemic penumbra but not within the infarcted core [11].

In support of a neuroprotective role for SUMOylation in brain ischemia, it has been recently demonstrated that transgenic mice overexpressing the conjugating enzyme Ubc9 showed higher basal levels of SUMO conjugated proteins and are less susceptible to the brain ischemic damage in a model of focal ischemia induced by permanent middle cerebral artery occlusion (pMCAO) [26]. On the other hand, SUMO-1 overexpression in primary cortical neurons increased cell survival under oxygen and glucose deprivation (OGD) while silencing of SUMO-1 and SUMO-2/3 expression increased cell death [9,24]. Accordingly, in a model of transient focal ischemia in rats, silencing of SUMO-1 significantly exacerbated the ischemic damage [3] and neuron-specific SUMO-1/3 knocked down (SUMO-KD) mice exhibited worse functional outcomes after transient forebrain ischemia. In these mice, a significant number of such genes that are positively regulated by SUMOylation are instead suppressed [27].

4. SUMOylation process during ischemic preconditioning

Several studies supported the concept that the increase in SUMOylated proteins induces tolerance to an ischemic injury. Indeed, brain protein SUMOylation may represent an endogenous neuroprotective response in models of brain tolerance [9–11,28]. For instance, a significant increase of SUMO-1- and SUMO-2/3-mediated SUMOylation has been shown in hibernating ground squirrels [23], where the reduction in cerebral blood flow and in oxygen and glucose levels in the hibernating brain represent an endogenous model of brain tolerance. Indeed, during hibernation torpor, ground squirrels can tolerate 90 % reduction in brain blood flow, a reduction very similar to that observed in the ischemic core during stroke [29].

The hibernation torpor has also been demonstrated to exert a neuroprotective action against a subsequent lethal ischemic insult, thus functioning as a preconditioning stimulus.

Moreover, also in rats exposed to another well known neuroprotective treatment such as hypothermia, an increase in nuclear accumulation of SUMO-2/3 in cortical neurons was observed in the brain [30]. More interestingly, the increase in SUMO conjugation may be considered as the main mediator of the hypothermia-induced neuroprotection both in *in vitro* models of hypoxia and in *in vivo* models of brain ischemia [26].

Interestingly, hibernation and hypothermia share several transductional pathways with ischemic preconditioning, a phenomenon in which a sublethal injurious stimulus was able to protect to a subsequent longer harmful ischemic injury [8,31].

The role of SUMOylation during ischemic preconditioning was first investigated *in vitro*. In particular, primary cortical neurons preconditioned through the exposure to a subthreshold protocol of OGD were less susceptible to a subsequent longer OGD stimulus. More interestingly, these neurons showed higher levels of SUMO-1 conjugation compared to unconditioned neurons. Accordingly, when SUMO-1 was silenced, preconditioned-induced neuroprotection failed [9]. Similar results were obtained also in an *in vivo* model of transient cerebral ischemia in rats [3]. Most of the published surgical protocols for preconditioning induction require the transient mechanical interruption of the same artery occluded during stroke. In an *in vivo* model of ischemic preconditioning in rats, the increase in protein SUMOylation participates to brain tolerance, particularly through SUMO-1 isoform. This finding was reinforced by the observation that silencing of SUMO-1 isoform, through a specific siRNA, prevented the protection mediated by ischemic preconditioning.

In the same study, the sodium calcium exchanger NCX3 has been proposed as one mediator of SUMO-1 protective effect during ischemic

preconditioning [3].

Collectively, up to now several proteins have been characterized as SUMO targets after brain ischemia and ischemic preconditioning including transcriptional factors [13,22,32], mitochondrial and extranuclear proteins, such as membrane receptors, transporters and channels as described below [13].

5. SUMOylation of ion channels and transporters during brain ischemia

Ion channels and transporters play a crucial role in ionic cell homeostasis and membrane excitability. Considering all the changes occurring after an ischemic brain injury, including disrupted ionic homeostasis and dysfunction of ionic transporters, it is not surprising that these proteins are subjected to tight regulation and are candidates for modulation at the post-translational level under hypoxic-ischemic conditions. In particular, ion channels and transporters represent an important class of SUMO-modified extranuclear proteins and several studies have identified SUMO signaling as a crucial mechanism of modulating multiple members of K^+, Ca^{2+} and Na^+ ion regulators under ischemic conditions [13].

The SUMO-mediated alterations of voltage-gated potassium channels is considered an important neuroprotective mechanism [33]. Indeed, SUMO-1 and SUMO-2/3 conjugation of $K_v2.1$ channels promotes channel opening and voltage-gating. The consequent reduction of excitability exerts a neuroprotective effect in neurons exposed to hypoxic-ischemic injury [22,34]. In addition, the de-SUMOylation of voltage-gated $K_v1.5$ potassium channels leads to a functional inactivation of these channels and its inactivation dramatically increases ischemic tolerance in neurons [13,35]. At the same time, the involvement of SUMOylation on sodium signaling under hypoxic conditions is a field of particular relevance. In fact, recent evidence reveals that SUMOylation of $Na_v1.2$ induces the increase in voltage-gated sodium inward current (I_{Na}) in cortical neurons under hypoxic-ischemic conditions [36]. Finally, SUMOylation of the sodium/calcium exchanger NCX3 by SUMO-1 participates in brain neuroprotection induced by ischemic preconditioning [3] as deeper described below.

6. Sodium Calcium Exchangers as SUMO targets in ischemic conditions

The Na^+/Ca^{2+} exchanger (NCX) is a plasmalemmal transporter composed of ten transmembrane domains which couples, in a bidirectional way, the influx/efflux of Ca^{2+} to the efflux/influx of Na^+ ions. The NCX family includes three different gene products, NCX1, NCX2 and NCX3 [37,38]. The sodium/calcium exchangers, acting in concert with ion channels, receptors and other ionic transporters play an important role in the control of ionic cell homeostasis. Several pieces of evidence reveal that the NCX family members represent crucial regulators of sodium and calcium homeostasis both in physiological and pathophysiological conditions [39,41,40]. Notably, it has been widely demonstrated that the member 3, NCX3, represents a downstream player of neuroprotection in stroke [40,42], since its knocking-down and knocking-out caused an enlargement of the focal ischemic damage in two different models of ischemia induced by permanent or transient middle cerebral artery occlusion [42,43]. In addition, NCX3 has been characterized as a new effector of the ischemic preconditioning [40].

Interestingly, it has been recently established that NCX3 represents a new putative target of SUMO-1 mediated neuroprotective action (Fig. 1). The reason for investigating NCX3 as a possible target for SUMO-mediated neuroprotective effect resided in its well known neuroprotective role in cerebral ischemia [42] and ischemic preconditioning [40].

In order to assess the role played by SUMO-1 conjugation during cerebral ischemia and ischemic preconditioning, SUMOylation expression profile was evaluated in the temporoparietal cortex and striatum of rats exposed to middle cerebral artery occlusion and sacrificed at different reperfusion time points [3]. The analyses revealed a significant upregulation of SUMOylation in the cortex and no changes in the striatum. The fact that this increase involved just the brain area less injured by the ischemic damage and not the striatum supports the idea that SUMOylation represents a cell protective response to ischemic injury. Immunohistochemical analyses performed on rat brain slices after preconditioning, tMCAO, and preconditioning plus tMCAO showed that NCX3 and SUMO-1 signals colocalized in the temporoparietal cortex. More important, bioinformatic analysis showed that NCX3 has nine possible consensus sites that might be targeted by the SUMO conjugation enzyme. The interaction between SUMO-1 and NCX3 was finally assessed by immunoprecipitation experiments on wild-type BHK cells not expressing NCX3 and on BHK cells stably transfected with a flag-tag NCX3 (NCX3F). Furthermore, to finally establish the possible SUMOylation site, the experiments were replicated on an NCX3F mutant lacking the f-loop (NCX3FΔf), since this region contained most of the Lysine residues with the highest probability of SUMO-1 binding. Since no immunoprecipitate was observed in NCX3FΔf-transfected BHK cells, the authors concluded that the putative SUMOylation site was located in the f-loop of the antiporter. In further support to this conclusion, the substitution of Lysine590 prevented the formation of the immunoprecipitate thus demonstrating that this Lysine residue is required for SUMOylation (Fig. 2).

In the same paper, the presence of a biochemical interaction between SUMO-1 and NCX3 was also analyzed and confirmed *in vivo*. Indeed, immunoprecipitation experiments on rat cortex from ischemic and preconditioned animals showed that NCX3 immunoprecipitated with SUMO-1. By contrast, another NCX member, NCX1, did not immunoprecipitate with SUMO-1, thus suggesting that the neuroprotective action of SUMO-1 is selectively exerted through the preservation of NCX3 levels.

The neuroprotective role of NCX3 SUMOylation was supported by the observation that SUMO-1 silencing, apart from enlarging the ischemic damage after ischemia and reverting the protective potential of ischemic preconditioning, caused also a downregulation of NCX3 levels in preconditioned animals. This observation suggested that the increase in SUMOylation levels during ischemic preconditioning prevented NCX3 degradation thus mediating neuroprotection. At the same time, NCX3 was further decreased by SUMO-1 silencing also after tMCAO alone, and consequently the ischemic damage increased, thus suggesting that NCX3 SUMOylation process also participates in the tMCAO phase.

Interestingly, the authors speculated that, since the SUMO-target residue is located in the main cytosolic f-loop, a region involved in the regulation of NCX stability and activity, NCX3 SUMOylation might influence such properties of the antiporter.

Collectively, these results suggest that SUMO-1 conjugation, by regulating the stability of NCX3, might influence sodium and calcium homeostasis during ischemia and ischemic preconditioning. In conclusion, NCX3 SUMOylation by SUMO-1 may be considered one of the neuroprotective mechanisms involved in ischemic preconditioning [3].

7. Conclusions

Several papers support the idea that SUMOylation plays an important role in the cellular response to hypoxic-ischemic injury. For most of the targets, SUMOylation seems to exert a neuroprotective role, however for some others rimental [4,44].

Another limitation of the already published studies is the fact that they widely describe the role of SUMOylation in neurons, and very less in other brain cell types. The deep evaluation of the SUMO pathways in astrocytes will be critical to clarify their supportive/detrimental function for the neuronal compartment. On the other hand, revealing the effects of SUMOylation also in post-ischemic oligodendrocytes, microglia, and invading immune cells will be of great interest. Despite the

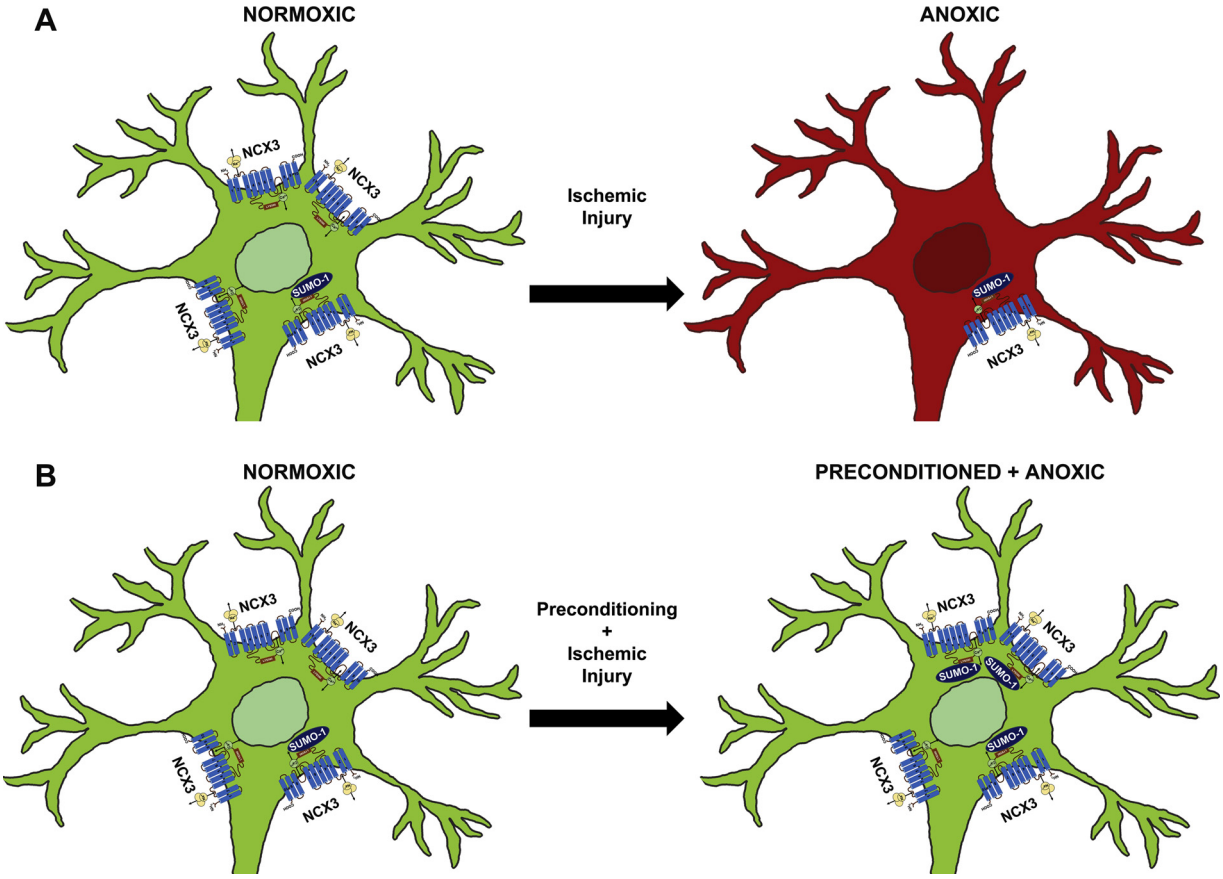

Fig. 1. SUMOylation of NCX3 by SUMO-1 participates in brain neuroprotection induced by ischemic preconditioning. (A) After an ischemic injury not-SUMOylated NCX3 was degraded and the anoxic neuron was damaged. (B) After ischemic preconditioning NCX3 SUMOylation increases, thus preventing its degradation and conferring neuronal protection to a subsequent ischemic damage.

number of ion channels and transporters currently shown to be SUMOylated is still small, the identification of additional SUMO targets involved in sodium and calcium homeostasis both in neuronal and glial cells will be fundamental to extend the reach of the SUMO conjugation machinery to the plasma membrane both in physiological and in pathological conditions.

In vitro and *in vivo* studies using models of ischemia, together with those using therapeutic hypothermia, strongly support the idea that the enhancement of SUMOylation may prevent ischemic damage within the brain.

However, no specific pharmacological modulators of SUMOylation process are now available. Only agents capable to aspecifically upregulate SUMOylation process have been so far described. Recently, a molecule able to activate the E1 enzyme of SUMOylation process has been described but need to be better characterized [45]. Interestingly, recent evidence identified small molecules capable of modulating global SUMOylation through the manipulation of miRNA [46]. Several molecules, such as histone de-acetylase (HDAC) inhibitors, synthetic retinoids and anti-miRNA are promising compounds potentially able to increase global SUMOylation and, consequently, capable to induce protection from anoxic and ischemic damage [4,46].

However, considering the evidence that SUMOylation inhibitors

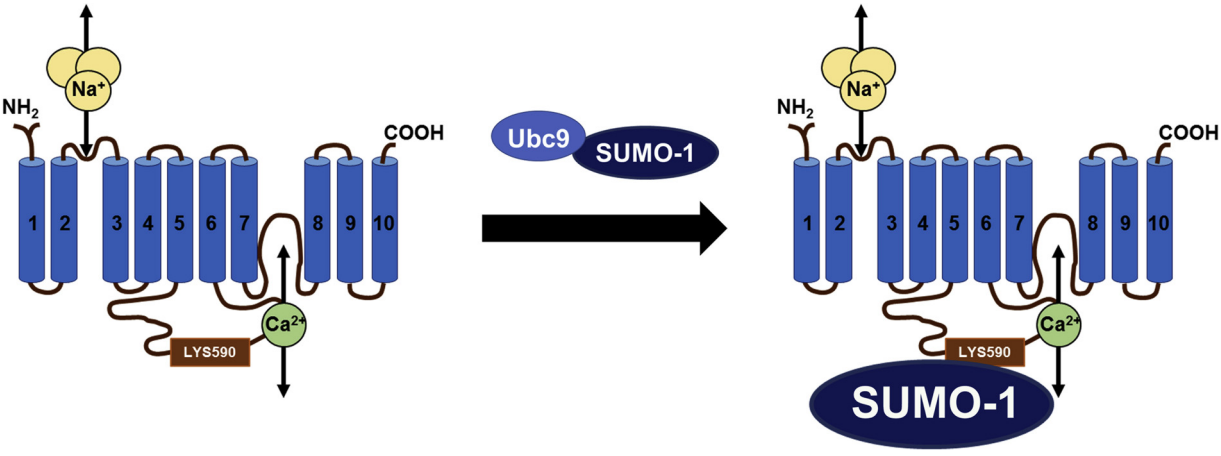

Fig. 2. Lys590 of NCX3 is SUMOylated. Mutagenesis studies revealed that Lys590 is the target residue for SUMO-1 conjugation of NCX3.

may be a putative novel drug for cancer therapy [4,44,47], the exact molecular mechanisms underlying the SUMO-mediated protective roles remain to be clarified.

In conclusion, to support the translatability of all the preclinical results to the clinical management of acute brain ischemia, the identification of new pharmacological compounds able to modulate brain SUMOylation will may have a very great clinical significance in a therapeutic approach against ischemic stroke.

Acknowledgments

This work was supported by the following grants: Programma Operativo Nazionale (PON_01602 and PON03PE_00146_1) from MIUR to L.A.; POR Campania FESR 2007 to 2013 FARMABIONET (B25C1300023007) to G.P.; POR Campania FESR 2007 to 2013 OCKEY (B25C13000280007).

CRediT authorship contribution statement

Ornella Cuomo: Supervision, Conceptualization, Writing - original draft, Writing - review & editing. **Antonella Casamassa:** Conceptualization, Writing - original draft, Writing - review & editing. **Paola Brancaccio:** Writing - original draft. **Giusy Laudati:** Writing - review & editing. **Valeria Valsecchi:** Writing - original draft. **Serenella Anzilotti:** Writing - review & editing. **Antonio Vinciguerra:** Writing - review & editing. **Giuseppe Pignataro:** Writing - review & editing. **Lucio Annunziato:** Writing - review & editing.

References

[1] J. Zhao, Sumoylation regulates diverse biological processes, Cell. Mol. Life Sci. 64 (23) (2007) 3017–3033, https://doi.org/10.1007/s00018-007-7137-4.
[2] G. Gill, Post-translational modification by the small ubiquitin-related modifier SUMO has big effects on transcription factor activity, Curr. Opin. Genet. Dev. 13 (2) (2003) 108–113, https://doi.org/10.1016/S0959-437X(03)00021-2.
[3] O. Cuomo, G. Pignataro, R. Sirabella, P. Molinaro, S. Anzilotti, A. Scorziello, M.J. Sisalli, G. Di Renzo, L. Annunziato, Sumoylation of LYS590 of NCX3 f-loop by SUMO1 participates in brain neuroprotection induced by ischemic preconditioning, Stroke 47 (4) (2016) 1085–1093, https://doi.org/10.1161/STROKEAHA.115.012514.
[4] J.D. Bernstock, W. Yang, D.G. Ye, Y. Shen, S. Pluchino, Y.J. Lee, J.M. Hallenbeck, W. Paschen, SUMOylation in brain ischemia: patterns, targets, and translational implications, J. Cereb. Blood Flow Metab. 38 (1) (2018) 5–16, https://doi.org/10.1177/0271678X17742260.
[5] K.M. Bohren, V. Nadkarni, J.H. Song, K.H. Gabbay, D. Owerbach, A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus, J. Biol. Chem. 279 (26) (2004) 27233–27238, https://doi.org/10.1074/jbc.M402273200.
[6] R.T. Hay, SUMO: a history of modification, Mol. Cell 18 (1) (2005) 1–12, https://doi.org/10.1016/j.molcel.2005.03.012.
[7] Y.C. Liang, C.C. Lee, Y.L. Yao, C.C. Lai, M.L. Schmitz, W.M. Yang, SUMO5, a novel Poly-SUMO isoform, Regul. PML Nuclear BodiesSci. Rep. 23 (6) (2016) 26509, https://doi.org/10.1038/srep26509.
[8] Flotho, F. Melchior, Sumoylation: a regulatory protein modification in health and disease, Annu. Rev. Biochem. 82 (2013) 357–385, https://doi.org/10.1146/annurev-biochem-061909-093311.
[9] Y.J. Lee, P. Castri, J. Bembry, et al., SUMOylation participates in induction of ischemic tolerance, J. Neurochem. 109 (1) (2009) 257–267, https://doi.org/10.1111/j.1471-4159.2009.05957.x.
[10] W. Yang, H. Sheng, D.S. Warner, W. Paschen, Transient focal cerebral ischemia induces a dramatic activation of small ubiquitin-like modifier conjugation, J. Cereb. Blood Flow Metab. 28 (5) (2008) 892–896, https://doi.org/10.1038/sj.jcbfm.9600601.
[11] W. Yang, H. Sheng, D.S. Warner, W. Paschen, Transient global cerebral ischemia induces a massive increase in protein sumoylation, J. Cereb. Blood Flow Metab. 28 (2) (2008) 269–279, https://doi.org/10.1038/sj.jcbfm.9600523.
[12] D. Tempè, M. Piechaczyk, G. Bossis, SUMO under stress, Biochem. Soc. Trans. 36 (Pt 5) (2008) 874–878, https://doi.org/10.1042/BST0360874.
[13] M. Peters, B. Wielsch, J. Boltze, The role of SUMOylation in cerebral hypoxia and ischemia, Neurochem. Int. 107 (2017) 66–77, https://doi.org/10.1016/j.neuint.2017.03.011.
[14] D.B. Anderson, C.A. Zanella, J.M. Henley, H. Cimarosti, Sumoylation: implications for neurodegenerative diseases, Adv. Exp. Med. Biol. 963 (2017) 261–281, https://doi.org/10.1007/978-3-319-50044-7_16.
[15] A. Shalizi, B. Gaudillière, Z. Yuan, J. Stegmüller, T. Shirogane, Q. Ge, Y. Tan, B. Schulman, J. Wade Harper, A. Bonni, A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation, Science 311 (5763) (2006) 1012–1017 DOI:10.1126/science.1122513.
[16] M. Feligioni, A. Nishimune, J.M. Henley, Protein SUMOylation modulates calcium influx and glutamate release from presynaptic terminals, Eur. J. Neurosci. 29 (7) (2009) 1348–1356, https://doi.org/10.1111/j.1460-9568.2009.06692.x.
[17] E.A. van Niekerk, D.E. Willis, J.H. Chang, K. Reumann, T. Heise, J.L. Twiss, Sumoylation in axons triggers retrograde transport of the RNA-binding protein, Proc. Natl. Acad. Sci. U. S. A. 104 (31) (2007) 12913–12918, https://doi.org/10.1073/pnas.0611562104.
[18] V. Dorval, P.E. Fraser, SUMO on the road to neurodegeneration, Biochim. Biophys. Acta 1773 (6) (2007) 694–706, https://doi.org/10.1016/j.bbamcr.2007.03.017.
[19] S. Martin, K.A. Wilkinson, A. Nishimune, J.M. Henley, Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction, Nat. Rev. Neurosci. 8 (12) (2007) 948–959, https://doi.org/10.1038/nrn2276.
[20] C. Loriol, J. Parisot, G. Poupon, C. Gwizdek, S. Martin, Developmental regulation and spatiotemporal redistribution of the sumoylation machinery in the rat central nervous system, PLoS One 7 (3) (2012) e33757, https://doi.org/10.1371/journal.pone.0033757.
[21] W. Yang, H. Sheng, J.W. Thompson, et al., Small ubiquitinlike modifier 3-modified proteome regulated by brain ischemia in novel small ubiquitin-like modifier transgenic mice: putative protective proteins/pathways, Stroke 45 (4) (2014) 1115–1122 DOI:10.1161/STROKEAHA.113.004315.
[22] V. Silveirinha, G.J. Stephens, H. Cimarosti, Molecular targets underlying SUMO-mediated neuroprotection in brain ischemia, J. Neurochem. 127 (5) (2013) 580–591, https://doi.org/10.1111/jnc.12347.
[23] Y.J. Lee, S. Miyake, H. Wakita, et al., Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells, J. Cereb. Blood Flow Metab. 27 (5) (2007) 950–962, https://doi.org/10.1038/sj.jcbfm.9600395.
[24] A.L. Datwyler, G. Lattig-Tunnemann, W. Yang, et al., SUMO2/3 conjugation is an endogenous neuroprotective mechanism, J. Cereb. Blood Flow Metab. 31 (11) (2011) 2152–2159, https://doi.org/10.1038/jcbfm.2011.112.
[25] H. Cimarosti, E. Ashikaga, N. Jaafari, et al., Enhanced SUMOylation and SENP-1 protein levels following oxygen and glucose deprivation in neurones, J. Cereb. Blood Flow Metab. 32 (1) (2012) 17–22, https://doi.org/10.1038/jcbfm.2011.146.
[26] Y.J. Lee, Y. Mou, D. Klimanis, J.D. Bernstock, J.M. Hallenbeck, Global SUMOylation is a molecular mechanism underlying hypothermia induced ischemic tolerance, Front. Cell. Neurosci. 8 (2014) 416, https://doi.org/10.3389/fncel.2014.00416.
[27] L. Zhang, X. Liu, H. Sheng, et al., Neuron-specific SUMO knockdown suppresses global gene expression response and worsens functional outcome after transient forebrain ischemia in mice, Neuroscience 343 (2017) 190–212, https://doi.org/10.1016/j.neuroscience.2016.11.036.
[28] H. Cimarosti, C. Lindberg, S.F. Bomholt, L.C. Rønn, J.M. Henley, Increased protein SUMOylation following focal cerebral ischemia, Neuropharmacology 54 (2) (2008) 280–289, https://doi.org/10.1016/j.neuropharm.2007.09.010.
[29] J.M. Gidday, Cerebral preconditioning and ischaemic tolerance, Nat. Rev. Neurosci. 7 (6) (2006) 437–448, https://doi.org/10.1038/nrn1927.
[30] L. Wang, Q. Ma, W. Yang, G.B. Mackensen, W. Paschen, Moderate hypothermia induces marked increase in levels and nuclear accumulation of SUMO2/3-conjugated proteins in neurons, J. Neurochem. 123 (3) (2012) 349–359, https://doi.org/10.1111/j.1471-4159.2012.07916.x.
[31] C.M. Hickey, N.R. Wilson, M. Hochstrasser, Function and regulation of SUMO proteases, Nat. Rev. Mol. Cell Biol. 13 (2012) 755–766, https://doi.org/10.1038/nrm3478.
[32] M.J. Lyst, I. Stancheva, A role for SUMO modification in transcriptional repression and activation, Biochem. Soc. Trans. 35 (Pt 6) (2007) 1389–1392, https://doi.org/10.1042/BST0351389.
[33] M. Stapels, C. Piper, T. Yang, M. Li, C. Stowell, Z.G. Xiong, J. Saugstad, R.P. Simon, S. Geromanos, J. Langridge, J.Q. Lan, A. Zhou, Polycomb group proteins as epigenetic mediators of neuroprotection in ischemic tolerance, Sci. Signal. 3 (111) (2010), https://doi.org/10.1126/scisignal.2000502 ra15.
[34] L.D. Plant, E.J. Dowdell, I.S. Dementieva, J.D. Marks, S.A. Goldstein, SUMO modification of cell surface Kv2.1 potassium channels regulates the activity of rat hippocampal neurons, J. Gen. Physiol. 137 (5) (2011) 441–454, https://doi.org/10.1085/jgp.201110604.
[35] M.D. Benson, Q.J. Li, K. Kieckhafer, D. Dudek, M.R. Whorton, R.K. Sunahara, J.A. Iniguez-Lluhí, J.R. Martens, SUMO modification regulates inactivation of the voltage-gated potassium channel Kv1.5, Proc. Natl. Acad. Sci. U. S. A. 104 (6) (2007) 1805–1810, https://doi.org/10.1073/pnas.0606702104.
[36] L.D. Plant, J.D. Marks, S.A. Goldstein, SUMOylation of $Na_V1.2$ channels me-diates the early response to acute hypoxia in central neurons, Elife 5 (2016) e20054, https://doi.org/10.7554/eLife.20054.
[37] L. Annunziato, G. Pignataro, F. Boscia, R. Sirabella, L. Formisano, M. Saggese, O. Cuomo, R. Gala, A. Secondo, D. Viggiano, P. Molinaro, V. Valsecchi, A. Tortiglione, A. Adornetto, A. Scorziello, M. Cataldi, GF Di Renzo.ncx1, ncx2, and ncx3 gene product expression and function in neuronal anoxia and brain ischemia, Ann. N. Y. Acad. Sci. 1099 (2007) 413–426, https://doi.org/10.1196/annals.1387.050.
[38] L. Annunziato, G. Pignataro, G.F. Di Renzo, Pharmacology of brain Na+/Ca2+ exchanger: from molecular biology to therapeutic perspectives, Pharmacol. Rev. 56 (4) (2004) 633–654, https://doi.org/10.1124/pr.56.4.5.
[39] G. Pignataro, E. Esposito, O. Cuomo, R. Sirabella, F. Boscia, N. Guida, G. Di Renzo, L. Annunziato, The NCX3 isoform of the Na+/Ca2+ exchanger contributes to neuroprotection elicited by ischemic postconditioning, J. Cereb. Blood Flow Metab. 31 (1) (2011) 362–370, https://doi.org/10.1038/jcbfm.2010.100.
[40] G. Pignataro, F. Boscia, E. Esposito, R. Sirabella, O. Cuomo, A. Vinciguerra, G. Di Renzo, L. Annunziato, NCX1 and NCX3: two new effectors of delayed preconditioning in brain ischemia, Neurobiol. Dis. 45 (1) (2012) 616–623, https://doi.

org/10.1016/j.nbd.2011.10.007.
[41] F. Boscia, G. Begum, G. Pignataro, R. Sirabella, OCuomo, A. Casamassa, D. Sun, L. Annunziato, Glial Na(+)-ependent ion transporters in pathophysiological conditions, Glia. 64 (10) (2016) 1677–972016, https://doi.org/10.1002/glia.23030.
[42] P. Molinaro, O. Cuomo, G. Pignataro, F. Boscia, R. Sirabella, A. Pannaccione, A. Secondo, A. Scorziello, A. Adornetto, R. Gala, D. Viggiano, S. Sokolow, A. Herchuelz, S. Schurmans, G. Di Renzo, L. Annunziato, Targeted disruption of Na+/Ca2+ exchanger 3 (NCX3) gene leads to a worsening of ischemic brain damage, J. Neurosci. 28 (5) (2008) 1179–1184, https://doi.org/10.1523/JNEUROSCI.4671-07.2008.
[43] G. Pignataro, R. Gala, O. Cuomo, A. Tortiglione, L. Giaccio, P. Castaldo, R. Sirabella, C. Matrone, A. Canitano, S. Amoroso, G. Di Renzo, L. Annunziato, Two sodium/calcium exchanger gene products, NCX1 and NCX3, play a major role in the development of permanent focal cerebral ischemia, Stroke 35 (11) (2004) 2566–2570, https://doi.org/10.1161/01.STR.0000143730.29964.93.
[44] R. Li, J. Wei, C. Jiang, D. Liu, et al., Akt SUMOylation regulates cell proliferation and tumorigenesis, Cancer Res. 73 (2013) 5742–5753, https://doi.org/10.1158/0008-5472.CAN-13-0538.
[45] C. Kho, A. Lee, D. Jeong, jg Oh, P.A. Gorski, K. Fish, R. Sanchez, R.J. De Vita, G. Christensen, R. Dahl, R.J. Hajjar, Small-molecule activation of SERCA2a SUMOylation for the treatment of heart failure, Nat. Commun. 6 (2015) 7229, https://doi.org/10.1038/ncomms8229.
[46] J.D. Bernstock, Y.J. Lee, L. Peruzzotti-Jametti, et al., A novel quantitative high-throughput screen identifies drugs that both activate SUMO conjugation via the inhibition of microRNAs 182 and 183 and facilitate neuroprotection in a model of oxygen and glucose deprivation, J. Cereb. Blood Flow Metab. 36 (2) (2016) 426–441, https://doi.org/10.1177/0271678X15609939.
[47] J.D. Bernstock, D. Ye, F.A. Gessler, et al., Topotecan is a potent inhibitor of SUMOylation in glioblastoma multiforme and alters both cellular replication and metabolic programming, Sci. Rep. 7 (2017) 7425, https://doi.org/10.1038/s41598-017-07631-9.