Proper progression of neurogenesis relies on a defined pattern of SUMO-modified proteins

Mario García-Domínguez

Neurogenesis is a complex process involving the orchestration of many transcription factors and other proteins. Fine regulation of their activities is crucial for proper progression of neurogenesis. A few decades ago, covalent attachment of Small Ubiquitin-like Modifier (SUMO) to other proteins was revealed as a major regulator of protein activities, constituting an essential posttranslational modification system in vertebrates. Since then, hundreds of proteins have been shown to be targets of SUMO, which is implicated in controlling many relevant processes in eukaryotic cells. These include the development and function of the nervous system, with SUMO tightly linked to synopsis and neurodegenerative diseases (Yau et al., 2020). The SUMO protease SENP7 has been involved in neurogenesis (Juárez-Vicente et al., 2016), while sumoylation of BRAF35, a subunit of the LSD1 histone demethylase complex, assures the undifferentiated state of neural progenitors (Ceballos-Chávez et al., 2012). In addition, increased sumoylation in mouse brain-derived neural stem cells results in enhanced neuronal differentiation (Bernstock et al., 2019). However, in general, the implication of SUMO in neuronal differentiation and nervous system development has been poorly studied. We have recently developed a proteomic study aimed at identifying SUMO targets associated either with proliferation or with neuronal differentiation conditions, which has highlighted the relevance of SUMO modification for proper progression of neurogenesis. In this Perspective, we discuss our recent results, present specific SUMO targets that are key to the process, and indicate future research directions to uncover the molecular mechanisms underlying SUMO modification of relevant neurogenesis-associated factors. SUMO is a small polypeptide of approximately 100 amino acids, similar to ubiquitin, that is able to covalently attach to other proteins through the ε-amino group of a lysine (K) residue to modulate its function/activity (Flohto and Melchior, 2013). Target K is often included in the consensus ψKxE (ψ, large hydrophobic residue; x, any residue). Three major functional properties of SUMO have been described in vertebrates, SUMO1–3. While most SUMO1 in the cell appears conjugated to proteins, a good fraction of the closely related paralogs SUMO2 and SUMO3 (SUMO2/3) pool is free to be rapidly conjugated to proteins in response to a variety of stress conditions. In addition to specific activating (SAE1-UBA2) and conjugating (UBC9) enzymes, SUMO ligases and proteases participate in SUMO attachment and detachment from targets, respectively, making the modification a regulated and reversible process.

The involvement of SUMO in regulating most relevant cellular processes anticipates its participation in the control of neurogenesis. To shed light on this aspect, we recently developed a SILAC-based proteomic approach to compare the SUMO proteome of proliferating and neuronal differentiating pluripotent embryonic carcinoma P19 cells (Correa-Vázquez et al., 2021). From this analysis, we identified up to 318 differentially sumoylated proteins between the two conditions. They included proteins that were similarly expressed under both conditions but sumoylated in one of them, as proteins expressed in one of the conditions in which they were sumoylated. Thus, we uncovered distinct patterns of sumoylated proteins associated with proliferation and neuronal differentiation (Figure 1A). For a selection of proteins, we made use of sumoylation mutants in gain-of-function experiments to gain insight into the role the modifications plays during neurogenesis. Mutants consisted of substitution of target K residues by arginine (KR). We analyzed the effects of wild-type (WT) and mutant proteins in cellular and embryonic models of neurogenesis in which we induced neurogenesis. Thus, the effect on neurogenesis by NeuroD2 and Neurogenin2, respectively. In all cases, the effects of mutants significantly differed from those of WT proteins. For most proteins, sumoylation was associated with proper progression of neurogenesis (Figure 1A). This was the case of Prospero homeobox 1, essentially expressed and sumoylated under differentiation conditions. SUMO was necessary for its previously reported function in cell cycle withdrawal of neural progenitors (Misra et al., 2008), indicating that SUMO-dependent modification of the protein is required for neurogenesis. Interestingly, although the pluripotency-associated factors Spalt-like transcription factor 4 and undifferentiated embryonic cell transcription factor 1 (UTF1) (Rao et al., 2010; Raina et al., 2021) were expressed and sumoylated under proliferation conditions, their modification was also required for neuronal differentiation. This illustrates how sumoylation of proliferation-associated factors is also key to neurogenesis. In turn, tripartite motif containing 24 expression remained unchanged between conditions, and sumoylation under differentiation was required for neurogenesis. Thus, regardless of whether these proteins are expressed under one or the other condition, SUMO-mediated modification of them is associated with proper neurogenesis.

We also studied potassium channel tetramerization domain containing 15 (KCTD15) (Correa-Vázquez et al., 2021). This protein was expressed and sumoylated under differentiation conditions, and unexpectedly, overexpression of the WT protein was associated with impaired neurogenesis. Thus, the effect of neuronal differentiation on KCTD15 sumoylation was opposite to that of other analyzed proteins, suggesting that KCTD15 slows neurogenesis in a SUMO-dependent manner for accurate timing and successful accomplishment of the process. Moreover, the newly displayed neurogenic activity in the developing neural tube in the absence of induced neurogenesis.
global sumoylation in neurodevelopment. We have previously shown that enhancing selective abolition of SUMO modification may lead to opposite effects. In turn, global differences in stabilities between WT and KR mutants indicated that sumoylation may result in both positive (+) and negative (–) effects on neurogenesis. UTF1 stands out among the key SUMO targets whose modification participates in the progression of neurogenesis. Sumoylation (S) of WT UTF1, by modulating UTF1 affinity for chromatin, limits sumoylated UTF1 desumylation and downregulation at very early stages of differentiation, when it recruits DCP1A for degradation of leaking mRNAs, resulting in a poised expression state of bivalent genes. Nonsumoylated (KR) mutant UTF1 displays enhanced localization to promoters and fails to recruit DCP1A, leading to altered gene expression. NGN2: Neurogenin 2.

Our proteomic analysis revealed that UTF1 is strongly downregulated after four days of differentiation, but the rate of desumoylation was greater than the rate of protein decrease. A possibility is that desumylation leads to protein degradation, since SUMO attachment has been proposed to protect against ubiquitin/proteasome-mediated degradation in certain cases (Ulrich, 2005). This would mean that nonsumoylated UTF1 would have limited opportunity to interfere with the recruitment of other factors/complexes. We did not observe differences in stabilities between WT and KR protein short times. However, even if it is the case, faint and transitory interference at very early stages of differentiation, when desumoylated UTF1 is still abundant, might have a major impact on neurogenesis at later stages. It would be of interest to establish the kinetics of UTF1 desumylation and downregulation following the induction of neuronal differentiation. UTF1 is a key transcription factor associated with stemness and development in placental mammals and is also implicated in cancer (Raina et al., 2021). Thus, detailed dissection of its operating mechanisms is of high interest.

Undoubtedly, as occurs in the case of many other relevant cellular processes, sumoylation is critical for neurogenesis. Many of the players of the process use SUMO to modulate their activities. Thus, depending on the target, selective ablation of SUMO modification may lead to opposite effects. In turn, global alteration of sumoylation should lead to unique general effects. In relation to this, we have previously shown that enhancing global sumoylation in neurodevelopmental models, either by overexpressing different SUMO species or by knocking down the SUMO protease SENP7, leads to impaired neurogenesis (Juárez-Vicente et al., 2016). Conversely, it has been reported that UBC9 overexpression in adult mouse-derived neural stem cells leads to increased global sumoylation and enhanced neuronal differentiation (Bernstock et al., 2019). This might suggest that the association of SUMO with a neural undifferentiated state would be restricted to development. However, far from this interpretation, it probably means that establishment of highly specialized differentiation programs in stem cells requires fine tuning of a number of specific factors and that minor alteration of the activity of any of them suffices to abrogate neurogenesis. In other words, if just one key factor whose activity depends on sumoylation fails to be modified, underlying control systems should assure interruption of such a complex process. Therefore, it appears critical to precisely study the consequences of blocks in sumoylation of each isolated key factor. In this context, it would be desirable in upcoming research to approach this topic by substitution of endogenous genes by their corresponding sumoylation mutant versions, for instance, by making use of CRISPR/Cas9 technology. However, discrete mutations impairing sumoylation may not result in dramatic phenotypes. Although milder effects at early developmental stages do not exclude severe consequences at later stages, in some cases, this probably results from additional overlapping control levels of protein activities. Nonetheless, a fundamental role of SUMO relies on the maintenance of complex stability, especially in the case of chromatin-associated complexes (García-Domínguez and Reyes, 2009). Thus, compared with moderate outcomes of discrete mutations, more severe phenotypes would be expected from simultaneous mutation of several proteins in a complex or pathway.

References

Bernstock JD, Peruzzotti-Jametti L, Leonardi T, Vicario N, Ye D, Lee YI, Maric D, Johnson KR, Mou Y, Van Den Bosch A, Winterbone M, Friedman GK, Franklin RJM, Hallenbeck JH, Puchino S (2019) SUMOylation promotes survival and integration of neural stem cell grafts in ischemic stroke. EBioMedicine 42:214-224.

Ceballos-Chávez M, Rivero S, García-Gutiérrez P, Rodríguez-Paredes M, García-Domínguez M, Bhattacharya SS, Reyes JC (2012) Control of neuronal differentiation by Braf35 sumoylation. Proc Natl Acad Sci U S A 109:8085-8090.

Correa-Vázquez JF, Juárez-Vicente F, García-Gutiérrez P, Barysh SV, Melchior F, García-Domínguez M (2021) The Sumo proteome of proliferating and neuronal-differentiating cells reveals UTF1 among key SUMO targets involved in neurogenesis. Cell Death Dis 12:308.

Florea A, Melchior F (2013) Sumoylation: a regulatory protein modification in health and disease. Annu Rev Biochem 82:357-385.

García-Domínguez M, Reyes JC (2009) SUMOylation with repressor complexes, emerging routes for transcriptional control. Biochem Biophys Acta 1789:451-459.

Jia J, Zheng X, Hu G, Cui K, Zhang J, Zhang A, Jiang H, Lu B, Yates III J, Liu C, Zhao K, Zheng Y (2012) Regulation of pluripotency and self-renewal of ESCs through epigenetic-threshold modulation and mRNA pruning. Cell 151:576-589.

Juárez-Vicente F, Luna-Peláez N, García-Domínguez M (2016) The Sumo protease Senp7 is required for proper neuronal differentiation. Biochem Biophys Acta 1863:1490-1498.

Mista K, Gui H, Matise MP (2008) Proxl regulates a transitory state for interneuron neurogenesis in the spinal cord. Dev Dyn 237:393-402.

Raina K, Dey C, Thoir M, Sudhagar S, Thummer RP (2021) An insight into the role of UTF1 in development, stem cells, and cancer. Stem Cell Rev Rep doi: 10.1007/s12015-021-1017-9.

Rao S, Zhen S, Roumiantsev S, McDonald LT, Yuan GC, Orkin SH (2010) Differential roles of Sall4 isoforms in pluripotency and self-renewal of ESCs through the Sall4-Salt4 regulatory network. Dev Dyn 239:2939-2950.

Juárez-Vicente F, Luna-Peláez N, García-Domínguez M (2017) The Sumo protease Senp7 is required for proper neuronal differentiation. Biochem Biophys Acta 1863:1490-1498.

Mista K, Gui H, Matise MP (2008) Proxl regulates a transitory state for interneuron neurogenesis in the spinal cord. Dev Dyn 237:393-402.

Raina K, Dey C, Thoir M, Sudhagar S, Thummer RP (2021) An insight into the role of UTF1 in development, stem cells, and cancer. Stem Cell Rev Rep doi: 10.1007/s12015-021-1017-9.

Rao S, Zhen S, Roumiantsev S, McDonald LT, Yuan GC, Orkin SH (2010) Differential roles of Salt4 isoforms in embryonic stem cell pluripotency. Mol Cell Biol 30:5364-5380.

Ulrich HD (2005) Mutual interactions between the SUMO and ubiquitin systems: a plea of no contest. Trends Cell Biol 15:525-532.

Yau TY, Molina O, Courey AJ (2020) SUMOylation in development and neurodegeneration. Development 147:175703.