Destroy to create: E3 ubiquitin ligases in neurogenesis

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Abstract

The ubiquitin proteasome system (UPS) has drawn tremendous attention in the field of neuroscience. In recent years, we have gained insights into UPS-dependent mechanisms in brain development and disease. Several interesting studies over the past two years have highlighted the role of distinct E3 ubiquitin ligases in neurogenesis. Here, we will review the major findings in these studies and discuss their implications.

Introduction and context

The major function of the ubiquitin proteasome system (UPS) is to provide the regulated degradation of proteins [1]. The UPS consists of sets of enzymes that mark a protein destined for degradation with a polyubiquitin chain. In an enzymatic cascade, ubiquitin, which is a small 8.5-kDa protein, is first covalently linked to the E1 ubiquitin-activating enzyme in an energy-dependent manner. Subsequently, ubiquitin is transferred onto the E2 ubiquitin-conjugating enzyme. Finally, the E3 ubiquitin ligase recruits both the E2 ubiquitin-conjugating enzyme and substrate, thereby facilitating the transfer of ubiquitin to the substrate. The processivity of the ubiquitination reaction leads to the formation of a polyubiquitin chain. Polyubiquitinated substrates are recognized by the proteasome, leading to the degradation of the substrate. While there are two characterized E1 enzymes and approximately 35 E2 enzymes, E3 ligases are quite numerous, with more than 600 enzymes [2]. Although E3 ligases confer substrate specificity, individual E3 ligases can recruit more than one substrate. Conversely, substrates may be targeted by more than one E3 ligase [3,4]. Among E3 ligases, RING (really interesting new gene)-type ligases represent the largest group and are composed of a single subunit or multisubunit complexes [2]. The RING-type E3 ligases operate as scaffolds to recruit both the E2 and the substrate to facilitate ubiquitination.

In contrast, members of the smaller family of HECT (homologues to E6AP C-terminus) ligases harbor enzymatic activity, which mediates the transfer of ubiquitin to the target [5]. Besides the control of protein abundance, ubiquitination regulates diverse aspects of cellular signaling and physiology [6,7].

Over the past few years, a number of E3 ubiquitin ligases have been reported to play critical roles in diverse aspects of neuronal development, including axon, dendrite, and synapse morphogenesis (for reviews, see [8-10]). In this review, we will focus on recent studies implicating E3 ubiquitin ligases in the regulation of neurogenesis (Figure 1).

Major recent advances

E3 ubiquitin ligase pathways regulating neurogenesis

Neurogenesis requires the precise and sophisticated orchestration of many signaling events [11]. One way of determining the cell fate is to epigenetically program the genome to restrict the expression of genes that favor one cell fate over another. In this regard, a prominent factor is the repressor element 1 (RE-1)-silencing transcription factor (REST), also known as NRSE (neuron-restrictive silencing factor), which is a DNA-binding protein that recognizes a conserved motif present in many neuronal genes [12,13]. To ensure permanent gene silencing, REST associates with the

Figure 1. E3 ubiquitin ligases in neurogenesis

The E3 ubiquitin ligases SCF-βTRCP, Huwe-1, TRIM32, or TRIM11 promote neurogenesis by targeting their respective substrates REST, N-Myc, c-Myc, or PAX6 for degradation by the proteasome. βTRCP, beta-transducin repeat containing protein; REST, repressor element 1-silencing transcription factor; SCF; Skp, Cullin, F-box containing; TRIM, tripartite motif.

co-repressors mSin3 and CoREST, which in turn recruit HDACs (histone deacetylases) to target genes [12]. The REST-dependent silencing mechanism is found in all non-neuronal cells, including those within the nervous system, raising the question of how precursor cells escape this silencing program to acquire a neuronal cell fate.

A recent study by Westbrook and colleagues [14] provides insight into this problem. The authors found that REST is polyubiquitinated and hence subjected to proteasomal degradation in embryonic stem (ES) cells. The authors then identified SCF^{βTRCP} as the E3 ubiquitin ligase that targets REST for degradation. SCF^{βTRCP} is an SCF complex consisting of the adaptor proteins Skp1 and Cullin1, the RING component Roc1, and the F-box protein βTRCP (beta-transducin repeat containing protein), which confers substrate specificity [15]. The recruitment of most F-box proteins, including βTRCP, requires a phosphorylated recognition motif within the substrate [15]. Correspondingly, REST is recruited to SCF^{βTRCP} in a phosphodegron-dependent manner. To test the effect of the REST/SCF^{βTRCP} interaction on neuronal gene expression, ES cells harboring a Sox1 reporter gene as a marker of early-stage neurogenesis were transfected with REST, SCF^{βTRCP}, or control short interfering RNAs (siRNAs). Knockdown of βTRCP led to significantly fewer Sox1-positive cells as compared with control cells. In contrast, knockdown of REST strongly induced Sox1 expression. A comparably strong induction of Sox1 expression was obtained by simultaneous knockdown of REST and βTRCP. In other experiments, ES cells containing the Sox1 reporter were transfected with wild-type or degen-

mutant REST and placed in differentiation media. Expression of mutant REST, but not wild-type REST, dramatically reduced Sox1 expression. Taken together, these findings identify REST as a novel target of the E3 ubiquitin ligase SCF^{βTRCP} and support the conclusion that SCF^{βTRCP} acts upstream of REST to control neurogenesis. While REST imposes a non-neuronal cell fate at an epigenetic level, controlled clearance of REST favors a neuronal cell fate by derepression and subsequent induction of neuronal genes.

The HECT domain E3 ubiquitin ligase Huwe1 employs a strategy similar to that of SCF^{βTRCP} to promote neurogenesis. The transcription factor N-Myc maintains the proliferation of precursor cells in the nervous system [16,17]. Previous studies have revealed that N-Myc undergoes proteasome-dependent degradation [18,19]. In an effort to determine how N-Myc is regulated, Lasorella and colleagues [20] used an unbiased biochemical approach to identify proteins that form a physical complex with N-Myc. The authors identified the E3 ubiquitin ligase Huwe1 as a new N-Myc-interacting protein. Huwe1 triggered the polyubiquitination and subsequent degradation of N-Myc [20]. To determine the functional consequence of Huwe1-mediated degradation of N-Myc in ES cells, wild-type and Huwe1 knockout ES cells were placed in differentiating conditions. While differentiated wild-type ES cells expressed neuronal genes, including *neurogenin*, *n-cadherin*, and *MAP2*, Huwe1 knockout ES cells failed to express these markers. Furthermore, knockout of Huwe1 in ES cells led to the accumulation of N-Myc protein. In other experiments, *ex utero* electroporation analyses of the cerebral cortex revealed that knockdown of N-Myc significantly decreased the number of proliferating cells and increased the number of neurons, suggesting that N-Myc knockdown leads to cell cycle exit of precursor cells. In contrast, knockdown of Huwe1 restored the proliferative nature of the cells and prevented differentiation along the neuronal lineage. In epistasis analyses, knockdown of N-Myc suppressed the Huwe1 knockdown-induced phenotype of increased cell proliferation and impaired differentiation, supporting the conclusion that N-Myc acts downstream of Huwe1 in precursor cells in the cerebral cortex.

In a follow-up study, Zhao and colleagues [21] reported conditional knockout of Huwe1 in the cerebral cortex of mice. The authors also provided evidence suggesting that the gene encoding the Notch ligand Delta-like 3 (Dll3) may act as a target of N-Myc. Accordingly, Dll3 levels are elevated in Huwe1-null brains as compared with wild-type brain. By silencing N-Myc and Dll3 in Huwe1 knockout animals, the loss-of-Huwe1 phenotype of hyperproliferation and defective differentiation was reversed. Huwe1 ensures a low level of N-Myc at

a critical time point of development, when neural precursors are scheduled to differentiate. Destruction of N-Myc results in the suppression of the *dll3* gene. The authors reasoned that inactivation of the Dll3-notch pathway is critical for neural precursors to acquire a neuronal cell fate and that Huwe1 acts in a critical developmental window to target N-Myc for degradation and to induce neurogenesis.

Prior to acquiring a neuronal cell fate, the multipotential neural precursor cell undergoes asymmetric cell division, giving rise to a multipotential precursor cell and a restricted neuronal progenitor cell [22]. The latter inherits cell fate-determining proteins that initiate the production of neurons [22]. An asymmetrically distributed determinant is the ubiquitin ligase TRIM32 (tripartite motif-containing 32) [23,24]. TRIM32 is a member of the tripartite interaction motif family of single-subunit E3 ligases. These proteins typically harbor a RING domain, a B-box domain (BB), and a coiled-coil domain (CC). The family consists of at least 65 members, which are implicated in diverse cellular functions and pathological conditions [25]. The *Drosophila* orthologue of TRIM32, Brat, has been identified as a determinant of cell fate. Once inherited by the daughter cell, Brat triggers neuronal differentiation of *Drosophila* neuroblasts [23]. In addition, *Drosophila* Brat has been identified as an interaction partner of the RNAase Argonaute 1 (Ago1) [26]. Also, the transcription factor dMyc was found to be regulated post-transcriptionally downstream of Brat. The exact regulatory mechanism, however, remained unexplored until recently [23].

Schwamborn and colleagues [24] investigated TRIM32 function in the mammalian cerebral cortex. TRIM32 displayed an asymmetrical distribution in dividing precursor cells and was enriched in one of the two daughter cells [24]. In overexpression analyses of precursor cells, TRIM32 potently induced neuronal cell fate as monitored by neuron-specific *class III β -tubulin* expression. In addition to the conserved proneuronal function of TRIM32, the binding to Ago1 is conserved across species. Interestingly, the TRIM32/Ago1 complex was found to bind to and activate the microRNA (miRNA) *let-7a*, which promoted the expression of *class III β -tubulin* in precursor cells. This proneuronal function of TRIM32, however, is independent of its ligase activity. To establish a functional relationship of TRIM32 and c-Myc based on the authors' previous findings [23], TRIM32 was subjected to immunoprecipitation analyses and found to interact with c-Myc. In addition, the RING domain of TRIM32 was required for the polyubiquitination and consequent degradation of c-Myc. Importantly, the degradation of c-Myc triggered

cell cycle exit of mitotic cells, and expression of RING-defective TRIM32 or TRIM32 together with c-Myc failed to induce neuronal differentiation in the cerebral cortex. Hence, the authors proposed a model in which TRIM32 triggers at least two events that promote neurogenesis: TRIM32 activates miRNAs and targets c-Myc for proteasomal degradation. Thus, the degradation of Myc family members appears to be a recurring concept in cells that differentiate along the neuronal lineage.

TRIM11 represents another member of the TRIM family that has been implicated in the regulation of neurogenesis. Tuoc and Stoykova [27] recently reported that TRIM11 may regulate the abundance of the transcription factor Pax6 in neural precursor cells. TRIM11 binds to and polyubiquitinates Pax6, thus triggering its degradation by the proteasome. Intriguingly, TRIM11 clears the cell of insoluble Pax6. Overexpression of TRIM11 in cortical precursor cells inhibits neuronal differentiation. Using siRNAs to silence TRIM11, the authors showed that TRIM11 knockdown led to the accumulation of Pax6 in cortical precursor cells, leading to apoptosis. While TRIM11 gain-of-function impaired neurogenesis, TRIM loss-of-function induced apoptosis. Interestingly, TRIM11 itself is a Pax6-controlled gene, providing an elegant feedback mechanism of substrate and E3 ligase [27]. In summary, Tuoc and Stoykova [27] present a model in which TRIM11 regulates Pax6 levels to ensure the correct gene dosage of Pax6-controlled genes to promote neurogenesis.

Future directions

The featured studies in this review explore the role of the UPS in neurogenesis. Future studies in this area should provide further insights into the role of ubiquitin ligases in neurogenesis. The physiologic role of each of the E3 ubiquitin ligases in the development and function of the nervous system remains to be elucidated. In addition, how each of the E3 ubiquitin ligases, SCF^{TRCP}, Huwe1, TRIM32, and TRIM11, is regulated in neural precursor cells will be a fruitful avenue for future research. Finally, it will be interesting to determine whether and how these distinct ubiquitin signaling pathways might interact with each other in neural stem cells and in the course of neuronal differentiation.

In view of the large number of E3 ubiquitin ligases, we are just beginning to gain an understanding of the role of these enzymes in the early events of neuronal development. Recent studies have implicated the major cell cycle ubiquitin ligases Cdh1-anaphase-promoting complex (Cdh1-APC) and Cdc20-APC in distinct aspects of neuronal development [9,10,28]. In particular, Cdh1-APC has been implicated in the control of axon growth and patterning, whereas Cdc20-APC has been implicated

in the control of dendrite morphogenesis and presynaptic differentiation in the mammalian brain [29-33]. Cdh1-APC has also been implicated in the control of synapse development in invertebrates [34,35]. Recent studies have broadened the roles of Cdh1-APC beyond development to the control of glycolysis and synapse function in the mature nervous system [36-38]. Interestingly, the roles of Cdh1-APC and Cdc20-APC in neurogenesis in the mammalian brain have yet to be elucidated. Since Cdh1-APC and Cdc20-APC are critical regulators of cell cycle progression, these ligases are anticipated to have important functions in the early events of neuronal development.

Beyond the APC, it will be interesting to explore the role of additional E3 ubiquitin ligases in neurogenesis. Screens might be a useful approach to identify novel E3 ligases that regulate neurogenesis. Since deregulation of mechanisms of neurogenesis is thought to play a critical role in disease pathogenesis [39], the identification of ubiquitin ligase pathways that control neurogenesis will advance our understanding of brain development and diseases. These studies also raise the prospect that therapies that target E3 ligases in order to stimulate neurogenesis in the adult brain may be developed. It will be an exciting and worthwhile endeavor to shed light on the complexity of E3 ubiquitin ligase function in neurogenesis.

Abbreviations

Ago1, Argonaute 1; APC, anaphase-promoting complex; β TRCP, beta-transducin repeat containing protein; Cdc20-APC, Cdc20-anaphase-promoting complex; Cdh1-APC, Cdh1-anaphase-promoting complex; Dll3, Delta-like 3; ES, embryonic stem; HECT, homologues to E6AP C-terminus; miRNA, microRNA; REST, repressor element 1-silencing transcription factor; RING, really interesting new gene; SCF complex; Skp, Cullin, F-box containing complex; siRNA, short interfering RNA; TRIM, tripartite motif; UPS, ubiquitin proteasome system.

Competing interests

The authors declare that they have no competing interests.

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