The role of post-translational modifications of huntingtin in the pathogenesis of Huntington's disease

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Abstract: Post-translational modifications are rapid, effective and reversible ways to regulate protein stability, localization, function, and their interactions with other molecules. Post-translational modifications usually occur as chemical modifications at amino acid residues, including SUMOylation, phosphorylation, palmitoylation, acetylation, *etc.* These complex biochemical modifications tightly regulate and control a variety of cellular processes. Several forms of post-translational modifications of huntingtin (Htt) have been described. These modifications affect Htt metabolism, protein-protein interactions and cellular toxicity. Cleavage and clearance of mutant Htt, and the interactions between mutant Htt and other cellular proteins are important biochemical events leading to Huntington's disease (HD). Therefore, identifying signaling pathways of Htt modification and evaluating the significance of Htt modifications would lead to a better understanding of the normal function of wild-type Htt and the pathogenic mechanisms of mutant Htt.

Keywords: Huntington's disease; huntingtin; modification; SUMOylation; phosphorylation; palmitoylation; acetylation

1 Introduction

Post-translational modification indicates the chemical modification of a protein. It is one of the later steps of biosynthesis of polypeptides. During protein synthesis, 21 different amino acids can be incorporated into proteins. The post-translational modification extends the functions of the protein by attaching the biochemical functional groups, such as acetate, phosphate, various lipids and carbohydrates, to the polypeptide, through changing the chemical nature of an amino acid or making structural changes (such as the formation of disulfide bridges). Also, enzymes may remove amino acids from the amino end of the protein, or cut the peptide

chain in the middle. In most nascent polypeptides, the post-translational modifications start at the amino acid methionine, since the "start" codon on mRNA also codes for this amino acid. Therefore, this amino acid is usually taken off during post-translational modification. Other modifications, such as phosphorylation, are common mechanisms underlying the control of the behavior of a protein, such as activating/inactivating of an enzyme or a kinase.

Huntington's disease (HD) is a neurodegenerative disease caused by an expanded polyglutamine (polyQ) tract in the N-terminal of huntingtin (Htt) protein, and is characterized by the accumulation of a pathogenic protein that contains this abnormal polyQ expansion^[1]. Htt can be cleaved by caspases, calpains and aspartic proteases to form short N-terminal toxic fragments with polyQ expansion both *in vitro* and in the brain of HD patients^[2]. An important pathological feature of HD and other polyQ disorders is the protein

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aggregation, which has been found in the nucleus, the cytoplasm and the neurites^[3]. The conformational changes of protein can cause aggregation, and allow abnormal protein interactions and sequestration of other normal cellular proteins, thus leading to neurotoxicity. Protein interactions and post-translational modifications regulate normal functions of wild-type Htt or neurotoxicity of mutant Htt. Therefore, identifying signaling pathways that act on Htt modifications is critical for understanding Htt functions under normal and pathological conditions^[4].

Post-translational modifications, such as SUMOylation, ubiquitination, phosphorylation, palmitoylation, and acetylation, play important roles in the pathogenesis of HD, probably by modulating clearance and toxicity of polyQ-expanded Htt, or by modulating the physiological function of wild-type Htt^[4]. For example, SUMOylation promotes the capacity of Htt to repress transcription^[1], while phosphorylation reduces the toxicity of mutant Htt^[5,6]. Besides, palmitoylation alters the normal distribution and function of Htt^[4]. Thus, understanding the post-translational modification of Htt in the context of polyQ expansion may provide an insight into HD pathogenesis.

2 Types of post-translational modifications

2.1 SUMOylation Small Ubiquitin-like Modifier (SUMO) or SUMO proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their functions^[7]. Four different SUMO isoforms, termed SUMO-1, SUMO-2, SUMO-3 and SUMO-4, have been detected in mammalian cells^[8]. SUMO-1 is mainly found in the nuclear membrane while SUMO-2 and SUMO-3 are predominantly localized in the cytoplasm and the nuclear bodies, respectively^[9]. SUMOylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, protein stability, apoptosis, transcriptional regulation, response to stress, and progression through the cell cycle^[10].

SUMO modification can affect many biological functions of proteins and appears to play essential roles in diverse processes by attaching SUMO to the lysine residues in target proteins. The process of SUMO attachment to the

target is similar to that of ubiquitination, during which 4 enzymatic reactions (mediated by SUMO protease, E1, E2 and E3 enzymes) are involved, to form an isopeptide bond between the carboxy-terminal glycine of SUMO, and relies on a single E2 enzyme, Ubc9. SUMO firstly binds to a heterodimer E1 enzyme, and then passes to an E2 which is a conjugating enzyme (Ubc9)^[11]. Finally, it is attached to one of a small number of E3 ligating proteins. SUMOylation of target proteins has been shown to produce a number of different outcomes, including alterations of localization and binding partners. Typically, of a given protein, only a small fraction is SUMOylated and this modification can be rapidly reversed by the action of deSUMOylating enzymes^[12].

The SUMO-1 modification of RanGAP1 (the first identified SUMO substrate) leads to its trafficking from the cytosol to the nuclear pore complex^[13,14]. The SUMO modification of hNinein leads to its movement from the centrosome to the nucleus^[15]. In many cases, SUMO modifications of transcriptional regulators correlate with the inhibition of transcription^[16]. This reversible pathway provides a rapid and efficient way to modulate the subcellular localization, activity and stability of various substrates^[7].

2.2 Phosphorylation Reversible phosphorylation of protein is an important regulatory mechanism in both prokaryotic and eukaryotic organisms^[17,18]. Phosphorylation commonly regulates the proteolysis^[19] and nuclear transport^[20] of many proteins. Kinases (phosphorylation) and phosphatases (dephosphorylation) are involved in this process^[21]. Many enzymes and receptors are switched “on” or “off” by phosphorylation or dephosphorylation. Reversible phosphorylation results in a conformational change in the structure, leading to the activation or deactivation of many enzymes and receptors. Phosphorylation usually occurs at serine, threonine, and tyrosine residues in eukaryotic proteins, catalyzed by their corresponding kinases^[22]. When receiving the deactivating signal, the protein becomes dephosphorylated and stops working. This mechanism underlies many forms of signal transduction.

2.3 Palmitoylation Protein palmitoylation was first described 30 years ago, when it represented the first example of covalent lipid modification of proteins in eukaryotic cells. Palmitoylation

is the covalent attachment of fatty acids, such as palmitic acid, to cysteine residues of membrane proteins^[23]. The precise function of palmitoylation varies based on the specific proteins. Palmitoylation enhances the hydrophobicity of proteins and contributes to their membrane association. Palmitoylation also appears to play a significant role in the subcellular trafficking of proteins between membrane compartments, and in modulating protein-protein interactions^[66]. Since palmitoylation is reversible, it allows the dynamic regulation of the location of specific proteins.

2.4 Acetylation Acetylation describes a reaction that introduces an acetyl functional group into an organic compound, while deacetylation is the removal of the acetyl group. For histone, acetylation and deacetylation occur at lysine residues in the N-terminal, and serve as one of the mechanisms of gene regulation. Typically, these reactions are catalyzed by enzymes with “histone acetyltransferase (HAT)” such as CREB-binding proteins (CBP), or “histone deacetylase (HDAC)”, although HATs and HDACs can also modify the acetylation status of non-histone proteins^[24]. Like the phosphorylation by kinases or the dephosphorylation by phosphatases, the regulation of transcription factors, effectors, molecular chaperones, and cytoskeletal proteins by acetylation/deacetylation has emerged as a significant post-translational regulatory mechanism^[25].

3 Htt post-translational modifications and their significance in HD pathogenesis

3.1 Htt SUMOylation and HD SUMO proteins have been implicated in HD and many other polyQ diseases, suggesting that SUMO modification contributes to the neurodegeneration in polyQ diseases^[7]. SUMO modification of the polyQ proteins is important for their toxicity. Although it still needs to be clarified whether other polyQ proteins are SUMOylated, some studies have reported that a pathogenic fragment of Htt can be modified either by SUMO-1 or by ubiquitin at identical lysine residues^[1]. The SUMO-1 conjugation sites are located within the N-terminal domain of the Htt peptide, and overlap with the ubiquitin-targeted lysine residues, which may lead to the competition between the 2 modifiers.

Some studies have indicated that expression of a stable

Htt-SUMO conjugate in a neuronal cell line results in increases of protein stability and Htt accumulation, a decrease in aggregate formation, and enhancement of Htt-mediated transcriptional repression^[1,7]. The latest research indicates that Rhes overexpression can markedly reduce the aggregation of mutant Htt and increase the levels of soluble mutant Htt, by enhancing the SUMOylation of mutant Htt^[26]. Rhes, which is specifically localized in the striatum, binds physiologically to mutant Htt, and this interaction can account for the localized neuropathogenesis of HD. Rhes binds to Ubc9, the cellular E2 ligase, and enhances the SUMOylation of mutant Htt, but not the wild-type Htt, in a time- and concentration-dependent manner^[26]. This indicates that the availability of the target lysine is essential for degradation of Htt and that SUMOylation at these sites results in a more significant pathological response, as compared to the ubiquitination-associated slight amelioration.

SUMOylation could increase the nuclear or subnuclear localization of Htt. Nuclear localization of Htt is essential for HD pathogenesis. SUMOylation may alter the cytoplasmic activity of Htt, and induce the release of a nuclear repressor that translocates to the nucleus, or the cytoplasmic retention of proteins that are necessary to activate transcription. The N-terminal domain of Htt is responsible for cytoplasmic targeting, and therefore SUMO conjugation within this domain could mask a cytoplasmic retention signal and promote its nuclear localization^[1]. This potential mechanism can explain the SUMO-mediated increase in Htt transcriptional repression, which is a direct pathological implication of SUMO in polyQ diseases^[1]. Many polyQ-interacting partners that are recruited into neuronal inclusions including the nuclear bodies, protein PML^[27], p53^[28] and c-jun^[27], are also SUMO substrates. SUMOylation may, therefore, modulate their abilities to interact with polyQ mutant proteins and/or to be recruited into transcriptional complexes. A recent report has demonstrated the role of SUMO in synapses through the regulation of transcription factor MEF2A^[29]. It is therefore plausible that SUMO is involved in transcriptional deregulation in neurodegenerative diseases.

In summary, Htt can be SUMOylated. And SUMOylation can increase Htt accumulation, influence its subcellular lo-

calization and promote nuclear repression of transcription. Therefore, SUMOylation exacerbates the pathogenesis of HD.

3.2 Htt phosphorylation and HD Phosphorylation of Htt at certain sites can inhibit mutant Htt cleavage, aggregation and cell death^[30]. Htt can be phosphorylated at serine 421 by protein kinase Akt (also known as PKB) and serum- and glucocorticoid-induced kinase (SGK). *In vitro* and *in vivo* studies indicate that this phosphorylation can protect against the toxicity of polyQ-expanded Htt^[5,31-34]. Another crucial kinase involved in the pathway leading to HD pathogenesis is cyclin-dependent kinase 5 (CDK5), a serine-threonine kinase. It has been reported that Htt can be phosphorylated at serine 434 by CDK5.

3.2.1 The Akt signaling pathway The Akt signaling pathway is of particular interest in investigating neurodegenerative disorders. Akt, a serine-threonine kinase, is a potent pro-survival kinase that exerts its survival effect on neurons by phosphorylating several substrates including Htt^[35]. A previous report has shown that phosphorylation of Htt at serine 421 by Akt is neuroprotective against HD cellular toxicity^[34,36]. Phosphorylation at serine 421 plays an important role in inhibiting toxicity of mutant Htt^[8]. Phosphorylation of polyQ-expanded Htt at serine 421 by Akt leads to the decreased formation of nuclear inclusions and reduced toxicity in cultured striatal neurons^[5] and in a rodent model of HD^[32]. Additionally, this phosphorylation is reduced in the cortex and the striatum of HD mice expressing mutant N-terminal Htt^[6]. The importance of this pathway in HD is further proved by the finding that inhibition of phosphatase calcineurin increases the phosphorylation of mutant Htt at serine 421 and reduces striatal neuronal death^[32].

Recently, Colin *et al.* have shown that Htt regulates vesicle axonal transport^[31]. This function is weakened by the expansion of polyQ, but restored by phosphorylation at serine 421^[36]. Axonal transport is responsible for the transportation of molecules destined for degradation from the axon back to the cell body, where they are broken down by lysosomes. Axonal Htt aggregates can physically block intracellular transport in cultured neurons^[37]. Similar findings have also been observed in *Drosophila*, where aggregates

formed by mutant Htt disrupt the axonal transport^[38]. Indeed, Htt has been reported to be involved in axonal transport in rat striatum nerve^[39]. The transport of vesicles in neurons is a highly regulated process, during which vesicles move either anterogradely or retrogradely, depending on the nature of the molecular motors, kinesins or dynein, respectively. These proteins propel vesicles along microtubules. When phosphorylated, Htt recruits kinesin-1 to the dynactin complex on vesicles and microtubules. Using brain-derived neurotrophic factor (BDNF) as a marker for vesicular transport, Colin *et al.* demonstrate that Htt phosphorylation can promote the anterograde transport. Conversely, when Htt is not phosphorylated, kinesin-1 detaches and vesicles are more likely to undergo retrograde transport. This also applies to the movement of other vesicles, suggesting an essential role of Htt in the control of vesicular transport directionality in neurons^[31]. In sum, both loss and gain of function of Htt affect axonal transport^[40], and phosphorylation of wild-type Htt at serine 421 is also crucial to control the direction of vesicle movement in neurons.

3.2.2 The SGK pathway Phosphorylation at serine 421 plays an important role in inhibiting polyQ-Htt-induced toxicity. Thus, identifying the kinases that phosphorylate Htt at serine 421 is crucial for understanding the cellular mechanisms that control toxicity. Besides Akt, SGK can also phosphorylate Htt at serine 421, and SGK can inhibit polyQ-Htt-induced toxicity in striatal neurons. The role of SGK in the context of HD has been firstly revealed and further demonstrated by the up-regulation of SGK in HD postmortem brain samples and in the central nervous system cells derived from HD mice. Some experiments have also identified p38/MAPK as a regulator of SGK level, indicating a novel link between stress and polyQ toxicity^[33].

3.2.3 The CDK 5 pathway Htt can also be phosphorylated at other nearby residues, including serines 434, 536, 1181, 1201, 2076, 2653 and 2657^[41]. Among these sites, serines 1181, 1201 and 434 have been shown to be phosphorylated by CDK5^[30,42]. CDK5 is a serine-threonine kinase, whose activity is mostly restricted to post-mitotic and mature neurons. However, CDK5 is also associated with cell death under pathological conditions^[43]. Phosphorylation of polyQ-expanded Htt at serines

1181 and 1201 protects against toxicity.

The phosphorylation of Htt at serine 434 by CDK5 reduces caspase-mediated Htt cleavage at residue 513. Reduced cleavage of mutant Htt can attenuate the aggregation of mutant Htt and the toxicity in cells expressing mutant Htt. CDK5 activity is reduced in the brain of HD transgenic mice, compared with that of control. These data suggest that the ability of CDK5 to phosphorylate Htt to protect against cleavage, aggregation, and toxicity, is compromised in cells expressing toxic fragments of mutant Htt^[30]. In addition, CDK5 has also been reported to suppress polyQ-expanded Htt toxicity through a phosphorylation-independent mechanism^[44].

3.3 Htt palmitoylation and HD Post-translational modification at cysteine residues by palmitate is reversible, and is important for the trafficking and functional modulation of different membrane proteins and their signaling pathways^[45-47]. Some experimental results show that Htt is normally palmitoylated at cysteine 214, which is essential for its trafficking and function. The palmitoylation and distribution of Htt are regulated by the palmitoyl transferase Htt interacting protein 14 (HIP14).

HIP14 is mainly localized in the Golgi's complex^[48]. The sorting of some neuronal proteins, such as Ras, requires palmitoylation for trafficking to Golgi membranes and delivery to transporting vesicles. Palmitoylated Ras shows a pronounced Golgi localization and a faster retrograde trafficking from the plasma membrane to the Golgi^[47]. HIP14 alters the distributions of a subset of palmitoylated proteins in a palmitoylation-dependent manner^[49]. Some researchers find that HIP14 overexpression results in the redistribution of endogenous Htt and to a lesser extent, mutant Htt, to the Golgi. This redistribution does not occur in palmitoylation-resistant Htt, suggesting Htt trafficking to the Golgi is at least partially regulated by palmitoylation. All these findings raise a possibility that HIP14 is involved in Htt palmitoylation *in vivo*. The palmitoylation of wild-type and mutant Htt was significantly increased by overexpressing HIP14, demonstrating that HIP14 catalyzes Htt palmitoylation. Some studies show further evidence for the role of palmitoylation in the normal distribution of Htt, by examining the effect of HIP14 on the formation of inclusions in neurons. Overexpression of

HIP14 can significantly reduce the number of Htt inclusions in polyQ expansion, but not in palmitoylation-resistant mutant Htt, highlighting the importance of Htt palmitoylation by HIP14 in the intracellular distribution and trafficking of Htt.

In summary, the role of palmitoylation in polyQ disease HD has been proposed^[50]. Expansion of the polyQ tract reduces the interaction between Htt and HIP14 and decreases the amount of palmitoylated polyQ Htt. Importantly, loss of Htt palmitoylation exacerbates the toxicity, resulting in increased protein aggregation and cell death. Mutation of the palmitoylation site of Htt which makes Htt resistant to palmitoylation, accelerates inclusion formation and increases neuronal toxicity. Downregulation of HIP14 in mouse neurons expressing wild-type and mutant Htt can increase the formation of mutant Htt inclusion, while overexpression of HIP14 substantially reduces the inclusions. Expansion of the expanded polyQ tract in Htt results in decreased palmitoylation, which contributes to the formation of inclusion bodies and the enhancement of neuronal toxicity^[49].

3.4 Htt acetylation and HD Recently, acetylation has been implicated in the removal of accumulated protein in HD through targeting Htt for degradation by autophagy. Post-translational modification of the mutant Htt by acetylation at lysine 444 (K444)^[51] enhances autophagic clearance of mutant Htt. CBP is a resident nuclear protein that can acetylate histone proteins^[53]. Mutant Htt interacts directly with the HAT domain of CBP^[52], indicating that CBP is responsible for mutant Htt acetylation. While CBP is primarily localized in the nucleus, acetylated Htt is predominantly found in the cytoplasmic compartment, suggesting that upon acetylation by CBP in the nucleus, mutant Htt is available for autophagic degradation in the cytoplasm. Increased acetylation at K444 by CBP facilitates the trafficking of mutant Htt into autophagosomes, leading to neuroprotection. There are 3 forms of autophagic degradation: microautophagy, chaperone-mediated autophagy, and macroautophagy^[54]. The latter form, through which cytosolic constituents and organelles are engulfed by multilamellar vesicles and then fuse to the lysosome, has been implicated in a wide array of neurological disorders including HD^[55-57].

Moreover, it has been shown that mutation of Htt at K444 leads to the formation of a larger complex of proteins that may serve as a substrate for lysosomal degradation. In the other hand, lysosomal inhibition causes the specific accumulation of high molecular weight (HMW) Ac-Htt, while the monomeric form is unchanged, indicating that the larger complex of proteins may serve as a substrate for autophagic degradation. As a protein that co-localizes with mutant Htt inclusions, p62^[58] has been previously implicated in modulating the level of mutant exon 1 Htt^[59]. Some studies have indicated that the HMW species of acetylated Htt may be subject to p62-mediated degradation, and the precise nature of the interaction between p62 and acetylated Htt has also been elucidated, suggesting a link between protein acetylation and targeted degradation by autophagy.

Since acetylation of non-histone proteins has already emerged as an important mechanism underlying the regulation of cellular functions^[60], it will be of interest to further examine whether acetylation represents a more general regulatory mechanism for selective targeting of proteins for lysosomal degradation.

4 Therapeutic points

At present, HD is an incurable polyQ-expanded neurodegenerative disease, and the underlying mechanism still remains obscure. Since HD is caused by neuronal accumulation of mutant Htt, improving the clearance of mutant Htt has been expected to prevent cellular dysfunction and neurodegeneration in HD^[13]. Besides, understanding the role of Htt modification in the pathogenesis of HD will be impor-

Fig. 1 Post-translational modifications of Htt play important roles in the pathogenesis of HD. A: Htt can be SUMOylated. SUMOylation of Htt decreases the formation of Htt aggregates, masks a cytoplasmic retention signal of Htt, and promotes nuclear repression of transcription. B: Htt can be phosphorylated by protein kinases Akt and SGK, and this phosphorylation has been shown to be protective against the toxicity of polyQ-expanded Htt *in vitro* and *in vivo*. Another phosphorylation pathway is mediated by CDK5. CDK5-mediated Htt phosphorylation reduces mutant Htt cleavage, aggregation, and cell death. C: Htt can be palmitoylated by palmitoyl transferase HIP14. Overexpression of HIP14 reduces the formation of Htt inclusion, while downregulation of HIP14 expression increases the formation of Htt inclusions. D: Htt can be acetylated by CBP and then exported to the cytoplasm for autophagic degradation.

tant for early intervention of HD.

It is possible that the neuronal level of SUMOylated Htt will be decreased by inhibiting the expression of the SUMO-1 precursor or the activity of SUMO-1 ligases, or by increasing isopeptidase activity to remove SUMO-1, thus suppressing HD pathogenesis. The E3 ligase specific for the attachment of SUMO-1 to Htt may be a particularly attractive therapeutic target. Moreover, Akt mediates the phosphorylation of Htt at serine 421 and inhibits the toxicity of polyQ-expanded Htt in cell cultures, suggesting that phosphorylation-enhancing compounds are of therapeutic interest. Furthermore, the increase in the expression of HIP14, which is accompanied by an increase in palmitoylation of mutant Htt, partially restores the trafficking and distribution of Htt. Once the enzymes involved in the depalmitoylation of Htt are identified, inhibiting the function of these enzymes could result in an increase in palmitoylation of mutant Htt. Potentially, this could restore normal trafficking and alleviate cellular defects induced by polyQ expansion in Htt. Since the identified inhibitors of CBP and HDAC are not sufficiently specific as therapeutic agents, developing more selective compounds to specifically promote acetylation and clearance of mutant Htt is needed^[13]. Acetylation of mutant Htt at K444 promotes the clearance of mutant protein via an autophagic-lysosomal pathway. In other words, autophagy has emerged as an important cellular defense mechanism in HD and acts as a potential therapeutic target^[61-63].

5 Conclusion

Post-translational modifications have been recognized to be remarkably versatile regulators of the functions of Htt protein. Fig.1 is a schematic illustration of post-translational modifications of Htt. However, many fundamental questions concerning the biological functions of post-translational modifications and the underlying mechanisms still need to be solved. Post-translational modifications and a variety of biological processes can interact with each other. For instance, Htt phosphorylation by CDK5 at serine 434 reduces caspase-mediated cleavage at aspartate 513^[30]. In addition, phosphorylation of Htt at serine 536, a residue located in the proteolytic susceptibility domain, prevents the cleavage of Htt

by calpain and reduces the toxicity of polyQ protein^[64]. Moreover, phosphorylation of the polyQ proteins can influence SUMOylation and ubiquitination, while SUMOylation and ubiquitination can compete for the same lysine residues in Htt^[1, 65]. As Htt modification may play a significant role in HD pathogenesis, dissecting key regulators of Htt post-translational modification may offer novel strategies for HD treatment.

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亨廷顿蛋白的翻译后修饰在亨廷顿病发病机制中的作用

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摘要: 翻译后修饰能快速、有效且可逆地调节蛋白的稳定性、分布位置、功能及其与其它分子之间的相互作用。翻译后修饰主要包括氨基酸残基的 SUMO 化、磷酸化、棕榈化以及乙酰化等。这些复杂的生化修饰能严格、规范地调节各种细胞过程。亨廷顿蛋白(Htt)的几种翻译后修饰方式已见报道。这些翻译后修饰会影响 Htt 的代谢、Htt 与其它蛋白质的相互作用以及 Htt 的细胞毒性。突变 Htt 的剪切、清除以及与其它细胞蛋白质的相互作用是导致亨廷顿病的重要生化事件。因此, 了解 Htt 修饰的信号转导及其意义, 可以帮助我们更好地理解野生型 Htt 的正常功能和突变 Htt 的致病机制。

关键词: 亨廷顿病; 亨廷顿蛋白; 修饰; SUMO 化; 磷酸化; 棕榈酰化; 乙酰化