Post-translational modification by SUMO

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\textbf{Abstract}

Post-translational modifications (PTMs) are chemical alterations to a protein following translation, regulating stability and function. Reversible phosphorylation is an example of an important and well studied PTM involved in a number of cellular processes. SUMOylation is another PTM known to modify a large number of proteins and plays a role in various cellular processes including: cell cycle regulation, gene transcription, differentiation and cellular localisation. Therefore, understanding the role of SUMOylation in cell biology may allow the development of more efficient models, important in streamlining the drug discovery process. This review will focus on protein SUMOylation and its role in stem cell and somatic cell biology.

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\section*{1. Post-translational modifications}

Post-translational modifications (PTMs) involve the addition of a chemical group following protein translation (Walsh et al., 2005). PTMs are essential for a variety of cellular processes and provide another level of protein regulation, which is usually reversible. There are a large number of PTMs that take place in the cell such as phosphorylation (Burnett and Kennedy, 1954), methylation (Grewal and Rice, 2004), acetylation (Glozak et al., 2005) and glycosylation (Spiro, 2002); regulating various biological activities such as transcriptional regulation (Waby et al., 2008) and protein degradation (Orford et al., 1997).

\section*{2. SUMO—small ubiquitin like modifiers}

SUMOylation, another type of PTM, has a diverse range of effects within the living cell (Johnson, 2004). SUMO proteins are highly conserved in a large number of species and have been shown to be...
important in many eukaryotic cell processes (Hayashi et al., 2002) including: cell cycle regulation, transcription, cellular localisation, degradation and chromatin organisation (Müller et al., 2001; Seeler and Dejean, 2003; Verger et al., 2003). Despite the name, SUMO only shares ∼18% homology with ubiquitin (Müller et al., 2001) and is approximately 11 kDa in size, comparable to the 8 kDa ubiquitin molecule (Müller et al., 2001). SUMO has been found to bind to the lysine residue on the following consensus sequence: KxE (where ψ corresponds to a large hydrophobic amino acid, K is a lysine residue, x is any amino acid and E is a glutamic acid residue) on the target protein. Three homologues exist in mammals, SUMO-1, -2 and -3. SUMO-2 and -3 share 95% homology with each other, but only share 50% identity with SUMO-1 (Johnson, 2004). SUMO-2 and -3 have the ability to form polySUMO chains, covalently binding to themselves via the lysine residue at the N terminus consensus motif KxE. SUMO-1 lacks this consensus site and as a consequence is unable to form polychains (Kroetz, 2005) and acts as a polySUMO chain terminator (Ulrich, 2009).

3. SUMO conjugation

3.1. Pathway overview

The SUMO conjugation pathway has a lot in common with the ubiquitination pathway. Both processes involve the use of three enzymes: E1: activating enzyme, E2: conjugating enzyme and E3: ligase (Fig. 1) (Takahashi et al., 2001). SUMO is bound to its target protein via an isopeptide bond formed between an ϵ-amino group on the lysine residue on the target protein and the C terminal carboxyl group on the SUMO protein (Desterro et al., 1997). The pro-form of SUMO needs to be cleaved prior to protein conjugation. This is carried out by isopeptidases, also known as the SENP SUMO deconjugating enzymes (Mukhopadhyay and Dasso, 2007). The SUMO activating enzyme (E1), SAE1/2, commences the reaction process by interacting with SUMO (activated by SENP enzymes—Fig. 1), to form a high energy thiolester bond. The SUMO conjugating enzyme (E2) then binds SUMO via its cysteine residue in its active site. This intermediate provides a highly reactive species, important in the final conjugation, usually facilitated by an E3 ligase (Kroetz, 2005). SUMO E3 ligases act to either activate Ubc9 or bring Ubc9 and the target protein within close proximity of each other, thus enhancing SUMOylation (Ulrich, 2009). They can be regarded as E3 enzymes as they are able to bind to the E2 and the substrate and facilitate the formation of the bond formed between SUMO and the target protein. It has also been shown that a large number of proteins (∼40%) can be SUMOylated without the presence of the consensus sequence (ψKxE), demonstrating differences in substrate specificity (Ulrich, 2009).

3.2. The enzymes involved

3.2.1. E1

Unlike the ubiquitin (Ub) E1, the SUMO E1 exists as a heterodimer; with each monomer corresponding to a particular region of the Ub E1. The SAE subunit Aos1 (SAE1) shares similarity with the N terminus of the Ub E1, whilst Uba2 (SAE2), the second component of the SAE complex, is similar to the C terminus of the Ub E1 (Johnson et al., 1997). The monomers are never found individually and hence it is assumed that they are unable to function independently (Azuma et al., 2001). The SAE complex is responsible for preparing SUMO for transfer to the SUMO conjugating enzyme, Ubc9 (Walden et al., 2003).

3.2.2. E2

Ubc9 is the only known SUMO conjugating enzyme, unlike the ubiquitination pathway where each E2 has a specific set of target proteins (Hayashi et al., 2002). Ubc9 contains an active site with a cysteine residue which is responsible for binding the SUMO molecule directly to the ψKxE sequence found on the target protein (Sternsdorf et al., 1999).

3.2.3. E3

In contrast to SUMO E2s, a larger number of SUMO E3 liga- ses have been discovered and have been categorized into three types: the protein inhibitor of activated STAT—signal transducer and activator of transcription (PIAS) family (Hochstrasser, 2001), the nuclear pore proteins Ran binding protein 2 and nucleoporin 358/RanBP2/Nup358 (Pichler et al., 2002) and the polycytoplasm group protein Pc2 (Kagey et al., 2003). E3 ligases are usually substrate specific with little redundancy found within the system.
has been found that the different PIAS proteins SUMOylate distinct proteins (Rytinki et al., 2009). It has previously been stated that SUMOylation of Oct4 is a POU transcription factor associated with the undifferentiated state (Cai et al., 2006). It was recently shown by Hoof et al. (2009; Hietakangas et al., 2009) that SUMO modification affects its transcriptional activity (Hoof et al., 2009; Hietakangas et al., 2009). It has been suggested that SUMO modification of SOX2 affects its transcriptional activity (Hoof et al., 2009; Hietakangas et al., 2009). It has been suggested that SUMOylation of heat shock factor 1 (HSF1). After its translation, HSF1 is phosphorylated prior to its SUMOylation, which enhances its DNA binding ability (Hong et al., 2001). It is also widely recognised that SUMO alters protein activity by modulating other PTMs, such as phosphorylation and ubiquitination. For example, SUMOylation of bKcO, an important factor in the inflammatory response, prevents its ubiquitination, and therefore inhibits its degradation and subsequent NF-kB activation and nuclear translocation (Desterro et al., 1998). SUMO can also regulate protein activity by modulating its interactions with other macromolecules or proteins. Various models have been proposed such as the addition of SUMO by altering protein configuration, creating a new interaction motif affecting its function (Johnson, 2004). An interesting example of interaction motifs is arsenic induced RNF4 mediated degradation of promyelocytic leukemia (PML) bodies. In the presence of arsenic, PML is polySUMOylated, and following the recruitment of RNF4, an E3 Ub ligase, PML is ubiquitinated and degraded (Tatham et al., 2008).

5. SUMO modification plays an important role in development and cell biology

Various studies have shown that disruption of the SUMO pathway causes abnormal cellular differentiation. Moreover, disruption of the SUMO pathway during embryogenesis may lead to embryonic lethality (Nacerddine et al., 2005; Nowak and Hammerschmidt, 2006), demonstrating the requirement for SUMOylation during development. Due to the lethal nature of Ubc9 knock outs during development, other experimental strategies are necessary to determine the precise role of SUMOylation. In vitro, there has been a focus on the role of SUMOylation in a number of cell types, human embryonic stem cells (hESCs) and representatives of all three germ layers. These models, although not in vivo, provide a good developmental surrogate.

5.1. Human embryonic stem cells

Human embryonic stem cells (hESCs) are isolated from the inner cell mass of blastocyst stage embryos and are self-renewing cells capable of forming cell types from the three germ layers: mesoderm, endoderm and ectoderm (Fletcher et al., 2008). The ability to culture hESCs under standardized conditions and differentiate these cells into a variety of cell types using highly efficient and reproducible protocols may provide an inexhaustible resource for clinical and industrial application (Hannoun et al., 2010a,b; Hay et al., 2008). SUMOylation plays an important role in both hESC self-renewal and pluripotency (Wei et al., 2007). Oct4 is a POU transcription factor associated with the undifferentiated and pluripotent status of embryonic stem cells (Hay et al., 2004; Hardeland et al., 2002; Nichols et al., 1998). It is known to be SUMO modified, which results in its increased stability, DNA binding and transcriptional activity (Wei et al., 2007) (Fig. 3). Sex determining region Y box 2 (SOX2) is another important transcription factor required for embryonic stem cell self-renewal in an undifferentiated state (Cai et al., 2006). It was recently shown by Hoof and colleagues that SOX2 is SUMO modified as a result of phosphorylation. It has been suggested that SUMOylation of SOX2 affects its transcriptional activity (Hoof et al., 2009; Hietakangas et al., 2009).
Fig. 3. The effect of SUMO modification in various cell types. SUMO modification affects a number of cellular processes. (A) In human embryonic stem cells (hESCs), SUMO binds to Oct4 in the nucleus (blue), enhancing its stability and transcriptional activity which is an important regulatory mechanism in hESC self-renewal and pluripotency. SUMO modification also plays an important role in somatic cell biology. (B) In cardiomyocytes, SUMOylation regulates the properties of the Kv1.5 potassium voltage channel located at the plasma membrane. These channels play an essential role in cardiomyocyte membrane potential. The inhibition of SUMO modification to the Kv1.5 channel results in the opening of membrane channels, exporting potassium ions, which results in cellular hyperpolarisation. (C) SUMO conjugation in hepatocytes regulates the transcriptional activity of C/EBP impacting on albumin (ALB) expression within the nucleus (blue). SUMO modification of C/EBP inhibits its ability to form a complex with BRG1/SW1/SNF essential for high level albumin expression. SW1/SNF is a chromatin remodelling complex and BRG is a core subunit of the complex. (D) SUMOylation has also been shown to regulate the activity of the GluR6 receptor in neural cells by endocytosis. Kinate induced receptor internalisation on the cell plasma membrane is dependent on SUMO modification of GluR6, thus affecting neurone excitability.

al., 2006) but further investigation is required. The role of SUMOylation has also been determined in cell types representative of the three germ layers: endoderm, mesoderm and ectoderm.

5.2. Endoderm

The endoderm layer is formed during embryogenesis and is the precursor of liver, pancreas and lung amongst others (Tam et al., 2003). SUMOylation plays an important role in hepatocyte biology regulating C/EBPα, a crucial factor in hepatic differentiation (Pedersen et al., 2001; Sato et al., 2006). SUMOylation of C/EBPα prevents its association with BRG1, a core subunit in the SW1/SNF chromatin remodelling unit, leading to the inhibition of albumin expression (Sato et al., 2006) (Fig. 3). In line with this, it has been shown that there is a decrease in levels of SUMOylation as rat hepatocytes mature (Sato et al., 2006), suggesting an inhibitory effect of SUMOylation in hepatocyte terminal differentiation. The mitochondria are an essential component of hepatocytes, the main cell type in the liver, and are required for efficient liver function. Mitochondrial levels in the cell are dynamic and continuously undergo fusion and fission (Twig et al., 2008; Frazier et al., 2006). It has been shown that an increase in SUMO-1 expression results in an increase in mitochondrial fragmentation by stabilising the GTPase dynamin-related protein 1 (DRP1) (Harder et al., 2004). Further investigation of this pathway has revealed that SENP5, a SUMO deconjugating enzyme, is required for normal mitochondrial morphology and levels of reactive oxidative species within the cell, partly by SUMO deconjugation of DRP1 (Zunino et al., 2007). In the pancreas, SUMO modification of islet cell autoantigen 512 (ICA512) has been shown to disrupt its binding to STAT5.
and inhibit insulin and granule related gene transcription (Mziaut et al., 2006).

5.3. Mesoderm

During development the mesoderm differentiates into muscle, cartilage, bone, blood and connective tissue (Biggers and Borland, 1976). The heart significantly relies on the coordination of various ion channels for regular function. One such voltage gated channel is the potassium channel Kv1.5 found in atrial myocytes which modulate membrane potential of smooth muscle cells (Lagrutta et al., 2006). Benson and colleagues have shown that Kv1.5 has two conserved consensus SUMOylation motifs, which play an important role in hyperpolarisation (efflux of potassium ions) (Benson et al., 2007) (Fig. 3). At the initial stages of development, the polycym 2 protein (PC2), part of the polycym repressor complex 1 (PRC1), is SUMOylated. This allows efficient complex formation and its recruitment to methylated histone 3 for controlled gene silencing. On mesoderm formation, SENP2 is recruited to PRC1, deSUMOylates the PC2 protein and allows the expression of GATA4 and 6 transcription factors essential for normal cardiac formation (Kang et al., 2010). Interestingly, in adult cardiomyocytes, SUMO modification of GATA4 results in increased transcriptional activity, and promotes cardiogenic gene activity (Wang et al., 2004).

5.4. Ectoderm

Ectodermal differentiation results in the formation of the skin and nervous system (Pelton et al., 1998). SUMOylation also has a vital role in the nervous system. GluR6 is a highly expressed kainate receptor found in the brain, and is concentrated in the hippocampus (Nasu-Nishimura et al., 2010). The receptor is known to regulate neuronal excitability and as such is involved in learning, memory and synaptic plasticity (Barberis et al., 2008). It has been shown that the internalisation of the receptor upon kainate stimulation is regulated by SUMOylation (Fig. 3). GluR6 is internalised via kainate or N-methyl-D-aspartate (NMDA) induced endocytosis. Only kainate induced internalisation requires GluR6 SUMOylation. The mutation of the SUMO consensus motif in GluR6 results in a large reduction in kainate induced GluR6 internalisation and disrupts regular synaptic function (Martin et al., 2007). Another factor important for both brain development and neuronal differentiation is MEZFA. MEZFA and its associated family members have been shown to be involved in the proliferation, differentiation and apoptosis of cells found in the developing brain (McKinsey et al., 2002). SUMOylation of MEZFA decreases its transcriptional activity, suppressing Nurr77 function; and promotes dendritic claw differentiation (Shalizi et al., 2006).

6. Conclusion

SUMOylation is an important PTM known to play roles embryonic stem cell and somatic cell biology. Given its importance in cell biology, it is critical that we understand SUMOylation in order to generate stable and high fidelity models that predict human drug toxicity. These models will not only be useful tools for toxicology, but will also provide a system whereby we can investigate the role(s) of SUMO modification in response to numerous stimuli. This will undoubtedly provide information on novel mechanisms of action with the possibility of developing new medicines and clinical intervention strategies.

Conflict of interest statement

The authors report no conflict of interest.

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