Sumoylation in Development and Differentiation

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Abstract

Tissue morphogenesis is a fascinating aspect of both developmental biology and regeneration of certain adult organs, and timely control of cellular differentiation is a key to these processes. During development, events interrupting cellular differentiation and leading to organ failure are embryonic lethal; likewise, perturbation of differentiation in regenerating tissues leads to dysfunction and disease. At the molecular level, cellular differentiation is orchestrated by a well-coordinated cascade of transcription factors (TFs) and chromatin remodeling complexes that drive gene expression. Altering the localization, stability, or activity of these regulatory elements can affect the sequential organization of the gene expression program and result in failed or abnormal tissue development. An accumulating body of evidence shows that the sumoylation system is a critical modulator of these regulatory cascades. For example, inhibition of the sumoylation system during embryogenesis causes lethality and/or severe abnormalities from invertebrates to mammals. Mechanistically, it is now known that many of the TFs and components of chromatin remodeling complexes that are critical for development and differentiation are targets for SUMO modification, though the specific functional consequences of the modifications remain uncharacterized in many cases. This chapter will address several of the models systems that have been examined for the role of sumoylation in differentiation and development. Understanding the profound regulatory role of SUMO in different tissues should lead not only to a better understanding of developmental biology, stem cell linage control,

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and the mechanisms of cellular differentiation, but may also lead to the identification of new targets for drug therapy and/or therapeutic manipulation of damaged organs and tissues.

Keywords
Ubc9 • SENP • Keratinocytes • Gonads • Germ cells • Hematopoietic cells • Neural cells • Stem cells

12.1 Introduction

Embryonic development and post-embryonic differentiation are complex processes that depend on exquisitely coordinated networks of gene expression. Orchestrating this network relies on diverse regulatory mechanisms that control the expression, localization, and activity of the pertinent transcription factors (TFs), co-regulators, and chromatin modifying complexes that collectively determine global patterns of transcription. Among these mechanisms is post-translational modification of the TFs and their co-factors. Over the last 20 years, sumoylation has emerged as a significant functional modifier of TFs, their co-activators and co-repressors, and components of the chromatin remodeling machinery (see Chaps. 2, 3, and 5). This broad target range is consistent with reports that sumoylation has a global impact protein networks, at least under certain conditions (Heaton et al. 2012; Xiao et al. 2015; Hendriks et al. 2014, 2015). There is also growing evidence that sumoylation can provide fine tuning to these networks by altering and coordinating activities of network components, and several example pertaining to development and differentiation are presented in the following sections, as well as in more detail in Chaps. 14, 15, and 19.

Initially, the importance of sumoylation in development and differentiation was underscored by studies using knockouts or knockdowns of the sumoylation system in various model organisms. Many studies have focused on Ubc9 as loss of this sole SUMO conjugating enzyme totally abrogates sumoylation. In *C. elegans*, RNAi knockdown of Ubc9 results in severe developmental defects and embryonic arrest after gastrulation (Jones et al. 2001). Likewise, deletion of smo-1, the sole SUMO encoding gene in the *elegans* genome, results in sterile adults with severe defects in the reproductive system (Broday et al. 2004). Interestingly, overexpression of SUMO also perturbed the reproductive system, suggesting that precise levels of sumoylation are critical for normal development (Rytinki et al. 2011). Developmental defects are also observed for Ubc9 mutation in zebrafish (Nowak and Hammerschmidt 2006). In *Drosophila*, dysregulation of sumoylation by targeting Ubc9 (Huang et al. 2005), SUMO (Nie et al. 2009; Kanakousaki and Gibson 2012), the E1 activating enzyme (Kanakousaki and Gibson 2012), or an E3 SUMO ligase (Betz et al. 2001) all yielded developmental defects [reviewed in (Smith et al. 2012)]. Both SUMO (Yukita et al. 2007) and SUMO proteases (Wang et al. 2009) have been shown to be essential for normal *Xenopus* development. Many of these sumoylation effects are even more pronounced during mouse development where loss of Ubc9 function leads to apoptosis and early embryonic lethality (Nacerddine et al. 2005). Embryonic lethality in mice was also seen for PIAS1 knockouts (Constanzo et al. 2016), SENP1 mutants (Sharma et al. 2013), and SENP2 knockouts (Kang et al. 2010). This critical role for sumoylation in development is not confined to animals, and is likewise observed for plants (see Chap. 14). Thus, the combined literature supports a critical role for sumoylation in development, though the individual targets and mechanisms appear to vary highly between species. The following sections will discuss the role of sumoylation in several diverse systems.

12.2 The Reproductive System

As cited in the previous section, reproductive tract defects were one of the first developmental abnormalities associated with defective sumoylation. Subsequent publications have reported abundant expression of SUMO and SUMO proteins in male and female germ cells of several species. High concentrations of sumoylation components were detected in testis and sperm cells in worms, mice, rats, and humans, emphasizing a conserved mechanism across species in the development of the male reproductive organs (Broday et al. 2004; Kim et al. 2000; Vigodner et al. 2006; Vigodner and Morris 2005; Brown et al. 2008; Santti et al. 2003; La Salle et al. 2008; Metzler-Guillemain et al. 2008; Yan et al. 2003). Likewise, SUMO proteins has also been reported in female germ cell oocytes (Li et al. 2006), and sumoylation appears to be critical for oocyte maturation (Wang et al. 2010; Yuan et al. 2014), though the contribution of sumoylation to the male reproductive system remains better characterized (Vigodner 2011). Together these observations underscore the importance of sumoylation for sex organ development and gamete maturation is a wide variety of species as described in more detail below.

12.2.1 Vuval Morphogenesis

To investigate the role of SUMO (SMO-1) in *C. elegans* development, a deletion mutant was constructed and analyzed (Broday et al. 2004). While earlier *smo-1* RNAi studies showed 100% embryonic lethality (Fraser et al. 2000; Jones et al. 2002), the *smo-1* −/− mutants survived, but were sterile. This mutation is associated with physiological disturbance of vulval uterine connection in *C. elegans* and also with somatic gonad and germ line abnormal differentiation. These results resembled the phenotype seen with mutations in LIN-11, a transcription factor which demonstrates important regulatory properties for vulval precursor cell division as well as uterine morphogenesis (Newman et al. 1999). Broday et al. showed that LIN-11 can be SUMO modified at lysines 17 and 18, and that the double mutant form of LIN-11 could partially rescue vulva formation in a LIN mutant background, but was still significantly impaired for uterine seam cell (utse) formation. They additionally showed that expression of a SUMO-LIN11 fusion in the *smo-1* mutant *C. elegans* background rescues π-cell differentiation, but accentuates impairment of late stages in vulval development. These combined results support the conclusion that sumoylation is critical for normal vulval development and that LIN-11 is an important sumoylation target in this system. Subsequent work showed that sumoylation of LIN-1 promoted transcriptional repression and interaction with MEP-1, a component of the NuRD transcriptional repressive complex which may be an important pathway in controlling vulval development (Leight et al. 2005). Nevertheless, it is likely that LIN-11 is not the only factor whose sumoylation state plays an essential role for gonadal uterine-vulval morphogenesis in worms. For example, recent work showed that the nuclear hormone receptor, NHR-25, is sumoylated and that this modification is critical for normal vulval formation (Ward et al. 2013). While these results underscore the impact of sumoylation on the formation of the *elegans* reproductive system, similar observations in vertebrates are lacking. In contrast, a large literature supports a role for sumoylation in vertebrate gametogenesis.

12.2.2 Sperm Differentiation

Spermatogenesis is a process of the reproductive system by which male germ cells enter into meiosis, divide, and differentiate into mature spermatozoa. During meiosis homologous chromosome pairs, including the heterochromosome XY, are distributed equally to the daughter cells. The timing, sub-cellular recruitment, and assembly of chromatin remodeling proteins are crucial for proper synapsis and chromosomal recombination during spermatogenesis. Initial reports indicated the presence of SUMO1 in mouse, rat, and human

spermatids (Rogers et al. 2004; Vigodner and Morris 2005; Vigodner et al. 2006), suggesting an active role for the sumoylation process.

Two groups described the dynamic expression pattern of SUMO during SC formation in the mouse and human models, respectively. La Salle et al. (La Salle et al. 2008) examined the sumoylation genes and proteins during prophase meiosis I in male mouse germ cells. They compared localization and expression levels of SUMO1 versus SUMO2/3 by immunolabeling on surface spread chromatin and RT-PCR, respectively. SUMO1 clearly localized to the XY body and the chromocenter of pachytene spermatocytes, as did SUMO2/3. However, as prophase progressed, SUMO1 and SUMO2/3 presence decreased from both the XY body and the chromocenter. Interestingly, while the presence of SUMO1 was completely absent in metaphase I, SUMO2/3 could still be detected in centromeres, suggesting a functional distinction for these 2 SUMO types. To further characterize the implication of the sumoylation in the division and development of male sperms, they determined that the only known SUMO conjugation enzyme (UBE2I) showed overlapping localization with SUMO1 and SUMO2/3 in prophase and with SUMO2/3 in metaphase. This strongly suggests that conjugation of proteins by SUMO is taking place at these locations, and thus infers that sumoylation plays a functional role in sperm maturation.

To further characterize the dynamics of this system, La Salle et al. looked at the relative transcription levels of 19 sumoylation system genes using quantitative RT-PCR. *Sumo1* transcript levels peaked in early prophase (Zygotene) and then quickly decreased as prophase progressed. *Sumo2/3*, *Sae1/2*, and *Ube2i* showed a similar pattern though with a peak expression at the adult pachytene spermatocyte stage and decreased expression in mature spermatids. The SUMO proteases showed a more varied pattern of expression with *Senp1*, *2*, and *6* having patterns similar to *Sae1/2* and *Ube2i*, while *Senp5* was similar to *Sumo1*. *Senp3* and *7* were distinct in that they had highest expression at the leptotene/zygotene stage followed by declining expression with subsequent sperm maturation, a pattern shared by the *Pias3* SUMO ligase. In contrast to *Pias3*, the other ligases (*Pias1*, *2*, and *4*) had low expression at the leptotene/zygotene stage with a dramatic increase in expression in spermatids. Unlike the changing expression patterns of the sumoylation system genes, expression of *Senp8*, a NEDD8 protease, was relatively constant throughout spermatogenesis, suggesting that changes in the sumoylation gene expressions is biologically significant. Overall, two main patterns emerged from RNA and protein expression studies: high expression during meiosis follow by low expression post-meiosis for SUMO1/2/3, SAE1/2, UBE2i, and SENP1/2/5/6; low expression during meiosis and high expression post-meiosis for PIAS 1, 2, and 4. These differential expression patterns clearly indicate a dynamic process during spermatogenesis and are consistent with a requirement for the sumoylation during this process. It is not yet clear if specific proteins modified by SUMO and it enzymes regulate chromosome dynamics during meiosis in male germ cells, although the co-localization and common expression patterns of SUMO and its enzymes does suggest that protein modification takes place on the chromatin. Consistent with this possibility, studies in yeast indicate that sumoylation of TOP2 is most important for proper chromosome segregation in mitosis, suggesting that sumoylation is important in maintaining proper cell division (Bachant et al. 2002) (Azuma et al. 2003, 2005).

In contrast to mouse, human spermatocytes show a much different distribution of SUMO1 (Metzler-Guillemain et al. 2008). Human pachytene spermatocytes showed the presence of SUMO1 in constitutive heterochromatin, but lacked SUMO1 on the XY body, underscoring a different regulatory regime associated with human compared to mouse spermatogenesis; why these XY results differ from those of Vigodner et al. is not yet clear (Vigodner et al. 2006). Additionally and consistent with mouse studies, there was no SUMO1 detected on synaptonemal complex (SC) structures, so unlike yeast there may be no role for SUMOs in mammalian SC assembly. In addition to SUMO1

localization to the constitutive heterochromatin, Guillemain et al. demonstrated that increased SUMO1 staining correlates with decreased histone H4-K20me3 staining. This result suggests a competition between sumoylation and methylation at lysine 20 and raises the possibility that sumoylation of lysine 20 is an important epigenetic mark for constitutive heterochromatin in human spermatocytes.

In summary, numerous bioimaging studies have localized SUMO1 versus SUMO2/3 throughout spermatogenesis and have shown differences in their distributions that suggests different functional roles [reviewed in (Vigodner 2011; Rodriguez and Pangas 2016)]. While some discrepancies exist in the literature, in general SUMO1 is found in association with the sex chromosomes of meiotic spermatocytes and with centrosome in spermatids (Brown et al. 2008; Vigodner and Morris 2005). All three SUMO are also found co-localized with XY bodies in spermatocytes (La Salle et al. 2008; Vigodner and Morris 2005) Interestingly, SUMO has been found at double-strand DNA break sites, indicating a possible role in meiotic recombination (Shrivastava et al. 2010; Vigodner 2009). While the numerous localization studies are supportive of a biological role for sumoylation in spermatogenesis, these studies are mostly observational, and functional evidence has been limited by the constraints of this cell system, including the paucity of identified SUMO targets in sperm cells. Three more recent reports are beginning to provide evidence for a functional role. First, it was reported that defective spermatozoa have excessive sumoylation in the tail and neck regions compared to normal sperm, a result that hints strongly towards a requirement for finely balanced sumoylation in normal sperm development (Vigodner et al. 2013). Second, cigarette smoke extract exposure which is known to cause oxidative stress in sperm results in desumoylation of many sperm proteins which may be at least partially responsible for the reduce sperm function (Shrivastava et al. 2014). Third, a large scale isolation and identification of sumoylated proteins from spermatocytes and spermatids revealed 120 substrates, including many with unique roles in spermatogenesis (Xiao et al. 2016). Having specific substrates proteins to evaluate for sumoylation effects should greatly accelerate the understanding of mechanisms and pathways through which SUMO modification affect the spermatogenesis process.

12.2.3 Oocyte Maturation

The role of sumoylation during oocyte formation is less studied than for spermatogenesis, but a few studies have examined the SUMO pathway and its components (Ihara et al. 2008; Wang et al. 2010; Yuan et al. 2014). SUMOs 1–3 can be detected throughout oocyte maturation and there is agreement that SUMO 2/3 localizes to the nucleoplasm (Ihara et al. 2008; Yuan et al. 2014). Likewise, Ubc9 is mostly found in the nucleoplasm (Ihara et al. 2008). In contrast, the location of SUMO1 has been reported to be either nuclear membrane associated (Ihara et al. 2008) or in germinal vesicle in meiotically competent oocytes (Yuan et al. 2014). During oocyte maturation there are differences in the localization of SUMO1 versus SUMO2/3 suggestive of different substrates and functional differences in their roles.

To begin to address the biologic function of sumoylation in oocytes, individual components of the sumoylation system have been overexpressed or inhibited. Overexpression of the SUMO protease, SENP2, led to defects in spindle organization, consistent with an important role of sumoylation (Wang et al. 2010). Consistent with the SENP2 result, blocking SUMO1 with antibodies or reducing Ubc9 levels with siRNA also led to spindle disruption and altered subcellular localization of gamma-tubulin, a known spindle organization protein (Yuan et al. 2014). Surprisingly, SUMO1 overexpression had no discernible effect. Similar studies with SUMO2/3 are not available so little is known about its mechanistic role in oocytes. Identification of specific SUMO substrates in these cells, such as was recently done for sperm cells, would greatly facilitate further functional characterization.

12.3 Embryonic Development

As discussed in Sect. 12.1, a general requirement for sumoylation during embryonic development is common to many organisms as loss of Ubc9, the sole SUMO conjugating enzyme, is typically lethal. In zebrafish, deficiency in any of the three SUMO paralogs is well tolerated during development, but loss of all three is led to severe defects, consistent with a requirement for sumoylation though with considerable redundancy between the paralogs (Yuan et al. 2010). Interestingly, even though all three SUMO paralogs are widely expressed throughout embryonic development in the mouse model, the essential SUMO paralog appears to be SUMO2 (Wang et al. 2014). It was initially reported that knocking out SUMO1 was embryonic (Alkuraya et al. 2006), but subsequent studies failed to find a phenotype for SUMO1 null mice suggesting that SUMO2/3 compensated for the loss of SUMO1 (Zhang et al. 2008). By constructing separate SUMO2(−/−) and SUMO3(−/−) mice, Wang et al. demonstrated that loss of SUMO2 was embryonic lethal while SUMO3(−/−) mice were viable (Wang et al. 2014). Somewhat surprisingly based on the SUMO2 result, SENP1 which specifically desumoylates SUMO1 conjugates was also found to be essential for mouse embryogenesis (Sharma et al. 2013). One possible role that might explain this requirement is removal of SUMO1 from poly-SUMO2/3 chains. Inability to degrade these chains could account for the accumulation of SUMO2/3 product observed. Embryonic lethality was also observed for SENP2 null mice (Kang et al. 2010). In these embryos there was a significant cardiac defect due to accumulation of the sumoylated form of the Pc2/CBX4 subunit of the polycomb repressive complex which led to reduced transcription of two genes essential for cardiac development, Gata4 and Gata6. Similar defects in cardiac development were also seen using a condition SENP2 knockout mouse model (Maruyama et al. 2016). Consistent with this result, the SUMO E3 ligase, PIAS1, is also critical for cardiac development during embryogenesis (Constanzo et al. 2016). Gata4 is sumoylated (Collavin et al. 2004), and PIAS1 co-localizes with Gata4 (Constanzo et al. 2016) suggesting that it may be the SUMO ligase that promotes modification of Gata4 by SUMO. A critical role for sumoylation is also seen in neural crest and muscle development where SUMO modification of the Pax7 transcription factor is essential proper morphogenesis (Luan et al. 2013). Thus, while many questions remain unaddressed, the evidence to date clearly indicates a critical role for sumoylation in the embryonic development of a wide range of organ systems.

12.4 Stem Cells

Stem cells, whether embryonic or adult, are relatively pluripotent cells with the capacity to differentiate into one or more cell types. These cells are critical for normal development and for certain maintenance needs in adult tissues. Additionally, stem cells have enormous potential for therapeutic applications in human disease and injury, so understanding their biology and function is a major focus in the medical sciences today (Sayed et al. 2016; Wang and Zhou 2016). It is becoming clear that sumoylation has significant roles in stem cell propagation and differentiation, and specific examples of SUMO modification in stem cell populations are beginning to be identified. As in the reproductive system, sumoylation in stem cells appears important for regulation of critical TFs that contribute to the differentiation decision switches that control cell fate. Better understanding of the role sumoylation plays in stem cell biology may provide new means for controlling and directing stem cell growth and differentiation.

12.4.1 Embryonic Stem Cells

One of the initial observations connecting sumoylation with embryonic stem cells was the phenotype of the Ubc9 null mice (Nacerddine et al. 2005). While the Ubc9 knockout is embryonic lethal, normal appearing blastocysts can be isolated at E3.5. However, the endogenous pluripotent stems cells in the blastocyst do not

expand and exhibit apoptosis, consistent with a requirement for sumoylation to develop beyond this stage. Further evidence for a role of sumoylation in development has come from studies examining the distribution and expression of sumoylation components during murine brain development. Loriol et al. demonstrated high levels of SUMO1 modified proteins in neuronal nuclei early in development (Loriol et al. 2012). As development progressed there was an overall reduction in sumoylation but an increase at synapses. They also noted developmental-dependent changes in SENP1 and SENP2 levels that may in part account for changing sumoylation levels in various regions of the brain. A subsequent study also observed developmental regulation of SUMO1, SUMO2/3, and Ubc9 in developing mice brains, again with a decrease in total sumoylated proteins as development proceeded (Hasegawa et al. 2014). Strong signal was observed for SUMO1 and SUMO2/3 in neural stem cells with persistence of the SUMO2/3 signal, suggestive of different functional roles for SUMO1 versus SUMO2/3 modification in neural stem cell differentiation. Analysis of SENP2 function in mouse embryos revealed a similar important role for sumoylation in trophoblast development (Chiu et al. 2008). In this system, SENP2 is required for desumoylation of Mdm2, a key regulator of p53 (Jiang et al. 2011). In the absence of SENP2, Mdm2 remains sumoylated and interferes with p53 degradation. The increased levels of p53 cause cellular stress and disrupt the G-S phase transition.

The sumoylation system is also critical for hematopoiesis in zebrafish via hematopoietic stem/progenitor cells (HSPCs). The tango(hkz5) zebrafish mutant is defective for hematopoiesis and this mutation maps to the gene encoding the SAE1 subunit of the SUMO E1 activating enzyme (Li et al. 2012). Embryos with this mutation show drastically reduced numbers of HSPCs, and this phenotype could be reproduced with an Ubc9 knockdown, strongly linking the HSPC decrease to defective sumoylation. Similar effects on hematopoiesis were seen with mopholino-mediated knockdown of either SUMOs or Ubc9 (Yuan et al. 2015). In this study, the CCAAT/enhancer-binding protein α (C/EBPα) was shown to be a critical sumoylation target as a SUMO- C/EBPα fusion could rescue the hematopoietic defect in embryos deficient for SUMO.

While the accumulating literature reveals a potent role for sumoylation during embryonic development, the specific mechanistic pathways in different organ systems are still mostly undefined. One general effect of sumoylation is likely to involve direct modification of the small pool of transcription factors that are critical for regulating embryonic stem (ES) cells. Octamer4 (Oct4) is known to be required for maintenance of stem cell pluripotency and their undifferentiated state; even slight variation in expression levels significantly impacts embryonic cellular differentiation (Niwa et al. 2000). Oct4 is a POU transcription factor which can act as a repressor or activator controlling over 600 genes in the genome. High expression of Oct4 in ES cells leads to differentiation commitment to endoderm or ectoderm. Persistence of high Oct4 expression leads to embryonic carcinoma while down regulation of Oct4 transactivation leads to mesoderm differentiation (Kuijk et al. 2008; Looijenga et al. 2003). Therefore, transient regulation of Oct4 is crucial for cell fate commitment and proper embryonic cell differentiation. Mouse Oct4 has 3 lysines that have a SUMO consensus sequence and can be modified *in vivo* and *in vitro* (Wei et al. 2007). Two of these lysines are conserved in human Oct4, including lysine 118. Lys118 is located near the N-terminal DNA binding domain and is poly-sumo modified *in vitro* and *in vivo*. Surprisingly, Oct4 sumoylation with SUMO1 doesn't decrease its transcriptional activity, but instead increases its stability, its DNA binding, and its transactivation. Although these combined studies support a role for sumoylation in the regulation of Oct4 activity, only suggestive data have been generated on the actual contribution of SUMO-Oct4 towards cell differentiation commitment. Similar to Oct4, other factors critical for stem cell regulation such a KLF4 (Du et al. 2010), Nanog (Wu et al. 2012), and Sox2 (Tsuruzoe et al. 2006) are sumoylated, suggesting that sumoylation may provide a complex coordination of the activity of these factors dur-

ing development. Much further work will be needed to define the precise functional roles of sumoylation in control of ES cell differentiation.

In addition to being SUMO modified, Oct4 can bind to other sumoylated proteins without being itself SUMO modified, and this may also account for changes in its transactivation as well. For instance, Sox2, another stem cell marker, forms a tight complex with Oct4 and regulates its transactivation as well (Rodda et al. 2005). Sox2 is also conserved from mice to humans. Sox2 is sumoylated (Tsuruzoe et al. 2006), but how sumoylation of either Sox2 or Oct4 affects complex formation or function of the complex in ES cell differentiation is unknown. Also of interest is the observation that Oct4 is sumoylated only by SUMO1 and not by SUMO2 (Wei et al. 2007). This observation may imply specific functional regulation through SUMO1 that could be tied to distinct patterns of SUMO1 versus SUMO2/3 expression in developing ES cells.

12.4.2 Post-natal Stem Cells

In addition to its role in embryonic development and ES cell regulation, numerous examples are accumulating that demonstrate an important role for sumoylation in adult stem cells such as bone marrow hematopoietic stem cells. These adult stem cells can split into two lineages: the myeloid and lymphoid lineages. Myeloid progenitor cells can be further divided into sub-classes of blood cells including monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, and dendritic cells. Their differentiation is regulated by lineage specific TFs leading multipotent cells to become specialized blood cells. For instance, expression of the MafB transcription factor in myeloid progenitor cells forces differentiation into macrophages and prevents myeloid lineage differentiation toward erythroid or dendritic cell types (Tillmanns et al. 2007). Conversely, high level of myb expression preserves immature myeloid cell proliferation, controlling timing of the differentiation process (Emambokus et al. 2003). Thus MafB and c-myb, two transcription factors, act as antagonists in the balance of the hematopoietic system. Moreover, induction of myb in macrophages leads to rapid de-differentiation (Beug et al. 1987).

To determine the mechanism driving the antagonist effect between MafB and v-myb, Tillmanns et al. investigated the sumoylation control of those two transcription factors and found that MafB is SUMO modified at two lysine residues *in vivo* and *in vitro* (Tillmanns et al. 2007). Interestingly, preventing MafB sumoylation led to macrophage differentiation and inhibition of myeloid progenitor expansion. Furthermore, the MafB SUMO site mutant could not be repressed by v-myb and committed to macrophage differentiation even with expression of v-myb, suggesting that repression of MafB by v-myb is dependent on the MafB sumoylation state. Likewise, c-myb is itself sumoylated via TRAF7, a SUMO ligase. Sumoylated c-myb is sequestered by TRAF in the cytoplasm, and therefore, negatively regulated by SUMO (Morita et al. 2005). The dual negative effect on both TFs suggests a finely tuned regulation of these competing activities by the sumoylation system. Consequently, the presence or absence of effector proteins such as SUMO ligases may be a key determinant in the balance between cellular differentiation versus proliferation, and the degree of sumoylation may coordinate the antagonist transcription factors to control hematopoietic cell differentiation. Evidence in support of a role for SUMO ligases in hematopoiesis was recently reported (Liu et al. 2014). PIAS1 was shown to control the switch for HSPCs between self-renewal and differentiation through another member of the Gata family, Gata1.

To more generally examine the role of sumoylation in adult mice, Demarque et al. developed an inducible knockout mouse line (Demarque et al. 2011). The major phenotypic effect was observed in the small intestine where the stem cell population was rapidly depleted leading to death within 6 days. At the subcellular level, defects were observed in nuclear positioning and in polarization of actin, with keratin 8 identified as a major SUMO target. The combined molecular defects resulted in diminished proliferative capacity and detachment of enterocytes

from the basal lamina. A critical role for Ubc9, and hence sumoylation, was likewise observed for reprogramming of mouse embryonic fibroblasts into induced pluripotent stem (iPS) cells and for survival of embryonic stem (ES) cells (Tahmasebi et al. 2014). In the absence of Ubc9, iPS induction was greatly impaired and ES cells underwent apoptosis. Interestingly, reduced Ubc9 was correlated with decreases in the protein levels for Nanog, Oct4, KLF4, and Sox2, all critical transcription factors for stem cell differentiation and themselves targets for sumoylation. While these two studies clearly demonstrate the requirement for sumoylation, adult stem cells, like embryonic stem cells, appear to require a delicate balance between sumoylation and desumoylation (Nayak et al. 2014). In human dental follicle stem cells, siRNA knockdown of the SENP3 desumoylating enzyme also prevented differentiation in the osteogenic pathway. A number of critical regulatory factors accumulated in the sumoylated form in the absence of SENP3, suggesting that inability to turn over the SUMO moieties on these substrates impaired the differentiation program in these cells. Thus, the reoccurring theme is that a delicate balance between sumoylation and desumoylation is essential for proper maintenance and/or differentiation of stem cells.

12.5 Tissue and Cellular Differentiation

In addition to its roles in embryonic development, SUMO also plays critical roles in differentiation in adult tissues. Several examples have been clearly identified and characterized in recent years, and while much remains unknown, the contribution of sumoylation to the differentiation process in distinct tissue types is now well established. In some cases, specific TFs serve as the critical sumoylation targets for differentiation, while in other cases the targets are unidentified and the mechanism by which sumoylation contributes to the differentiation process is undefined. The following subsections discuss the currently evaluated systems where sumoylation has a known effect on initiation or completion of differentiation.

12.5.1 Epithelial Tissue

The epidermis has been intensely studied both as a convenient model of tissue differentiation (Gandarillas 2000; Werner and Smola 2001) and for its medical importance in wounds, oncogenesis, congenital and acquired skin dysfunctions, and infections (Angel et al. 2001; Ghoreishi 2000). Human keratinocytes are easily induced to differentiate in culture so that state-specific differences can be explored at the biochemical and molecular level (Poumay and Leclercq-Smekens 1998). Furthermore, the development of organotypic cultures has allowed the recapitulation of nearly authentic epidermal histology and morphology *in vitro* (Benbrook et al. 1995). Nonetheless, regulation of epidermal differentiation is still poorly understood (Koster et al. 2002). Many studies have focused on changes in transcriptional programs that result from differentiation induction signals and have identified a number of TFs relevant to the differentiation process. More recently, Deyrieux et al. studied the role of sumoylation in skin biology using the human HaCaT line as a model system (Deyrieux et al. 2007). In both undifferentiated and differentiated HaCaT cells the sumoylation system was expressed and active with numerous substrates modified. Interestingly, at both the RNA and protein levels, expression of the sumoylation system components was transiently upregulated during the active differentiation process with a peak expression observed as late differentiation markers appeared. Immunohistochemical analysis of HaCaT cells stratified in organotypic cultures revealed that Ubc9 expression increased in the suprabasal cells, just beneath where keratin K1 expression commenced, and then waned in the upper layers, consistent with the transient expression increase seen in differentiating monolayer cultures. When sumoylation was prevented during differentiation the monolayer

HaCaT cells showed delayed and reduced expression of the late differentiation markers and grossly abnormal morphology, suggesting that sumoylation is needed for successful completion of the differentiation program. Global 2-dimensional gel analysis of the SUMO3 substrates during HaCaT differentiation revealed a complex profile (Heaton et al. 2012). The number of SUMO3-modified proteins was highest in basal cells with an abrupt decrease immediately following induction of differentiation followed by a gradual increase at 2–3 days post-induction. However, within this overall trend there was great variability in the level of sumoylation of individual proteins; some increased, some decreased, and some were unchanged. While the specific critical target(s) has not yet been identified, these results strongly support a role for sumoylation in the differentiation of skin, likely through modulatory effects on pertinent TFs.

A role for sumoylation in keratinocyte biology has also been observed through studies of the Cbx4 protein, a component of the polycomb repressive complex 1 (PCR1) (Luis et al. 2011; Mardaryev et al. 2016). Cbx4 has SUMO ligase activity that is important for its regulatory activity (Kagey et al. 2003; Wotton and Merrill 2007). In epidermal stem cells a ligase-minus mutant of Cbx4 stimulated proliferation and increased differentiation, suggesting that sumoylation of one or more targets contributes to restricting growth and keeping these cells in the undifferentiated state (Luis et al. 2011). Consistent with these observations in cultured cells, deletion of Cbx4 in mice results in altered epidermis with enhanced expression of differentiation markers and premature expression of these markers in the suprabasal layers (Mardaryev et al. 2016). Transfection studies with domain-deletion versions of Cbx4 confirmed that these effects on keratinocyte growth and differentiation were dependent on the SUMO-ligase activity. To further understand this pathway it will be critical to identify SUMO substrates for Cbx4.

Like keratinocytes, differentiation of ocular lens epithelial cells also requires sumoylation, with SUMO1 and SUMO2/3 exhibiting distinct functions (Gong et al. 2014). Differentiation in this system can be triggered by treatment with basic fibroblast growth factor (bFGF), and overexpression of SUMO2/3 inhibits this bFGF-induced differentiation while overexpression of SUMO1 has no effect. Conversely, knockdown of SUMO2/3 did not affect differentiation while knockdown of SUMO1 again inhibited the bFGF-induced differentiation. These results suggest that SUMO1 expression is required for differentiation and the SUMO2/3 is inhibiting this process. Mechanistically, the transcription factor Sp1 is known to be a major regulator of lens-specific gene transcription, and Sp1 was shown to be differentially regulated by the SUMO paralogs. Sp1 was activated by SUMO1 while it was repressed by SUMO2 conjugation at K683. Addition of the SUMO2 moiety at K683 reduced both DNA binding capacity of Sp1 and its ability to interact with the coactivator, p300. This antagonistic activity of SUMO1 versus SUMO2 on Sp1 function is consistent with the effects of these two paralogs on lens cell differentiation and suggests that varying levels of the different SUMOS may be a major pathway for regulating differentiation in this cell system.

12.5.2 Myocytes

Like basal keratinocytes, the muscle precursor cells known as myoblasts are proliferative cells that can stop replicating and enter terminal differentiation (Pownall et al. 2002). Upon differentiation the myoblasts start to fuse and form multinucleated myotubes, a process driven by the activity of the MyoD family of TFs in cooperation with the myocyte enhancer factor (MEF2) family (Tapscott 2005). Using the well-established C2C12 myoblast differentiation model, Riquelme et al. examined sumoylation during the differentiation process (Riquelme et al. 2006a). In contrast to keratinocytes, they showed that overall sumoylation of cellular targets declined for both SUMO1 and SUMO2/3 after induction of differentiation. Additionally, Ubc9, which is expressed in both myocytes and myotubes, changes its distribution during differentiation and became more homogenously

distributed throughout the nuclei of myotubes. Ubc9 knockdown with siRNA reduced global sumoylation, but had no effect on MyoD or myogenin expression, localization, or activity, suggesting that the effect of Ubc9 in not mediated directly through the MyoD family. Somewhat surprisingly since overall sumoylation decreases during myocyte differentiation, Ubc9 knockdown inhibited differentiation and resulted in decreased formation of myotubes. Neither apoptosis nor G2/M arrested cells increased under the knockdown conditions, so the mechanism of the Ubc9 effect is unclear, but must reflect subtle differences in target modification during knockdown compared to the sumoylation decrease seen during normal differentiation.

While the sumoylation of the MyoD family is uncertain, sumoylation of other myogenesis regulatory factors is now well documented. SnoN is an oncoprotein that also plays a role in muscle differentiation and was recently shown to be sumoylated at a single lysine residue in C2C12 cells (Wrighton et al. 2007). Mutation of the sumoylation site to arginine imbued SnoN with enhanced myogenic activity and enhanced transcriptional synergy with MyoD. During C2C12 cell differentiation, sumoylation of SnoN decreased slightly, consistent with decreased sumoylation promoting myocyte differentiation and myotube formation. Similarly, several members of the MEF2 family have been shown to be sumoylated, including MEF2A (Riquelme et al. 2006b), MEF2C (Gocke et al. 2005), and MEF2D (Gregoire et al. 2006), and at least for MEF2A (Riquelme et al. 2006b) and MEF2C (Kang et al. 2006) sumoylation is a negative regulator of transcriptional activity. While the role of MEF2 sumoylation in myocyte differentiation remains to be explored, the modification of these important regulatory factors by SUMO is clearly consistent with a functional role for sumoylation in growth and differentiation of this cell type. Furthermore, cross-talk between MEF2 sumoylation and other post-translational modifications such as phosphorylation (Gregoire et al. 2006) and acetylation (Gregoire et al. 2007) suggests exciting and complex regulatory feedback that may be critical for proper response to external stimuli and subsequent control of differentiation.

Several additional studies have begun to identify and characterize other sumoylation targets that are critical for muscle cell development. One of the members of the Pax family of transcriptional regulators, Pax7 is sumoylated on K85, and this modification is necessary to prevent myogenic differentiation of murine skeletal muscle cells (Luan et al. 2013). A lysine to arginine mutant of Pax7 at residue 85, which cannot be sumoylated, fails to transactivate known Pax7 target genes, which suggests that one or more of these gene products is critical for maintaining the cells in the undifferentiated state. Sharp-1 is another inhibitor of skeletal muscle differentiation that is also sumoylated, in this case at lysines 240 and 255 (Wang et al. 2013). Mutation of the SUMO addition sites or overexpression of SENP1 reduces the ability of Sharp-1 to repress differentiation, strongly linking this ability to SUMO modification. Mechanistically, the sumoylation of Sharp-1 promotes interaction with G9a, a histone methyltransferase with co-repressor activity. In the absence of Sharp-1 sumoylation G9a occupancy of muscle promoters is reduced, likely leading to transcription of genes promoting differentiation. A third inhibitor of muscle cell differentiation, BS69, is also a substrate for sumoylation at lysine 367, and in this case PIAS1 appears to be an important SUMO ligase to enhance BS69 sumoylation (Yu et al. 2009). However, sumoylation deficient mutants of BS69 showed no obvious phenotype so the role of sumoylation in the differentiation function of BS69 remains uncertain.

In contrast to Pax7, Sharp-1, and BS69 which act as inhibitors of muscle differentiation, skNAC appears to be a positive regulator of differentiation through a sumoylation-dependent process (Berkholz et al. 2014). skNAC bind to both the Mms21/Nse2 complex, which is known to function as a SUMO ligase (Potts and Yu 2005) and to a myogenic regulator known as Smyd1 (Li et al. 2009). Knockdown of Mms21/Nse2 partially inhibits myogenesis and decreases Smyd1 sumoylation muscle cells, suggesting that sumoylation regulates the activity of the skNAC/

Smyd1 complex to control muscle differentiation. The results with these different factors, Pax7, Sharp-1, BS69, and skNAC/Smyd1 all highlight a role for sumoylation in coordinating events that regulate the transition from undifferentiated to differentiated state in muscle cells. Pax7 and BS69 play a similar role in both muscle and neural cells (Luan et al. 2013), and contribution of sumoylation to neural cell differentiation is discussed in the next section.

12.5.3 Neuronal Cells

In addition to their role in myocyte differentiation, MEF2 proteins are also critical factors for neuronal biology (Heidenreich and Linseman 2004). The MEF2 family members are widely expressed in developing brains and have been implicated in control of proliferation, differentiation, and apoptosis [reviewed in (McKinsey et al. 2002)]. One important function of the MEF2 proteins is to function as integrators of calcium signals mediated through calmodulin and the calcium/calmodulin-dependent protein kinase (CaMK). CaMK stimulates MEF2 transcriptional activity and appears to act through disruption of MEF2 interactions with the HDAC transcriptional repressors (Lu et al. 2000). Functional regulation of the MEF2 family is known to involve phosphorylation, and several studies have now shown that at least MEF2A (Riquelme et al. 2006b), MEF2C (Gocke et al. 2005), and MEF2D (Gregoire et al. 2006) are sumoylated, implying that post-translational modifications will be an important mechanism for controlling MEF2 activity.

Among the MEF2 family members, the role of MEF2A in neuronal differentiation is the best characterized. MEF2A is required for postsynaptic differentiation of cerebellar dendrites into dendritic claws, and this activity is regulated by sumoylation (Shalizi et al. 2006). MEF2 is sumoylated on lysine 403 in a process that is promoted by phosphorylation at lysine 408. When lysine 408 is dephosphorylated by calcium-dependent activation of calcineurin, sumoylation at lysine 403 is reduced and acetylation of K403 is promoted. Sumoylation of MEF2A reduces its transcriptional activating function and represses Nur77, a factor that normally prevents dendritic claw formation. In a subsequent publication, Shalizi et al. demonstrated that PIASx (αor β) were the SUMO E3 ligases responsible for sumoylation of MEF2A, while the other 3 PIAS family members were inactive on MEF2 (Shalizi et al. 2007). PIASx knockdown reduced dendritic claw formation, but this reduction could be overcome by expression of a MEF2A-SUMO fusion protein, indicating that PIASx is normally acting through stimulation of MEF2A sumoylation. Thus, sumoylation is a key component of the regulatory switch that controls morphogenesis of the claw. Interestingly, a previous report indicated that PIAS1 could enhance sumoylation of MEF2A (Riquelme et al. 2006b), and while the reason for this discrepancy is unknown it does support a role PIAS proteins in MEF2 sumoylation. As very little is known about the expression patterns of PIAS proteins in neural tissues, it will be important to determine how PIAS expression is regulated and what effect that has on sumoylation of different MEF2 members and their isoforms.

The calcium/calmodulin-dependent serine protein kinase (CASK), a member of the membrane-associated guanylate kinase (MAGUK) family, is also important for dendritic spine stabilization or maintenance in hippocampal neurons (Chao et al. 2008). CASK appears to function by linking plasma membrane proteins with the actin skeleton through its interaction with the 4.1 protein. CASK is sumoylated at a single site, lysine 679, and sumoylated CASK shows reduced 4.1 binding and a more cytosolic location, suggesting that sumoylation may promote dissociation of CASK from the membrane. Expression of a CASK-SUMO fusion protein impaired spine formation which implies that sumoylation is a negative regulator of this event, in contrast to the positive regulation of cerebellar dendritic claw formation. Unfortunately, regulation of these neuronal processes is likely to be complex and difficult to resolve due to the numerous potential SUMO targets involved.

Consistent with several other of the systems described above, desumoylation is also critical for neuronal differentiation (Juarez-Vicente et al. 2016). Using a mouse teratocarcinoma cell line, Juarez-Vicente et al. demonstrated a generalized increase in free SUMO following neuronal induction with retinoic acid. Examination of the expression levels for the components of the sumoylation system found no changes except for upregulation of SENP5 and SENP7, whose desumoylation activity could account for the increase in free SUMO. Consistent with this deconjugation of SUMO during induction being functionally significant, overexpression of SUMO1 or SUMO2 impaired differentiation. Likewise, SENP7 knockdown impaired differentiation and reduced free SUMO levels, specifically of SUMO2/3, implicating these paralogs as the important regulators. Identification of specific SUMO2/3 targets and exploration of their functional roles will be highly informative for elucidating the pathways and molecular mechanisms that contribute to differentiation control in neuron cells.

12.5.4 Hematopoietic Cells

In the adult, hematopoietic stem cells (HSCs) give rise to multiple lineages, including B cells, T cells, neutrophils, and monocyte/macrophages (Wang and Ema 2016). Diverse studies have now shown that sumoylation plays a regulatory role in many of these pathways. For example, MafB is a transcription factor that promotes macrophage differentiation from myeloid precursors, and MafB is sumoylated at K32 and K297 (Tillmanns et al. 2007). Sumoylation is required for repression of MafB by v-Myb, so sumoylation-defective mutants of MafB exhibit increased differentiation and suggest that the level of sumoylation can control the switch between maintenance of the precursor and differentiation into macrophages. Sumoylation-dependent regulation was also observed for another critical factor controlling hematopoietic development, GATA-1 (Lee et al. 2009). Like MafB, GATA-1 is sumoylated, and in this case sumoylation is required for binding to FOG-1 and transcriptional activation of FOG-1-dependent genes. While not directly tested, loss of GATA-1 sumoylation would like cause significant disruption in the differentiation program. Similarly, GFI1 is another multipotent regulatory factor that plays important roles in mammalian neutrophil differentiation (van der Meer et al. 2010). GFI1 is sumoylated at K239, and SUMO conjugation at this residue is required for GFI1 to support granulocytic differentiation (Andrade et al. 2016). Interaction between GFI1 and its partner, the LSD1/CoREST lysine demethylase complex, is disrupted by mutation of lysine 239 to arginine, implicating this pathway as the critical step that is regulated by sumoylation.

Like macrophages and neutrophils, sumoylation has also been implicated in development of B and T cells (Van Nguyen et al. 2012). STAT5 is a key regulator that is critical for both B and T cells (Yao et al. 2006), and it is modified by SUMO (Van Nguyen et al. 2012). Sumoylation of STAT5 on K696 blocks acetylation at this same residue; lack of acetylation prevents STAT5 dimerization and results in transcriptionally inactive STAT5. Unless the SUMO moiety can be removed by SENP1, the inactive form of STAT5 accumulates resulting in impairment of B and T cell development. Thus, a cycle of sumoylation-desumoylation is essential for the normal function and regulation of B and T cell lineage development by STAT5.

In addition to a regulatory role in normal hematopoietic cell development, it has been noted that sumoylation is disrupted in several type of hematopoietic malignancies. Driscoll et al. observed that patients with multiple myeloma had enhanced overall levels of sumoylation (Driscoll et al. 2010). Levels of Ubc9 and PIAS1 were also elevated in many patients, and this elevation of the conjugation and ligase could explain the increase in sumoylation. High expression levels of these two components correlated with lower patient survival suggesting that increased sumoylation was advantageous for the tumors. Consistent with positive role for sumoylation in tumor cells, acute myeloid

leukemia (AML) cells have reduced levels of SENP5 compared to normal cells (Federzoni et al. 2015). While not addressed in the study, reduction in this desumoylating enzyme would be expected to elevate overall sumoylation. Knocking down SENP5 in an AML neutrophil model prevented differentiation, so this pathway may be contributing to the occurrence of the undifferentiated blast cells that are characteristic of this disease. While a great deal more work is needed to fully understand how sumoylation relates to hematopoietic cancers, there is great potential here for possible diagnostic and/or therapeutic approaches.

12.6 Conclusions

Numerous publications over the last 10 years have shown that the sumoylation system is an important regulator of cellular fate and differentiation, and several systems have been described in this chapter. Examples of both positive and negative effects on differentiation by sumoylation have been reported, so the possibility exists of opposing pathways that are co-regulated by sumoylation to provide fine control of the commitment to differentiate. At the molecular level, SUMO conjugation modulates transcriptional activity both for specific TFs and more globally via changes in chromatin structure. Through its covalent attachment to transcription factors and other chromatin regulatory proteins, such as the histones and chromatin remodeling enzymes, SUMO can influence the recruitment and formation of multi-protein complexes that are critical mediators of the cellular transcriptional program. Additionally, sumoylation of non-TFs, such as kinases or structural proteins, also appears to have important contributions to regulation of cell fate. Consequently, understanding the precise functions of sumoylation in different developmental and differentiation systems may provide new targets for specific or global modulation of these processes. Being able to exert subtle control on developmental and differentiation systems should have important therapeutic benefits for treatment of diseases and repair of injuries.

References

Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL (2006) SUMO1 haploinsufficiency leads to cleft lip and palate. Science 313:1751

Andrade D, Velinder M, Singer J, Maese L, Bareyan D, Nguyen H, Chandrasekharan MB, Lucente H, McClellan D, Jones D, Sharma S, Liu F, Engel ME (2016) SUMOylation regulates growth factor independence 1 in transcriptional control and hematopoiesis. Mol Cell Biol 36:1438–1450

Angel P, Szabowski A, Schorpp-Kistner M (2001) Function and regulation of AP-1 subunits in skin physiology and pathology. Oncogene 20:2413–2423

Azuma Y, Arnaoutov A, Dasso M (2003) SUMO-2/3 regulates topoisomerase II in mitosis. J Cell Biol 163:477–487

Azuma Y, Arnaoutov A, Anan T, Dasso M (2005) PIASy mediates SUMO-2 conjugation of Topoisomerase-II on mitotic chromosomes. EMBO J 24:2172–2182

Bachant J, Alcasabas A, Blat Y, Kleckner N, Elledge SJ (2002) The SUMO-1 isopeptidase Smt4 is linked to centromeric cohesion through SUMO-1 modification of DNA Topoisomerase II. Mol Cell 9:1169–1182

Benbrook DM, Rogers RS, Medlin MA, Dunn ST (1995) Immunohistochemical analysis of proliferation and differentiation in organotypic cultures of cervical tumor cell lines. Tissue Cell 27:269–274

Berkholz J, Michalick L, Munz B (2014) The E3 SUMO ligase Nse2 regulates sumoylation and nuclear-to-cytoplasmic translocation of skNAC-Smyd1 in myogenesis. J Cell Sci 127:3794–3804

Betz A, Lampen N, Martinek S, Young MW, Darnell JE Jr (2001) A Drosophila PIAS homologue negatively regulates stat92E. Proc Natl Acad Sci U S A 98:9563–9568

Beug H, Blundell PA, Graf T (1987) Reversibility of differentiation and proliferative capacity in avian myelomonocytic cells transformed by tsE26 leukemia virus. Genes Dev 1:277–286

Broday L, Kolotuev I, Didier C, Bhoumik A, Gupta BP, Sternberg PW, Podbilewicz B, Ronai Z (2004) The small ubiquitin-like modifier (SUMO) is required for gonadal and uterine-vulval morphogenesis *Caenorhabditis elegans*. Genes Dev 18:2380–2391

Brown PW, Hwang K, Schlegel PN, Morris PL (2008) Small ubiquitin-related modifier (SUMO)-1, SUMO-2/3 and SUMOylation are involved with centromeric heterochromatin of chromosomes 9 and 1 and proteins of the synaptonemal complex during meiosis in men. Hum Reprod 23:2850–2857

Chao HW, Hong CJ, Huang TN, Lin YL, Hsueh YP (2008) SUMOylation of the MAGUK protein CASK regulates dendritic spinogenesis. J Cell Biol 182:141–155

Chiu SY, Asai N, Costantini F, Hsu W (2008) SUMO-specific protease 2 Is essential for modulating p53-Mdm2 in development of trophoblast stem cell niches and lineages. PLoS Biol 6:2801–2816

Collavin L, Gostissa M, Avolio F, Secco P, Ronchi A, Santoro C, Del Sal G (2004) Modification of the erythroid transcription factor GATA-1 by SUMO-1. Proc Natl Acad Sci U S A 101:8870–8875

Constanzo JD, Deng M, Rindhe S, Tang KJ, Zhang CC, Scaglioni PP (2016) Pias1 is essential for erythroid and vascular development in the mouse embryo. Dev Biol 415:98–110

Demarque MD, Nacerddine K, Neyret-Kahn H, Andrieux A, Danenberg E, Jouvion G, Bomme P, Hamard G, Romagnolo B, Terris B, Cumano A, Barker N, Clevers H, Dejean A (2011) Sumoylation by Ubc9 regulates the stem cell compartment and structure and function of the intestinal epithelium in mice. Gastroenterol 140:286–296

Deyrieux AF, Rosas-Acosta G, Ozbun MA, Wilson VG (2007) Sumoylation dynamics during keratinocyte differentiation. J Cell Sci 120:125–136

Driscoll JJ, Pelluru D, Lefkimmiatis K, Fulciniti M, Prabhala RH, Greipp PR, Barlogie B, Tai YT, Anderson KC, Shaughnessy JD, Annunziata CM, Munshi NC (2010) The sumoylation pathway is dysregulated in multiple myeloma and is associated with adverse patient outcome. Blood 115:2827–2834

Du JX, McConnell BB, Yang VW (2010) A small ubiquitin-related modifier-interacting motif functions as the transcriptional activation domain of Kruppel-like factor 4. J Biol Chem 285:28298–28308

Emambokus N, Vegiopoulos A, Harman B, Jenkinson E, Anderson G, Frampton J (2003) Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. EMBO J 22:4478–4488

Federzoni EA, Gloor S, Jin J, Shan-Krauer D, Fey MF, Torbett BE, Tschan MP (2015) Linking the SUMO protease SENP5 to neutrophil differentiation of AML cells. Leuk Res Rep 4:32–35

Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J (2000) Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature 408:325–330

Gandarillas A (2000) Epidermal differentiation, apoptosis, and senescence: common pathways? Exp Gerontol 35:53–62

Ghoreishi M (2000) Heat shock proteins in the pathogenesis of inflammatory skin diseases. J Med & Dent Sci 47:143–150

Gocke CB, Yu HT, Kang JS (2005) Systematic identification and analysis of mammalian small ubiquitin-like modifier substrates. J Biol Chem 280:5004–5012

Gong L, Ji WK, Hu XH, Hu WF, Tang XC, Huang ZX, Li L, Liu M, Xiang SH, Wu E, Woodward Z, Liu YZ, Nguyen QD, Li DW (2014) Sumoylation differentially regulates Sp1 to control cell differentiation. Proc Natl Acad Sci U S A 111:5574–5579

Gregoire S, Tremblay AM, Xiao L, Yang Q, Ma KW, Nie JY, Mao ZX, Wu ZG, Giguere V, Yang XJ (2006) Control of MEF2 transcriptional activity by coordinated phosphorylation and sumoylation. J Biol Chem 281:4423–4433

Gregoire S, Xiao L, Nie J, Zhang X, Xu M, Li J, Wong J, Seto E, Yang XJ (2007) Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2. Mol Cell Biol 27:1280–1295

Hasegawa Y, Yoshida D, Nakamura Y, Sakakibara S (2014) Spatiotemporal distribution of SUMOylation components during mouse brain development. J Comp Nneurol 522:3020–3036

Heaton PR, Santos A, Rosas-Acosta G, Wilson VG (2012) Analysis of global sumoylation changes occurring during keratinocyte differentiation. PLoS One 7:e30165

Heidenreich KA, Linseman DA (2004) Myocyte enhancer factor-2 transcription factors in neuronal differentiation and survival. Mol Neurobiol 29:155–166

Hendriks IA, D'Souza RC, Yang B, Verlaan-de Vries M, Mann M, Vertegaal AC (2014) Uncovering global SUMOylation signaling networks in a site-specific manner. Nat Struct Mol Biol 21:927–936

Hendriks IA, Treffers LW, Verlaan-de Vries M, Olsen JV, Vertegaal AC (2015) SUMO-2 orchestrates chromatin modifiers in response to DNA damage. Cell Rep 10:1778–1791

Huang L, Ohsako S, Tanda S (2005) The lesswright mutation activates Rel-related proteins, leading to overproduction of larval hemocytes in *Drosophila melanogaster*. Dev Biol 280:407–420

Ihara M, Stein P, Schultz RM (2008) UBE2I (URC9), a SUMO-conjugating enzyme, localizes to nuclear speckles and stimulates transcription in mouse oocytes. Biol Reprod 79:906–913

Jiang M, Chiu SY, Hsu W (2011) SUMO-specific protease 2 in Mdm2-mediated regulation of p53. Cell Death Differ 18:1005–1015

Jones D, Crowe E, Stevens TA, Candido EPM (2001) Functional and phylogenetic analysis of the ubiquitylation system in *Caenorhabditis elegans*: ubiquitin-conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like proteins. Genome Biol 3:2.1–2.15

Jones D, Crowe E, Stevens TA, Candido EP (2002) Functional and phylogenetic analysis of the ubiquitylation system in *Caenorhabditis elegans*: ubiquitin-conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like proteins. Genome Biol 3:0002.0001–0002.0015

Juarez-Vicente F, Luna-Pelaez N, Garcia-Dominguez M (2016) The SUMO protease SENP7 is required for proper neuronal differentiation. Biochim Biophys Acta 1863:1490–1498

Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. Cell 113:127–137

Kanakousaki K, Gibson MC (2012) A differential requirement for SUMOylation in proliferating and non-proliferating cells during *Drosophila* development. Development 139:2751–2762

Kang J, Gocke CB, Yu H (2006) Phosphorylation-facilitated sumoylation of MEF2C negatively regulates its transcriptional activity. *BMC Biochem* **7**:1–14

Kang XL, Qi YT, Zuo Y, Wang Q, Zou YQ, Schwartz RJ, Cheng JK, Yeh ETH (2010) SUMO-specific protease 2 Is essential for suppression of polycomb group protein-mediated gene silencing during embryonic development. Mol Cell 38:191–201

Kim KI, Baek SH, Jeon YJ, Nishimori S, Suzuki T, Uchida S, Shimbara N, Saitoh H, Tanaka K, Chung CH (2000) A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. J Biol Chem 275:14102–14106

Koster MI, Huntzinger KA, Roop DR (2002) Epidermal differentiation: transgenic/knockout mouse models reveal genes involved in stem cell fate decisions and commitment to differentiation. J Invest Dermatol 7:41–45

Kuijk EW, Du Puy L, Van Tol HT, Oei CH, Haagsman HP, Colenbrander B, Roelen BA (2008) Differences in early lineage segregation between mammals. Dev Dyn 237:918–927

La Salle S, Sun F, Zhang XD, Matunis MJ, Handel MA (2008) Developmental control of sumoylation pathway proteins in mouse male germ cells. Dev Biol 321:227–237

Lee HY, Johnson KD, Fujiwara T, Boyer ME, Kim SI, Bresnick EH (2009) Controlling hematopoiesis through sumoylation-dependent regulation of a GATA factor. Mol Cell 36:984–995

Leight ER, Glossip D, Kornfeld K (2005) Sumoylation of LIN-1 promotes transcriptional repression and inhibition of vulval cell fates. Development 132:1047–1056

Li SS, Liu YH, Tseng CN, Singh S (2006) Analysis of gene expression in single human oocytes and preimplantation embryos. Biochem Biophys Res Commun 340:48–53

Li D, Niu Z, Yu W, Qian Y, Wang Q, Li Q, Yi Z, Luo J, Wu X, Wang Y, Schwartz RJ, Liu M (2009) SMYD1, the myogenic activator, is a direct target of serum response factor and myogenin. Nucleic Acids Res 37:7059–7071

Li X, Lan Y, Xu J, Zhang W, Wen Z (2012) SUMO1-activating enzyme subunit 1 is essential for the survival of hematopoietic stem/progenitor cells in zebrafish. Development 139:4321–4329

Liu B, Yee KM, Tahk S, Mackie R, Hsu C, Shuai K (2014) PIAS1 SUMO ligase regulates the self-renewal and differentiation of hematopoietic stem cells. EMBO J 33:101–113

Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, van Roozendaal KE, van Zoelen EJ, Weber RF, Wolffenbuttel KP, van Dekken H, Honecker F, Bokemeyer C, Perlman EJ, Schneider DT, Kononen J, Sauter G, Oosterhuis JW (2003) POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 63:2244–2250

Loriol C, Parisot J, Poupon G, Gwizdek C, Martin S (2012) Developmental regulation and spatiotemporal redistribution of the sumoylation machinery in the rat central nervous system. PLoS One 7:e33757

Lu J, McKinsey TA, Nicol RL, Olson EN (2000) Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. Proc Natl Acad Sci U S A 97:4070–4075

Luan Z, Liu Y, Stuhlmiller TJ, Marquez J, Garcia-Castro MI (2013) SUMOylation of Pax7 is essential for neural crest and muscle development. Cell Mol Life Sci 70:1793–1806

Luis NM, Morey L, Mejetta S, Pascual G, Janich P, Kuebler B, Cozutto L, Roma G, Nascimento E, Frye M, Di Croce L, Benitah SA (2011) Regulation of human epidermal stem cell proliferation and senescence requires polycomb- dependent and -independent functions of Cbx4. Cell Stem Cell 9:233–246

Mardaryev AN, Liu B, Rapisarda V, Poterlowicz K, Malashchuk I, Rudolf J, Sharov AA, Jahoda CA, Fessing MY, Benitah SA, Xu GL, Botchkarev VA (2016) Cbx4 maintains the epithelial lineage identity and cell proliferation in the developing stratified epithelium. J Cell Biol 212:77–89

Maruyama EO, Lin H, Chiu SY, Yu HM, Porter GA, Hsu W (2016) Extraembryonic but not embryonic SUMO-specific protease 2 is required for heart development. Sci Rep 6:20999

McKinsey TA, Zhang CL, Olson EN (2002) MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 27:40–47

Metzler-Guillemain C, Depetris D, Luciani JJ, Mignon-Ravix C, Mitchell MJ, Mattei MG (2008) In human pachytene spermatocytes, SUMO protein is restricted to the constitutive heterochromatin. *Chromosom Res* 16:761–782

Morita Y, Kanei-Ishii C, Nomura T, Ishii S (2005) TRAF7 sequesters c-Myb to the cytoplasm by stimulating its sumoylation. Mol Biol Cell 16:5433–5444

Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A (2005) The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell 9:769–779

Nayak A, Viale-Bouroncle S, Morsczeck C, Muller S (2014) The SUMO-specific isopeptidase SENP3 regulates MLL1/MLL2 methyltransferase complexes and controls osteogenic differentiation. Mol Cell 55:47–58

Newman AP, Acton GZ, Hartwieg E, Horvitz HR, Sternberg PW (1999) The lin-11 LIM domain transcription factor is necessary for morphogenesis of *C. elegans* uterine cells. Development 126:5319–5326

Nie MH, Xie YM, Loo JA, Courey AJ (2009) Genetic and proteomic evidence for roles of *Drosophila* SUMO in cell cycle control, Ras signaling, and early pattern formation. PLoS One 4:e5905

Niwa H, Miyazaki J, Smith AG (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24:372–376

Nowak M, Hammerschmidt M (2006) Ubc9 regulates mitosis and cell survival during zebrafish development. Mol Biol Cell 17:5324–5336

Potts PR, Yu HT (2005) Human MMS21/NSE2 is a SUMO ligase required for DNA repair. Mol Cell Biol 25:7021–7032

Poumay Y, Leclercq-Smekens M (1998) In vitro models of epidermal differentiation. Folia Med 40:5–12

Pownall ME, Gustafsson MK, Emerson CP Jr (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu Rev Cell Dev Biol 18:747–783

Riquelme C, Barthel KK, Qin XF, Liu X (2006a) Ubc9 expression is essential for myotube formation in C2C12. Exp Cell Res 312:2132–2141

Riquelme C, Barthel KKB, Liu XD (2006b) SUMO-1 modification of MEF2A regulates its transcriptional activity. J Cell Mol Med 10:132–144

Rodda DJ, Chew JL, Lim LH, Loh YH, Wang B, Ng HH, Robson P (2005) Transcriptional regulation of nanog by OCT4 and SOX2. J Biol Chem 280:24731–24737

Rodriguez A, Pangas SA (2016) Regulation of germ cell function by SUMOylation. Cell Tissue Res 363:47–55

Rogers RS, Inselman A, Handel MA, Matunis MJ (2004) SUMO modified proteins localize to the XY body of pachytene spermatocytes. Chromosoma 113:233–243

Rytinki MM, Lakso M, Pehkonen P, Aarnio V, Reisner K, Perakyla M, Wong G, Palvimo JJ (2011) Overexpression of SUMO perturbs the growth and development of *Caenorhabditis elegans*. Cell Mol Life Sci 68:3219–3232

Santti H, Mikkonen L, Hirvonen-Santti S, Toppari J, Janne OA, Palvimo JJ (2003) Identification of a short PIASx gene promoter that directs male germ cell-specific transcription in vivo. Biochem Biophys Res Commun 308:139–147

Sayed N, Liu C, Wu JC (2016) Translation of human-induced pluripotent stem cells: from clinical trial in a dish to precision medicine. J AmColl Cardiol 67:2161–2176

Shalizi A, Gaudilliere B, Yuan ZQ, Stegmuller J, Shirogane T, Ge QY, Tan Y, Schulman B, Harper JW, Bonni A (2006) A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. Science 311:1012–1017

Shalizi A, Bilimoria PM, Stegmuller J, Gaudilliere B, Yang Y, Shuai K, Bonni A (2007) PIASx is a MEF2 SUMO E3 ligase that promotes postsynaptic dendritic morphogenesis. J Neurosci 27:10037–10046

Sharma P, Yamada S, Lualdi M, Dasso M, Kuehn MR (2013) SENP1 is essential for desumoylating SUMO1-modified proteins but dispensable for SUMO2 and SUMO3 deconjugation in the mouse embryo. Cell Rep 3:1640–1650

Shrivastava V, Pekar M, Grosser E, Im J, Vigodner M (2010) SUMO proteins are involved in the stress response during spermatogenesis and are localized to DNA double-strand breaks in germ cells. Reproduction 139:999–1010

Shrivastava V, Marmor H, Chernyak S, Goldstein M, Feliciano M, Vigodner M (2014) Cigarette smoke affects posttranslational modifications and inhibits capacitation-induced changes in human sperm proteins. Reprod Toxicol 43:125–129

Smith M, Turki-Judeh W, Courey AJ (2012) SUMOylation in *Drosophila* development. Biomolecules 2:331–349

Tahmasebi S, Ghorbani M, Savage P, Gocevski G, Yang XJ (2014) The SUMO conjugating enzyme Ubc9 is required for inducing and maintaining stem cell pluripotency. Stem Cells 32:1012–1020

Tapscott SJ (2005) The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. Development 132:2685–2695

Tillmanns S, Otto C, Jaffray E, Du Roure C, Bakri Y, Vanhille L, Sarrazin S, Hay RT, Sieweke MH (2007) SUMO modification regulates MafB-driven macrophage differentiation by enabling Myb-dependent transcriptional repression. Mol Cell Biol 27:5554–5564

Tsuruzoe S, Ishihara K, Uchimura Y, Watanabe S, Sekita Y, Aoto T, Saitoh H, Yuasa Y, Niwa H, Kawasuji M, Baba H, Nakao M (2006) Inhibition of DNA binding of Sox2 by the SUMO conjugation. Biochem Biophys Res Commun 351:920–926

van der Meer LT, Jansen JH, van der Reijden BA (2010) Gfi1 and Gfi1b: key regulators of hematopoiesis. Leukemia 24:1834–1843

Van Nguyen T, Angkasekwinai P, Dou H, Lin FM, Lu LS, Cheng J, Chin YE, Dong C, Yeh ET (2012) SUMO-specific protease 1 is critical for early lymphoid development through regulation of STAT5 activation. Mol Cell 45:210–221

Vigodner M (2009) Sumoylation precedes accumulation of phosphorylated H2AX on sex chromosomes during their meiotic inactivation. Chromosom Res 17:37–45

Vigodner M (2011) Roles of small ubiquitin-related modifiers in male reproductive function. Int Rev Cell Mol Biol 288:227–259

Vigodner M, Morris PL (2005) Testicular expression of small ubiquitin-related modifier-1 (SUMO-1) supports multiple roles in spermatogenesis: silencing of sex chromosomes in spermatocytes, spermatid microtubule nucleation, and nuclear reshaping. Dev Biol 282:480–492

Vigodner M, Ishikawa T, Schlegel PN, Morris PL (2006) SUMO-1, human male germ cell development, and the androgen receptor in the testis of men with normal and abnormal spermatogenesis. Am J Physiol-Endocrinol Metab 290:E1022–E1033

Vigodner M, Shrivastava V, Gutstein LE, Schneider J, Nieves E, Goldstein M, Feliciano M, Callaway M (2013) Localization and identification of sumoylated proteins in human sperm: excessive sumoylation is a marker of defective spermatozoa. Hum Reprod 28:210–223

Wang Z, Ema H (2016) Mechanisms of self-renewal in hematopoietic stem cells. Int J Hematol 103:498–509

Wang J, Zhou Q (2016) Derivation and application of pluripotent stem cells for regenerative medicine. Sci China Life Sci 59:576–583

Wang YG, Mukhopadhyay D, Mathew S, Hasebe T, Heimeier RA, Azuma Y, Kolli N, Shi YB, Wilkinson KD, Dasso M (2009) Identification and developmental expression of *Xenopus laevis* SUMO proteases. PLoS One 4:e8462

Wang ZB, Ou XH, Tong JS, Li S, Wei LA, Ouyang YC, Hou Y, Schatten H, Sun QY (2010) The SUMO pathway functions in mouse oocyte maturation. Cell Cycle 9:2640–2646

Wang Y, Shankar SR, Kher D, Ling BM, Taneja R (2013) Sumoylation of the basic helix-loop-helix transcription factor Sharp-1 regulates recruitment of the histone methyltransferase G9a and function in myogenesis. J Biol Chem 288:17654–17662

Wang L, Wansleeben C, Zhao S, Miao P, Paschen W, Yang W (2014) SUMO2 is essential while SUMO3 is dispensable for mouse embryonic development. EMBO Rep 15:878–885

Ward JD, Bojanala N, Bernal T, Ashrafi K, Asahina M, Yamamoto KR (2013) Sumoylated NHR-25/NR5A regulates cell fate during *C. elegans* vulval development. *PLoS Genet* **9**:e1003992

Wei F, Scholer HR, Atchison ML (2007) Sumoylation of Oct4 enhances its stability, DNA binding, and transactivation. J Biol Chem 282:21551–21560

Werner S, Smola H (2001) Paracrine regulation of keratinocyte proliferation and differentiation. Trends Cell Biol 4:143–146

Wotton D, Merrill JC (2007) Pc2 and SUMOylation. Biochem Soc Trans 35:1401–1404

Wrighton KH, Liang M, Bryan B, Luo K, Liu M, Feng XH, Lin X (2007) Transforming growth factor-beta-independent regulation of myogenesis by SnoN sumoylation. J Biol Chem 282:6517–6524

Wu Y, Guo Z, Wu H, Wang X, Yang L, Shi X, Du J, Tang B, Li W, Yang L, Zhang Y (2012) SUMOylation represses Nanog expression via modulating transcription factors Oct4 and Sox2. PLoS One 7:e39606

Xiao Z, Chang JG, Hendriks IA, Sigurethsson JO, Olsen JV, Vertegaal AC (2015) System-wide analysis of SUMOylation dynamics in response to replication stress reveals novel small ubiquitin-like modified target proteins and acceptor lysines relevant for genome stability. Mol Cell Proteomics 14:1419–1434

Xiao Y, Pollack D, Andrusier M, Levy A, Callaway M, Nieves E, Reddi P, Vigodner M (2016) Identification of cell-specific targets of sumoylation during mouse spermatogenesis. Reproduction 151:149–166

Yan W, Santti H, Janne OA, Palvimo JJ, Toppari J (2003) Expression of the E3 SUMO-1 ligases PIASx and PIAS1 during spermatogenesis in the rat. Gene Expr Patterns 3:301–308

Yao Z, Cui Y, Watford WT, Bream JH, Yamaoka K, Hissong BD, Li D, Durum SK, Jiang Q, Bhandoola A, Hennighausen L, O'Shea JJ (2006) Stat5a/b are essential for normal lymphoid development and differentiation. Proc Natl Acad Sci U S A 103:1000–1005

Yu B, Shao Y, Zhang C, Chen YW, Zhong QL, Zhang J, Yang H, Zhang W, Wan J (2009) BS69 undergoes SUMO modification and plays an inhibitory role in muscle and neuronal differentiation. Exp Cell Res 315:3543–3553

Yuan H, Zhou J, Deng M, Liu X, Le Bras M, de The H, Chen SJ, Chen Z, Liu TX, Zhu J (2010) Small ubiquitin-related modifier paralogs are indispensable but functionally redundant during early development of zebrafish. Cell Res 20:185–196

Yuan YF, Zhai R, Liu XM, Khan HA, Zhen YH, Huo LJ (2014) SUMO-1 plays crucial roles for spindle organization, chromosome congression, and chromosome segregation during mouse oocyte meiotic maturation. Mol Reprod Dev 81:712–724

Yuan H, Zhang T, Liu X, Deng M, Zhang W, Wen Z, Chen S, Chen Z, de The H, Zhou J, Zhu J (2015) Sumoylation of CCAAT/enhancer-binding protein alpha is implicated in hematopoietic stem/progenitor cell development through regulating runx1 in zebrafish. Sci Rep 5:9011

Yukita A, Michiue T, Danno H, Asashima M (2007) XSUMO-1 is required for normal mesoderm induction and axis elongation during early Xenopus development. Dev Dyn 236:2757–2766

Zhang FP, Mikkonen L, Toppari J, Palvimo JJ, Thesleff I, Janne OA (2008) SUMO-1 function is dispensable in normal mouse development. Mol Cell Biol 28:5381–5390