Sumoylation in Craniofacial Disorders

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Abstract

Craniofacial development requires a complex series of coordinated and finely tuned events to take place, during a relatively short time frame. These events are set in motion by switching on and off transcriptional cascades that involve the use of numerous signalling pathways and a multitude of factors that act at the site of gene transcription. It is now well known that amidst the subtlety of this process lies the intricate world of protein modification, and the posttranslational addition of the small ubiquitin-like modifier, SUMO, is an example that has been implicated in this process. Many proteins that are required for formation of various structures in the embryonic head and face adapt specific functions with SUMO modification. Interestingly, the main clinical phenotype reported for a disruption of the *SUMO1* locus is the common birth defect cleft lip and palate. In this chapter therefore, we discuss the role of SUMO1 in craniofacial development, with emphasis on orofacial clefts. We suggest that these defects can be a sensitive indication of down regulated SUMO modification at a critical stage during embryogenesis. As well as specific mutations affecting the ability of particular proteins to be sumoylated, non-genetic events may have the effect of down-regulating the SUMO pathway to give the same result. Enzymes regulating the SUMO pathway may become important therapeutic targets in the preventative and treatment therapies for craniofacial defects in the future.

Keywords

SUMO • Craniofacial development • Cleft lip and palate • Transcription • Stress

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19.1 Key Role for Sumo in Development

Post-translational protein modifications can have many and variable consequences, but in general, they play a key role in regulating and expanding the diversity of function in the proteome. As documented in this book, the reversible conjugation of SUMO to protein substrates (sumoylation) has emerged as a major post-translational regulatory process. In the last two decades, numerous proteins have been identified that undergo SUMO modification and this list has been greatly expanded with the advent of mass spectroscopy approaches to study the SUMO proteasome (Seeler and Dejean 2003; Geiss-Friedlander and Melchior 2007; Eifler and Vertegaal 2015). The precise action of SUMO modification can vary considerably depending upon the substrate, but in many cases the specific functional effect still remains to be elucidated. For those proteins involved in regulation of gene transcription, SUMO modification usually plays an important role either with (sub)nuclear localisation or the functional activity of the transcription factor in the nucleus. It is therefore not surprising that sumoylation is now being increasingly recognised as a crucial regulator of embryonic morphogenesis. Overall the biological significance of the SUMO pathway in mammalian development can be judged as essential, based on observations of mice deficient for the key E2 conjugating enzyme Ubc9 (Nacerddine et al. 2005). Although heterozygous animals are essentially normal, null embryos die during the period between the early postimplantation stage and prior to embryonal day (E)7.5. In *C. elegans*, knock down of ubc-9 causes severe pharyngeal defects, partly resulting from an altered sub-nuclear distribution of the sumoylated transcription factor tbx-2 (Roy Chowdhuri et al. 2006; Crum and Okkema 2007). The ability to successfully sumoylate individual target proteins and precisely regulate this process is likely to be more subtle but will nevertheless be an essential part of embryonic development. As suggested by the *C. elegans* data and the over-representation of sumoylated proteins involved in craniofacial development, the most sensitive readout of this process in developmental terms may occur during formation of the embryonic head (Pauws and Stanier 2007).

19.2 Sumo1 Haploinsufficiency Causes Cleft Lip and/or Palate

The most direct evidence implicating a role for SUMO in craniofacial development came originally from the analysis of a female patient with a cleft lip and palate who was found to be carrying a balanced reciprocal translocation between human chromosomes 2q and 8q (Alkuraya et al. 2006). Mapping the breakpoint on chromosome 2 revealed an interruption within the gene encoding *SUMO1*, and was predicted to result in haploinsufficiency. The functional significance was then investigated in mice. In wild-type animals, strong *Sumo1* expression in the upper lip, primary palate and medial edge epithelia of the secondary palate was demonstrated by whole mount *in situ* hybridisation (Alkuraya et al. 2006). Next, a mouse with a GeneTrap mutation (RRQ016) in *Sumo1* that generated a null allele was investigated. A low penetrance (8.7%) of cleft palate (CP) was observed in heterozygote animals, while homozygote embryos were embryonic lethal prior to palate closure, indicating that SUMO1 is required for other important developmental functions. *EYA1* is a homolog of the *Drosophila* absent eyes gene, which is mutated in human patients with brachio-oto-renal syndrome (Abdelhak et al. 1997). *Eya1* is important for palate development as evidenced by the fact that it is expressed in the developing mouse palate and mice completely lacking Eya1 have a cleft palate (amongst other defects) (Xu et al. 1999b). This is in contrast to heterozygous animals that show normal palate development. The expression of *Eya1* was noted to overlap with that of *Sumo1* and it has been shown to be a SUMO1 substrate (Alkuraya et al. 2006). Moreover, a significant increase (36%) in the penetrance of CP was observed in compound heterozygous mutants of *Eya1* and *Sumo1*, suggesting a genetic interaction between the two.

This data is not without controversy though, since two independent reports describe how SUMO1 is dispensable throughout development and question the validity of the original findings in the gene trap model. In the first of these, Zhang et al. (2008), describe a mouse in which *Sumo1* was targeted by homologous recombination to make either heterozygous (haploinsufficient) or homozygous null animals. These null animals do not produce any SUMO1 protein, yet they do not have an overt palate defect, nor do they have any disruption to adipogenesis, postnatal growth rate, reproductive function or any other noticeable phenotype. Interestingly, RanGAP1, usually modified by SUMO1, was demonstrated to show increased modification by the SUMO2 paralog instead. Many proteins are specifically modified with one paralog or another, and mechanisms regulating this specificity are only just coming to light (Meulmeester et al. 2008). In the Zhang et al. study, it seems that SUMO2 is able to rescue the SUMO1 deficient mice. However, as the authors point out, their *Sumo1* knockout mice are on a different genetic background to the animals described by Alkuraya et al. (2006) and a different set of genetic modifiers might be involved. This is not unusual when comparing inbred laboratory strains as evidenced by the differences to palate defects seen in C57BL/6 J $Eya^{-/-}$ mice compared to those seen for 129/Sv and Balb/C $Eya1^{-/-}$ mice (Xu et al. 1999b). Perhaps most significantly, the type of gene disruption is also different in the two reports. Unlike the targeted homologous gene targeting strategy employed by Zhang et al. (2008), Alkuraya et al. (2006) used mice generated using a gene trapping strategy, which can be leaky through processes such as mis-splicing (Galy et al. 2004). Whilst it is possible that a gain of function mutant may have been generated, it is also possible that the level of available SUMO1 protein may impact on the ability of other SUMO paralogs to compensate. Alternatively, environmental variables such as diet or stress factors may differ between laboratories and are not taken into account.

These ideas were further brought into question by a third study, where Evdokimov et al. (2008) investigated an independent *Sumo1* GeneTrap (XA024). It was found that resulting homozygous mice were phenotypically normal. This could partially be explained by alternate splicing leading to leaky translation, albeit of a protein lacking 25 amino acids which was predicted to be a loss-of-function allele. Interestingly, like Zhang et al. (2008), these authors also found that RanGAP1 sumoylation could be compensated for by SUMO2/3 in the absence or down regulation of SUMO1 in the XA024 GeneTrap. In order to try to resolve the developmental inconsistencies, Evdokimov et al., went on to reinvestigate the original GeneTrap mice derived from the same RRQ016 ES cells used by Alkuraya et al. (2006). Surprisingly, they found that these mice were normal and fertile. However, a possible explanation to the lack of phenotype was a complex rearrangement at this locus, potentially disrupting the GeneTrap. This was supported by the detection of normal SUMO1-RanGAP1 conjugation in these animals. They surmise that an independent mutation of another gene may have been present and the fundamental cause in the mice analysed by Alkuraya et al. (2006). It now appears that SUMO2 is the most important isoform during development, where embryonic deficiency in mice resulted in severe developmental delay and death at around E10.5 (Wang et al. 2014). As previously suggested by Zhang et al. (2008) and Evdokimov et al. (2008), SUMO2 appears to have some ability to compensate for loss of other SUMO isoforms, all though the reciprocal arrangement is less obvious. Moreover, the precise role of the SUMO pathway in embryonic development still remains to be fully elucidated since embryos deficient for other components of the SUMO regulatory machinery are observed to result in lethality at different stages of embryonic development, presumably acting through different mechanisms (Nacerddine et al. 2005; Cheng et al. 2007; Kang et al. 2010; Sharma et al. 2013).

Despite the controversies over the effect of SUMO1 in mice, independent evidence for a role in cleft lip and palate has come from genetic studies in human CL/P cohorts. It was noted that 2q32-q33 where the *SUMO1* gene resides was previously reported as a region where copy

number variants or translocations were implicated in craniofacial dysmorphology (Brewer et al. 1998, 1999; Van Buggenhout et al. 2005; Shi et al. 2009). The 2q32-q35 locus was also was identified by a meta-analysis of GWAS studies for NSCL/P (Marazita et al. 2004). Therefore, along with the Alkuraya et al. (2006) report, these collective findings prompted a closer look at the *SUMO1* locus, primarily by association studies. The first of these was from Song et al. (2008), who reported a positive association with NSCLP especially between a common haplotype of 4 SNPs within the *SUMO1* gene. This was followed by several further reports finding either association (Carter et al. 2010; Jia et al. 2010; Guo et al. 2012), borderline association (Mostowska et al. 2010) or no association (de Assis et al. 2011; Carta et al. 2012). In addition, de Assis et al. Sanger-sequenced *SUMO1* in a cohort of NSCL/P patients as did Carta et al. who also included *SUMO2*, *SUMO3*, *PIAS1* and *PIAS2* but both failed to identify sequence variants that could be implicated as disease causing. To analyse these apparently conflicting results further, a meta-analysis including 1381 NSCL/P patients and 2054 controls reports empirical evidence implicating a role for *SUMO1* in the etiology of NSCL/P in both Caucasian and Asian populations (Tang et al. 2014).

19.3 Sumoylation Regulates Craniofacial Developmental Genes

The underlying cause of cleft lip and/or cleft palate (CL/P) has been the subject of a great deal of attention (Murray and Schutte 2004: Stanier and Moore 2004; Lidral and Moreno 2005; Setó–Salvia and Stanier 2014). In general, oral clefts can be classified as non-syndromic (NS) when they occur as isolated defects or syndromic, when they occur together with one or more other anomaly. The underlying cause of NSCL/P still remain elusive, partly because they appear to be a sensitive developmental effect accruing from many different genetic and environmental factors. Consequently, any large collection of patients is likely to be extremely heterogeneous and refractory to the standard techniques of genome wide association studies frequently employed to investigate their aetiology. The study of syndromic cases has been much more successful since it has been possible to categorise patients more accurately according to the presence of a second phenotypic feature, such as hypodontia, lip pits, ectodermal dysplasia or ankyloglossia (Stanier and Moore 2004). This has allowed specific genes and etiologic mutations to be identified, but has also had the bonus of identifying the molecular basis of some forms of NSCL/P too, most notably for IRF6 (Kondo et al. 2002) and TBX22 (Braybrook et al. 2001). In addition to the direct role of SUMO1 in lip and palate development described above, it is now becoming apparent that many of the proteins associated with clefts are targets of SUMO modification (Table 19.1).

The sumoylated protein SATB2 is a homeobox transcription factor that was first implicated in NS cleft palate (NSCP) in a patient with a translocation in 2q32-q33 interrupting the gene (FitzPatrick et al. 2003). More recently mutations in *SATB2* were found in syndromic patients with CP, osteoporosis and mental retardation (Leoyklang et al. 2007) as well as NSCP (Vieira et al. 2005). *Satb2* knockout mice also show a distinct CP phenotype combined with skeletal defects (Dobreva et al. 2006). SATB2 has been shown to require SUMO conjugation to mediate its sub-nuclear localisation, protein stability and its transcriptional activity as a repressor (Dobreva et al. 2006).

Another sumoylation target that can result in CL/P when mutated is the MSX1 homeobox transcription factor. Initially, a transgenic mouse devoid of Msx1 was found to have a CP phenotype as well as hypodontia (Satokata and Mass1994). As a result, this gene was considered a good candidate in a 3 generation Dutch family who presented with combinations of tooth agenesis and CP or CLP. This was confirmed by the finding of a nonsense mutation (S105X) which segregated with the affected family members (van den Boogaard et al. 2000). Since then, numerous studies have investigated *MSX1* as a

Table 19.1 Sumoylated proteins involved in mammalian craniofacial development

SUMO target	Protein function	Human disorder and/or phenotype	MIM	Mouse phenotype	SUMO effect
DNMTs3B	*de novo* DNA methylation	ICF – hypertelorism, micrognathia, no CL/P known	242,860	Growth impairment, rostral neural tube defects	Affects nuclear localisation
EYA1	Transcription factor (activator)	BOR - ear defects, branchial clefts, renal dysplasia, no CL/P known	113,650	*Eya1* null – cleft palate	Unknown
				Sumo1/Eya1 double heterozygotes significantly increase CP occurrence	
GSC	Transcription factor (repressor)	None known		Abnormal mandible, tongue, nasal pits	Regulates repressive activity
MSX1	Transcription factor (repressor)	Hypodontia and/or CL/P; NSCL/P	142,983	Homozygous knockout has CP and tooth agenesis	Activates transcriptional repression; sub-nuclear localisation
SATB2	Transcription factor (activator/ repressor)	NSCP	608,148	Knockout has CP and craniofacial abnormalities	Activates transcriptional repression; sub-nuclear localisation
SMAD4	TGFβ signal transduction	None known (associated with colorectal cancer)		Embryonic lethal, mutants lack embryonic germ layers	Interaction with Daxx upregulates Bmp signalling
SnoN	−ve regulator of TGFβ signalling	None known (upregulated in various cancers)		Not known	Activates transcriptional repression
SOX2	Transcription factor (activator)	Micropthalmia	206,900	Inner ear defect, abnormal anterior pituitary development	Down regulates target activation
SOX9	Transcription factor (activator)	CMPD - skeletal defects, CP, micrognathia	114,290	Skeletal defects, CP	Down regulates target activation

(continued)

Table 19.1 (continued)

SUMO target	Protein function	Human disorder and/or phenotype	MIM	Mouse phenotype	SUMO effect
SOX10	Transcription factor (activator)	WS4; Hirschprung – deafness, pigment, megacolon	277,580 609,136	Aganglionic distal bowl	Down regulates target activation
TP63	Transcription factor (activator/ repressor)	EEC - CL/P, ectrodactyly, ectodermal dysplasia; NSCL/P	603,273	Homozygous knockout has bilateral CL and CP	Loss of transcriptional activation; reduced protein stability
TBX22	Transcription factor (repressor)	CPX – CP, ankyloglossia; NSCP	303,400	Not known	Activates transcriptional repression
TRPS1	Transcription factor (repressor)	TRPS – high arched palate	190,350	*Trsp1* loss-of-function mutant mouse model exhibits high-arched palate	Activates transcriptional repression

candidate gene for NSCL/P, both by direct sequencing of patient DNA and in association studies (Lidral and Moreno 2005). It has been suggested that mutations in *MSX1* account for up to 2% of all CL/P (Jezewski et al. 2003). Like SATB2, MSX1 is a transcriptional repressor (Gupta and Bei 2006). Studies suggest that sumoylation is not only required for their repression activity but also plays an important role in sub-nuclear localisation (Lee et al. 2006). Thus, the mode of action might be through appropriate access to its target genes during the period of craniofacial development.

By contrast, TP63, a p53 homolog, is a transcriptional activator, which has several isoforms associated with different disorders affecting ectodermal dysplasia, limb malformations and CL/P (Ghioni et al. 2005). These include split hand/foot malformation (SHFM4), ectodermal dysplasia and CL/P syndrome (EEC3), ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC), Limb mammary syndrome (LMS) and Rapp-Hodgkin syndrome (RHS). Mice deficient for *Tp63* have previously been described with severe craniofacial, limb and skin abnormalities, reflecting loss of the ectodermal cell lineage (Mills et al. 1999; Yang et al. 1999). A recent description of the craniofacial defects in mice deficient for Tp63, showed that they had bilateral cleft lip and cleft palate, which at least in part resulted from downstream effects on *Bmp4*, *Fgf8* and *Shh* expression (Thomason et al. 2008). Numerous mutations have been identified throughout the gene, with some evidence of genotype-phenotype correlations (Rinne et al. 2007). The prevalence of a cleft phenotype varies from 30–80% between these syndromes, whereas mutations in *TP63* are also found in NSCL/P patients (Rinne et al. 2007). SUMO1 conjugation of TP63 regulates its transcriptional activity and protein stability but not its intracellular localization (Ghioni et al. 2005). Several studies have now shown that naturally occurring mutations alter its sumoylation potential thereby strongly upregulating its normal transcriptional activity (Ghioni et al. 2005; Huang et al. 2004).

TBX22 is another SUMO1 target, and this modification has a profound regulatory effect on its transcriptional activity (Andreou et al. 2007). Mutations in *TBX22* were first identified following the study of several large X-linked families (CPX) and then later in collections of isolated CP patients with insufficient family history to predict inheritance (Braybrook et al. 2001; Marçano et al. 2004). Mutations are found in 4–8% of all NSCP patients and, as expected for an X-linked condition, males carrying mutations are most severely affected although 17% of heterozygous females also exhibit CP (Marçano et al. 2004; Suphapeetiporn et al. 2007). TBX22 has been shown to function as a transcriptional repressor with SUMO1 conjugation a necessary requirement for this activity. Functional studies show that most missense mutations in the T-box interfere with DNA-binding, while sumoylation and transcriptional repression are also compromised (Andreou et al. 2007). None of the mutations were located close to the K63 site of SUMO attachment though, which suggests a more general mechanism may be involved. In this case, a more subtle effect on protein conformation might inhibit the process of SUMO conjugation, leading to loss of TBX22 function and the resulting CP phenotype. The recruitment of transcriptional co-factors by SUMO and/or the modified protein seems a likely mechanism, although SUMO interacting motifs (SIMs) haven't been identified in the TBX22 protein yet. This may affect the remodelling of the chromatin structure, resulting in loss of transcriptional repression. These proposed mechanisms might also explain why a low-level sumoylation can be sufficient (Geiss-Friedlander and Melchior 2007).

19.4 Sumo in Developmental Pathways and Syndromes

The importance of SUMO1 for normal craniofacial development in addition to lip and palate formation has also been demonstrated through effects both on specific genes and signalling pathways, For example, the *Xenopus* SUMO1 (XSUMO-1) specific knockdown, using a morpholino antisense oligonucleotide, showed a striking effect, significantly decreasing body axis

formation and causing microcephaly (Yukita et al. 2007). These results appeared to be associated with an inhibitory effect on activin/nodal signalling since injection of XSUMO-1-MO suppressed expression of activin-response genes such as *Xbra, XGoosecoid* and *Chordin*. Meanwhile the observed down regulation was clearly rescued by *myc-XSUMO-1* mRNA. Goosecoid (Gsc) has itself been identified as post-translationally modified by SUMO in mice, (Izzi et al. 2008), while it is known to be essential for the development of mesenchymal-derived craniofacial tissues, with its deletion mainly causing skeletal defects (Rivera-Perez et al. 1999).

The Wnt pathway is essential for correct migration of cranial neural crest cells during development. Wnt signalling molecules Axin, LEF1 and Tcf4 are all modified by Sumo, suggesting that Wnt signal transduction is directly regulated by sumoylation (Rui et al. 2002; Sachdev et al. 2001; Yamamoto et al. 2003). Axin, which acts as a scaffold protein in the canonical Wnt signaling, effectively downregulates β-catenin but fails to activate JNK when mutated at the SUMO attachment site (Rui et al. 2002). The Wnt activated transcription factors LEF1 and Tcf4 are oppositely affected, with sumoylation of LEF1 inhibiting its transcription activity, while sumoylation of Tcf4 promotes it (Sachdev et al. 2001; Yamamoto et al. 2003). More recently, over expression of the SUMO-specific protease XSENP1 was found to cause head defects in *Xenopus* embryos as a consequence of suppressing Wnt signaling (Yukita et al. 2004).

The process of sumoylation also plays an important role in the regulation of Tgfβ signalling and includes both Smad3 and Smad4 as direct targets (Lin et al. 2003). Ubc9 is known to promote the stability of Smad4 and the nuclear accumulation of Smad1 in osteoblast-like Saos-2 cells (Lin et al. 2003; Shimada et al. 2008) with overexpression of E3 ligases upregulating Smad4- or TGFβ -mediated transcriptional activity (Lin et al. 2003; Long et al. 2004; Liang et al. 2004). SUMO1 conjugation of Smad4 also recruits the binding of the transcriptional corepressor, Daxx through its SIM, which downregulates its transcriptional activity (Chang et al. 2005). In *Xenopus*, XPIASy interacts with XSmad2, which enhances its sumoylation, and suppresses its activity required for proper mesoderm induction (Daniels et al. 2004). These findings together suggested that sumoylation of Smads is important for mesoderm formation in *Xenopus* development. The oncoproteins, c-Ski and related SnoN potently repress Tgfβ signaling through interaction with Smads. Their overexpression can result in the induction of skeletal muscle differentiation. SnoN is now also known to be sumoylated (Hsu et al. 2006; Wrighton et al. 2007). However, SUMO modification itself does not alter its ability to repress Tgfβ signaling, instead, it is loss of sumoylation that activates muscle-specific gene expression. Sumoylation of the TGFβ receptor, TGFbRI, meanwhile, controls responsiveness to TGFβ (Kang et al. 2008), with implications for tumor progression, although its role in embryonic development is yet to be investigated.

There are also a number of other human syndromes with a craniofacial involvement that involve sumoylated proteins. *TRPS1*, named after tricho-rhino-phalangeal syndrome (TRPS) is also a transcriptional repressor whose function depends on sumoylation (Kaiser et al. 2007). Mutations in *TRPS1* result in characteristic skeletal and craniofacial malformations including a bulbous nose tip and a long and flat philtrum (Momeni et al. 2000). Mice that are heterozygous for deletions of the *Trps1* GATA-DNA binding domain display facial abnormalities that overlap with those seen in human patients, and consistently have a high-arched palate (Malik et al. 2002).

Several members of the Sox protein family are sumoylated and also function in craniofacial development include Sox2 which is important in eye development and can result in anophthalmia (Tsuruzoe et al. 2006), Sox9 and Sox10, which are both important for neural crest migration and inner ear development (Taylor and Labonne 2005).

The DNA methyltransferase 3B (*DNMT*DNMTs*3B*) gene is mutated in

immunodeficiency, centromere instability, and facial anomalies (ICF) syndrome (Xu et al. 1999a; Hansen et al. 1999). It has been demonstrated that Dnmt3b is post translationally modified by SUMO1 (Kang et al. 2001). Most reported ICF mutations of DNMT3B are missense changes in the C-terminal region, which directly reduce enzymatic activity, however, one exception is the S270P mutation, which has been shown to abrogate SUMO1 attachment (Park et al. 2008). It appears that S270 is important for a non-covalent interaction with SUMO1 and is also the location for interaction with the E3 ligase, PIAS1. Interestingly, the interactions between DNMT3B and either PIAS1 or SUMO1 are inversely affected by increasing concentrations of H_2O_2 treatment, emulating conditions of oxidative stress.

19.5 Sumo, Stress, and CL/P

An environmental component to orofacial clefts has long been recognised with an estimated 50–75% of cases having no recognisable familial history, and monozygotic twins are only concordant for the phenotype approximately 40% of the time (Murray 2002; Wyszynski et al. 1996). It is clear that although the interactions between genes and the environment that are crucial in CL/P development remain elusive, they do converge on the same developmental pathways (Chakravarti and Little 2003). Environmental risk factors thought to play a role in NSCL/P include maternal alcohol use and smoking, whereas exposure to environmental toxins, such as dioxin, folic acid deficiency and increased vitamin A intake during pregnancy have also been suggested to induce syndromic craniofacial abnormalities such as CL/P (Murray 2002). Among these, a study on the effects of maternal smoking in 1244 cleft patients supported a role for genetic-environmental interactions in the pathogenesis of CL/P and suggested that detoxification gene variants were possible risk factors (Shi et al. 2007).

Interestingly, the process of SUMO modification is known to be susceptible to environmental effects that are strikingly similar to some of the risk factors described for orofacial clefts. These include stresses such as heat shock, osmotic and oxidative stress conditions and viral infection, which all trigger changes to the cellular SUMO1 conjugation/deconjugation pathway (Bossis and Melchior 2006; Tempe et al. 2008). Severe oxidative stress is usually associated with an increase in SUMO1 conjugation but lower, more physiologically relevant concentrations of free radicals induce an almost complete loss of SUMO1 modification of target proteins (Bossis and Melchior 2006). A study into the stress response of the transcription factor c-Myb shows that SUMO2/3, rather than SUMO1 conjugation can rapidly inactivate the transcriptional activity of the SUMO target (Sramko et al. 2006). Although SUMO isoforms are similar, it is not clear whether SUMO1 and SUMO2/3 respond similarly to stress within cells. There appears to be a developing link resulting from the interrelationships of environmental stresses with both SUMO and CL/P risk. The finding that several genetic risk factors are regulated by SUMO modification, suggests that further investigation is warranted. This might initially focus on a destabilisation of the normal balance of expression and activity for genes such as *TBX22*, *MSX1*, *SATB2* and *TP63* during early pregnancy that might provide a high-risk environment for CL/P occurrence.

19.6 Conclusions

As described in this chapter and elsewhere in this book, sumoylation is required for many cellular functions. From a developmental perspective, evidence suggests that formation of various craniofacial structures, especially the upper lip and palate are sensitive to varying SUMO1 levels. Moreover, the efficiency of normal SUMO modification in response to local oxidative and osmotic conditions or infection status suggest a potential explanation as to how environmental factors may impact on this birth defect risk. These responses will need to be much more thoroughly investigated, starting with cell based systems and animal models. It is not clear why proteins involved

in craniofacial development are predominantly modified by SUMO1, as opposed to SUMO2/3 has also not yet been addressed, especially since all of these SUMO paralogs are ubiquitously expressed. As demonstrated for the SUMO1 knockout, SUMO2/3 do seem to be able to rescue the phenotype, at least in some circumstances (Zhang et al. 2008; Evdokimov et al. 2008). It is not yet known if these paralogs regularly share targets with SUMO1 or if there is a level of redundancy built in to act as a buffer against catastrophic developmental aberration. Another alternative explanation for discrepancies reported in different animal studies may include local exposure to stress factors such as pathogen load. Global analyses of sumoylated proteins at different stages and sites of development and under different environmental conditions can be used to investigate such effects. Nevertheless, taken together with current evidence from a variety of genes and networks, the process of SUMO protein modification can be seen to play an important role in fine-tuning developmental events required for normal craniofacial morphogenesis. Given the dependency on the SUMO pathway during development, it is likely that we will see future research investigating the regulation of SUMO pathway enzymes as a means of delivering therapeutic and preventative treatments, potentially targeting craniofacial defects specifically.

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