Reciprocal skeletal phenotypes of PRC2-related overgrowth and Rubinstein–Taybi syndromes: potential role of H3K27 modifications

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Abstract Within histone H3, lysine 27 (H3K27) is one of the residues that functions as a molecular switch, by virtue of being subject to mutually exclusive post-translational modifications that have reciprocal effects on gene expression. Whereas acetylation of H3K27 is associated with transcriptional activation, methylation at this residue causes transcriptional silencing; these two modifications are mutually exclusive. Establishment of these epigenetic marks is important in defining cellular identity and for maintaining normal cell function, as evidenced by rare genetic disorders of epigenetic writers involved in H3K27 post-translational modification. Polycomb repressive complex (PRC2)-related overgrowth and Rubinstein–Taybi syndrome (RSTS) are respectively associated with impaired H3K27 methylation and acetylation. Whereas these syndromes share commonalities like intellectual disability and susceptibility to cancers, they are generally divergent in their skeletal growth phenotypes, potentially through dysregulation of their opposing H3K27 writer functions. In this review, we discuss the requirement of H3K27 modifications for successful embryogenesis, highlighting data from relevant mouse knockout studies. Although such gene ablation studies are integral for defining fundamental biological roles of methyl- and acetyltransferase function in vivo, studies of partial loss-of-function models are likely to yield more meaningful translational insight into progression of PRC2-related overgrowth or RSTS. Thus, modeling of rare human PRC2-related overgrowth and RSTS variants in mice is needed to fully understand the causative role of aberrant H3K27 modification in the pathophysiology of these syndromes.

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INTRODUCTION

Although the body's somatic cells typically share the same genome, a plurality of cell types is required to support diverse biological functions. Such diversity demands specialization in the ways the genome is put to use. Successful histogenesis requires the coordinated activation and suppression of lineage-specific genes. To achieve this, cells modify their chromatin structure to allow or restrict these critical loci from transcriptional access. This process involves histones, DNA methylation, and noncoding RNA species (to name a few participants). In particular, core histone proteins undergo post-translational modifications (PTMs) at several key lysine residues; the relative importance of specific PTMs at specific sites is an area of active investigation.

Histone octamers are composed of two copies each of H2A, H2B, H3, and H4. When bound to DNA, they form nucleosomes, the fundamental unit of chromatin. A number

of histone PTMs alter chromatin state, including phosphorylation, ubiquitination, SUMOylation, ADP ribosylation, deamination, proline isomerization, methylation, and acetylation (Goyal et al. 2019). The combinatorial profile of these histone PTMs is the theoretical basis of the histone code (Strahl and Allis 2000), whereby their presence is linked to distinct biological events (Munshi et al. 2009). Lysine 27 of H3 appears to be a key molecular switch, because either an acetyl group or one or more methyl groups may be attached to it (Bedford et al. 2010; Deevy and Bracken 2019; Sneppen and Ringrose 2019). Acetyl and methyl groups are mutually exclusive at this residue, yet are also associated with opposite transcriptional outcomes. Specifically, addition of an acetyl group to H3K27 (H3K27ac) neutralizes positively charged amino-terminal lysine residues (Kalkhoven 2004), allowing chromatin to relax. Such loosening of DNA–histone and histone–histone contacts results in a more open conformation that permits transcription factor binding and transcriptional activation (Roth et al. 2001). Conversely, H3K27 can be mono-, di-, and trimethylated (H3K27me1–3), which enables tighter chromatin packing and transcriptional silencing (Schuettengruber et al. 2017). Deposition and maintenance of H3K27ac and H3K27me1–3 is controlled by key epigenetic writers and erasers that lay down or remove these moieties in a cell- and context-specific manner. Not surprisingly, functional variants in the enzymes responsible for regulating the H3K27ac mark, or H3K27me1–3 marks, have significant consequences for cells, tissues, and the whole organism, as evidenced by rare diseases associated with mutations of H3K27 writers and erasers. In this review, we focus on the role(s) of the main regulators of H3K27 acetylation and methylation during development, and how mutations that affect these epigenetic writers result in somewhat reciprocal disease phenotypes.

REGULATORS OF THE H3K27 TRANSCRIPTIONAL SWITCH

The two major groups of proteins that have opposing writer functions at H3K27 are Polycomb group (PcG) and CBP/EP300. Readers are referred to the excellent review by Schuettengruber et al. (2017) for a detailed discussion of all PcG components. Although an appropriate balance of H3K27 methylation and acetylation also requires demethylases and deacetylases that erase these histone marks, our focus here is on the writers at H3K27, specifically the Polycomb repressive complex (PRC2) and p300/CBP.

PRC2 Components and General Function

Multicellular organisms have PRC1 and PRC2, both of which control gene silencing despite having different histone substrates: PRC1 monoubiquitinates H2AK119, whereas PRC2 mono-, di-, and trimethylates H3K27 (Yu et al. 2019). Ultimately, their activity causes chromatin compaction, restricting the physical access of transcriptional machinery to target genes and preventing their expression. PRC2 reader and writer functions enable the inheritance of H3K27me1–3 during cell division; stable transmission of histone marks is required during lineage specification and maintenance, X-chromosome inactivation, genomic imprinting, and segmental patterning during embryogenesis (Wang et al. 2001; Cao et al. 2002; Plath et al. 2003; Silva et al. 2003; Lewis et al. 2004; Ringrose and Paro 2004). The PRC2 core complex is composed of enhancer of zeste (EZH2), embryonic ectoderm development (EED), suppressor of zeste (SUZ12), and retinoblastoma binding protein (RBBP4/7). Furthermore, PRC2 interacts with a combination of accessory proteins, including AE binding protein 2 (AEBP2), jumonji and AT-rich interaction domain containing 2 (JARID2), elongin BC and PRC2-associated protein (EPOP), and Polycomb-like proteins (PCLs) (Schuettengruber et al. 2017; Deevy and Bracken 2019), forming PRC2 subcomplexes (i.e., PRC2.1 and PRC2.2).

Chromatin binding and enzyme activity of PRC2 is conferred by the unique roles of its core components. EZH2 contains both a SANT domain, allowing it to bind histones, and a catalytic SET domain, containing its H3K27 methyltransferase activity (Laible et al. 1997). SUZ12 is a zinc finger protein responsible for both DNA and RNA binding, whereas EED performs PRC2's reader function by recognizing methylated H3K27 residues (Schuettengruber et al. 2017). Although EZH2 is PRC2's catalytic subunit, both EED and SUZ12 are important for maintaining PRC2 integrity and enzyme function (Pasini et al. 2004; Montgomery et al. 2007).

Each PRC2 core component is required for mammalian embryogenesis, as global loss of any one of them in mice results in embryonic lethality (Faust et al. 1995; O'Carroll et al. 2001; Pasini et al. 2004). Consistent with impaired cellular proliferation, homozygous-null embryos are smaller in size than wild-type embryos and do not survive past midgestation (Faust et al. 1995; O'Carroll et al. 2001; Cao and Zhang 2004; Pasini et al. 2004). Classically, PcG components were described in *Drosophila* as suppressors of the homeotic *HOX* gene cluster, which control body segmentation and morphology during embryogenesis (Lewis 1978; Pirrotta 1998). PRC2 has retained conserved functions across phyla, binding to genes involved in development, morphogenesis, organogenesis, and neurogenesis in both human and murine embryonic stem cells (Boyer et al. 2006; Bracken et al. 2006; Lee et al. 2006). Many of the pathways suppressed by PRC2 are conserved between fruit flies, mice, and humans, including genes important for Wnt, transforming growth factor-β, fibroblast growth factor, Notch, and Hedgehog signaling (Bracken et al. 2006). Given the number of developmental pathways regulated by PRC2, the mechanisms of dysfunctional embryogenesis in PRC2 mutant models described above are diverse and multifaceted. Although global knockout of core PRC2 components is lethal in mice, global "knockdown" causes a range of phenotypes in humans, as evidenced by the range of severities attributable to partial loss-of-function (LoF) mutations ascertained in human patients.

Disorders of H3K27 Methylation: PRC2-Related Overgrowth Syndromes

PRC2-related overgrowth syndromes (Table 1) consist of Weaver syndrome (OMIM #277590), Cohen–Gibson syndrome (OMIM #617561), and *SUZ12*-related overgrowth, the latter of which is the least well-characterized of the three. All are autosomal dominant disorders, although most cases are observed sporadically, without prior family history. Weaver

Table 1. Rare disorders of H3K27 post-translational modifications summary

Parameter	PRC2-related overgrowth	Rubinstein–Taybi syndrome
Rare disease subtypes (gene)	Weaver syndrome (*EZH2*), Cohen–Gibson syndrome (*EED*), *SUZ12*-related overgrowth (*SUZ12*)	RSTS1 (*CREBBP*), RSTS2 (*EP300*)
Canonical histone modification affecting transcription	H3K27me1–3	H3K27ac
Putative mechanism	Partial loss of function	Haploinsufficiency
General skeletal phenotype	Tall stature/macrocephaly, advanced bone age	Short stature, broad thumbs/halluces
Intellectual disability	Mild to severe	Mild to severe
Cancer predisposition	Acute myeloid leukemia, acute lymphoblastic leukemia, neuroblastoma	Diffuse large B-cell lymphoma, neuroblastoma, rhabdomyosarcoma, medulloblastoma, meningiomas, pilomatrixomas

syndrome is caused by pathogenic variants in *EZH2* (Tatton-Brown et al. 2011; Gibson et al. 2012), resulting in partial LoF of PRC2 methyltransferase activity (Cohen et al. 2016; Lui et al. 2018). All *EZH2* mutations currently described to cause Weaver syndrome are either missense variants or truncating variants of the last exon (Tatton-Brown et al. 2018). Rare de novo mutations in *EED* were recently reported to cause Cohen–Gibson syndrome (Cohen et al. 2015; Cohen and Gibson 2016), whereas *SUZ12*-related overgrowth (also referred to as Weaver-like syndrome) is caused by rare variants in its namesake gene (Imagawa et al. 2017; Imagawa et al. 2018; Cyrus et al. 2019). Similar to Weaver syndrome, reported mutations in *EED* and *SUZ12* impair H3K27me3 formation (Imagawa et al. 2017), in line with the role these core components play in stabilizing PRC2 enzyme function.

PRC2-related overgrowth syndromes present with accelerated pre- and postnatal growth (i.e., tall stature, macrocephaly), advanced bone age, distinctive facial features, and mild-to-severe intellectual disability. Whereas many of these features are common to all three, phenotypic differences do exist between Weaver syndrome, Cohen–Gibson syndrome, and *SUZ12*-related overgrowth; these differences are succinctly reviewed elsewhere for interested readers (Cyrus et al. 2019). It is challenging to reconcile pathophysiological insight from studies globally knocking out core PRC2 components in mice with their respective human overgrowth syndromes, because PRC2 knockout animals are typically not viable and invariably small, not large. Furthermore, conditional knockout of PRC2 function has brought forth some controversy regarding the cellular mechanism causing overgrowth. For example, loss of either *Ezh2* or *Eed* from murine chondrocytes severely retards skeletal growth by impairing cellular proliferation and hypertrophy (Lui et al. 2016; Mirzamohammadi et al. 2016). Although these studies have led some to suggest Weaver syndrome mutations cause skeletal overgrowth by a gain-of-function mechanism (Lui et al. 2016), this does not appear to be the case, because Weaver mutations display impaired methyltransferase activity in vitro and in vivo (Cohen et al. 2016; Lui et al. 2018).

Recently, Lui et al. (2018) made a Weaver syndrome mouse harboring a patient-derived c.1876G > A (p.V626M) missense mutation. Consistent with other variants, p.V626M was shown to be a partial LoF mutant, with heterozygous and homozygous embryos showing a dose-dependent reduction of H3K27me2-3 (Lui et al. 2018). Although homozygous Weaver syndrome mice were not viable, heterozygous mice survived and were moderately heavier than controls as they aged (Lui et al. 2018). Surprisingly, the overgrowth of the Weaver mice appears to be the result of organomegaly and not the skeletal overgrowth typical of Weaver syndrome patients (Lui et al. 2018). However, organomegaly was not initially reported in their patient, so it is currently unclear why p.V626M results in pathophysiological differences between mice and human. To our knowledge, mice harboring human mutations in *Eed* and *Suz12* have yet to be reported.

So why then does impaired PRC2 function cause a pronounced overgrowth phenotype only in humans? Ultimately, the answer may relate to the degree to which H3K27 methylation is disrupted and to the specific loci missing this histone mark. Mice missing just one copy of core PRC2 components may experience catastrophic growth failure because H3K27 methylation is missing at genes integral for maintaining cell identity, viability, and survival. Alternatively, a variety of genes that promote cellular differentiation may fail to be suppressed in the absence of sufficient H3K27 methylation. These mechanisms may account for the predisposition to hematological malignancies that is observed in Weaver syndrome (Table 1). The extent to which constitutional mutations in *EED* and *SUZ12* might predispose to hematological or other malignancies remains to be clarified.

The degree to which loss of function occurs in vivo for PRC2-related overgrowth syndromes (other than p.V626M) is unclear. Although a 50% reduction in PRC2 function might be lethal to most mice, partial loss of function from a missense allele might cause a redistribution of PRC2 across the genome, allowing maintenance of cell-specific identity at the

expense of depressing pathways controlling cell expansion and size. We imagine that complete LoF mutations in human PRC2 components are not compatible with life, because stop-gain mutations near the amino terminus have not been reported to date, although some patients with deletions that include one copy of EZH2 have been reported. Given that there is a reciprocal nature between H3K27 methylation and acetylation (Deevy and Bracken 2019), future studies modeling PRC2 variants should consider which genes missing H3K27me3 at their promoters subsequently acquire the acetylation mark.

p300 and CBP Are H3K27 Acetyltransferases

Acetylation of H3K27 is associated with transcriptional activation because it loosens DNA contacts from core histones, thereby enabling basal transcriptional machinery to interact with gene promoters. Although a number of histone acetyltransferases (HATs) exist with differing histone and protein substrate specificities, the ubiquitously expressed transcriptional coactivators CREB-binding protein (CBP) and p300 are responsible for writing H3K27ac (Jin et al. 2011; Lasko et al. 2017). CBP and p300 belong to the KAT3 family of HATs and are believed to be functionally homologous because they share ~57% structural similarity at the protein level, with 88% sequence homology between their HAT domains (Bedford et al. 2010; Lipinski et al. 2019).

CBP and p300 integrate signals from numerous biological pathways. They can physically interact with more than 400 different proteins, many of which are transcription factors (Bedford et al. 2010). Although CBP and p300 play a major role in chromatin remodeling by writing H3K27ac, recently H2B has also been shown to be a major target of their HAT activity, as have thousands of different sites on nonhistone proteins (Weinert et al. 2018), suggesting they are a control node for enzymes and transcription factors. Thus, CBP and p300 protein scaffolding and acetyltransferase functions coordinate biological programs with a stunning level of complexity, operating within a diversity of cell types (Bedford et al. 2010; Dyson and Wright 2016).

Mutations in EP300 and CREBBP Cause Rubinstein–Taybi Syndrome

Both p300 and CBP are integral for proper growth and development, as evident by the rare autosomal dominant disorder Rubinstein–Taybi syndrome (RSTS) (Table 1). RSTS affects 1:100,000 to 1:125,000 live births (Stevens 1993) and is classified as either RSTS1 (OMIM #180849) or RSTS2 (OMIM #613684) resulting from heterozygous pathogenic variants in either the *CREBBP* or *EP300* genes, respectively. RSTS is characterized by distinctive facial features, broad thumbs and halluces, and mild-to-severe intellectual disability, but unlike PRC2-related overgrowth syndromes, RSTS patients are typically short in stature (Stevens 1993). At some level, p300/CBP can be considered to act as tumor suppressors, because instances of both benign and malignant tumors have been reported in RSTS patients (Boot et al. 2018). Based on the increased incidence of tumors in RSTS patients, we can infer that not only are sufficient quantities of acetyl marks on H3K27, H2B, and other nonhistone proteins required during fetal development, but maintenance of these PTMs is required throughout postnatal life for cells to retain a fully differentiated and functional adult state.

As was found with deletion of PRC2 members, global deletion of p300 or CBP individually in mice causes lethality midgestation (Yao et al. 1998; Tanaka et al. 2000). These embryos are severely growth-retarded and show signs of defective neural tube closure, exencephaly, cardiac anomalies, and brain hemorrhaging. Importantly, these findings indicate that p300 and CBP function are not completely redundant, and that each HAT governs distinctive pathways critical for embryogenesis. One reason for this is that p300 and CBP have overlapping but distinct expression profiles throughout stages of mouse embryogenesis (Yao et al. 1998;

Bhattacherjee et al. 2009). Thus, CBP and p300 control a unique set of genes and nonhistone PTMs that are both spatially and temporally restricted. Furthermore, mice heterozygous for both *Crebbp* and *Ep300* display a similar embryologic phenotype to global single knockout embryos (Yao et al. 1998), suggesting that a full genomic complement of these HATs are essential for normal development.

Despite the early lethality caused by p300 or CBP deletion in mice, haploinsufficient mouse models are viable; however, they do have marked growth and morphological phenotypes (Tanaka et al. 1997, 2000; Oike et al. 1999). Initially, mice with a single copy of *Crebbp* were reported as smaller and recapitulated several features reported in patients with RSTS (albeit with variable penetrance), including enlarged anterior fontanels and abnormal skeletal patterning, but without hallmark RSTS features like broad first phalanges (Tanaka et al. 1997). Similarly, mice carrying one copy of a truncated form of CBP showed pre- and postnatal growth retardation, distinctive facial features (e.g., broad nasal bridge, short nose), and frequent cardiac anomalies (Oike et al. 1999).

To our knowledge, targeted deletion studies of p300 or CBP from mouse chondrocytes or osteoblasts in vivo (similar to those described above for PRC2 core members) have not been done. However, a number of in vitro and cell-based studies have shown p300/CBP are important for enhancing the transcriptional activity and/or gene expression of regulators of chondrocyte and osteoblast differentiation, such as Sox9 (Tsuda et al. 2003; Furumatsu et al. 2005; Imamura et al. 2005), mammalian Runt domain protein 2/core binding factor a1 (Sierra et al. 2003), and cartilage homeoprotein-1 (Iioka et al. 2003).

Determining the unique p300 and CBP-dependent genes and nonhistone PTMs responsible for hallmark features of RSTS will prove to be difficult. In addition to being associated with active gene promoters, H3K27ac in particular is a feature of active enhancers, enabling the occupancy of transcription factors at unique noncoding regions to control target genes via long-range chromatin interactions. Using p300 chromatin immunoprecipitation followed by sequencing (ChIP-seq) to predict tissue-specific enhancers, thousands of p300-binding sites have been identified in murine limb buds and neural tissues at midgestation alone (Visel et al. 2009). Determining the functional relevance of these regions in vivo with respect to normal growth and development will require targeted deletion followed by deep phenotyping. Although two enhancers (i.e., *M280* and *M1442*) previously identified by Visel et al. (2009) were predicted to affect limb development, neither of them were obligatory for proper limb formation and morphology, although loss of *M280* resulted in smaller mice (Nolte et al. 2014); this finding is in line with the generalized growth retardation of RSTS. It remains to be seen which genes *M280* regulates, which tissues it is important for forming/maintaining, and whether this enhancer is truly dependent on p300's HAT function. Thus, although CBP and p300 ChIP-seq will yield valuable information regarding the unique subsets of gene promoters that depend on their HAT function, we must also consider the role of CBP and p300 in forming active enhancers. Furthermore, future studies examining RSTS pathophysiology should also consider the role of H2B hypoacetylation and p300/CBP's various other nonhistone substrates.

Although the function of p300/CBP as coactivators of numerous signaling proteins makes it difficult to deconvolute the various role of H3K27ac in the molecular etiology of RSTS, some studies have attempted to overcome this by developing mice carrying site-specific mutations, such as those removing CBP HAT function while preserving its protein-binding domains (Korzus et al. 2004). Selective loss of HAT activity from forebrain neurons impairs the ability of these mice to form long-term memory (Korzus et al. 2004), a finding consistent with studies of both haploinsufficient RSTS mouse models (Oike et al. 1999; Alarcon et al. 2004) and total loss of CBP coactivator function from forebrain principal neurons (Valor et al. 2011). Together, these studies may directly implicate impaired chromatin remodeling in causing aspects of intellectual disability seen in RSTS.

Epigenetic Therapies Affecting H3K27 Modifications

Knowledge of the molecular mechanisms behind rare human genetic disorders is often presented as a necessary step toward targeted therapies to improve outcomes. However, the geographic dispersal of the patients makes case accrual and standardized medical assessment difficult. Similarly, the length of time required to observe a measurable difference in growth velocity and/or neurodevelopment makes proper placebo-controlled trials (even crossover trials) challenging, to say the least. It is likely, then, that the first personalized therapies for PRC2-related overgrowth and Rubinstein–Taybi syndrome will appear in the context of personalized oncogenomics. Such a scenario is likely to arise when treating physicians wish to make use of the prior knowledge of the underlying syndrome (e.g., Weaver syndrome or RSTS) to make educated additions to standardized treatment regimens for whatever neoplasms might arise in these patients.

Although EZH2 inhibitors are in development for a variety of indications, it seems unlikely that these drugs would confer obvious benefit to a patient with Weaver syndrome, because the preexisting partial loss of H3K27 methyltransferase activity would be exacerbated by an EZH2 inhibitor, rather than mitigated by it.

With respect to tumors or leukemias arising in RSTS patients, some clinical traction may be gained in considering the use of histone deacetylase (HDAC) inhibitors such as valproic acid (Phiel et al. 2001). The rationale for this indirect approach would involve the inhibition of histone deacetylases, in an attempt to preserve H3K27ac marks (and acetylation of other histone/nonhistone residues) that were already diminished by the preexisting reduction in HAT activity. Although speculative, the reciprocal nature of H3K27 methyl and acetyl marks may warrant consideration of HAT inhibition for Weaver syndrome in an attempt to dampen genes derepressed by impaired methyltransferase activity. Conversely, PRC2 inhibition may "lift the break" on loci indirectly suppressed in the absence of sufficient p300/CBP activity. Recently, the compound A-485 has been described as a highly selective p300/CBP HAT inhibitor capable of impairing H3K27/18ac formation along with proliferation of various solid state and hematological cancer-cell lines (Lasko et al. 2017; Michaelides et al. 2018). Whereas A-485 may hold therapeutic relevance in treating some malignancies, it will likely also prove useful in cell-based assays aimed at defining the specific genes regulated by p300/CBP HAT function that are causative of RSTS phenotypes. It may also be valuable for proof-of-concept studies designed to prevent excessive H3K27ac in the context of PRC2 LoF. Given that preclinical mouse models are expensive to generate, patient-derived induced pluripotent stem cells (iPSCs) will serve as an important model system to assess mutation-specific changes in cellular function and also as a test of the mechanisms that might be engaged by HDAC therapies. To date, only a few studies have generated iPCS-derived neurons from RSTS1 and RSTS2 patients (Alari et al. 2018a,b), so this represents a fruitful area to study the pathophysiology of aberrant H3K27ac deposition.

CONCLUSION

Proper embryogenesis and postnatal growth is dependent on reciprocal modifications to H3K27, which are catalyzed by PRC2 and p300/CBP. The importance of H3K27 as a molecular switch is evidenced by PRC2-related overgrowth disorders and Rubinstein–Taybi syndrome. These rare diseases have opposing skeletal growth phenotypes, which may be associated with impairments in their underlying epigenetic writers to properly silence or activate gene transcription, respectively. Although mouse knockout studies have revealed the requirement of PRC2 core components and p300/CBP for development, few have managed to accurately model their respective human syndromes. Although species-specific differences in PRC2 or HAT requirements may explain this, it is likely that even a 50% reduction in

their enzyme function is catastrophic for cellular viability in mice, preventing detailed studies throughout their lifespan. Instead, partial LoF variants may allow a redistribution of these epigenetic writers across the genome and proteome to maintain control of pathways governing cell survival at the expense of those regulating proliferation and size. Given their well-established role in gene expression, it will be of interest going forward to determine which gene sets depleted of H3K27ac or H3K27me1–3 become reciprocally repressed or activated in order to determine the direct and indirect effects PRC2 or p300/CBP mutations.

ADDITIONAL INFORMATION

Author Contributions

Both D.G. and W.T.G. conceived and wrote the manuscript.

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