Covalent Modifications of RUNX Proteins: Structure Affects Function

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Abstract

The RUNX family of transcription factors plays important roles in tissue-specific gene expression. Many of their functions depend on specific post-translational modifications (PTMs), and in this review, we describe how PTMs govern RUNX DNA binding, transcriptional activity, protein stability, cellular localization, and protein-protein interactions. We also report how these processes can be disrupted in disease settings. Finally, we describe how alterations of RUNX1, or the enzymes that catalyze its post-translational modifications, contribute to hematopoietic malignancies.

Keywords

RUNX1 • RUNX2 • RUNX3 • CBFβ • Post-Translational Modifications • Acetylation • Methylation • Phosphorylation • Ubiquitylation • Transcriptional Activation • Transcriptional Repression • Acute Myeloid Leukemia • FPD/AML • Cleidocranial Dysplasia

3.1 Contents of the Chapter (Including 1 Figure and 1 Table)

Post-translational modifications (PTMs) are key regulators of RUNX protein function. Together these PTMs govern the transcriptional activity of RUNX proteins by modulating their DNA binding, protein stability, cellular localization, and protein-protein interactions. The sensitivity of RUNX proteins to subtle changes in these properties allows extracellular signals to influence stem cell, progenitor cell, and differentiated cell biology. Furthermore, RUNX protein modifications help create scaffolds that facilitate

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the recruitment of proteins that either promote or inhibit transcription. Among RUNX proteins, the functional loss of RUNX1 activity in the myeloid malignancies, driven by genomic alterations, indicates its critical role as a suppressor of myeloid transformation. In this section, we describe how PTMs affect RUNX functions in normal and malignant cells.

3.2 RUNX1 Post-translational Modifications in Transcriptional Activation

Lineage specific gene expression requires the complex interplay between transcription factors with DNA binding ability and histone modifying enzymes (Fig. 3.1, Table 3.1). The transcription factor RUNX1 associates with chromatin modifiers, cofactors, and other transcription factors at the regulatory regions of target genes critical for myeloid and lymphoid differentiation, such as *SPI1* Zhao et al. (2008) and *EBF1* Seo et al. (2012). Genetic alterations involving *RUNX1* are common in acute myeloid leukemia (AML), occurring in approximately 15% of patients (The Cancer Genome Atlas Research Network 2013). They include point mutations, truncating mutations, amplifications, and chromosomal translocations that generate fusion proteins, most commonly RUNX1-ETO, also known as AML1-ETO. RUNX1 binds DNA with high affinity only when bound to its heterodimeric protein partner CBFβ, which is encoded by a gene that is also

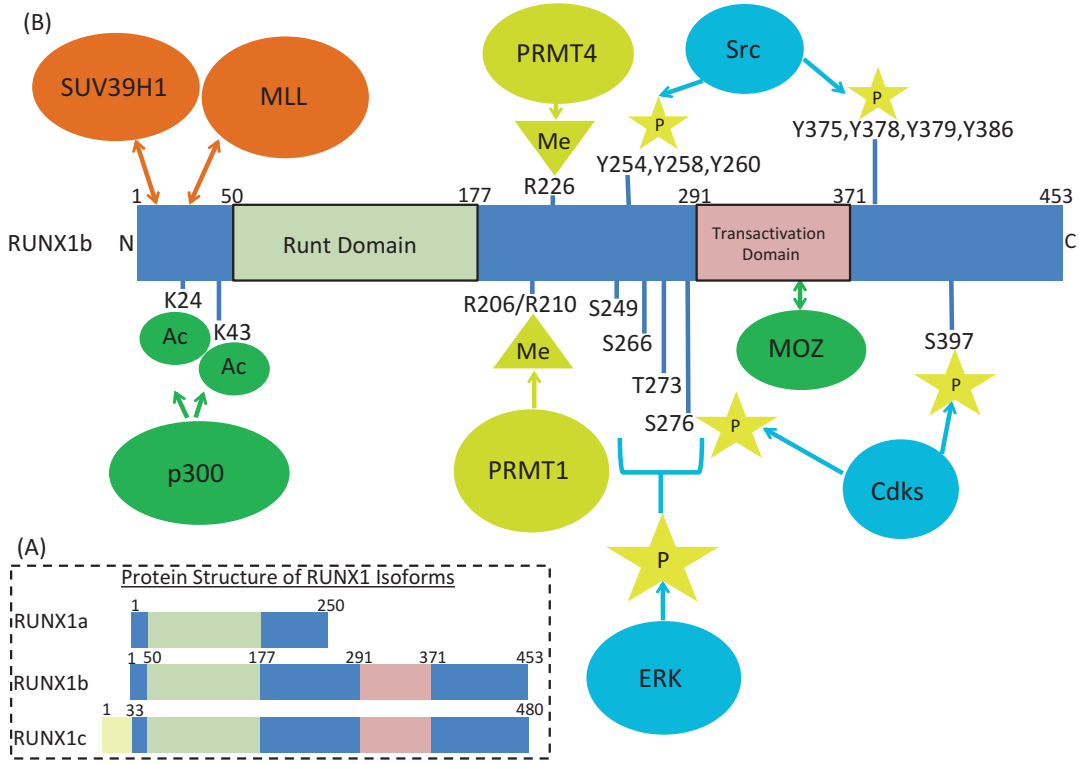

Fig. 3.1 (**a**) Protein structure of RUNX1 isoforms. The DNA-binding Runt domain (*light green*) is conserved across all RUNX1 isoforms. The transactivation domain (*red*) is found only in RUNX1b and RUNX1c. (**b**) RUNX1b: selected protein interactions and post-translational modifications. Kinases (*light blue*) interact with the RUNX1 C-terminus following cytokine stimulation and regulate cell cycle progression. The lysine acetyltransferases p300 and MOZ (*green*) generally promote transcriptional activation by RUNX1. Arginine methyltransferases and lysine methyltransferases (mustard and orange, respectively) both stimulate and inhibit transcription by RUNX1 and play important roles in hematopoietic differentiation

Table 3.1 List of RUNX interacting proteins

Protein	Interacting partner	Function	Reference
RUNX1	p300/CBP	Lysine Acetyltransferase	Yamaguchi et al. (2004)
	MOZ	Lysine Acetyltransferase	Kitabayashi et al. (2001)
	MLL	Lysine Methyltransferase	Huang et al. (2011), Koh et al. (2013)
	PRMT1	Arginine Methyltransferase	Zhao et al. (2008)
	PRMT4	Arginine Methyltransferase	Vu et al. (2013)
	PRMT6	Arginine Methyltransferase	Reed-Inderbitzin et al. (2006)
	SUV39H1	Lysine Methyltransferase	Herglotz et al. (2013) and Chakraborty et al. (2003)
	ERK	Kinase	Tanaka et al. (1996), Zhang et al. (2004) and Yoshimi et al. (2012)
	CDKs	Kinase	Biggs et al. (2006) and Zhang et al. (2008)
	PIM1	Kinase	Aho et al. (2006)
	HIPK2	Kinase	Wee et al. (2008) and Aikawa et al. (2006)
	IKK	Kinase	Nakagawa et al. (2011)
	HDACs	Deacetylase	Guo and Friedman (2011), Lutterbach et al. (2000) and Chakraborty et al. (2003)
	Sin3a	Transcriptional Regulator	Zhao et al. (2008), Imai et al. (2004) and Lutterbach et al. (2000)
	Groucho	Transcriptional Regulator	Lutterbach et al. (2000)
	BMI1	Polycomb Protein	Yu et al. (2012)
	SHP2	Tyrosine Phosphatase	Huang et al. (2012)
	Src	Kinase	Huang et al. (2012)
	APC/C	Ubiquitin Ligase	Biggs et al. (2006)
	SMADs	Transcription Factors	Coco Lo et al. (1997)
	CHIP/Stub1	Ubiquitin Ligase	Shang et al. (2009)
RUNX2	p300/CBP	Lysine Acetyltransferase	Sierra et al. (2003)
	MOZ	Lysine Acetyltransferase	Shang et al. (2009)
	MORF	Lysine Acetyltransferase	Pelletier et al. (2002)
	HDAC4, HDAC5	Deactetylase	Jeon et al. (2006)
	SMADs	Transcription Factor	Jeon et al. (2006) and Zhang et al. (2000)
	ERK	Kinase	Franceschi et al. (2003) and Qiao et al. (2004)
	PKA	Kinase	Selvamurugan et al. (2000)
	PKC	Kinase	Kim et al. (2006)
	GSK3β	Kinase	Kugimiya et al. (2007)
RUNX3	p300/CBP	Lysine Acetyltransferase	Jin et al. (2004)
	BRD2	Transcriptional Regulator	Li et al. (2002)
	SirT2	Deacetylase	Lee et al. (2013)
	Smurf	Ubiquitin Ligase	Jin et al. (2004)
	SMADs	Transcription Factors	Zaidi et al. (2002)
	HDAC1,-2,-4,-5	Deacetylases	Jin et al. (2004)

involved in a chromosomal translocation, one that generates the CBFβ-SMMHC fusion protein (Kamikubo et al. 2010). Taken together, AML1-ETO and CBFβ-SMMHC expressing leukemias are referred to as CBF-AML.

3.2.1 Acetylation

The recruitment of coactivator proteins to specific promoters is a critical step for transcriptional activation. Many of these transcriptional coactivators possess histone acetyltransferase activity. RUNX proteins are rich in lysine residues, and are modified by multiple members of the lysine acetyltransferase (KAT) family. In general, the acetylation of RUNX proteins stimulates their transcriptional activity. Members of the KAT family that bind and acetylate RUNX proteins include p300 and MOZ, while P/CAF and GCN5 can modify the RUNX1 fusion protein AML1/MDS1/EVI1 (Yamaguchi et al. 2004; Jin et al. 2004; Kitabayashi et al. 2001; Senyuk et al. 2003). The KAT proteins p300 and CBP are transcriptional coactivators with distinct roles in normal hematopoiesis (Rebel et al. 2002). The p300-mediated acetylation of lysines 24 and 43 on RUNX1 augments RUNX1 DNA binding and transcriptional activation. Mutation of these sites to arginine does not disrupt the interaction with p300 but rather impairs RUNX1 DNA binding (Yamaguchi et al. 2004). In t(8;21) AML, the AML1-ETO fusion protein is acetylated by p300 on lysine 24 and 43, which promotes its ability to activate key self-renewing genes; the absence of K43 acetylation significantly abrogates AML1-ETO mediated leukemogenesis in vivo (Wang et al. 2011).

The *Myst* acetyltransferase family member *Moz* is another coactivator of RUNX1-mediated transcription. RUNX1 binds MOZ through its C-terminal transactivation domain, which drives the expression of genes involved in monocyte/macrophage differentiation (Kitabayashi et al. 2001). The MYST domain of MOZ has KAT activity, however, it is neither required for interacting with RUNX1 nor essential for activating transcription, implying that other domains within MOZ regulate transcriptional activation by RUNX1. MOZ can also be found in a fusion protein that contains the p300 homolog CBP, and in fact MOZ, CBP and MOZ-CBP can all acetylate RUNX1 in vitro. Acetylation by MOZ or CBP has been shown to promote RUNX1 transactivation, while acetylation by MOZ-CBP attenuates RUNX1-driven gene expression (Kitabayashi et al. 2001). Although the mechanism remains unclear, it is possible that MOZ-CBP disrupts the interaction between RUNX1 and CBP or MOZ, or another cofactor necessary for transactivation. In sum, the KAT proteins p300, CBP and MOZ play important roles in the RUNX1-mediated transcriptional program, and their dysregulation could contribute to aberrant gene regulation in hematopoietic malignancies.

3.2.2 Methylation

Protein methyltransferases are another group of histone modifying enzymes that regulate RUNX1 transcriptional activity and recruitment to its target gene promoters. We have shown that the lysine methyltransferase MLL physically interacts with the N-terminal region of RUNX1, and promotes the deposition of H3K4me3 activating marks upstream of the critical RUNX1 target gene PU.1. This interaction also appears to stabilize RUNX1 by inhibiting its poly-ubiquitination (Huang et al. 2011). PRMT1, an arginine methyltransferase that targets histone H4R3, methylates RUNX1 at R206 and R210, which abrogates its association with the corepressor SIN3A, enhances its transcriptional activity, and facilitates the binding of RUNX1 to its target gene promoters including *ITGA2B* and *SPI1* (Zhao et al. 2008). Interestingly, knock-in mice with arginine-to-lysine mutations at R206/R210 primarily display impaired peripheral T-cell maintenance (Mizutani et al. 2015). PRMT1 also methylates AML1-ETO (and a truncated isoform AE9a) affecting its transcriptional activating properties (Shia et al. 2012). Thus, multiple methyltransferases are involved in the control of RUNX1-mediated transcriptional activation.

3.2.3 Phosphorylation

[Note: Amino acid positions in this section refer to RUNX1c].

RUNX1 is also subject to phosphorylation by kinase signaling cascades; these include kinases activated by hematopoietic cytokines and growth factors, and kinases that function as cell cycle regulatory proteins. In response to cytokine stimulation, extracellular signal-regulated kinase (ERK) phosphorylates the C-terminus of RUNX1 at S276, S293, T300, S303, and S462 (Tanaka et al. 1996; Zhang et al. 2004; Yoshimi et al. 2012), which increase RUNX1 mediated transactivation by preventing its interaction with Sin3A (Imai et al. 2004). Mutation of four RUNX1 ERK phosphorylation sites (S276/S293/T300/S303) impairs T-cell differentiation, although the mutants retain the ability to rescue early hematopoiesis (Yoshimi et al. 2012). The sensitivity of T-lymphocytes to specific changes in RUNX1 phosphorylation and methylation suggests a tissue-specific role of ERK signaling in RUNX1 function.

RUNX1 directly regulates the G1 to S transition, a process that is inhibited by the leukemia-associated CBFβ-SMMHC and AML1-ETO fusion proteins. While RUNX1 RNA levels remain constant throughout the cell cycle, RUNX1 protein levels increase at the G1 to S transition, and then decrease due to ubiquitin-mediated degradation during G2/M. Serine phosphorylation of RUNX1 occurs during G_2/M by cyclin B/Cdk1 and cyclin A/Cdk2 on S276, and S303, triggering RUNX1 ubiquitination by the APC-CDC20 complex (Biggs et al. 2006). Cyclin B/CDK1 and cyclin D3/CDK6 also phosphorylate RUNX1 on S48 and S424; while S48 is phosphorylated throughout the cell cycle, S303 and S424 are phosphorylated most prominently during G2/M and G1, respectively (Zhang et al. 2008). These modifications reduce the interaction of RUNX1 with HDAC1 and HDAC3, further promoting transcriptional activation (Guo and Friedman 2011). PIM1 kinase, another regulator of cell cycle progression, also interacts with RUNX1 to enhance its transactivation activity following cytokine stimulation (Aho et al. 2006).

Cross talk between serine/threonine phosphorylation and histone acetylation can synergistically enhance the transcriptional activity of RUNX1. Homeodomain-interacting protein kinase 2 (HIPK2) is a nuclear kinase that forms a complex with both RUNX1 and p300, and it initiates a phosphorylation cascade that stimulates transcriptional activation (Wee et al. 2008; Aikawa et al. 2006). *Hipk1/2* double-deficient mice show impaired phosphorylation of both p300 and RUNX1, and defects in definitive hematopoiesis (Aikawa et al. 2006).

Taken together, the phosphorylation of RUNX1 provides for its dynamic regulation in response to extracellular signals and cell cycle progression. Serine/threonine phosphorylation serves to increase RUNX1 transcriptional activity while also decreasing its protein stability. As we describe below, tyrosine phosphorylation of RUNX1 also regulates its protein-protein interactions and diminishes its DNA binding ability.

3.3 RUNX1 Post-translational Modifications in Transcriptional Repression

Covalent modifications of RUNX family members can also have negative effects on transcription. BMI1, a component of the polycomb repressive complex 1 (PRC1), has been shown to be recruited to chromatin at key RUNX1 binding sites in megakaryocytes and lymphocytes (Yu et al. 2012). Transcriptional repression by RUNX1 is critical for normal hematopoietic development. For example, RUNX1 and RUNX3 are required for CD4 silencing in vivo, a necessary process for cytotoxic T-cell development and maturation (Levanon et al. 2002). Analysis of *Runx1* knockout mice demonstrates that RUNX1 suppresses the nuclear translocation of NF-kB. Normally, NF-kB is bound to IkB in the cytoplasm. Phosphorylation of IkB by IkB kinase (IKK) degrades IkB, leading to nuclear import of NF-kB, which induces expression of NF-kB target genes. RUNX1 inhibits the enzymatic activity of IKK by binding to it in the cytoplasm, thereby suppressing the nuclear shuttling of NF-kB (Nakagawa et al. 2011).

3.3.1 Deacetylation

Histone deacetylase (HDAC) complexes classically participate in chromatin remodeling and gene repression, and several members of HDAC complexes have been found to interact with RUNX1 including HDAC1, SIN3A and Gro/TLE. While SIN3A also interacts with RUNX2, RUNX3, and RUNX1-ETO, mutation of the SIN3A binding domain in RUNX1 reduces the recruitment of histone deacetylases and impairs RUNX1-mediated repression of the CDKNIA promoter (Lutterbach et al. 2000). Trichostatin A, a broad-spectrum histone deacetylase inhibitor, impairs RUNX1-guided gene repression, further highlighting the contribution of histone deacetylases to the negative regulation of gene expression by RUNX1 (Lutterbach et al. 2000). Interestingly, chromosomal translocations involving *RUNX1* generate RUNX1 fusion proteins that show enhanced recruitment of co-repressors, compared to wildtype RUNX1. The ALL associated t(12;21) fusion protein TEL/AML1 binds SIN3A with higher affinity than RUNX1 alone. It appears that in pathologic settings, RUNX1 fusion proteins may more potently repress RUNX1 target gene expression, acting at least in part by suppressing wildtype RUNX1 function (Guidez et al. 2000).

3.3.2 Methylation

Methylation of RUNX1 can also promote its repression of transcription. Hematopoietic stem cells express high levels of PRMT4 (CARM1), a Type-I arginine methyltransferase that methylates RUNX1 on an evolutionarily conserved residue, arginine 223. RUNX1-R223me2 drives the assembly of a repressive complex that inhibits transcription of mir-223, a driver of myeloid differentiation, and other RUNX1 target genes. AML patient samples significantly overexpress PRMT4, suggesting that R223-methyl-RUNX1 contributes to the block in differentiation that is characteristic of human AML (Vu et al. 2013).

RUNX1 has also been shown to form a corepressor complex with PRMT6 (and SIN3A and HDAC1) to mediate repression of its target genes prior to megakaryocytic differentiation (Herglotz et al. 2013). The histone H3 lysine 9 specific methyltransferase SUV39H1 binds RUNX1 at its N-terminus. H3K9me is a histone mark that recruits heterochromatin protein-1 (HP1) to silence gene expression. The interaction between RUNX1 and SUV39H1 decreases the affinity of RUNX1 for DNA and also impairs transcription of the RUNX1 target gene CSF1R (Chakraborty et al. 2003; Reed-Inderbitzin et al. 2006).

3.3.3 Phosphorylation

Similar to methylation, phosphorylation of RUNX1 can alter its effects on gene expression. RUNX1 tyrosine phosphorylation was recently implicated in impairing megakaryocyte development, as the level of tyrosine phosphorylation inversely correlates with the extent of differentiation. RUNX1 interacts with the non-receptor tyrosine kinase c-Src and the tyrosine phosphatase SHP2; its tyrosyl phosphorylation decreases the interactions of RUNX1 with CBFβ and GATA1 and FLI1, but increases its affinity for the chromatin remodeling SWI/SNF subunits BRG1 and SNF5 (Huang et al. 2012; Neel and Speck 2012). Additionally, the phosphorylated tyrosine residues in RUNX1 impair its ability to bind and transactivate DNA. These findings suggest that while serine/threonine phosphorylation events promote transcriptional activation by RUNX1, tyrosine phosphorylation may promote gene repression.

3.4 Protein Stability and Localization of RUNX1

3.4.1 Ubiquitination

The levels of RUNX1 are tightly regulated at both the transcriptional and post-translational level. RUNX1 degradation is mediated by the ubiquitin-proteasome system, yet multiple protein-protein interactions can stabilize RUNX1 by shielding it from ubiquitin ligases. For example, the interaction of RUNX1 with its obligate binding partner CBFβ stabilizes RUNX1 through

inhibition of ubiquitination (Huang et al. 2001). The mixed lineage leukemia (MLL) protein also binds RUNX1 to produce a similar stabilizing effect (Huang et al. 2011). The anaphase promoting complex (APC) is an E3 ubiquitin ligase known to regulate mitosis. The CDC20 subunit of APC targets RUNX1 for degradation following RUNX1 phosphorylation by CDK1 or CDK2 (Biggs et al. 2006). The heat shock protein-binding co-chaperone protein CHIP/STUB1 impairs protein stability by promoting the ubiquitination and degradation of misfolded or unfolded proteins. While reports have suggested that its E3 ligase activity depends on its interaction with HSP70/90 (Murata et al. 2003), CHIP/Stub1 is capable of promoting the degradation of RUNX1 independently of HSP70 (Shang et al. 2009).

It appears that several leukemia-associated RUNX1 mutant proteins are relatively resistant to ubiquitin-mediated degradation, allowing them to function as dominant negatives. For example, in-vitro assays demonstrate that the RUNX1 L117P and I150T mutants are ubiquitinated to a lesser extent than wildtype RUNX1. These runt domain mutants lack transcriptional activity; they can competitively bind to CBFβ and/or DNA and suppress transactivation by wildtype RUNX1 (Koh et al. 2013). The runt domain also harbors the nuclear localization signal, which is critical for the nuclear translocation of RUNX1. Wildtype RUNX1 is exclusively localized to the nucleus, but many leukemia-associated runt domain mutants exhibit both cytoplasmic and nuclear staining (I150T, P156A, R170Q), or distinct cytoplasmic staining (Y260X) (Koh et al. 2013). In summary, runt domain mutations impair the transcriptional activity of RUNX1 through stabilization of dominant-negative mutants or disruption of nuclear localization.

3.5 Post-translational Modifications of RUNX2 and RUNX3

Runx2 (*Aml3*) is a critical regulator of bone development and it also interacts with multiple acetyltransferases, including p300, MOZ, and MORF (Sierra et al. 2003; Pelletier et al. 2002). Acetylation of RUNX2 generally favors bone growth; for instance, following ERK activation (e.g. in response to BMP2), p300 acetylates RUNX2 which enhances its transcriptional activity and stability, triggering osteoblast differentiation and bone formation (Jeon et al. 2006). The interaction between p300 and RUNX2 is promoted by the SMAD proteins but blocked if the phosphorylation sites in the C-terminus of RUNX2 are mutated; these mutations inhibit responsiveness of cells to TGF-β/BMP signaling (Zhang et al. 2000). The H3K36 trimethyltransferase, WHSC1, also promotes the interaction between RUNX2 and p300, which drives expression of the *Spp1* and *Collagen type 1a* genes (Lee et al. 2014). Loss of one *Whsc1* allele results in Wolf-Hirschorn Syndrome (WHS), a disease characterized by skeletal abnormalities and hypo-ossification.

Histone deacetylases (HDACs) such as HDAC4 and HDAC5, can reverse RUNX2 acetylation and impair osteoblast differentiation, decreasing bone formation (Jeon et al. 2006). As predicted, the use of HDAC inhibitors can promote osteoblast maturation and bone growth, as can overexpression of miR-29a, which blocks HDAC4 expression, stabilizes acetyl-RUNX2 and rescues defects in osteoblast differentiation (Ko et al. 2015).

RUNX2 activity is modulated throughout osteoblast differentiation by multiple signaling pathways including MAPK/ERK, cAMP/PKA, and DAG/PKC (Franceschi et al. 2003). IGF-1 stimulates the PI3K and ERK pathways to enhance RUNX2 DNA binding and transactivation (Qiao et al. 2004). Similarly, FGF2 activates ERK signaling and RUNX2 phosphorylation to enhance expression of osteocalcin (Franceschi et al. 2003). FGF2 also activates PKC which phosphorylates S247 on RUNX2, promoting its transcriptional activity (Kim et al. 2006). PKA is stimulated by parathyroid hormone to phosphorylate RUNX2 on S347 which drives *Collagenase-3* transcription (Selvamurugan et al. 2000). Clearly, RUNX2 is a critical point of integration for a variety of signaling pathways that augment the expression of genes essential for osteoblast activity and bone development.

Similar to RUNX1, phosphorylation can also negatively regulate the transcriptional activity of RUNX2. Phosphorylation on serine 104 disrupts its binding to CBFβ, which subjects RUNX2 to proteolytic degradation. Notably, the S104R mutant, which mimics constitutive phosphorylation, is seen in patients with cleidocranial dysplasia, a hereditary congenital disorder characterized by underdeveloped bone and teeth (Wee et al. 2002). Phosphorylation of S451 occurs within the C-terminal inhibitory domain of RUNX2 to diminish transcriptional activity, although the mechanism remains unclear (Wee et al. 2002). GSK3β-mediated phosphorylation of RUNX2 inhibits its transactivation; therefore inhibiting GSK3β activity may be useful for those with catabolic bone disorders. Indeed, mice with heterozygous loss of GSK3β, or wildtype mice treated with lithium chloride, an inhibitor of GSK3β, exhibit increased bone mass (Kugimiya et al. 2007).

Evidently, PTMs and protein-protein interactions are crucial to RUNX2 activity and highlight its bone-specific functions. Indeed, the loss of key RUNX2 interactions result in profound dysostosis as seen in developmental disorders like WHS and cleidocranial dysplasia. Given its essential role in bone development, an expanded understanding of the PTMs that influence RUNX2-driven gene expression may have implications for treating other bone disorders in the future.

Runx3 appears to be essential for neurogenesis (Levanon et al. 2002; Inoue et al. 2002) and it appears to function as a tumor suppressor gene in bladder cancer, gastric cancer, and lung cancer development (Kim et al. 2005; Li et al. 2002; Lee et al. 2013). The stability of the RUNX3 protein results from a dynamic equilibrium of RUNX3 acetylation, deacetylation and ubiquitination. Upon TGF-β stimulation, p300 acetylates RUNX3, impairing its ubiquitination and promoting its stabilization (Jin et al. 2004). Acetyl-RUNX3 has a higher affinity for the bromodomain-containing protein, BRD2, than its unmodified counterpart and the RUNX3-BRD2 complex positively regulates transcription of *p21* and *ARF* genes, preventing K-Ras induced transformation (Lee et al. 2013). The HDAC SIRT2 deacetylates RUNX3, which allows RUNX3 to be ubiquitinated by E3 ligase SMURF and subsequently degraded (Jin et al. 2004; Kim et al. 2011). Since a number of cancers have abnormally low expression of p300 or inactivating mutations in p300 (Iyer et al. 2004), a reduction in acetyl-RUNX3 may contribute to a decline in transcription of tumor suppressor genes. As all of the RUNX proteins have been associated with cancer (Ito et al. 2015), further study of their protein-protein interactions and PTMs will clarify their contribution to normal and aberrant gene expression.

3.6 RUNX1 as a Tumor Suppressor

As mentioned above, *RUNX1* can be altered through chromosomal rearrangement, copy number variation, point mutation, and internal tandem duplication (Ferro et al. 2004). In general, loss of RUNX1 function impairs early hematopoiesis and differentiation. However, it is clear that RUNX1 can act as a tumor suppressor or tumor promoter depending on the cellular context and its expression level. Several oncogenic mouse model systems show a requirement for at least low levels of *Runx1* in the hematologic malignancies; indeed, complete knockout of *Runx1* inhibited leukemia development in an MLL-AF9 driven AML model (Goyama et al. 2013). Examining alterations in RUNX1 in the context of PTMs, and the enzymes that catalyze them, will provide insight into its context dependent roles.

Inactivating mutations of *RUNX1* are frequently found in patients with MDS and cytogenetically normal AML, and implicate its role as a canonical tumor suppressor. Heterozygous germ line mutations of *RUNX1* are associated with familial platelet disorder with predisposition to AML (FPD/AML), a disease where approximately 35% of carriers develop AML (Owen et al. 2008). In several RUNX1 fusion proteins, the gene rearrangement eliminates functional domains in RUNX1 that affect its transcriptional regulatory properties. Several of these fusion pro-

teins have lost the C-terminal regulatory region of RUNX1 but retain their ability to bind to RUNX1 consensus sequences. As a result, these fusion proteins are able to compete with wildtype RUNX1 for target gene occupancy. The absent C-terminus of RUNX1 mediates critical interactions with the chromatin modifying enzymes MOZ and MLL, and includes the sites of extensive phosphorylation that follow extracellular signals. Therefore, point mutations that disrupt the C-terminal PTM code may phenocopy the physical loss of this region by attenuating essential protein interactions and the ability of RUNX1 to respond to mitogenic or antiproliferative signaling pathways.

Mutations in chromatin modifying enzymes may also contribute to inactivation of RUNX1 by modifying its protein level in the nucleus or affecting its overall protein stability. MLL fusion proteins induce abnormal RUNX1 ubiquitination and downregulate its expression (Zhao et al. 2014). Somatic RUNX1 mutations have also been identified in 15% of cytogenetically normal AML, with specific mutations correlated with mislocalization of the RUNX1 protein and defective ubiquitination (Licht 2001). Thus, it appears that there are multiple mechanisms for the disruption of RUNX1 activity in cancer cells, either by direct inactivating mutations, or by indirect post-translational regulation.

Taken in a larger context, many mechanisms exist to fine tune RUNX1 activity and there are many opportunities for disruption of its PTM code. While MOZ and MLL proteins normally promote transcription activation through interaction with RUNX1, the MOZ-CBP and MLL fusion proteins block RUNX1 mediated transcription. The CBFB-SMMHC translocation formed by inv(16) sequesters the transcription activating kinase HIPK2 in the cytoplasm, preventing the phosphorylation of RUNX1 and p300. Mutations have also been noted in HIPK2 itself in AML patients, leading to disrupted subcellular localization of RUNX1 (Wee et al. 2008; Calzado et al. 2007). Thus, the overall activity of RUNX1 in hematopoietic cells depends on multiple factors, including the enzymes that catalyze its PTMs.

Increased *RUNX1* gene copy numbers and protein levels have been cited as evidence for an oncogenic role of RUNX1 in human cancer. Patients with Trisomy 21 have an increased susceptibility to AML due to augmented gene dosage (De Vita et al. 2010), while amplification of RUNX1 occurs in a subset of patients with T-ALL and B-ALL (Grossmann et al. 2011). However, increased gene dosage may not be correlated with increased RUNX1 activity and may mask the contribution of other genes located in the same region. The mouse studies in which knockout of RUNX1 inhibited leukemia development can also be viewed in the context of overall RUNX1 regulation, namely that cancer cells retain low levels of RUNX1 even in the context of inactivating mutations and that this may be required for leukemogenesis. In summary, RUNX1 activity is highly regulated and often disrupted through genetic alteration and PTMs, generally suggesting a tumor suppressive role in hematopoietic malignancies.

3.7 Conclusion

Post-translational modifications regulate the function of RUNX proteins by affecting their DNA binding, cellular localization, stability, and protein-protein interactions. RUNX proteins often act as scaffolds to mediate the formation of activating or repressive complexes that regulate tissue-specific gene expression. As pharmacologic targeting of transcription factors has proven challenging, an understanding of the enzymes that catalyze RUNX modifications and their biological roles may have implications for developing new therapies for disorders such as cleidocranial dysplasia or t(8;21) RUNX1-ETO AML. For example, blocking RUNX2 phosphorylation through the inhibition of GSK3β may provide a paradigm by which RUNX2 activity can be partially restored in patients with catabolic bone disorders. Moreover, as the leukemogenicity of t(8;21) AML is dependent on the acetylation of AML1-ETO, the use of KAT inhibitors may overcome the aberrant gene

expression and self-renewal that is seen in this context.

The roles of RUNX proteins in development and disease have been under intense study since the cloning of *AML1* in 1991 (Coco Lo et al. 1997). As the use of next generation sequencing continues to expand our knowledge of the RUNX transcriptome, we must continue to integrate these approaches with traditional biochemistry and cell biology to further our understanding of RUNX PTMs and RUNX-related diseases.

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