MRTFA: A critical protein in normal and malignant hematopoiesis and beyond

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Myocardin-related transcription factor A (MRTFA) is a coactivator of serum response factor, a transcription factor that participates in several critical cellular functions including cell growth and apoptosis. MRTFA couples transcriptional regulation to actin cytoskeleton dynamics, and the transcriptional targets of the MRTFA–serum response factor complex include genes encoding cytoskeletal proteins as well as immediate early genes. Previous work has shown that MRTFA promotes the differentiation of many cell types, including various types of muscle cells and hematopoietic cells, and MRTFA's interactions with other protein partners broaden its cellular roles. However, despite being first identified as part of the recurrent t(1;22) chromosomal translocation in acute megakaryoblastic leukemia, the mechanisms by which MRTFA functions in malignant hematopoiesis have yet to be defined. In this review, we provide an in-depth examination of the structure, regulation, and known functions of MRTFA with a focus on hematopoiesis. We conclude by identifying areas of study that merit further investigation.

Myocardin-related transcription factor A (MRTFA), which has also been named MKL1, MAL, or BSAC, is expressed in most cells. First identified as a member of the fusion product resulting from the recurrent t(1;22)(p13;q13) chromosomal translocation found uniquely in pediatric acute megakaryoblastic leukemia (AMKL), its primary function lies in its ability to coactivate the transcription factor serum response factor (SRF) and thereby induce the transcription of genes affecting cell migration, adhesion, and structure (1, 2). Through binding to SRF, MRTFA serves as a key regulator not just of hematopoietic differentiation but also muscle and myofibroblast maturation and solid cancer metastasis (3–7). This wide range of functions in both normal and pathological processes, as well as in multiple tissue types, makes MRTFA a worthy candidate for further research and investigation.

Roles for the other two members of the myocardin-related transcription factor family (myocardin and MRTFB, alias MKL2) in both cytoskeletal reorganization and cell differentiation have been described (7). Myocardin's role is best characterized for cardiac and smooth muscle cells, where it is predominantly expressed. MRTFB is more widely expressed than myocardin and is also critical for cardiac muscle and blood vessel development; *Mrtfb* knockout (KO) in mice is embryonic lethal because of defective cardiovascular development (8–10). In the adult, MRTFB has not been widely studied, with most reported functions of MRTFB largely redundant with those of MRTFA. In contrast, MRTFA has been widely studied in the adult (4, 7). Here, we focus on MRTFA and its critical role in hematopoiesis.

In addition to its primary role as a transcriptional coactivator for SRF, MRTFA also interacts with other proteins (*e.g.*, SMADs) in an SRF-independent manner. While interactions between MRTFA and proteins other than SRF have been observed, the relative impact of these interactions on cellular and cytoskeletal function is not yet clear. This promising area of research may shed more light on the role of MRTFA in hematopoietic cell differentiation and AMKL.

The significance of a better understanding of MRTFA is multifold. In addition to a role in AMKL, MRTFA has been clearly implicated in normal hematopoiesis, immune function, wound healing, and cancer metastasis (10, 11). Elucidating the mechanisms by which MRTFA drives normal differentiation in the many cell types in which it is expressed could lead to the identification of novel therapeutics not only for AMKL but other diseases as well. In this review, we aim to provide a comprehensive overview of the current state of research surrounding MRTFA, with a focus on what is known about its role in hematopoietic cells, and conclude with areas that are worthy of further exploration.

MRTFA protein structure

Protein structure and domain functions

Figure 1 shows the exon organization and subsequent protein isoforms encoded by the different transcriptional start site and splice variants of MRTFA. MRTFA has five principal functional protein domains and is encoded by 15 exons. The N terminus of the protein contains a number of RPEL motifs. Canonical RPEL motifs have the amino acid sequence RPxxxEL, where x can be any amino acid. The first RPEL-like sequence of MRTFA, referred to as RPEL1 and encoded by exon 4, has the sequence RRxxxEL and is thus not a "true" RPEL motif. RPEL2 and RPEL3 are canonical and are encoded by exons 6 and 7. These RPEL domains are required for the

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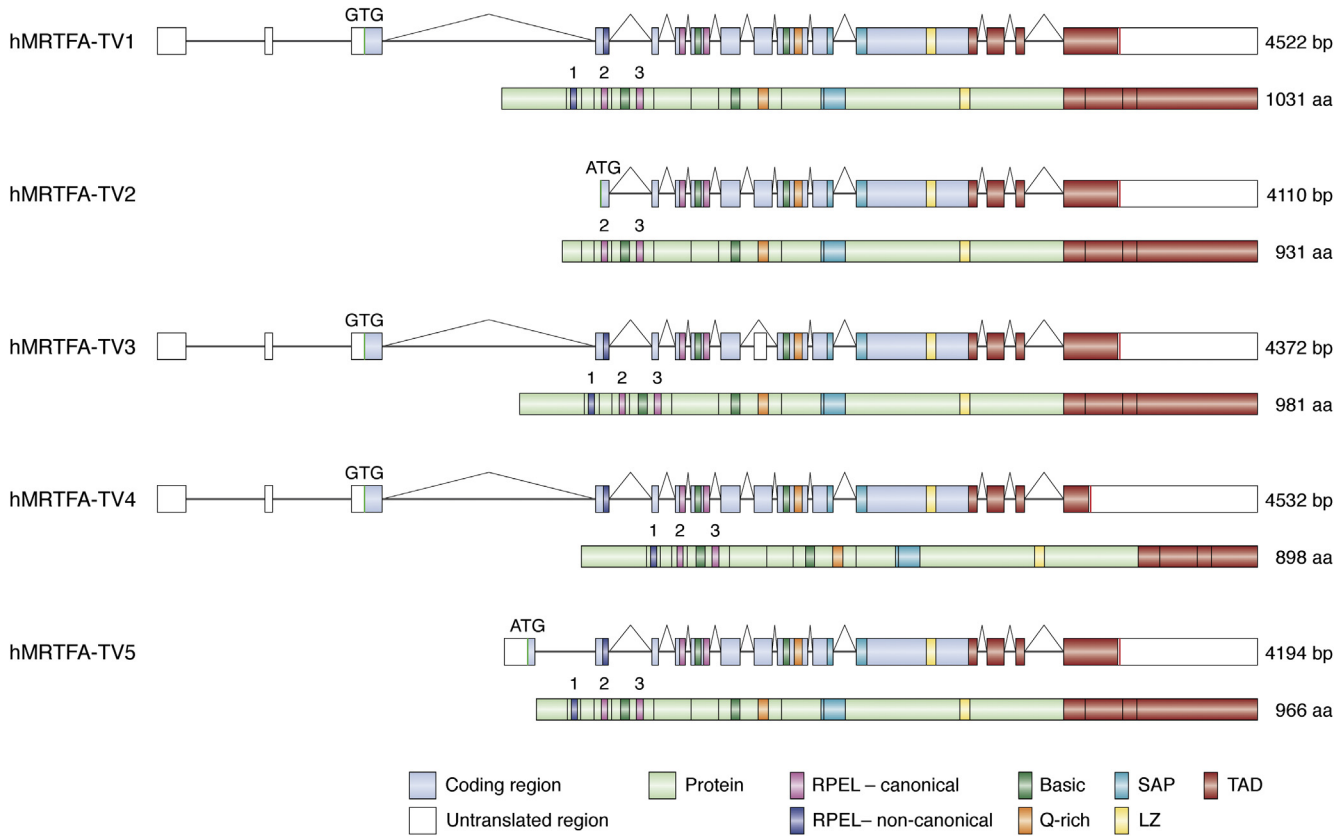


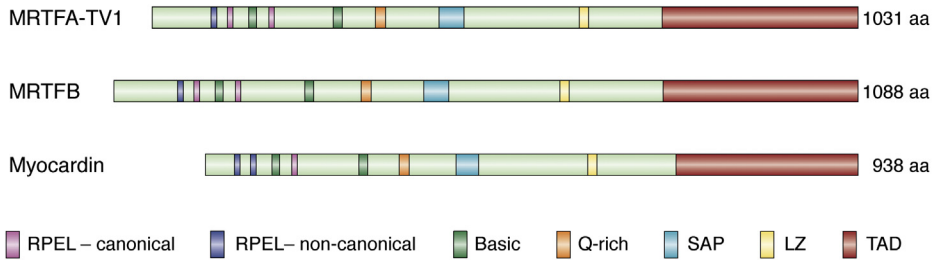
Figure 1. The five transcript variants of human MRTFA, including translation start and stop sites as well as locations of each of the five domains and their corresponding exons. Below each transcript is a schematic of the protein encoded by the variant with critical domains indicated. *hMRTFA*, human *MRTFA*; MRTFA, myocardin-related transcription factor A; TV, transcript variant.

interaction between MRTFA and monomeric G-actin that regulates the cellular localization and activity of MRTFA. Highly disordered in the unbound protein, RPEL2 and RPEL3 are induced to form stable alpha helices upon binding to G-actin (12). Deletion of the RPEL domains renders the protein predominantly nuclear and constitutively active (13). While one transcript variant of MRTFA contains only RPEL2 and RPEL3, the others also contain the noncanonical RPEL1 (Fig. 1) (6, 13). Although the significance of these transcript variants is not yet clear, Guettler *et al.* showed that in transcript variants containing three RPEL motifs, RPEL2 and RPEL3 are most critical for actin binding with RPEL1 playing a lesser role (14).

MRTFA contains two regions enriched for basic residues (*e.g.*, lysine and arginine), located on either side of RPEL3. The first basic domain, encoded by exon 7 upstream of RPEL3, contains a nuclear localization signal, and promotes MRTFA nuclear localization (6, 13). The second basic domain, encoded within exon 10, also plays a role in nuclear localization, but its primary function is to bind SRF. SRF binding occurs *via* an interaction between a hydrophobic β-sheet in MRTFA and a hydrophobic pocket in the DNA-binding domain of SRF (6, 14). The second basic domain is critical for most known functions of MRTFA. Deletion of the two basic regions prevents MRTFA from accumulating in the nucleus (6). Downstream of the basic domains is a glutamine-rich region canonically referred to as the "Q-rich" domain. Though no function for the Q-rich domain of MRTFA has been identified, the Q-rich domain of the related protein myocardin binds SRF (13, 15, 16).

Of potential interest is the SAP domain, named for the SAF-A/B, Acinus, and PIAS proteins in which it was discovered and which mediates binding to DNA in those three proteins. SAP domains form amphipathic helices, which are required for this DNA-binding ability (17). DNA binding *via* the SAP domain has not been convincingly demonstrated for MRTFA. The coding sequence of the SAP domain starts at the 3′ end of exon 11 and continues into exon 12. Deletion of the SAP domain has no effect on the ability of MRTFA to activate SRF-mediated transcription in transient transfection assays in HeLa cells (6). However, it has been suggested that the SAP domain may be required for MRTFA to activate transcription independent of SRF (18, 19).

Following the SAP domain, also encoded by exon 12, is a conserved leucine-zipper (LZ) domain. Found in many proteins, including others of the myocardin family, the LZ domain enables MRTFA homodimerizeration as well as heterodimerization with MRTFB or myocardin (3, 11). Deletion of the LZ domain decreases, but does not abolish, activation of SRF target genes, indicating that MRTFA preferentially binds SRF as a dimer, but this is not obligatory (11).



Domain	% Identity MRTFA-MRTFB	% Identity MRTFA-Myocardin
Entire protein	51	35
RPEL2, RPEL3	86,100	N/A, 86
B1, B2	100,83	100, 86
Q-rich	83	85
SAP	77	66
Leucine zipper	67	50
TAD	41	32

Figure 2. Comparison of MRTFA, MRTFB, and myocardin structures, displaying the location of each conserved domain described in the text. Note that noncanonical RPEL-like domains have little to no actin binding. Amino acid sequence similarities (% identity) between myocardin family proteins within the key domains are shown. *hMRTFA*, human *MRTFA*; MRTFA, myocardin-related transcription factor A; TV, transcript variant.

At the C terminus of MRTFA is a transcriptional activation domain (TAD), beginning in exon 12 and continuing through exon 15. Deletion of this domain, from amino acid residue 630 onward, prevents the activation of MRTFA target genes and acts as a dominant negative form of MRTFA (6). Record *et al.* described a patient with a homozygous nonsense mutation in this domain that resulted in decreased transcription of actin in neutrophils, deleteriously affecting the process of phagocytosis and inducing severe immunodeficiency (20).

mRNA transcript variants

Some or all of the five domains described above (RPEL, basic, SAP, LZ, and TAD) are present in all reported isoforms of MRTFA. To date, five mRNA transcript variants (TVs) of human *MRTFA* (*hMRTFA*) have been reported, each containing between 12 and 15 exons. In all forms of the protein, exons 1 and 2 are not translated. Translation of *hMRTFA-TV1* (referred to as MKL1_L by Scharenberg *et al.*), *hMRTFA-TV3*, and *hMRTFA-TV4* are initiated by a nontraditional GTG start codon in exon 3 (21). Kozak *et al.* define a flanking consensus sequence required for GTG to function as a start codon, which is present in exon 3; an alternative tRNA is utilized in this context, which translates the alternative start codon as methionine (22). As *hMRTFA-TV1*, *hMRTFA-TV3*, and *hMRTFA-TV4* each contain a complete exon 4, which encodes RPEL1, each of these isoforms contain all three RPEL domains. TVs *hMRTFA-TV1* and *hMRTFA-TV3* have alternative splicing and/or stop codons as depicted in Figure 1.

The translated protein encoded by *hMRTFA-TV2*, which begins with a traditional ATG in exon 4, is the only isoform to lack RPEL1. *hMRTFA-TV5* (referred to as MKL1_S by Scharenberg *et al.*) begins with an alternate exon 3, termed exon 3′, but proceeds to canonical exon 4, resulting in a translated protein with three RPEL motifs (21).

The differential expression of these TVs and their relative significance to the function of MRTFA remains unclear. Scharenberg *et al.* confirm expression of at least *hMRTFA-TV1*, *hMRTFA-TV2*, and *hMRTFA-TV5* in human tissues and also indicate that each of these TVs can activate SRF; however, thus far, only *hMRTFA-TV1* and *hMRTFA-TV2* have been found to be expressed in mice (13, 21). Scharenberg *et al.* also postulate that exon 3′, the start site of *hMRTFA-TV5*, is linked to a different promoter (21). Ishikawa *et al.* propose that because of the missing RPEL1 in *hMRTFA-TV2*, this isoform of MRTFA may have decreased affinity for actin and thus increased nuclear localization, but this is disputed by the assessment by Guettler *et al.* that RPEL1 does not play a significant role in actin binding (14, 23). Further investigation into functions of each of these TVs in discrete cell types and developmental stages is critical to elucidating the role of MRTFA in cell maturation and leukemogenesis.

Relationship to other myocardin family proteins

Overall, the three myocardin family proteins (Fig. 2) have an average of 35% similarity, with increased homology in the five conserved domains (6, 7). Although myocardin contains three RPEL-like motifs, only RPEL3 has a canonical RPEL sequence. The lack of the canonical consensus sequence in the first 2 RPEL-like domains (RPEL1 and RPEL2) in myocardin likely underlies myocardin's low affinity for G-actin and constitutively nuclear localization (14). The RPEL1-like sequences of both MRTFA and MRTFB, while highly similar, are noncanonical, containing RR rather than RP, and in the case of MRTFB, RPEL1 terminates with QL rather than EL (6, 12, 13).

Overall, MRTFA and MRTFB are 51% similar; the addition of 71 amino acids to the N-terminal end of MRTFB may increase its transcriptional activation activity (5). All three coactivate SRF in a basic domain-dependent manner, though myocardin also requires the Q domain (6, 7). Additionally, all myocardin family proteins can form homodimers or heterodimers with each other with the ability to dimerize being more critical for myocardin function than either MRTFA or MRTFB (24, 25). While myocardin is expressed primarily in cardiac and smooth muscle cells, MRTFA and MRTFB are ubiquitously expressed (6, 7). As well as sharing more than half of their amino acid sequence, MRTFA and MRTFB can compensate for one another functionally which is of particular importance to hematopoietic development (see MRTFA in megakaryopoiesis).

Posttranslational regulation of MRTFA

Most of what is known regarding regulation of MRTFA function occurs at the protein level; there is little to no information in the literature about transcriptional or

translational regulation of MRTFA expression. Investigation of MRTFA gene regulation could be an area of fruitful research shedding light on its wider expression relative to myocardin.

Dependence on G-protein–mediated actin polymerization

Posttranslational regulation of MRTFA includes its binding to G-actin, which affects cellular localization and function. Upon activation by membrane receptors and/or mechanical stimuli, signaling *via* RhoA and/or other G-proteins indirectly regulates MRTFA localization by promoting formin-mediated actin polymerization (13, 26, 27). MRTFA binds monomeric G-actin in the cytoplasm *via* its RPEL motifs until actin polymerization is induced *via* RhoA signaling (26, 28). Unable to bind F-actin, MRTFA is released from G-actin and undergoes a conformational change revealing the nuclear localization signal in the basic region, resulting in importin α/β-dependent translocation of MRTFA to the nucleus—see Figure 3 (13, 29). Once in the nucleus, MRTFA is generally considered free to bind and activate SRF. However, it can be kept in an inactive state by binding to nuclear G-actin (30). Increased nuclear G-actin inhibits SRF-mediated gene expression, with effects on cell proliferation and migration that mimic those of non-polymerizable nuclear actin mutants (31, 32).

It is important to note that MRTFA cycles between nucleus and cytoplasm even in the absence of RhoA signaling with nuclear import requiring the basic domains and nuclear export, mediated by XPO1, requiring MRTFA binding to G-actin (33). MRTFA cycling is also regulated by MAPK/ERK-directed phosphorylation of several key MRTFA residues. Phosphorylation at S454 increases the association between MRTFA and G-actin, thereby decreasing SRF activation and promoting nuclear export (30, 34). In contrast, phosphorylation of S498 (also mediated by MAPK/ERK) prevents the association between MRTFA and G-actin, resulting in increased localization of MRTFA to the nucleus as well as functional activation of SRF (35). The mechanisms by which differential serine phosphorylation is regulated are not yet known (30).

Additional regulators of MRTFA include filamin A and Four and a Half LIM domain protein 2. Filamin A enhances MRTFA activity directly *via* enhanced nuclear actin polymerization (36). MRTFA is protected from degradation by the proteasome *via* interaction with Four and a Half LIM domain protein 2 (37).

Mechanosensitive signaling

In recent years, the role of mechanosensitive signaling in MRTFA activation has become increasingly well-defined. As the cellular location of MRTFA is dependent on actin polymerization, it follows that changes in cellular morphology that alter the actin cytoskeleton may also affect MRTFA. Cell stretching and other morphological changes that occur when cells migrate can induce nuclear accumulation of MRTFA and subsequent transcription of SRF targets (38, 39). However, nuclear localization is maintained only while the cells are experiencing mechanical stress. When internal and external forces on the cytoskeleton are balanced, MRTFA is predominantly cytoplasmic (40). Extracellular mechanical stress–mediated MRTFA activation is critical for myofibroblast maturation. Mechanical stress in myofibroblasts caused by

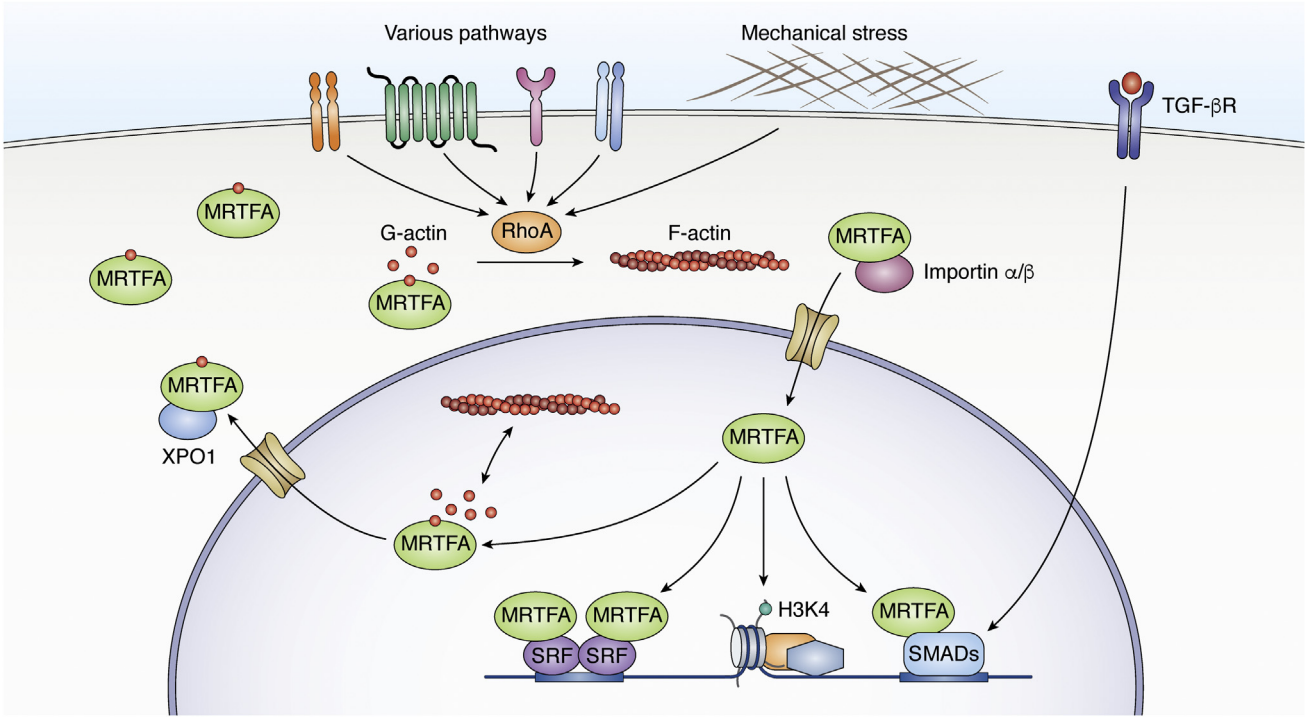


Figure 3. Schematic representation of MRTFA activation and regulation as well as interactions with its various transcriptional partners. H3K4, histone 3 lysine 4; MRTFA, myocardin-related transcription factor A; SRF, serum response factor.

increased matrix stiffness results in MRTFA–SRF–dependent activity, and lack of MRTFA in these cells not only renders them unresponsive to mechanical stress but also prevents cell maturation (41).

Alterations in nuclear shape also affect MRTFA activity. Increased nuclear stiffness in embryonic stem cells is associated with nuclear accumulation of MRTFA in a manner dependent on lamins A and C (42). Linker of nucleoskeleton and cytoskeleton (LINC) complexes, which couple nuclear morphology to extracellular signaling *via* the cytoskeleton may enhance RhoA (42). Increased RhoA signaling leads to upregulated SRF–MRTFA–dependent gene expression, including expression of the LINC complex member Sun2, which, *via* LINC-mediated enhancement of RhoA activation, may create a positive feedback loop (43). Further supporting a connection between MRTFA, chromatin, and the LINC complex, the inhibition of reprogramming of somatic cells to induced pluripotent stem cells by enhanced MRTFA activity requires Sun2, a key component of the LINC complex (44).

Role in transcription

Regardless of the regulatory signals that result in release of G-actin and translocation of MRTFA to the nucleus, once localized there, MRTFA is able to activate SRF-dependent gene expression in part by helping to recruit epigenetic modifiers and may also regulate other signaling pathways including those mediated by SMAD family proteins.

Interaction with SRF

The transcription factor SRF stimulates expression of immediate early genes, including c-Fos, as well as genes encoding cytoskeletal proteins (28). SRF binds to the genomic DNA *via* consensus binding domains known as serum response elements. These serum response elements are further classified as CArG boxes because of their CCAT-richGG sequence. As a MADS-box transcription factor, SRF contains a DNA-binding MADS domain, so named for the transcription factors in multiple species in which it was identified (MCM1, AGAMOUS, DEFICIENS, and SRF), each of which can bind to CArG sequences (6). SRF can be coactivated by MRTFA or by the ternary complex factor (TCF) family of transcriptional coactivators, which are a subfamily of Ets transcription factors including Elk1 and SAP1. In contrast to MRTFA, which binds SRF in response to Rho signaling and actin polymerization, TCF-mediated SRF activation is dependent on MAPK/ERK signaling (6, 13). Though MRTFA/SRF and TCF/SRF target genes overlap to some extent, MRTFA is considered to promote cytoskeletal gene expression and cellular maturation, whereas TCF/SRF is considered to stimulate proliferation associated genes.

Targets of MRTFA–SRF transcriptional activity

MRTFA/SRF complexes bind to their target genes as a quaternary unit with two MRTFA and two SRF molecules (6). This induces a 55.5 degree DNA bend, enabling MRTFA to contact the DNA in such a way that it flanks SRF on either side (45). Although SRF binds DNA on its own, in the absence of MRTFA, SRF binds DNA less efficiently, and the expression of SRF targets is significantly reduced (46). MRTFA-dependent targets of SRF tend to be involved in cell adhesion, cell motility, and migration [*e.g.*, myosin regulatory light polypeptide 9 (MYL9), myosin heavy chain 9 (MYH9), and actin] as well as include immediate early genes (*e.g.*, c-Fos and Jun-B) (13). It has been suggested that MRTFA engages in homeostatic regulation of actin levels within the cell; an increase in G-actin reduces the amount of free MRTFA in the nucleus, decreasing MRTFA–SRF–mediated transcription of actin, and vice versa (47, 48).

SRF-independent mechanisms of transcriptional activation

MRTFA may play a role in transcription that is independent of SRF by direct DNA association *via* its SAP domain. SAP domains in other proteins bind DNA and are associated with chromosomal organization, DNA repair, and RNA post-transcriptional modification (17). Asparuhova *et al.* suggest that the application of cyclic strain in fibroblasts and mammary epithelial cells induces expression of tenascin-C in an MRTFA-dependent yet SRF-independent manner; their data suggest that MRTFA binds directly to the tenascin-C promoter *via* the SAP domain (18). Additional studies from this same group suggest that mechanical stress, independent from the RhoA pathway, may be responsible for this SRF-independent activity (19, 49). However, as Gau *et al.* conclude, MRTFA and SRF regulate other genes known to be sensitive to mechanical strain, so the idea that stress induces MRTFA to switch to another mode of gene activation seems unlikely (50). Further replication of these experiments as well as research into the SAP domain is required; nowhere else in the literature has a function for the SAP domain of MRTFA been described.

MRTFA likely regulates gene expression *via* coordinated induction of epigenetic changes including activation of histone 3 lysine 4 (H3K4) methylation. Cheng *et al.* (51) observed that H3K4 methylation surrounding the promoter of matrix metallopeptidase 9, a known MRTFA target that plays a role in cell motility through tissue including metastasis, is reduced in the absence of MRTFA. The mechanisms underlying MRTFA's associations with the H3K4 complex are not clear, but MRTFA can associate with WDR5, a member of the H3K4 methylase complex, and assist in recruiting other members of the complex, including SET1, which increases histone methylation at target genes (52, 53). MRTFA can also interact with SMAD1, 3, and 4, downstream effectors of TGF-β, independently from SRF (54). While the broad physiological significance of this interaction is not yet known, MRTFA inhibits differentiation to mature muscle myocytes through interaction with SMAD1/4 (55).

Recent evidence supports an interaction between the TAD of MRTFA and STAT5b, which suggests a role in JAK/STAT signaling. It is not yet clear whether this occurs independently from interactions between MRTFA and SRF, which are

enhanced by this process (56). Together, these studies show that MRTFA interacts with multiple factors to regulate transcription downstream of different signaling pathways and that some of these effects may be independent from SRF.

Control of cell maturation and function

While many questions remain regarding MRTFA's multiple protein–protein interactions, its importance in cell maturation and differentiation is well established. In the hematopoietic system, MRTFA plays a critical role in hematopoietic stem cell (HSC) homing and megakaryocyte maturation, as well as in regulating the function of cells in the innate immune system.

MRTFA in megakaryopoiesis

Megakaryocytes are cells of the myeloid lineage found primarily in the adult bone marrow and fetal liver and to a lesser extent in the spleen and lung. They can arise from bipotent megakaryocyte-erythroid progenitors and perhaps also from a megakaryocyte-biased subpopulation of hematopoietic stem/progenitor cells (57, 58). Megakaryocytes are ultimately responsible for producing platelets—approximately 10^{11} per day in humans. Mature megakaryocytes are large and highly polyploid with multilobated nuclei, and their maturation requires multiple rounds of endomitosis with massive cytoskeletal reorganization (59, 60). Common cell surface markers include CD41 and CD61—also known as platelet glycoproteins IIb and IIIa—which form a heterodimeric complex that binds fibrinogen. More mature megakaryocytes also express the multiprotein CD42 complex that binds von Willebrand factor (60, 61). Maturation is promoted by thrombopoietin, which binds to the thrombopoietin receptor, first identified as the myeloproliferative leukemia protooncogene and abbreviated as c-MPL (62). For more information on the role of non-MRTFA transcription factors in megakaryopoiesis, see Szalai *et al.* and Tijssen *et al.* (63, 64).

Cheng *et al.* first identified MRTFA as a potential actor in megakaryopoiesis by showing that MRTFA KO bone marrow exhibits increased numbers of megakaryocytes with dramatically reduced polyploidization and decreased platelet output, pointing to a role for MRTFA in polyploidization and/or maturation (52). Furthermore, MRTFA expression increases in murine fetal liver–derived megakaryocytes throughout maturation, and MRTFA overexpression in murine bone marrow-derived megakaryocytic progenitors increases megakaryocyte polyploidization as well as the expression of megakaryocyte markers CD41, CD42, and CD61. Gilles *et al.* showed that siRNA-mediated knockdown of MRTFA during the early stages of differentiation reduced filopodia and proplatelet formation, and introduced abnormalities in the structure of the demarcation membrane (5). These data indicate that MRTFA primarily affects the later stages of megakaryocyte maturation, including platelet production.

The ability of MRTFA to promote megakaryocyte maturation is dependent on its function as a coactivator of SRF. Conditional KO of SRF in the megakaryocyte lineage similarly results in low ploidy megakaryocytes and thrombocytopenia in mice; these megakaryocytes also exhibit abnormal actin cytoskeletons and reduced or altered proplatelet formation (52, 65). SRF conditional KO results in a more severe maturation defect than MRTFA KO, suggesting that MRTFA may have a degree of functional redundancy with other myocardin family proteins (66). Consistent with this, Smith *et al.* found that the double KO of MRTFA and MRTFB increases the severity of the thrombocytopenic phenotype relative to MRTFA KO alone.

Consistent with the requirement for SRF, epigenetic studies reveal that overexpression of MRTFA promotes megakaryocyte maturation by outcompeting TCF coactivators for SRF, resulting in increased SRF binding at CArG box-containing promoters and heightening the transcription of genes essential for megakaryopoiesis (67). Important targets of MRTFA-SRF in megakaryopoiesis include actin, vinculin, MYL9, MYH9, and myomesin-1 (5, 52).

MRTFA/SRF-induced downregulation of gene expression appears to be required for megakaryocyte polyploidization by enhancing endomitosis of megakaryoblasts. During the first round of endomitosis, wherein the 2N megakaryocyte becomes 4N, the cleavage furrow fails to fully close thereby preventing cytokinesis (68). ARHGEF2, a guanine exchange factor, must be downregulated to prevent completion of cytokinesis (69, 70). Through mechanisms that are not yet fully understood, MRTFA, *via* SRF, downregulates ARHGEF2 transcription to prevent endomitosis and promote megakaryocyte polyploidization. MRTFA KO megakaryocytes, which fail to undergo polyploidization, have increased ARHGEF2 expression relative to wild-type megakaryocytes. In these MRTFA KO cells, ARHGEF2 knockdown is sufficient to restore normal ploidy levels (70).

Other hematopoietic cell types affected by loss of function of MRTFA

The role of MRTFA in the expression of actin and other cytoskeletal genes has far-reaching impacts on the differentiation and function of diverse cell types, and its dysregulation can have significant clinical consequences. Record *et al.* describe a patient with a loss of function mutation in the MRTFA TAD that initially manifested as severe immunodeficiency coupled with thrombocytopenia as well as abnormal scarring because of defective fibroblast migration. Patient neutrophils exhibited impaired phagocytosis and chemotaxis, a phenotype that is recapitulated by shRNA knockdown of MRTFA in HL60 cells (20). Sprenkeler *et al.*, in reporting a second clinical case of severe neutrophil dysfunction caused by MRTFA deficiency, did not observe impaired fibroblast migration and suggest that this is a result of MRTFB acting in a redundant and compensatory manner because of the fact that MRTFB is expressed in fibroblasts (15). Although this seemingly conflicts with the observations by Record *et al.*, deletion of the MRTFA TAD domain confers a dominant negative effect on the protein, possibly precluding the rescue ability of MRTFB (15). Regardless, these observations suggest that MRTFA, in its role as a mediator of cytoskeletal gene transcription, is required for normal neutrophil function.

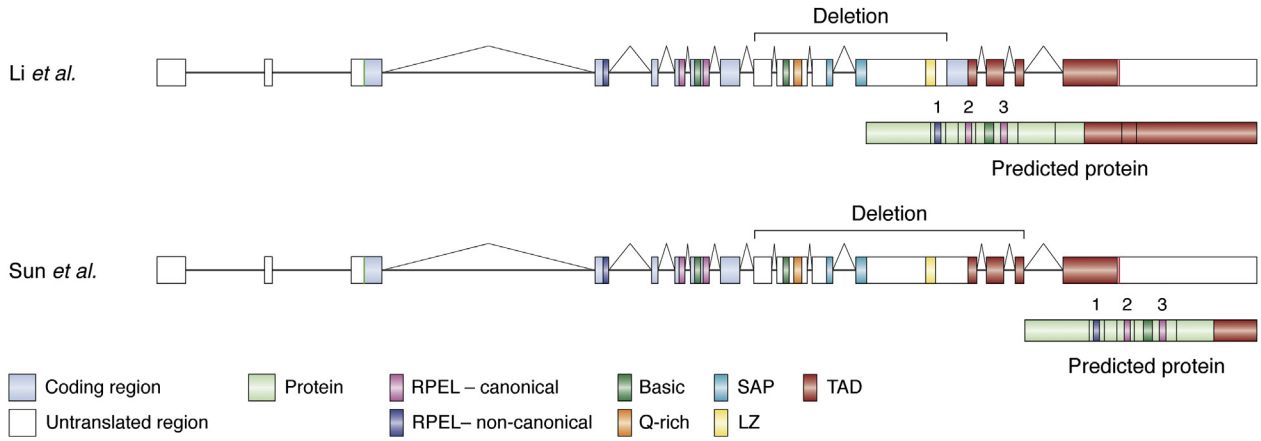


Figure 4. Overview of MRTFA KO mice developed by Li *et al* (71) and Sun *et al.* (72), including their respective exon deletions. Below each transcript is a schematic of the predicted protein encoded by the variant with critical domains indicated. LZ, leucine-zipper; MRTFA, myocardin-related transcription factor A; TAD, transcriptional activation domain.

Gene expression programs initiated by MRTFA are also involved in HSC chemotaxis and migration (71, 72). Murine fetal liver HSCs lacking SRF exhibit impaired motility and response to chemotactic signaling, ultimately failing to colonize the bone marrow during development (73). A similar effect is observed in MRTFA/B double KO cells, and HSCs lacking MRTFA/B or SRF fail to engraft when transplanted into irradiated recipients (73).

In macrophages, MRTFA, MRTFB, and SRF regulate cytoskeletal gene expression programs and appear to promote macrophage function with specificity for proinflammatory macrophages (74). Yu *et al.* report that MRTFA is necessary for the activation of proinflammatory transcription by helping to recruit members of the H3K4 methylation complex to these inflammatory promoters (53). Further, Zhang *et al.* found reduced IL-6 secretion from murine macrophages when MRTFA is inhibited, a process which may be regulated directly by p38 phosphorylation of several key residues of MRTFA (75). Whether these processes of MRTFA-directed epigenetic modification, or its ability to activate the transcription of inflammatory-related genes, occur independently of SRF remains to be confirmed.

In addition to its important roles in the hematopoietic system, KO models (Fig. 4) have revealed the effects of *Mrtfa* KO on multiple other cell types, briefly discussed here. Consistent with the finding that loss of MRTFA impairs the differentiation of smooth muscle from embryonic stem cells, *Mrtfa* KO impacts lactation in mice (3, 71). In their paper describing the first *Mrtfa* KO mouse, which expresses a truncated version of MRTFA missing exons 9 through 14, Sun *et al.* observe that MRTFA KO mothers are unable to nurse their pups. Mothers produce milk normally, but defective mammary myoepithelial cells are unable to contract properly and cannot release it (72). Li *et al.* observe the same phenomenon in their KO mouse model—which lacks only exons 9 and 10—and similarly report an inability to maintain differentiation of mammary myoepithelial cells resulting in premature involution of the mammary glands (71). While Sun *et al.* report decreased megakaryocyte ploidy and thrombocytopenia in their model, Li *et al.* do not, perhaps due to the fact that in their model the entire TAD remains intact despite the deletion of exons 9 and 10 (for a schematic representation of these deletions and summary of phenotypes, see Fig. 4 and Table 1). In addition to nursing defects, MRTFA KO pups are not born in Mendelian ratios because of occasional failure of cardiac muscle cell differentiation in utero and subsequent death of the embryo (72).

Consistent with its role in activating cytoskeletal genes, MRTFA also mediates cell migration in pathological contexts. For example, deficiency of the MRTFA/SRF complex, and thus its target genes *MYH9*, *MYL9*, and *MMP9,* reduces the invasiveness and speed of cell migration in solid tumors (4, 76). MRTFA expression positively correlates with invasive tumor migration and is dependent on TGF-β signaling (50, 77). Excellent reviews on the subject of MRTFA in metastasis and migration are Gau *et al.* and Scharenberg *et al.* (50, 78).

Acute megakaryoblastic leukemia

MRTFA was originally identified as part of the recurrent t(1;22) translocation in AMKL. AMKL is a subtype of acute myeloid leukemia (AML) characterized by the proliferation and accumulation of abnormal megakaryoblasts in the bone marrow and peripheral blood (79). AMKL is exceedingly rare in adults, accounting for approximately 1% of adult AML cases, but occurs in about 10% of pediatric AML cases and frequently presents with extensive fibrosis of the bone marrow (79, 80). This disease is subdivided into two major groups, AMKL in patients with Down Syndrome (DS-AMKL) and AMKL in patients without Down Syndrome (non-DS-AMKL). Both types of AMKL are treated with nonspecific chemotherapies which have limited efficacy and can be highly toxic. Although patients in both of these subgroups present with similar phenotypes, these two groups of disease are biologically and clinically distinct and the putative mechanism of leukemogenesis differs between them. Non-DS-AMKL progress rapidly and has a poorer prognosis than DS-AMKL, though survival varies depending on the causative mutation (80, 81). Non-DS-AMKL has been found to be associated with several

Table 1
Summary of phenotypic differences between MRTFA KO mice created in different laboratories

Phenotype	Li *et al.* (71)	Sun *et al.* (72)
Mammary gland involution	Yes	Yes
Decreased platelet count	Yes	Yes
Increased mean platelet volume	Yes	Yes
Decreased megakaryocyte ploidy	No	Yes
Increased megakaryocyte number	No	Yes

MRTFA, myocardin-related transcription factor A.

different recurrent chromosomal translocations including t(1;22)(p13;q13), which encodes the RBM15-MRTFA fusion protein (79-81). For a more in-depth review of AMKL, see Khan *et al.* and Laurent *et al.* (82, 83).

RBM15-MRTFA fusion protein

The t(1;22)(p13;q13) translocation is highly specific to infantile AMKL and is nearly always diagnosed in patients younger than 6 months of age (79). Owing to the high specificity of the t(1;22)(p13;q13) translocation to infant AMKL, it is hypothesized that this translocation may occur in utero affecting a developmentally unique hematopoietic stem or progenitor population. The fusion protein encoded by t(1;22) is RBM15-MRTFA (also known as OTT-MAL or RBM15-MKL1) (1, 2). The breakpoint in chromosome 1 occurs in the 4 kilobase *RBM15* (RNA binding motif 15) intron 1; the breakpoint in the *MRTFA* gene on chromosome 22 can occur in an intron either upstream or downstream (more common) of exon 3 (2, 80, 84). This rearrangement generates an in-frame fusion that contains almost the entire full-length coding region and all known functional domains of *RBM15*. When the translocation occurs upstream of exon 3, the entire *MRTFA* coding sequence is included, plus an additional 20 amino acids encoded by in frame codons upstream of the first ATG of *MRTFA* (2, 80). Although both *RBM15-MRTFA* and the reciprocal fusion mRNA *MRTFA-RBM15* occur in patient samples, only RBM15-MRTFA retains all of the functional regions of both *MRTFA* and *RBM15*, making it the candidate oncoprotein (1). Additionally, the predicted MRTFA-RBM15 peptide is severely truncated, containing only 17 to 25 amino acids, and does not contain any known protein motifs, suggesting it is nonfunctional (1). The molecular mechanisms by which the RBM15-MRTFA chimeric protein promotes leukemic transformation are still unknown. However, clues to the functions of this chimeric protein are revealed by the normal functions of the fusion partners.

RBM15 encodes a nuclear protein with three RNA recognition motifs (RRMs) and a Spen paralog & ortholog C-terminal (SPOC) domain (1, 79, 80). RBM15 exhibits significant homology to the *Drosophila* Spen protein, an RRM protein that plays a role in cell fate specification and cell survival (80). The activity of METTL3, the enzyme responsible for methylating target adenines for N6-methyladenosine (m6A) RNA modification, requires the presence of RBM15 (85). The primary function of RBM15 is to serve as a linker between the RNAs that are bound to its RRM domains and this m6A RNA methylation complex. It is highly likely that the phenotype of hematopoiesis-specific inducible *Rbm15* KO mice, which lose long-term reconstituting HSC function, is because of loss of m6A RNA modification as hematopoiesis-specific inducible *Mettl3* KO mice also lack functional long-term reconstituting HSCs (86). Whether m6A RNA modification accounts for the published roles of RBM15 in RNA splicing, chromatin remodeling, and nuclear export of RNA is not yet known (87).

As in other SPEN family members, the SPOC domain of RBM15 can interact with the SMRT (silencer of retinoid and thyroid receptors) and N-CoR (nuclear receptor corepressor) complexes. These interactions suggest that RBM15 may act as a transcriptional repressor when associated with DNA. However, to date, it has not been shown whether the fusion protein, RBM15-MRFTA, is capable of interacting with the SMRT/N-CoR complex *via* the SPOC domain of RBM15. In addition, Ma *et al.* show that RBM15 affects Notch signaling by binding to RBPJk (recombination signal binding protein for immunoglobulin kappa J region), a transcription factor critical for Notch signaling (80). RBM15 has cell type–specific stimulatory and inhibitory effects on Notch signaling in transient transfection assays (88).

RBM15 is differentially expressed during myelopoiesis; additionally, suppression of *Rbm15* enhances myeloid differentiation, while enforced expression inhibits this process (80). This evidence indicates that *RBM15* plays a key role in normal hematopoiesis and its aberrant regulation may promote the development of AMKL (86, 89).

Mouse model of t(1;22) AMKL

A mouse model to study the RBM15–MTRFA fusion protein was produced to assess the mechanisms underlying transformation to AMKL (2, 89). In this model, a cDNA encoding full-length human MRTFA was inserted downstream of exon 1 of endogenous murine Rbm15, which is analogous to what occurs in the t(1;22) translocation. Homozygotes are embryonic lethal (personal communication, Krause Lab). Heterozygotes are viable, have a modest increase in megakaryoblasts in the fetal liver and, *in vitro*, their spleen-derived hematopoietic progenitors have an increased colony replating capacity (81). About 5% of heterozygous *Rbm15-MRTFA* transgenic mice develop leukemia after a long latency period (~16 months) (89). Critical, though, to the link with human AMKL, the leukemia that they develop is characteristic of AMKL. Thus, the mouse model is informative, but does not parallel the presentation of human AMKL in infants.

Dysregulation of Notch signaling

Although much remains to be discovered, published data have revealed several potential mechanisms by which the *RBM15–MRTFA* fusion could promote AMKL. As with RBM15, the RBM15–MRTFA fusion protein binds to and activates RBPJk-mediated gene expression *in vivo* and *in vitro* in a dose-dependent manner suggesting that AMKL could result at least in part from dysregulated Notch signaling (80, 81).

This activity is mediated by the TAD of MRTFA and the RRM domains of RBM15 (81). Activation of Notch signaling has been implicated in HSC self-renewal, and Notch signaling inhibits myeloid differentiation under certain conditions, indicating that dysregulation of this pathway may be important in megakaryoblast transformation in AMKL (80, 81, 84). Studies performed with Notch inhibitors demonstrated that in the presence of RBM15-MRTFA, activation of RBPJk target genes is independent of the upstream Notch signaling pathway and may occur as a result of direct interactions between the fusion protein and RBPJk (81). Although deregulated Notch signaling may play a key role in the pathogenesis of AMKL, alone this dysregulation is insufficient to induce leukemia. Because Notch signaling may also act to promote normal megakaryocyte maturation, Mercher *et al.* suggest that stimulation of Notch signaling by RBM15-MRTFA may underlie the megakaryocytic phenotype of t(1;22) AMKL (81).

Aberrant regulation of SRF target genes

In addition to aberrant Notch signaling, RBM15–MRTFA may also exert its oncogenic effects *via* aberrant regulation of SRF target genes. RBM15–MRTFA has been demonstrated to have an increased ability over MRTFA alone to activate SRF-dependent gene transcription, even at very low expression levels. However, nonintuitively, simultaneous overexpression of RBM15–MRTFA with MRTFA leads to inhibition of SRF target gene expression, suggesting that the relative ratio of fusion protein to endogenous MRTFA differentially perturbs SRF-dependent transcription (7, 12). Like RBM15, the RBM15–MRTFA fusion protein is constitutively nuclear, and RBM15–MRTFA fails to respond to cytochalasin D, which induces SRF activity by dissociating MRTFA from G-actin (84). Consistent with a lack of actin-dependent nuclear shuttling, RBM15-MRTFA does not bind G-actin despite the presence of intact RPEL domains (84).

The Rho-independent nature of RBM15–MRTFA also suggests that a potential mechanism of leukemogenesis is the uncoupling of SRF activation from upstream signaling pathways. RBM15–MRTFA also activates SRF responsive promoters that are usually considered to be TCF-dependent and MRTFA-independent, thus presenting another possible mechanism of dysregulation leading to the development of AMKL (84). Interestingly, RBM15–MRTFA has a cytotoxic effect and leads to decreased proliferation in the HEK293 cell line, indicating that RBM15–MRTFA–expressing AMKL cells have undergone other modifications to be able to survive (84). Taken together, these data indicate that RBM15–MRTFA likely leads to AMKL *via* aberrant regulation of both Notch and SRF target genes. Finally, while it is known that m6A RNA modification is critical for leukemogenesis, the extent to which RBM15–MRTFA promotes AMKL by modifying the m6A epitranscriptome is not yet known (86).

Epigenetic regulation

Another potential mechanism by which the *RBM15–MRTFA* fusion gene may promote transformation is through dysregulation of epigenetic modifications leading to abnormal gene expression. Several critical regulators of epigenetic markers, including the Set1-like enzymes, Setd1a and Setd1b, and mixed-lineage leukemia (MLL) 1, MLL2, and MLL3 are implicated in leukemias, solid tumors, and other hematopoietic diseases (89). Both RBM15 and RBM15-MRTFA, but not MRTFA alone, coimmunoprecipitate with Setd1b in 6133 cells, a murine cell line expressing the fusion protein (89). The association of RBM15 and RBM15–MRTFA with the Setd1b H3K4 histone methyltransferase (HMT) complex suggests that in the presence of the RBM15–MRTFA protein, the normal targeting of the complex is altered, leading to aberrant epigenetic regulation and leukemogenesis. In the 6133 cell line, which was derived from an AMKL that occurred in a 16-month-old *Rbm15-MRTFA* transgenic mouse, overexpression of exogenous Rbm15-MRTFA promotes cellular proliferation, including cytokine-independent proliferation, and supports cell survival, possibly *via* interactions with the Setd1b HMT complex (89). Taken together, these findings suggest that leukemic transformation may be a consequence of abnormal interactions between Rbm15-MRTFA and HMT complexes, including the Setd1b HMT complex. Further studies must be conducted to determine the role of epigenetic regulation in the development of AMKL.

Cooperating mutations

In the murine *Rbm15-MRTFA* transgenic mice (on a C56Bl6 background), addition of MPL^{W515L}, an activating mutant of the thrombopoietin receptor induces a fully penetrant, early onset, and fatal disease that closely recapitulates human AMKL (81). Increased phosphorylation of ERK1/2 and cell proliferation were also observed, further indicating that this MPL mutation could enhance leukemic transformation (81). In patients with t(1;22), several additional cooperating mutations in tyrosine kinases and other signaling molecules have been identified (90). It is vital that future endeavors determine the functionality of cooperating mutations that promote transformation to AMKL.

Directions of future research

We have described here numerous functions for MRTFA in megakaryopoiesis, muscle cell differentiation, and leukemogenesis. A comprehensive study of each structural domain of MRTFA and their functions in various hematopoietic cells should be undertaken to understand how these processes are driven. To supplement this information, the tissue-specific expression levels of each TV of MRTFA should be characterized. Additional information about MRTFA-SRF transcriptional targets in megakaryopoiesis is desired; how does the activation of cytoskeletal gene expression by MRTFA-SRF lead to proplatelet formation? Furthermore, it should be investigated how RBM15–MRTFA interferes with or otherwise alters normal megakaryopoiesis beyond acting as a constitutive activator of SRF.

The potential ability of MRTFA to bind and regulate transcription factors other than SRF (*e.g.*, SMADs) is of particular

interest as this may be an alternate avenue by which MRTFA drives the development of megakaryocytes or other hematopoietic cells. Because MRTFA appears to interact with members of the H3K4 methylation complex in various cell types, a comprehensive study of this process is needed. Further characterization of the SAP domain and its ability to bind DNA independently of SRF could augment this information. Finally, though it is known that the RBM15–MRTFA fusion protein plays a role in AMKL progression by way of epigenetic modifications, this is an area that warrants further investigation. We anticipate that further definition of the molecular mechanisms by which MRTFA controls cell differentiation and maturation will provide potential avenues of investigation for novel AMKL-specific therapies as well as treatments for other diseases associated with improper regulation of cell maturation and development.

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Abbreviations—The abbreviations used are: AML, acute myeloid leukemia; AMKL, acute megakaryoblastic leukemia; DS-AMKL, AMKL in patients with Down Syndrome; H3K4, histone 3 lysine 4; HMT, histone methyltransferase; *hMRTFA*, human *MRTFA*; HSC, hematopoietic stem cell; LINC, linker of nucleoskeleton and cytoskeleton; LZ, leucine-zipper; MLL, mixed-lineage leukemia; MRTFA, myocardin-related transcription factor A; MYH9, myosin heavy chain 9; MYL9, myosin regulatory light polypeptide 9; non-DS-AMKL, AMKL in patients without Down Syndrome; SRF, serum response factor; TAD, transcriptional activation domain; TCF, ternary complex factor; TV, transcript variant.

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