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REVIEW

SRF'ing and SAP'ing – the role of MRTF proteins in cell migration

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ABSTRACT

Actin-based cell migration is a fundamental cellular activity that plays a crucial role in a wide range of physiological and pathological processes. An essential feature of the remodeling of actin cytoskeleton during cell motility is the *de novo* synthesis of factors involved in the regulation of the actin cytoskeleton and cell adhesion in response to growth-factor signaling, and this aspect of cell migration is critically regulated by serum-response factor (SRF)-mediated gene transcription. Myocardin-related transcription factors (MRTFs) are key coactivators of SRF that link actin dynamics to SRF-mediated gene transcription. In this Review, we provide a comprehensive overview of the role of MRTF in both normal and cancer cell migration by discussing its canonical SRF-dependent as well as its recently emerged SRF-independent functions, exerted through its SAP domain, in the context of cell migration. We conclude by highlighting outstanding questions for future research in this field.

KEY WORDS: Cell migration, Actin, MRTF, SRF, SAP, Cancer

Introduction

Cell migration is a highly regulated event that has an important role in both physiological and pathological processes, ranging from embryonic development to angiogenesis and tumor metastasis (Le Clainche and Carlier, 2008). Cells migrate either as isolated entities (for example mesenchymal or hematopoietic cells) or collectively as a cohesive group comprising leader cells at the front followed by follower cells (e.g. movement of epithelial cell sheets or clusters). Mesenchymal cell motility, the most widely studied form of single-cell motility, requires four fundamental steps: (i) membrane protrusion at the leading edge driven by actin polymerization, (ii) stabilization of membrane protrusion through integrin-mediated cell-extracellular matrix (ECM) adhesions, (iii) forward translocation of the cell body powered by actomyosin contractile forces and, (iv) detachment of the rear of the cell (facilitated by contractile force as well as proteolysis of cell–ECM adhesion components) (Sheetz et al., 1999). Cells can also undertake amoeboid motility – a prevalent form of immune and tumor cell motility – that involves quick expansion and intensive membrane blebbing in order to invade through the ECM with the main driving force being the contractile cortical actomyosin network. This type of motility does not require ECM proteolysis (Mierke et al., 2008; Paňková et al., 2010). Dynamic remodeling of the actin cytoskeleton is an essential feature of all motile cells. Upon sensing and transducing different micro-environmental cues (e.g. chemical gradients, haptotatic cues), cells initiate membrane protrusions through *de novo* actin nucleation and filament elongation proximal to the leading edge by concerted actions of an ensemble of actin-binding proteins (ABPs) (Small et al., 2002). Briefly, the Arp2/3 complex – activated by Wiskott–Aldrich syndrome protein (WASP) – and formins are the key initiators of actin nucleation, whereas filament elongation is aided by enabled (Ena)/vasodilator-stimulated phosphoprotein (VASP) and formins in cooperation with the profilin (a prominent nucleotide exchange factor for actin) family of ABPs (Rotty et al., 2015). The growth of actin filaments is also balanced and spatially restricted by the actions of actin-depolymerizing factors and/or actin-severing factors (cofilin, gelsolin) and actin-capping proteins, respectively. Rho-family GTPases, kinases, phosphatases and membrane phosphoinositides functionally control many of these ABPs and their upstream regulators (Bezanilla et al., 2015).

Signal-induced transcriptional regulation of several actin cytoskeleton-associated genes through the action of serum-response factor (SRF) – a ubiquitously expressed and highly conserved transcription factor – is another essential feature of the remodeling of the actin cytoskeleton during cell motility. SRF binds to the $CC[AT]_6GG$ (CArG) consensus sequence – originally discovered in the transcription regulatory sequences of the serum-inducible genes *Fos* and *Egr1* (Khachigian and Collins, 1997; Norman et al., 1988) – that is located in several of its target genes, including SRF itself and many genes related to the actin cytoskeleton, cell–ECM or cell–cell adhesion and cellular contractility, such as actin, cofilin, Arp2, myosin, vinculin, cadherin and integrin to name a few; the CArG element in aggregate within the genome is also known as the CArGome (Benson et al., 2011; Olson and Nordheim, 2010; Sun et al., 2006a). SRF activity is mainly regulated by two broad classes of transcriptional coactivator: ternary complex factors (TCFs), i.e. the ETS domain-containing proteins 1, 3 and 4 (Elk1, Elk3 and Elk4, respectively, Hill et al., 1995; Zinck et al., 1993), and the myocardin-family proteins (Miralles et al., 2003; Wang et al., 2003). However, SRF can be also regulated by members of zinc-finger (i.e. GATA; Belaguli et al., 2000) and homeobox-domain (i.e. Nkx2-5; Chen et al., 2002) transcription factors. Myocardin, the founding member of the myocardin family of proteins, is expressed exclusively in cardiac and smooth muscle cells, and in two alternatively spliced forms with the longer and the shorter variants expressed in heart and smooth muscle cells, respectively (Imamura et al., 2010; Parmacek, 2007). The two more widely expressed myocardin-family proteins are myocardin-related transcription factor A (officially known as MRTFA) that is also known as megakaryoblastic leukemia 1 (MKL1) because of its genetic rearrangement associated with acute megakaryoblastic leukemia in children (Ma et al., 2001) and MRTFB (also known as MKL2; Wang et al., 2002; Wei et al., 2007). Because TCFs and myocardin proteins compete for a common surface on the DNA-binding domain of SRF, they interact with SRF in a mutually exclusive manner and activate different sets of SRF-target genes (Esnault et al., 2014; Gualdrini et al., 2016; Miralles et al., 2003; Wang et al., 2004; Zaromytidou et al., 2006). TCF-dependent SRF-mediated gene expression is promoted by signaling via Ras and mitogen-activated protein kinases (MAPKs), whereas the MRTF arm of SRF-mediated transcription responds primarily to

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Rho-GTPase-induced fluctuations in the actin monomer concentration in cells (Buchwalter et al., 2004; Hill et al., 1995; Olson and Nordheim, 2010). Genomic recruitment of SRF occurs both constitutively and in a manner induced by growth factors, and the vast majority of signal-inducible SRF target genes are controlled by MRTF (Esnault et al., 2014). Because it is primarily the MRTF-SRF gene expression signature that is associated with the regulation of cell motility (Esnault et al., 2014), MRTF will be the primary focus of this Review. Although TCFs can indirectly suppress MRTF-SRF target genes through competition for SRF, TCF-dependent SRF target genes are mostly associated with cell proliferation, signaling and transcription (Gualdrini et al., 2016).

In this Review, we will first provide a brief overview of MRTF structure and regulation. We will then discuss the role of MRTFs in cell motility, emphasizing their canonical SRF-dependent functions, as well as emerging SRF-independent functions, in both physiological and pathological contexts. Readers are also encouraged to refer to several excellent general reviews on MRTF-SRF signaling (Cen et al., 2004; Gasparics and Sebe, 2018; Hendzel, 2014; Olson and Nordheim, 2010; Pipes et al., 2006; Posern and Treisman, 2006).

Structural features and regulation of MRTF

Basic structural features

Myocardin proteins are encoded by genes located on different chromosomal loci (human myocardin 17p11.2, MRTFA: 22q13.2, MRTFB 16p13.12) but share common structural motifs (Fig. 1). The three RPEL motifs (RPEL1, RPEL2, RPEL3) at the N-terminus of MRTFs interact with G-actin; the affinity for actin is weakest for the R3 motif (Mouilleron et al., 2008). Crystallography studies have revealed that the two spacer regions (S1 and S2) between the RPEL domains also contribute to actin binding, allowing trivalent (actin binding the RPEL1, RPEL2 and S1 regions) aa well as pentavalent (two additional actin molecules also binding the S2 and RPEL3 regions) complexes between actin and the RPEL domain for MRTFA (Mouilleron et al., 2011). Because of conserved residues in the spacer region, MRTFB presumably also interacts with actin in a similar manner. An extended bipartite basic region comprising basic boxes 3 and 2 (B3 and B2) is located within RPEL2 and the N-terminal side of RPEL3 contains a nuclear localization signal (NLS) (Pawłowski et al., 2010). The other basic region B1 harbors an additional NLS and the SRF-binding site. Further important regions are a glutamine (Q)-rich domain that is important for the regulation of nuclear localization by promoting nuclear export and the interaction with SRF; the 35-residue SAF-A/B, acinus, PIAS (SAP) motif, a putative DNA-binding domain found in other nuclear proteins involved in chromatin remodeling (Aravind and Koonin, 2000) that is used by MRTFs to transcribe genes in an SRF-independent manner; a leucine zipper (LZ) motif that allows homo- and hetero-dimerization; and a C-terminal transcriptional activation domain (TAD) (Zaromytidou et al., 2006). Because of structural similarities, MRTF isoforms generally have overlapping functions, which explains why developmental defects in several organs – such as liver (Sun et al., 2009), heart (Parlakian et al., 2004), muscle (Li et al., 2005) and brain (Stringer et al., 2002) – induced by conditional knockout of SRF in mice – can be recapitulated only when both MRTF genes are deleted. However, there are certain exceptions. For example, MRTFA knockout mice, although viable, exhibit defects in myoepithelial differentiation of mammary epithelial cells as well as in lactation, suggesting that loss of MRTFA function in the mammary gland cannot be compensated by that of residual MRTFB (Li et al., 2006; Sun et al., 2006b). Similarly, global knockout of MRTFB alone is sufficient to cause vascular malformation and embryonic lethality in mice (Oh et al., 2005). Therefore, MRTF isoforms also have unique functions in specific contexts.

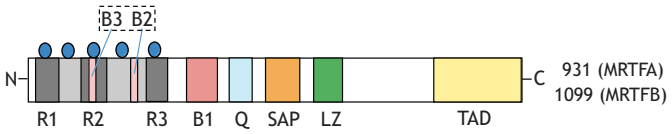

Fig. 1. Main structural features of MRTF. Shown are the main domains of MRTFs. Located N-terminally are the three RPEL domains (R1, R2 and R3; dark gray) separated by spacer regions (light gray), all of which are bound in a pentavalent complex to actin (blue ovals). The extended basic region comprising boxes B3 and B2 contains a bipartite nuclear localization signal (NLS); a second basic region (B1) with another NLS and an SRF-binding site; a glutamine (Q)-rich domain that promotes nuclear export and regulates SRF binding; a putative DNA-binding motif – the SAP domain – that allows MRTF to transcribe genes in SRF-independent manner; a leucine-zipper (LZ) that facilitates homo- and hetero-dimerization of the protein; and the transcriptional activation domain (TAD). Notice that myocardin has similar structural motifs.

Regulation of MRTF

Cellular localization and activation of MRTFs are tightly regulated in response to several intracellular signals.

Actin-dependent regulation

MRTF-SRF signaling is highly sensitive to the monomeric (G-) actin concentration in cells. Accordingly, increases in the overall cellular ratio of G-actin to F-actin – either by inhibiting Rho or in response to the actin-depolymerizing drug latrunculin – leads to a cytoplasmic accumulation of MRTFs, thereby suppressing serum-dependent SRF activation (Fig. 2). By contrast, nuclear accumulation of MRTFs and activation of SRF are promoted when actin polymerization is stimulated by either constitutive activation of Rho or jasplakilonide-induced stabilization of F-actin (Cen et al., 2003; Duggirala et al., 2015; Miralles et al., 2003; Mouilleron et al., 2008; Muehlich et al., 2008; Posern et al., 2004; Sotiropoulos et al., 1999; Sun et al., 2006b; Vartiainen et al., 2007). Artificial stimulation of actin polymerization in the nuclear compartment, either by overexpression of a depolymerization-resistant and nuclear-constrained mutant of actin (Kokai et al., 2014) or depletion of nuclear G-actin by microtubule-associated monooxygenase calponin and LIM domain-containing 2 (MICAL2) (Lundquist et al., 2014), also promotes MRTF-SRF activity, suggesting that the state of actin polymerization in the nucleus also influences MRTF and/or SRF function. Formins, actin-nucleating and -elongating factors, also have a key role in nuclear actin polymerization, leading to MRTF and SRF activation (Baarlink et al., 2013; Plessner et al., 2015). The RPEL domains are responsible for actin-dependent negative regulation of MRTFs. For instance, a MRTFA mutant that lacks RPEL domains – and is, therefore, impaired in its interaction with actin – constitutively localizes to the nucleus and activates SRF even in the absence of serum (Miralles et al., 2003; Vartiainen et al., 2007). This is because binding to G-actin not only prevents MRTF to interact with the importin family of nuclear import factors (Pawłowski et al., 2010) but also promotes CRM1-dependent nuclear export of MRTF (Vartiainen et al., 2007). Notice that importin-mediated nuclear import of MRTFA is also promoted by its interaction with the mRNA export factor Ddx19 (DDx19B) – an interaction that is not regulated by actin. This suggests that the subcellular localization of MRTF can be also regulated in an actin-independent manner

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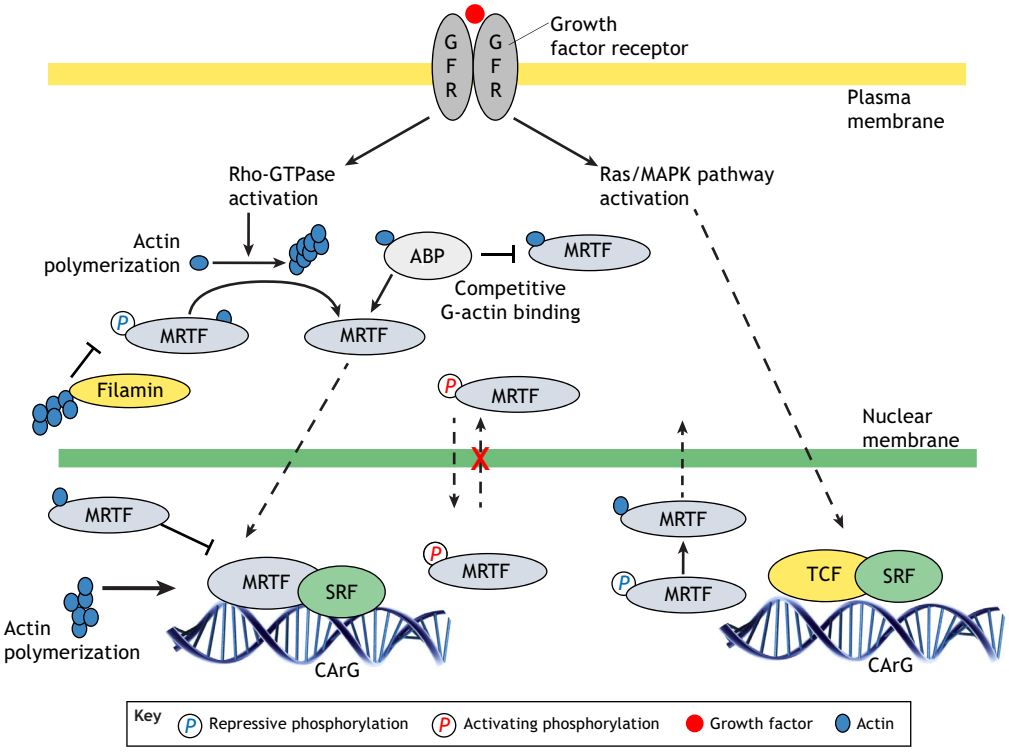

Fig. 2. Regulation of MRTF by actin and phosphorylation. Binding of growth factors to growth factor receptors (GFRs) activates Rho-GTPase or Ras-MAPK signaling pathways, leading to activation of SRF and its subsequent association with the MRTF or TCF families of coactivators, respectively. Activation of Rho-GTPase initiates actin polymerization and promotes the dissociation of MRTF from monomeric actin (G-actin). MRTF then translocates to the nucleus where, together with SRF, it promotes the expression of SRF target genes. Dissociation of MRTF from G-actin is also facilitated by sequestration of actin by other actin-binding proteins (ABPs) through competitive inhibition. Nuclear import and export of MRTF is regulated by either repressive or activating phosphorylation. MRTF-SRF signaling is also promoted through polymerization of nuclear actin or through interaction of the ABP filamin with MRTF, thereby inhibiting MRTF phosphorylation (i.e. repression) and attenuating its interaction with actin.

(Rajakylä et al., 2015). Although in unstimulated (serum-starved) cells MRTFs are predominantly localized in the cytoplasm, MRTFA continuously shuttles between the nuclear and the cytoplasmic compartments (Vartiainen et al., 2007). Rho-dependent G-actin depletion in response to serum stimulation leads to downregulation of interaction between MRTFA and actin in both the nucleus and the cytoplasm, thereby allowing the nuclear accumulation of MRTFA and SRF activation (Vartiainen et al., 2007). On the basis of subcellular localization studies that involved MRTFA mutants harboring alanine substitutions in the various RPEL and spacer regions, it has been postulated that the trivalent complex between actin and MRTF may readily form in cells, undergoing constant nucleocytoplasmic shuttling. It is, however, the pentameric actin–MRTF complex that is mainly sensitive to fluctuations of the G-actin concentration, thereby impacting the nuclear localization of MRTF (Mouilleron et al., 2011); however, this requires direct experimental validation. Dissociation of MRTF from actin can also occur independent of changes in the G-actin concentration. For example, several WH2-domain-containing actin-polymerizing factors (N-WASP, WAVE2, Spire and Cobl) compete with MRTFA for actin-binding and promote MRTFA activity by sequestering actin from MRTF (Weissbach et al., 2016). Note that myocardin cannot be efficiently retained in the cytoplasm and, therefore, tends to be constitutively active. This is despite myocardin being structurally similar to MRTFs because – compared with MRTFs – myocardin has much weaker affinity for G-actin (Guettler et al., 2008; Wang et al., 2001) and CRM1 (Hayashi and Morita, 2013), and stronger affinity for importin (Nakamura et al., 2010). In summary, nuclear localization of MRTFs and activation of MRTF-SRF are negatively regulated by the availability of G-actin to interact with MRTFs, which can be affected either by direct changes to actin polymerization or indirectly through other actin-binding proteins.

Given the critical importance of actin in regulating the interaction between MRTFs and nuclear import/export factors, as well as the localization and function of actin, an important question is how these interactions are modulated. Earlier studies have shown that growth-factor signaling via both Rho- and Ras-MAPK-dependent pathways leads to MRTF phosphorylation (Kalita et al., 2006; Miralles et al., 2003; Muehlich et al., 2008). A recent study has now identified at least 26 serum-induced Ser/Thr phosphorylation sites within MRTFA, many of which targets of MAPK – at least in *in vitro* kinase assays (Panayiotou et al., 2016). Abolition of all 26 phosphorylation sites diminished SRF activation, suggesting that phosphorylation (which is also inhibited by G-actin binding) is required for transcriptional activation of MRTFA. In particular, phosphorylation of Ser98 inhibited the formation of the MRTFA–actin complex, hence, promoting nuclear import of MRTFA. By contrast, phosphorylation of Ser33 activated the nuclear export sequence of MRTFA, thereby facilitating its CRM1-dependent nuclear export (Panayiotou et al., 2016). Binding to nuclear actin, which leads to nuclear export of MRTF, is facilitated by MAPK-mediated phosphorylation of MRTFA on Ser454 (Muehlich et al., 2008). The actin-binding protein filamin, which interacts with MRTFA, plays an important role in preventing MRTFA phosphorylation and, so, switches MRTFA from its repressed G-actin-bound state to its activated state (Kircher et al., 2015). Finally, MRTFA phosphorylation by GSK3β promotes its ubiquitylation and subsequent degradation, suggesting that cellular levels of MRTF can be also regulated by phosphorylation (Charbonney et al., 2011). In summary, MRTF is regulated both positively and negatively by phosphorylation, depending on the site of phosphorylation.

Dual regulation through cell–cell adhesion and TGFβ signaling

A series of elegant studies by Kapus and colleagues have shown that cross-talk between cell–cell adhesion and the transcription factor SMAD3, a central mediator of TGFβ signaling, can regulate MRTFA function (Fig. 3). For example, disruption of cell–cell adhesion in epithelial cells leads to p38MAPK-dependent nuclear translocation and MRTFA function through activation of the

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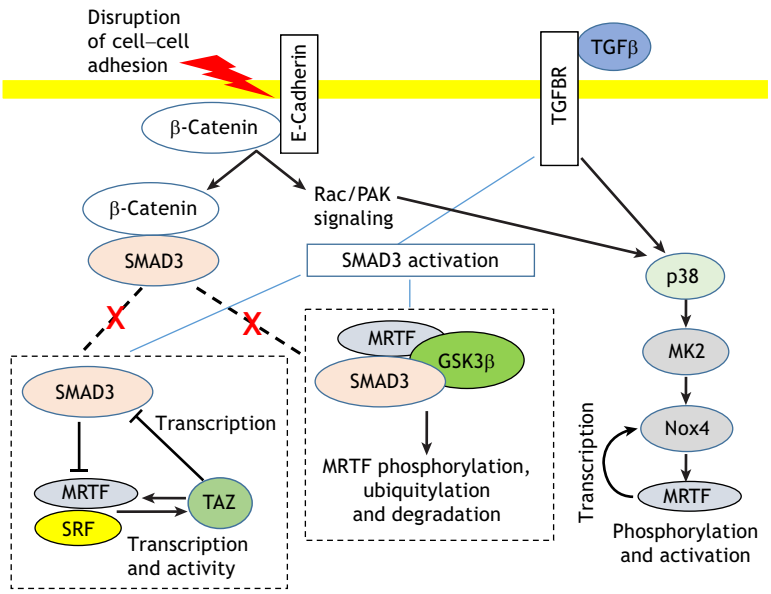

Fig. 3. Dual regulation of MRTF through cell–cell adhesion and TGFβ signaling. MRTF function can be positively or negatively regulated by loss of cell–cell adhesion and SMAD3. Binding of SMAD3 to MRTF promotes GSK3β-mediated phosphorylation of MRTF, leading to MRTF ubiquitylation and degradation. The inhibitory effect of SMAD3 on MRTF can be relieved by β-catenin-mediated sequestration of SMAD3 upon its dissociation from E-cadherin following the disruption of cell–cell junctions. This process is further reinforced by MRTF-SRF-mediated stimulation of TAZ expression, a negative regulator of *SMAD3* gene expression. There is also a mutual dependence between MRTF-SRF and TAZ signaling. Disruption of cell–cell adhesion also triggers MRTF-SRF activation through the activation of a Rac–PAK–p38MAPK signaling axis. p38MAPK, which is also activated by TGFβ signaling, promotes MRTF-SRF activity through MRTF-dependent NOX-mediated phosphorylation and activation of MRTF in a SMAD3-independent manner.

Rac–PAK (p21-activated kinase) signaling axis (Sebe et al., 2008). SMAD3, which is activated upon stimulation with TGFβ, is a key inhibitory factor of MRTFA as its binding to SMAD3 leads to the recruitment of GSK3β, with subsequent GSK3β-mediated phosphorylation of MRTFA, resulting in its ubiquitylation and degradation. SMAD3-mediated inhibition of MRTFA can be reversed upon disruption of cell–cell adhesion through sequestration of SMAD3 by cytoplasmic β-catenin, which leads to dissociation of the complex between MRTFA and SMAD3, resulting in activation of MRTFA-SRF (Charbonney et al., 2011; Masszi et al., 2010; Speight et al., 2013). This process is further augmented by MRTF-dependent transcription of the main mechanosensitive transcription factor *TAZ*, as TAZ activity can lead to downregulation of *SMAD3* gene expression (Miranda et al., 2017). MRTFA-mediated *TAZ* gene expression is facilitated by TGFβ in a SMAD3-independent manner through activation of the p38MAPK–MK2 (MAPKAPK2)–NADPH oxidase (NOX) signaling pathway, and NOX-mediated phosphorylation and activation of MRTFA (Miranda et al., 2017). Furthermore, since *NOX* can be also transcriptionally induced by activation of MRTFA-SRF, it creates a feed-forward loop in this signaling circuit (Rozycki et al., 2016). MRTF and TAZ can also functionally antagonize each other through their direct interactions. Moreover, depending on the context, i.e. whether cells are subjected to mechanical stimuli alone or to mechanical stimuli in combination with TGFβ, MRTF function can be fine-tuned through its binding with TAZ and SMAD3 (Speight et al., 2016). Finally, there is evidence of a synergy between TAZ and SMAD3 in gene regulation as these transcription factors can bind to adjacent cis-elements in the promoters of certain genes (Hiemer et al., 2014), as well as of mutual dependence between MRTFA-SRF and Yes-associated protein (YAP)–TAZ signaling. MRTF-SRF activates YAP-TAZ activity through cell contractility and YAP-TAZ promotes MRTF-SRF activity through potentiation of TGFβ signaling (Foster et al., 2018). Collectively, these studies suggest that there is a context-specific crosstalk between MRTF-SRF, YAP-TAZ and TGFβ-SMAD3 signaling pathways. Recent evidence also indicates that cell–cell contact is necessary for RhoA-mediated actin cytoskeletal control, MRTFA-SRF-mediated transcriptional activity and the maintenance of cardiomyocyte lineage (Dorn et al., 2018), suggesting that cell–cell interaction can have context-specific effects on MRTF-SRF signaling.

MRTF-SRF signaling in normal and cancer cell migration

Numerous studies have demonstrated that loss of function of either MRTF or SRF causes defects in the actin cytoskeleton (marked by reduced amounts of F-actin) and migration of a diverse range of both non-mammalian (i.e. *Drosophila* border cells in the ovaries during oogenesis and tracheal branching during development) (Han et al., 2004; Somogyi and Rørth, 2004) and mammalian cells in certain physiological contexts, such as development, angiogenesis, hematopoiesis and immune function (Box 1). Note that, unlike the studies in *Drosophila* – an organism comprising only one myocardin gene (*mal-d*) that is homologous to mammalian MRTFA – most of the loss-of-function studies in mammalian cells required simultaneous inhibition of both MRTF isoforms owing to the functional redundancy of the isoforms. MRTF-SRF signaling also contributes to motility, invasion and metastasis of tumor cells. For example, the inhibitory action of the suppressor of cancer cell invasion (SCAI) protein on human breast cancer cell invasion was linked to its ability to form a ternary complex with MRTF and SRF, and inhibit the MRTF-SRF complex in the nucleus (Brandt et al., 2009). Knockdown of either MRTFA isoform or SRF reduced directed migration and invasion of human breast cancer (MDA-MB-231) and mouse melanoma (B16) cells, both *in vitro* and *in vivo* (Medjkane et al., 2009). Likewise, pharmacological inhibition of MRTF-SRF signaling through the MRTF inhibitor CCG-1423 (Hayashi et al. 2014) or its derivative CCG-203971 inhibited migration and invasion of melanoma (Watanabe et al., 2015) and prostate (Evelyn et al., 2010, 2016) cancer cells *in vitro*, and experimental lung metastasis of melanoma cells *in vivo* (Haak et al., 2017). By contrast, overexpression of MRTFA stimulated motility of the non-invasive MCF-7 breast cancers (Luo et al., 2014; Zhang et al., 2013). Several other studies correlated the alteration in either expression or localization of MRTFs to phenotypes of cell motility in response to different molecular and pharmacological perturbations. For example, treatment with Tranilast that blocks Ca^{2+}-permeable ion channels, led to attenuation of MRTFA protein levels with concomitant reduction of breast cancer cell migration (Subramaniam et al., 2011). Overexpression of miRNA-200c, which suppresses

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Box 1. Reported roles of mammalian MRTF-SRF complexes in normal migration of different cell types

- **Neuronal cells:** Brain-specific deletion of SRF causes defects in neurite outgrowth, axon guidance and neuronal migration in mice. These effects are also mimicked upon conditional co-ablation of both MRTF isoforms (Alberti et al., 2005; Knöll et al., 2006; Mokalled et al., 2010).
- **Stem cells:** Hematopoietic stem cell (HSC)-specific knockout of SRF is associated with reduced adhesion, F-actin assembly and chemotactic migration of HSCs, leading to failure of HSC seeding in the bone marrow in mice. MRTFA and/or MRTFB knockout also caused impaired bone colonization, phenocopying SRF deletion (Costello et al., 2015).
- **Immune cells:** MRTFA deficiency causes dysfunction of the actin cytoskeleton, impaired migration and phagocytic ability of myeloid lineage-immune cells, including neutrophils and primary dendritic cells (Record et al., 2015).
- **Cardiac and smooth muscle cells:** Embryonic disruption of MRTFB is lethal in mice between embryonic day 13.5 and 14.5 because of defects in cardiac neural crest cell migration, vascular patterning, cardiac outflow tract and smooth muscle differentiation (Oh et al., 2005). MRTFA knockdown inhibits motility of rat aortic vascular smooth muscle cells (Minami et al., 2012). CTRP6 (officially known as C1QTNF6) inhibits TGFβ-mediated promotion of cardiac fibroblast migration and myofibroblast differentiation by targeting the RhoA–MRTFA pathway (Lei et al., 2015). Migration of epicardial cells also exhibits a dependency on MRTF-SRF signaling (Trembley et al., 2015). Loss of SMAD3 promotes migration of human pulmonary arterial smooth muscle cells and endothelial cells through increased activation of MRTFA (Zabini et al., 2017).
- **Endothelial cells:** Selective disruption of either *SRF* or the *MRTFA* and *MRFTB* genes in endothelial cells, when conditionally induced in neonatal and adult mice, causes a prominent reduction in actin-based filopodial processes, invasion of tip cells and angiogenesis in the retina (Franco et al., 2013; Weinl et al., 2013). In line with defective angiogenesis *in vivo* under MRTF-deficient or SRF-deficient conditions, knockdown of either SRF or MRTFs causes defects in migration and angiogenic ability of both human and rodent endothelial cells *in vitro* (Franco et al., 2013, 2008). Pro-angiogenic growth factor stimulation promotes nuclear accumulation of MRTFA (Franco et al., 2013) and also forces angiogenesis stimulated through expression of MRTFA in the heart in mice (Hinkel et al., 2014). Finally, consistent with the pro-migratory and pro-angiogenic role of MRTF, the inhibitor of MRTF-SRF activity CCG-1423 suppresses migration of human dermal microvascular endothelial cells *in vitro*, angiogenic ability of human and mouse endothelial cells *in vitro* and *ex vivo,* and developmental angiogenesis in zebrafish embryos *in vivo* (Gau et al., 2017).
- **Epithelial cells:** Loss of function of SRF reduces plasma membrane blebbing, whereas that of either MRTFA and SRF or MRTFB and SRF reduces entosis of mammary epithelial cells (Hinojosa et al., 2017).

epithelial-to-mesenchymal transformation (EMT) through targeting proteins containing the zinc-finger E-box-binding homeoboxes 1 and 2 (ZEB1 and ZEB2), or of miRNA-206 caused cytoplasmic localization or degradation of MRTFA, respectively, thereby suppressing migration and invasion of breast cancer (Jurmeister et al., 2012) or thyroid cancer (Zhang et al., 2015) cells, respectively. Consistent with the role of formins in promoting nuclear accumulation of MRTFA, knockdown of the formin FHOD1 increased the levels of cytoplasmic MRTFA, thereby inhibiting the motility of melanoma cells (Peippo et al., 2017). Furthermore, increased MRTFA activity has been correlated with increased migration, invasion and lung metastasis of 4T1 breast cancer cells (Asparuhova et al., 2015). In line with these findings, which mostly pertained to single-cell migration, there is evidence for the ability of MRTFB to promote the collective migration of lung cancer cells (Kato et al., 2014). Finally, MRTF and/or SRF function is, in mammary epithelial cells, also important for plasma membrane blebbing and bleb-dependent entosis, the invasion of one cell into another (Hinojosa et al., 2017). Thus, it will be interesting to explore whether MRTF-SRF activity promotes other types of blebbing-associated invasive motility, such as ameboid motility of tumor cells. Collectively, these studies underscore a general pro-migratory role of MRTF-SRF signaling in both physiological and pathological contexts.

MRTF-SRF signaling also plays an important role in the metastatic colonization of tumor cells, another critical aspect of cancer metastasis. For example, experimental lung colonization of breast cancer (Medjkane et al., 2009) and melanoma (Haak et al., 2017) cells are impaired upon knockdown of MRTF (or SRF) and pharmacological inhibition of MRTF-SRF signaling, respectively. The gene expression signature of MRTFA is enhanced in lung cancer cells downstream of the cell-adhesion molecule L1CAM that has a key role in the emergence from metastatic latency and subsequent colonization of extravasated tumor cells. Moreover, MRTFA knockdown alone is sufficient to cause significant defect in brain colonization of lung cancer cells (Er et al., 2018). Overall, these findings underscore a pro-metastatic role of the MRTF-SRF transcription axis in several cancer contexts. Interestingly, a recent study has shown that MRTFA-SRF plays a key role in the drug resistance of basal cell carcinoma (BCC) of skin cells through activation of glioma-associated oncogene (GLI1) – a key mediator of hedgehog pathway. Also, CCG-203971 has therapeutic effect in mouse and human breast cancer cell that are otherwise resistant to inhibition of Smoothened (a receptor for hedgehog) (Whitson et al., 2018). Therefore, the MRTF SRF pathway could also be a promising therapeutic target in certain drug-resistant malignancies.

Mechanisms of MRTF-dependent regulation of cell migration

SRF-dependent mechanisms

The general similarity between cell motility phenotypes, depending on whether MRTF or SRF is being modulated in most biological contexts, clearly suggests that the SRF-mediated gene expression plays a key role in MRTF-dependent regulation of cell motility. The MRTF-SRF transcriptional axis impacts on all main aspects of cell motility, such as protrusion, adhesion and contractility. Indeed, a dysfunctional actin cytoskeleton and impaired actin-based protrusions are key hallmarks of MRTF- or SRF-deficient cells (Weinl et al., 2013). Consistent with actin being one of the main transcriptional targets of the MRTF-SRF transcription axis, MRTFs are required for homeostatic regulation of actin; and restoration of actin level rescues the motility defect that is induced by MRTF deficiency in both *Drosophila* and human breast cancer cells (Salvany et al., 2014). Therefore, actin homeostasis is a key function of MRTF in the context of regulation of cell motility. MRTF-SRF activity can also affect the actin cytoskeleton and cell motility by regulating the expression of proteins that control the state of actin polymerization and F-actin reorganization, such as certain components of the actomyosin contractile apparatus. For example, defects in neuronal cell migration resulting from MRTF deficiency have been associated with reduced gene expression of the actin-severing proteins gelsolin and cofilin, and a dramatic upregulation of inhibitory phosphorylation of cofilin (Mokalled et al., 2010). Increased cofilin phosphorylation has been linked to the diminished expression of *Pctaire1* (officially known as *Cdk16*) an MRTF-SRF target gene and upstream regulator of Cdk5, as well as the suppression of Cdk5 kinase activity, resulting in activation of the PAK–LIMK signaling axis (PAK being inhibited by Cdk5-mediated phosphorylation and able to activate LIMK that directly phosphorylates cofilin) (Mokalled et al., 2010). Disrupted actin

cytoskeleton, reduced contractility and impaired motility of MRTFA-deficient neutrophils were correlated with the reduced expression of several actin regulators and actin-cytoskeleton-related proteins, including CDC42BPA and B, cortactin, FNBP1L (a positive regulator of actin polymerization depending on Cdc42 and/or N-WASP) and Myl9 (a component of myosin II) (Record et al., 2015). At least in MCF7 breast cancer cells, Myl9 upregulation was partly responsible for a MRTFA-induced hypermotile phenotype (Luo et al., 2014; Zhang et al., 2013). The ability of MRTFA to regulate the expression of factors that affect actomyosin contractility, such as Myl9, explains why it has a pivotal role in contractility-inducing cellular transitions, including epithelial-to-myofibroblast transformation (EmyT) and fibroblast-to-myofibroblast transformation (FMyT) (Lighthouse and Small, 2016; O'Connor and Gomez, 2013; Small, 2012; Trembley et al., 2015; Velasquez et al., 2013). Finally, MRTF-SRF activity promotes membrane blebbing and entosis by transcriptionally upregulating the gene expression of ezrin, a key member of the ERM (ezrin–radixin–moesin) family of proteins (Hinojosa et al., 2017). Thus, MRTF-SRF function also presumably plays a role in modulating the link between actin and the cytoskeletal membrane during bleb-associated invasive cell motility.

Cell–ECM adhesion, another important feature of cell migration, is also impacted by perturbation of MRTF-SRF function. Conditional knockout of either MRTFA, MRTFB or SRF reduced cell adhesion in hematopoietic stem cells (HSCs) (Costello et al., 2015). β1-integrin is an SRF target gene that is transcriptionally downregulated when MRTF-SRF function is inhibited, resulting in the suppression of an invasive phenotype of metastatic breast cancer cells (Brandt et al., 2009). There is also evidence for the ability of MRTFB to post-transcriptionally promote protein synthesis of β1-integrin by regulating the stability and translation of β1-integrin mRNA; this is mediated by repression of miRNA-124 (a microRNA that targets the 3′ UTR region of β1-integrin mRNA) in the leader cells during collective migration of lung cancer cells (Kato et al., 2014). Interestingly, higher levels of αV- and β1-integrins promote the nuclear localization of MRTFA and activation of MRTF-SRF, which in turn, upregulates transcription of the ubiquitin-like modifier of focal adhesion and cytoskeletal proteins ISG15 to enhance breast cancer cell motility, thus suggesting a synergistic action between integrin and MRTF-SRF signaling in the context of cell motility (Hermann et al., 2016). Although optimal cell–ECM adhesion is required for cell motility, excessive adhesion is counterproductive for cell motility. This explains why overexpression of MRTFA promotes migration of weakly adhesive breast cancer cells under low-adhesive but not high-adhesive culture conditions (Leitner et al., 2011). Furthermore, the motility of strongly adhesive cells, such as mouse fibroblasts and mammary epithelial cells, is actually suppressed by MRTFA with concomitant transcriptional upregulation of certain genes involved in cell–cell interactions (e.g. *Pkp2*) and cell–ECM interactions (e.g. *Itga5*, *FHL1*). Importantly, the anti-migratory effect of MRTFA on mammary epithelial cells can be reversed by silencing of Pkp2 and FHL1 (Leitner et al., 2011). Similarly, overexpression of constitutively active MRTFA suppresses migration and invasion of melanoma cells through integrin-dependent hyperactivation of focal adhesion kinase (FAK) and enhanced cell adhesion (Kishi et al., 2016). Therefore, the intrinsic cellular adhesiveness appears to be an important determinant for how MRTF affects cell motility, which partly provides the underlying basis for the context specificity of cell migration effects.

Finally, MRTF-SRF activity also regulates the expression of many secreted proteins that are involved in cell migration. For example, increased MRTFA-SRF activity and transcriptional upregulation of the secreted proteins CCN1 and CCN2 (officially known as CYR61 and CTGF, respectively), which are transcriptional targets of MRTF-SRF, were linked to the effect of the actin-monomer sequestering protein thymosin β4 in promoting angiogenesis and endothelial cell motility (Hinkel et al., 2014). Relevant to cell migration in a 3D environment, the ECM molecules tenascin C (TNC), collagen I and ECM-degrading proteases, such as MMP1, MMP2, MMP9 and MMP4, are transcriptionally regulated by MRTF-SRF (Asparuhova et al., 2015; Luchsinger et al., 2011; Parreno et al., 2014; Yu-Wai-Man et al., 2014). Tubulin acetylation, which protects microtubules from mechanical stress, promotes microtubule-dependent transport of matrix-degrading enzymes and plays a role in cell motility (Bouchet and Akhmanova, 2017; Castro-Castro et al., 2012; Palazzo et al., 2003). Because MRTF-SRF is capable of promoting tubulin acetylation by stimulating transcription of *ATAT1* (Fernández-Barrera et al., 2018), the MRTF-SRF axis also potentially controls cell migration through its effect on the microtubule cytoskeleton and microtubule-dependent transport processes.

SRF-independent mechanisms

Emerging evidence in the literature suggests that the role of MRTFs in cell motility extends beyond its SRF-mediated activity. First, MRTFA can promote cell migration through cooperation with other transcription factors, apart from SRF. For example, interactions of MRTFA with STAT3 (Liao et al., 2014; Xiang et al., 2017a,b; Xing et al., 2015; Zhuang et al., 2017) and YAP (Kim et al., 2017) enhance migration, invasion and metastatic competency of breast cancer cells. MRTFA can also form a complex with SMAD3, which binds to the cis-element GCCG-like motif of target genes, such as Slug (officially known as *Snai2*) and induces expression of Slug to drive TGFβ1-induced repression of E-cadherin protein levels suppression of and EMT, a key transformation of epithelial cells in order to induce cell motility (Morita et al., 2007).

Second, MRTFA can also transcribe genes in an SRF-independent manner by utilizing its SAP domain. Gene expression studies in non-transformed HCC1 mammary epithelial cells that overexpressed wild-type MRTFA (WT), MRTFA comprising point mutations in its B1 domain to disrupt SRF interaction (SRF-mutant) or a MRTFA mutant whose SAP domain was deleted (ΔSAP-MRTFA) identified ∼200 genes that exclusively depend on the SAP domain of MRTFA and do not depend on the ability of MRTF to bind to SRF; however, specific core sequences of SAP-targeted cis-elements have, so far, not been identified (Gurbuz et al., 2014). It is worth noting that a subset of the MRTF-target genes requires both the SAP domain and SRF. Interestingly, Gurbuz and colleagues also found that chemotactic migration of HCC1 cells is either unaffected or only modestly increased upon overexpression of WT or the SRF-mutant, respectively. However, chemotaxis was dramatically suppressed when the ΔSAP-MRTFA was overexpressed. As these experiments were performed in an overexpression setting, it is still unclear whether overexpression of ΔSAP-MRTFA inhibits migration by suppressing the expression of pro-migratory SAP-domain-target genes, inhibits SRF-mediated functions of MRTFA through a dominant-negative effect (an important issue because MRTF can form homo- as well as heterodimers) or both. However, the last option, although it cannot be ruled out formally, fails to explain the increased migration of HCC1 upon overexpression of the SRF-mutant (Gurbuz et al., 2014). The elevated expression of MRTFA SAP-domain function-dependent MRTFA target genes,

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which occurs most prominently in triple-negative breast cancer cells and reflects a poor prognosis (Asparuhova et al., 2015), strongly correlates with a putative pro-migratory effect of the SAP-domain-directed action of MRTFA. Although several candidate target genes that are dependent on SAP-domain activity of MRTFA are associated with cell motility, such as for example TNC (promoting cell adhesion and pro-migratory EGF signaling), keratin 5 (Krt5; controlling cell deformability) Kif26B (controlling cell polarity and adhesion) and Adamts26 (involved in ECM proteolysis) (Gurbuz et al., 2014), it is not known to what extent these proteins actually contribute to MRTF-dependent modulation of cell migration. We have recently reported that MRTFs play a crucial role in co-regulating the cellular abundance of PFN1 and PFN2 – the two main isoforms of the actin-binding protein profilin, a main regulator of actin dynamics and cell migration – by utilizing their SAP-domain-mediated transcriptional activity in an SRF-independent manner (Joy et al., 2017). Interestingly, MRTF activity promotes the cellular retention of profilin and, so, inhibits its release into the extracellular environment, rather than inducing its transcription, through an intermediate step that involved the regulation of the expressions of certain STAT isoforms. We have shown that overexpression of PFN1 can partly reverse the effect of MRTF knockdown on breast cancer cell motility (Joy et al., 2017); therefore, control of the externalization of selective actin-binding proteins is a potential novel mechanism for regulation of cell motility that is dependent of SAP-domain function of MRTF.

The studies summarized above demonstrate that the role of MRTFs in cell migration is complex and context specific, and that they can regulate cell migration through both SRF-dependent and -independent activities (see Fig. 4). The complexity and context specificity of MRTF-induced changes in cell migration could be owing to several reasons. First, MRTF-SRF has a broad range of transcriptional targets spanning from cytoskeletal to regulatory molecules of cell–cell and cell–ECM interactions, signaling and ECM-modifying molecules (Esnault et al., 2014), the integrated output of which can be cell-type specific conferring context specificity with regard to the overall readout of cell migration. Second, there could be context-specific differences in the status of the other signaling pathways that either crosstalk with (e.g. SMAD, YAP-TAZ) or indirectly affect (e.g. Ras-MAPK-TCF) MRTF-SRF signaling. Third, as recently demonstrated by higher levels of MRTF activation in cancer-associated fibroblasts, which reside in ECM that is stiffer than normal fibroblasts (Foster et al., 2018), the respective mechanical stiffness of the ECM could play a role in modulating MRTF-SRF signaling in a cell-type-specific manner. Interestingly, a recent study has shown an increased nuclear localization of MRTFA but not MRTFB in response to increasing substrate stiffness in stem cells (Hadden et al., 2017), suggesting that there may be isoform-specific differences in how mechanical signals are transduced to regulate MRTF function. Therefore, cell-type-dependent variations regarding relative expression levels of MRTFA and MRTFB could be another factor that contributes to context-specific differences in how signaling from the ECM is transduced into gene expression patterns in order to regulate cell migration.

Conclusions and outlook

The MRTF-SRF pathway is a crucial signaling axis that connects the dynamic actin organization with the transcriptional control of factors capable of impacting the actin cytoskeleton. There is overwhelming evidence for MRTF-SRF-mediated regulation of cell migration in both physiological and pathological contexts. However, recent studies have opened up a new area of MRTF function by highlighting the ability of MRTF to regulate cell migration in an SRF-independent manner through SAP-domain-dependent induction of transcription. So far, the function of the SAP domain of MRTF has not been studied well and is, thus, an exciting

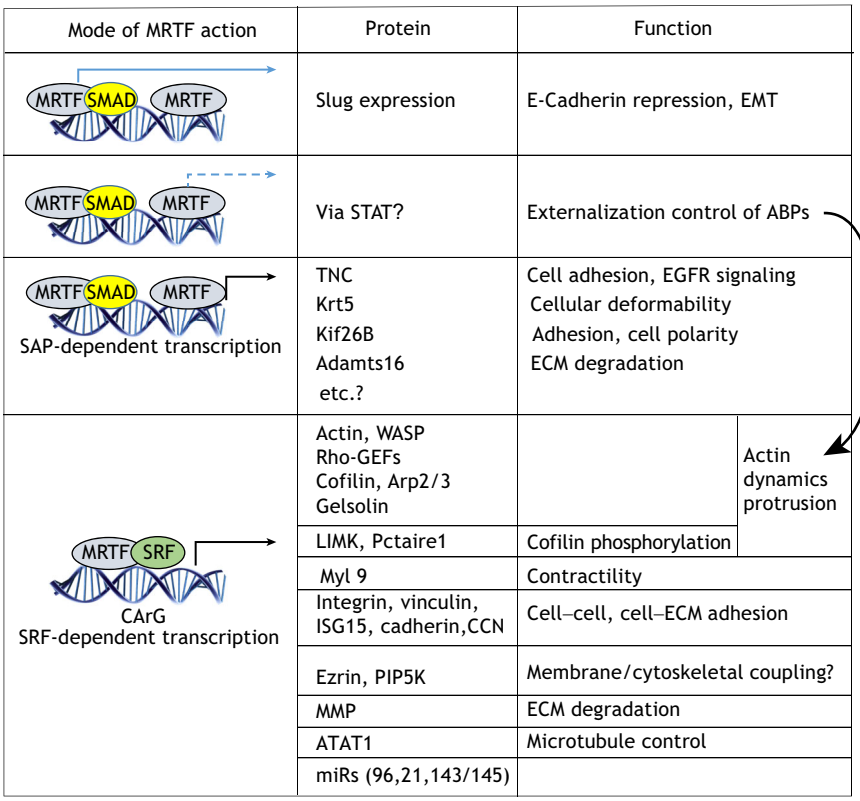

Fig. 4. Illustration of the different pathways linking MRTF to cell motility. MRTF potentially regulates all major aspects of cell migration, including EMT, dynamic control of actin polymerization and membrane protrusion, contractility, cell–cell adhesion and cell–ECM adhesion, cell polarity, cell deformability, microtubule acetylation, membrane-cytoskeletal linkage and ECM proteolysis. Shown here are some of the prominent protein-coding and microRNA genes that are important for cell motility and are regulated by MRTF- and SRF-dependent transcription, as well as those that are independent of SRF and mediated by the SAP domain of MRTF and cooperative interactions with other transcription factors, for example SMAD3. See main text for detailed discussion. ATAT1, tubulin acetyltransferase 1; PIP5K, phosphatidylinositol-4-phosphate-5-kinase. The arrow indicates that externalization of ABPs can also lead to altered actin dynamics and protrusion.

area of future research. First, the relative contributions of SRF-dependent and SRF-independent, SAP-domain-driven functions of MRTF should be re-examined in the context of cell migration of normal versus that of cancer cells. Ideally, these studies should be performed in knockdown-rescue settings in order to better assess the impact of disrupting a specific functionality of MRTF at the endogenous level. Second, whether and how SAP-domain-mediated transcription of MRTF target genes is regulated by intrinsic biochemical signals is not clearly understood. The mechanical environment of the extracellular milieu appears to have an important role in regulating MRTF functions that depend on its SAP domain because the transcription of certain mechanosensitive genes (e.g. *TNC*), mechanical strain-stimulated 4T1 breast cancer cell migration *in vitro* and tumor progression of 4T1 mammary tumors *in vivo* – particularly when tumors are irradiated, which induces tumor ECM stiffening – all exhibit dependency on the SAP domain of MRTFA (Asparuhova et al., 2011, 2015; Gurbuz et al., 2014). However, mechanosensitive genes (e.g. *TAZ*) are also regulated by MRTF-SRF (Esnault et al., 2014). It is, therefore, unlikely that distinct upstream biochemical pathways exist that switch MRTF from SRF-dependent to SRF-independent mode of transcription and vice versa. Nevertheless, there might be mechanisms that fine-tune the mechanosensitivity of MRTF, a possibility that needs to be examined in the future. In this context, the mechanistic basis underlying the differential response of the MRTF isoforms to the mechanics of the ECM is another exciting topic for future research.

Future efforts should also focus on defining the mechanisms underlying MRTF-mediated control of cell migration. As outlined above, a control of cellular retention of selective actin-binding proteins (e.g. profilin) downstream of MRTF (Joy et al., 2017) would offer a new means of how MRTF might regulate cell migration; nevertheless, how exactly MRTF might mediate this control is unclear and warrants further investigation. Moreover, several other molecular pathways that, potentially, link MRTF with cell migration are also worth investigating. For example, MRTF regulates the expression of NOX4, a key controller of the cellular redox state (Rozycki et al., 2016). Given that actin polymerization, activity of Rho-GTPases, such as Rac, as well as expression of surface integrins, are all sensitive to perturbations of the redox state (Nimnual et al., 2003; Yan and Smith, 2000), MRTF might impact on cell migration through modulation of the redox state of the cell. Finally, MRTF isoforms have also been shown to regulate the expression of several microRNAs that are involved in cell migration, including miR200, miR96, miR21, as well as miR143 and miR145, to name a few (Davis-Dusenbery et al., 2011; Li et al., 2017; Song et al., 2016). Thus, the regulation of the expression of certain non-coding RNAs is another mechanism of MRTF-mediated control of cell migration that benefits from further investigation. We anticipate that studies along these lines will not only lead to a better understanding of the unconventional functions of MRTFs in the context of cell motility but also reveal insights into their role in mechanotransduction and the progression of diseases associated with deregulated cell motility, such as cancer.

Competing interests
The authors declare no competing or financial interests.

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