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REVIEW

5q– myelodysplastic syndromes: chromosome 5q genes direct a tumor-suppression network sensing actin dynamics

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Complete loss or interstitial deletions of chromosome 5 are the most common karyotypic abnormality in myelodysplastic syndromes (MDSs). Isolated del(5q)/5q– MDS patients have a more favorable prognosis than those with additional karyotypic defects, who tend to develop myeloproliferative neoplasms (MPNs) and acute myeloid leukemia. The frequency of unbalanced chromosome 5 deletions has led to the idea that 5q harbors one or more tumor-suppressor genes that have fundamental roles in the growth control of hematopoietic stem/progenitor cells (HSCs/HPCs). Cytogenetic mapping of commonly deleted regions (CDRs) centered on 5q31 and 5q32 identified candidate tumor-suppressor genes, including the ribosomal subunit RPS14, the transcription factor Egr1/Krox20 and the cytoskeletal remodeling protein, α-catenin. Although each acts as a tumor suppressor, alone or in combination, no molecular mechanism accounts for how defects in individual 5q candidates may act as a lesion driving MDS or contributing to malignant progression in MPN. One candidate gene that resides between the conventional del(5q)/5q– MDS-associated CDRs is *DIAPH1* (5q31.3). *DIAPH1* encodes the mammalian Diaphanous-related formin, mDia1. mDia1 has critical roles in actin remodeling in cell division and in response to adhesive and migratory stimuli. This review examines evidence, with a focus on mouse gene-targeting experiments, that mDia1 acts as a node in a tumor-suppressor network that involves multiple 5q gene products. The network has the potential to sense dynamic changes in actin assembly. At the root of the network is a transcriptional response mechanism mediated by the MADS-box transcription factor, serum response factor (SRF), its actin-binding myocardin family coactivator, MAL, and the SRF-target 5q gene, *EGR1*, which regulate the expression of PTEN and p53-family tumor-suppressor proteins. We hypothesize that the network provides a homeostatic mechanism balancing HPC/HSC growth control and differentiation decisions in response to microenvironment and other external stimuli.
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Introduction

Myelodysplastic syndromes (MDSs) are a heterogeneous collection of clonal hematopoietic disorders that arise because of the defects in the control and differentiation of hematopoietic stem cells and/or hematopoietic progenitor cells (HSCs/HPCs) (Malcovati and Nimer, 2008). Myelodysplastic syndromes are characterized by ineffective formation of hematopoetic cell lineages with dysplastic features (Nimer, 2008a, b). The clinical picture in MDS ranges from a spectrum of anemias, leuko- or thrombocytopenias to severe transfusion-dependent peripheral pancytopenias. Thrombocytosis and leukocytosis occur in certain MDS subtypes (Nimer, 2008b). Often the bone marrow is normo- to hypercellular with paradoxically increased apoptosis (Nimer, 2008a). Patients with MDS have an increased risk of progression to acute myeloid leukemia (AML) (Malcovati and Nimer, 2008). Median onset of MDS is $\geqslant$65 years with a male predominance (Nimer, 2008b).

The most common karyotypic defects in MDS are loss of all or part of chromosome 5 ((del)5 or 5q–), chromosome 7, the Y chromosome and trisomy of chromosomes 20 and 8 (Nolte and Hofmann, 2008). Most patients have large interstitial deletions of 5q, and when these deletions occur in the context of more complex karyotypes, the prognosis is poor (Nimer, 2008b). The 5q deletions can be either more focused, such as in MDS with isolated del(5q) (Nolte and Hofmann, 2008), or quite large, including loss of the entire long arm of chromosome 5 or monosomy of chromosome 5 (5q–) (Olney and Le Beau, 2007). Aberrations in chromosome 5 (either (del)5 or 5q–) are frequently found in AML as well; however, often these harbor different break points and deletion sizes (Nolte

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and Hofmann, 2008), and are associated with additional cytogenetic changes and portent a worse prognosis (Nimer, 2008b; Vardiman *et al*., 2009). A separate MDS entity involving only chromosome 5q, and the focus of the actin-sensing mechanism discussed below, is the former 5q– syndrome, now called MDS with isolated del(5q) (herein referred to as ((del)5 or 5q–)) (Vardiman *et al*., 2009). This syndrome is characterized by interstitial deletions in chromosome 5q (different than those found in AML) and commonly presents as anemia, mild leukopenia and thrombocytosis with a female predominance, and has a more benign clinical course and good response to lenalidomide (List *et al*., 2005).

Myeloproliferative neoplasms (MPNs) belong to the group of clonal myeloid disorders with mainly proliferative changes in one or more hematopoetic lineages such as thrombo-, leuko- or erythrocytes. Many oncogenic events were shown to drive proliferation of these myeloid cells (Tefferi and Gilliland, 2007). An overlapping group of MDS–MPN exists, which shows both dysplastic and proliferative features (Neuwirtova *et al*., 1996; Vardiman *et al*., 2009). MPNs can transform into aggressive phenotypes such as AML and this process is frequently associated with additional 5q–/del(5q) chromosomal aberrations found in certain MPN subtypes such as primary myelofibrosis (Santana-Davila *et al*., 2008).

Chromosome 5 abnormalities are among the most common and frequent in MDS and in AML, respectively, as well as at progression of MPN to AML; therefore, 5q has been postulated to harbor one or more tumor-suppressor genes whose loss of function triggers the progression to malignancy in a multi-step carcinogenesis program (Van den Berghe *et al*., 1985; Giagounidis *et al*., 2006).

Cytogenetic studies have attempted to classify commonly deleted regions (CDRs) to pinpoint candidate genes and/or to identify a common chromosome 5 break point (Le Beau *et al*., 1993; Boultwood *et al*., 2007). Thus far, in (del)5 or 5q– MDS, no biallelic deletions or point mutations have been identified in genes associated with the 5q CDRs, leading to the idea that genes residing at 5q are behaving as haploinsufficient tumor suppressors (Shannon and Le Beau, 2008). The connection between changes in expression of (del)5 or 5q– MDS tumor-suppressor candidate genes and defects in growth control *in vivo* is largely correlative and limited to associations in gene expression in HPCs from (del)5 or 5q– patients. In this review, we will discuss the molecular evidence and mouse models supporting specific 5q candidates as tumor suppressors of (del)5 or 5q– MDS and their roles in growth control.

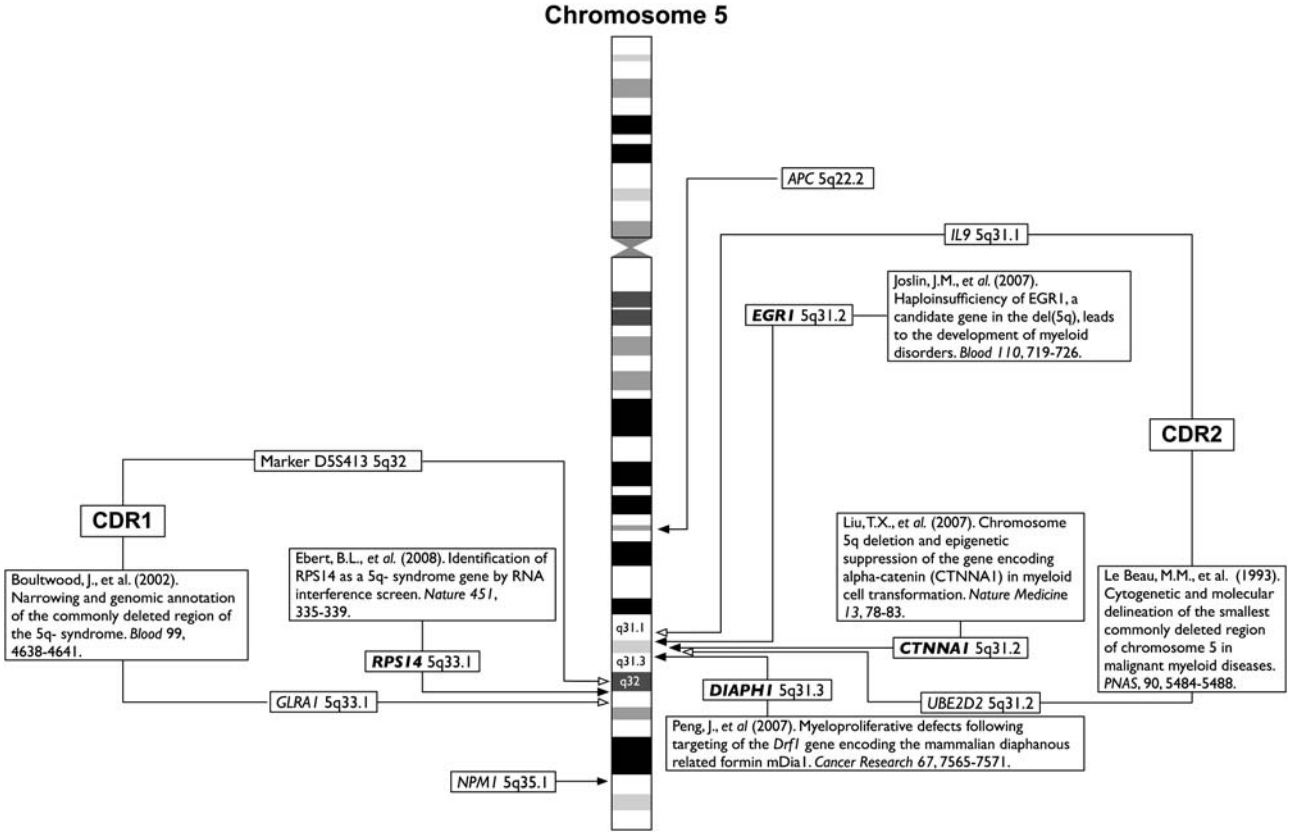


Figure 1 Commonly deleted regions (CDRs) and marker genes defined by conventional cytogenetics. The two CDRs (Le Beau *et al*., 1993; Boultwood *et al*., 2002) mapped by G-banding flank the human gene *DIAPH1* (*DRF1*) encoding human mammalian Diaphanous-related formin (mDia1) and located at 5q31.3. The CDR boundaries are indicated by the open arrowheads; the *RPS14* gene is located in CDR1 at 5q33.1, and both *EGR1* (5.31.2) and *CTNNA1* (5q31.2) genes are in CDR2.

Assessing the 5q tumor-suppressor candidates

CDRs in 5q

Despite study-to-study and patient-to-patient variability, different groups have identified a number of putative tumor-suppressor genes within the CDRs indicated in Figure 1 (Boultwood *et al*., 2002; Liu *et al*., 2007). The working list of genes defined by the CDRs is relatively limited and includes *EGR1* (5q31.2), *CTNNA1* (5q31.2) and *RPS14* (5q33.1). Conventional cytogenetic and array-based comparative genomic hybridization analyses point to CDRs in aggressive MDS and AML centered on 5q31, as well as a CDR associated with the 5q– subset of MDS localized to 5q32 (Van den Berghe *et al*., 1985; Le Beau *et al*., 1993; Boultwood and Fidler, 1995; Horrigan *et al*., 2000; Boultwood *et al*., 2002; Crescenzi *et al*., 2004; Giagounidis *et al*., 2004; Evers *et al*., 2007; Herry *et al*., 2007).

The emergence of array-based, high-resolution, DNA copy number analysis has allowed the chromosome 5q region to be examined in more detail (Evers *et al*., 2007). In addition, array-based approaches allow the determination of gene expression changes that accompany the progression to acute leukemia. Interestingly, array-based comparative genomic hybridization studies reinforce previous cytogenetic mapping studies and highlight the deletion of a fairly large region of chromosome 5 (5q31–5q33; Figure 2a). Coupled with the lack of evidence for a recurrent chromosome 5q break point, these studies suggest that deregulation of one or more candidate genes that map within the region of frequent 5q deletion contributes to (del)5 or 5q– MDS development.

However, the identification of specific candidate genes that lie within the del(5q) region is complicated by the global effects that chromosomal abnormalities have on gene transcription. Gene expression profiling studies of MDS and other tumors have shown that chromosome losses lead to dramatic gene expression defects within the deleted region (Greer *et al*., 2000). For example, on the basis of transcriptional profiling, when cells isolated from normal individuals were compared with cells from (del)5 or 5q– MDS patients, 146 of the 644 genes (23%) mapping to chromosome 5q were significantly downregulated in the (del)5 or 5q– MDS cells (Pellagatti *et al*., 2006). In contrast, no genes within this same region were significantly downregulated in MDS samples that contain a balanced chromosome 5q. Array-based comparative genomic hybridization and other microarray approaches have the potential to reveal other genes that possess tumor-suppressor properties. In each case, further functional studies are warranted to determine which gene or network of genes are 'drivers' of MDS or simply 'passengers' of the chromosome 5q deletion. Data emerging from multiple laboratories, using diverse approaches including targeted knockdown by interfering RNA (RNAi) and gene knockout mice, are beginning to suggest that multiple 5q gene candidates harbor tumor-suppressor function.

The two CDRs mapped to 5q by conventional cytogenetics flank the *DRF1*/*DIAPH1* (5q31.3) gene (Figure 1), which encodes the mammalian Diaphanous-related formin mDia1, a canonical member of the formin family of filamentous (F-) actin assembly proteins (discussed in more detail in following sections). Furthermore, detailed microarray gene expression profiling of samples from MDS patients with detectable 5q loss have shown that *DIAPH1* expression is diminished as significantly as other notable candidate 5q– tumor suppressors, including *RPS14*, *EGR1* and *CTNNA1* (Figure 2b). These data suggested a potential role for loss of mDia1 function in the etiology of (del)5 or 5q– MDS. This led us to target the murine *Drf1* gene for knockout (Peng *et al*., 2007); as previously published and described in detail below, $Drf1^{-/-}$ mice developed an age-dependent myelodysplasia, similar in phenotype to other knockout mice targeting genes residing in the 5q CDRs, including *EGR1* and *RPS14*. First, let us consider the 5q candidates historically associated with the (del)5 or 5q– subset of MDS.

RPS14: a role for defective translation in MDS?

Ribosomal proteins have a critical function in protein translation, and their dysregulation can promote tumorigenesis. RPS14 is an essential component of the 40S ribosome. Consistent with tumor-suppressor function, *RPS14* gene (5q33.1) expression is diminished in (del)5 or 5q– MDS patients (Boultwood *et al*., 2007; Lehmann *et al*., 2007; Ebert *et al*., 2008; Pellagatti *et al*., 2008; Valencia *et al*., 2008), and RPS14 re-expression in $CD34^+$ HSCs from affected patients slows proliferation and rescues protein synthesis defects (Ebert *et al*., 2008). It is noted that, Diamond-Blackfan anemia, which shares several clinical features with MDS, is characterized by loss-of-function mutations or deletion of the ribosomal components, RPS19, RPS24, RPS17 and RPL35A (Gazda *et al*., 2006; Cmejla *et al*., 2007; Farrar *et al*., 2008; Shannon and Le Beau, 2008).

Translational control is a critical target of common oncogenes and tumor suppressors (reviewed in detail by Bilanges and Stokoe (Bilanges and Stokoe, 2007)). Interestingly, ribosomal proteins associated with Diamond-Blackfan anemia and (del)5 or 5q– MDS have been proposed to function in p53 activation in response to nucleolar stress. Indeed, RPS proteins have been shown to inhibit p53 degradation by suppressing its ubiquitination; conversely, haploinsufficiency of RPS proteins could adversely affect ribosomal biogenesis itself, consequently affecting the expression of tumor suppressors such as p53 (Dai and Lu, 2008). Furthermore, RPS14 is thought to lie downstream of the phosphoinositide 3-kinase (PI3K) signaling pathway, which has a central role in governing translation. PI3K is a *bona fide* oncogene, as one of its catalytic subunits is either amplified or mutated in various solid tumors (Shayesteh *et al*., 1999; Samuels *et al*., 2004). The tumor suppressor PTEN (phosphatase and tensin homolog) is a phospholipid phosphatase that antagonizes PI3K by dephosphorylating phosphatidylinositol triphosphate (Tamguney and Stokoe, 2007).

PTEN is mutated in many late-stage tumors, including those arising in the brain, prostate and endometrium

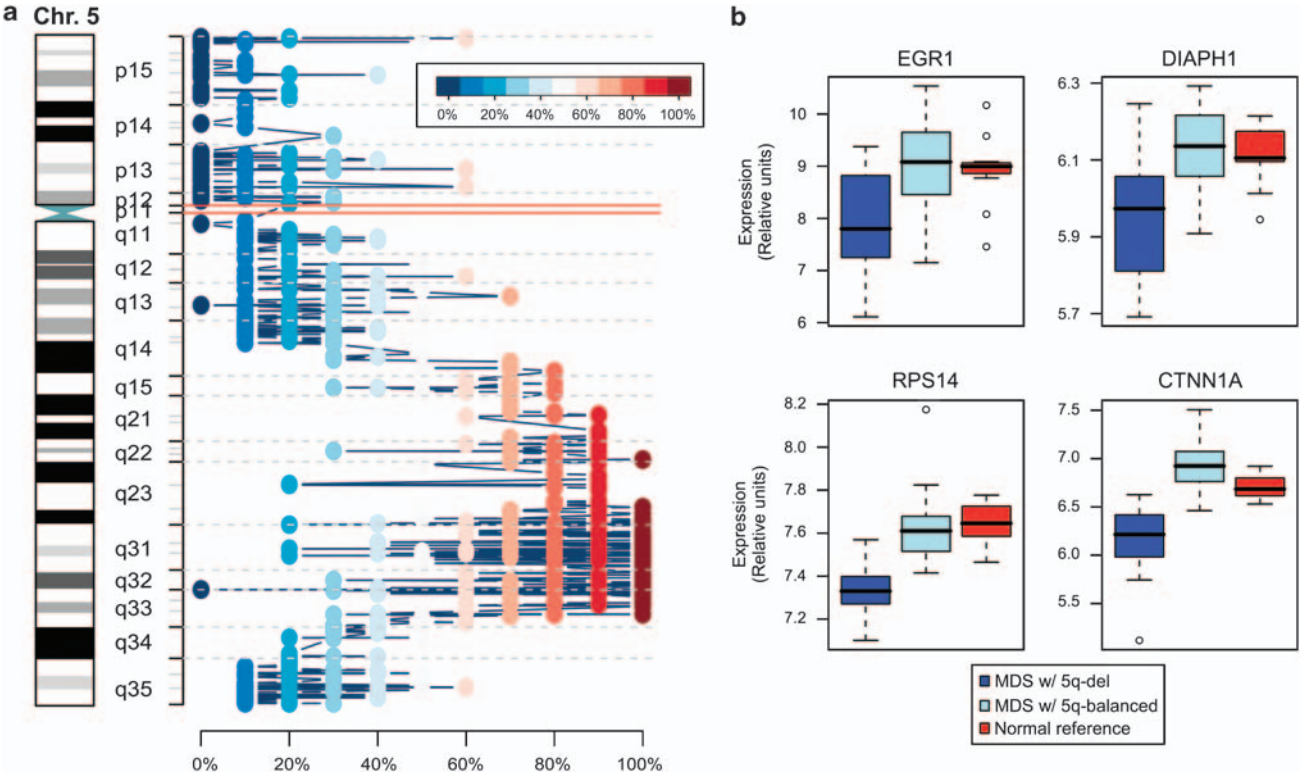


Figure 2 Gene expression analysis of chromosome 5q in (del)5 or 5q– myelodysplastic syndromes (MDSs): comparison of *DIAPH1*/*DRF1* expression versus other candidate 5q tumor suppressors. (**a**) The percentage of patients having a copy number loss, computed for 374 genomic locations on chromosome 5 (red lines indicate the centromere). MDS cells with chromosome 5q deletions were examined by high-resolution array comparative genomic hybridization ($n=10$) as described in Evers *et al.* (2007). The region of most frequent deletion maps between 5q23.3 and 5q33.3. To construct this plot, the Evers *et al.*'s DNA copy number data was obtained from the Gene Expression Omnibus database (GEO, GSE8804; http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GSE8804), replicate data was averaged, and genomic regions that had DNA copy number ratios <0.8 were considered regions of deletion. (**b**) Relative gene expression as determined by microarray analysis for several candidate genes that map within the del(5q) region. Gene expression values were obtained from CD34+ cells isolated from normal individuals ($n=11$), from MDS patients who do not have a detectable 5q loss (MDS, $n=25$) and from balanced and unbalanced del(5q) MDS patients ($n=20$). Significant decreases in expression are found in all of these genes in the 5q– versus normal individuals ($P<0.05$). To construct this plot, gene expression data generated by Pellagatti *et al.* (2004, 2006, 2008) was obtained from GEO (GSE4619), replicate gene expression measurements were averaged and data were plotted as log2-transformed intensity values.

(Li *et al.*, 1997; Steck *et al.*, 1997). The PI3K pathway seems to be activated in AML, but thus far, no mutations of *PTEN* or other components such as *AKT* have been found in AML (Tibes *et al.*, 2008). Upregulation of PI3K activity contributes to malignant alterations in proliferation, survival, metabolism, migration and membrane trafficking (Tamguney and Stokoe, 2007). In addition to *RPS14*, other 5q genes are functionally connected to PI3K, p53 and PTEN, including *EGR1* and *CTNNA1*.

Early growth response-1 (Egr1) as a tumor suppressor in (del)5 or 5q– MDS

Residing within the 5q CDR is the gene encoding Egr1, a zinc-finger protein belonging to the WT1 family of transcriptional regulators. *EGR1* is an early response gene and can mediate cellular responses to mitogens and growth factors. Such activity is typically associated with oncogenes, but interestingly, Egr1 possesses significant tumor-suppressor properties through its ability to directly regulate key target genes, including *TGFβ1*, *PTEN* and *p53* (*TPR53*) (Baron *et al.*, 2006). *Egr1*$^{+/-}$ or *Egr1*$^{-/-}$ mouse embryonic fibroblasts bypass senescence and, therefore, Egr1 was suggested to be an upstream regulator or 'gatekeeper' of p53-dependent growth regulation (Krones-Herzig *et al.*, 2003). Egr1 is known to regulate the promoters of both the *PTEN* and *p53*/*TP53* tumor-suppressor genes (Yu *et al.*, 2007) and was shown to physically interact with the p53 protein itself (Liu *et al.*, 2001). Egr1 upregulates PTEN expression in response to both radiation and calyculin A (Virolle *et al.*, 2001, 2003). In addition, Egr1-mediated control of p53 and PTEN was shown to have a role in DNA damage-induced apoptosis in both prostate and breast cancer cells (Adamson and Mercola, 2002; Adamson *et al.*, 2003; Yu *et al.*, 2007). Collectively, these data suggest a critical role for Egr1 in tumor suppression.

Beyond adhesion: a role for α-catenin in hyperproliferation in myeloid progenitors

CTNNA1, the gene encoding α-catenin, resides at 5q31.2, within a CDR linked to (del)5 or 5q– MDS.

Through an association with its well-known counterparts β-catenin and E-cadherin, the canonical roles for α-catenin were to promote the assembly of cell-cell junctions and to stabilize the actin cytoskeleton by directly binding actin (reviewed extensively in Benjamin and Nelson, (2008)). The quaternary complex then stabilized cell-cell linkages to promote cell adhesion.

However, recent data have prompted a modification of this classic model. In the updated model, α-catenin is maintained at a low concentration proximal to the plasma membrane and binds directly (albeit weakly) to β-catenin, and subsequently complexes with E-cadherin. Cell-cell adhesions drive E-cadherin clustering, and α-catenin dissociates from β-catenin; the juxtamembrane α-catenin concentration is increased sufficiently to promote its homodimerization. The α-catenin homodimer undergoes a conformational change, preferentially binds to F-actin (as opposed to β-catenin/E-cadherin) and promotes the bundling of actin filaments (Rimm *et al*., 1995). Furthermore, the homodimer negatively regulates the Arp2/3 complex, impeding actin filament nucleation and elongation as well as the formation of dynamic Arp2/3-dependent lamellae (Gates and Peifer, 2005). Cell-cell adhesions would be predicted to strengthen, potentially inhibiting cell migration and invasion.

This model strongly suggests that changes in the expression or subcellular localization of α-catenin may influence disease progression. Diminished (or complete loss of) α-catenin expression is observed in a host of primary cancers (for example, breast, colorectal, prostate), as well as in cancer cell lines derived from primary tumors (for example, leukemia, leukocyte, colon, prostate) (Benjamin and Nelson, 2008). Furthermore, human samples from MDS patients revealed a loss of α-catenin protein expression within myeloid progenitor cells (Desmond *et al*., 2007; Liu *et al*., 2007), and, in some cancers, diminished α-catenin is a strong predictor of invasive and metastatic behaviors, increased survival and proliferation.

Recent studies suggest that α-catenin also functions in disease progression through the regulation of cell survival and apoptotic signaling pathways, independent of its traditional association with E-cadherin. Conditional knockout of α-catenin in mouse epidermis showed not only adhesion and migration defects in the skin, but marked increases in Ras/MAPK signaling, hyperproliferation, and a significant presence of multinucleated cells (Vasioukhin *et al*., 2001). Conditional deletion of α-catenin in the mouse central nervous system led to severe hyperproliferation and dysplasia in the brain at E13.5 that was attributed to decreased cellular apoptosis and an accelerated cell cycle (Vasioukhin *et al*., 2001; Lien *et al*., 2006). Re-introduction of α-catenin into HL-60 myeloid leukemic cells (which harbor a 5q31 deletion encompassing *CTNNA1*) suppressed cellular proliferation though enhancing apoptotic death (Liu *et al*., 2007). Collectively, these data implicate α-catenin in the control of cellular proliferation and cell death pathways and imply that loss of α-catenin protein may also adversely affect the ability of HSCs to migrate correctly from the bone marrow.

The exact mechanism(s) of action that α-catenin utilizes to specifically maintain proper hematopoiesis is unclear. Is perturbation of α-catenin-dependent HSC migration to and from the bone marrow sufficient to promote disease? This mechanism would presumably involve disruption of the actin-binding/-bundling activities of α-catenin. This proposed role for actin bundling proteins such as α-catenin in hematopoiesis is consistent with a recent study from Qian *et al*. (Qian *et al*., 2008) demonstrating a role for APC in hematopoietic stem and progenitor cell survival . In that study, conditional knockout of APC in the hematopoietic compartment led to rapid and dramatic hematopoietic failure; specifically, Apc-depleted mice experienced exhaustion of the myeloid progenitor pool. Like α-catenin, Apc directly binds to and bundles actin filaments (Moseley *et al*., 2007), suggesting a role for actin bundling proteins in the maintenance of the hematopoietic stem and progenitor compartments.

An expanding role for mDia1 in cytoskeletal regulation

The *RPS14*, *EGR1* and *CTNNA1* genes reside within CDRs historically associated with (del)5 or 5q– MDS, yet recent evidence suggests that *DIAPH1*/*DRF1* influences the etiology of the disease. The *DIAPH1* gene resides at 5q31.3, between the two most oft-cited CDRs, and encodes the formin mDia1. Mining of the pre-existing expression data shows that *DIAPH1* expression is diminished to a similar degree as the other 5q– candidates (Figure 2b). We hypothesize that, in conjunction with other proteins possessing tumor-suppressor activity, mDia1 and its flanking neighbors operate together as a functional node sensing cytoskeletal dynamics and whose deletion or dysfunction promotes the development of (del)5 or 5q– MDS. First, we provide a brief primer of the multiple functions of mDia proteins.

Structure and function of mDia proteins

Structurally, all formin proteins share a formin homology-2 (FH2) domain (Higgs, 2005) (Figure 3a). The FH2 domain nucleates and processively elongates actin filaments by associating with growing barbed ends and creating a biochemical environment favoring actin monomer addition (Kovar, 2006) (Figure 3b). mDia family formins are tightly regulated (Goode and Eck, 2007) by a Rho-controlled autoregulatory mechanism mediated by the interaction between their N-terminal (Dia-inhibitory, or DID) and C-terminal (Dia-autoregulatory, or DAD) domains (Alberts, 2001; Li and Higgs, 2005). Activated GTP-bound Rho proteins bind to the GTPase-binding domains and occlude DAD binding to DID, thus alleviating its inhibitory influence over the FH2 domain (Goode and Eck, 2007). Newly generated actin filaments provide the underlying structures that drive changes in cell morphology to facilitate events as divergent as cell division, intracellular trafficking and chemotaxis (Chhabra and Higgs, 2007).

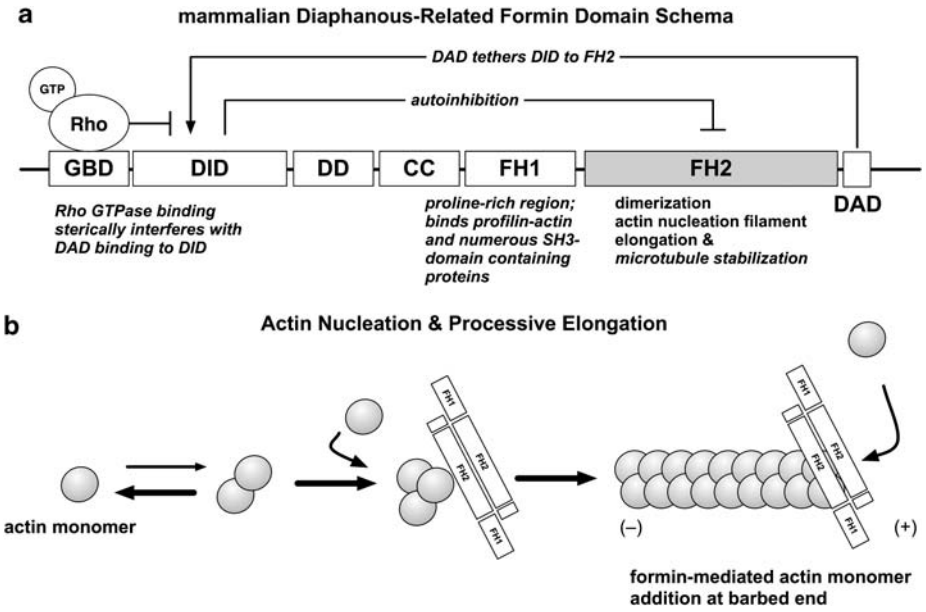


Figure 3 Schematics of mammalian Diaphanous-related formin (mDia1) domain structure and mDia1-mediated actin filament assembly. (**a**) Like all Diaphanous-related formins, mDia1 is autoregulated (Higgs, 2005). Dia-inhibitory domain (DID) weakly binds to and inhibits the actin-nucleating formin homology-2 (FH2) domain (Li and Higgs, 2003). Dia-autoregulatory domain (DAD) acts as a high-affinity anchor or catch that is released on Rho binding to the GTPase-binding domain (Alberts, 2001; Wallar *et al*., 2006). Bound GTP-Rho sterically interferes with DAD binding, thus releasing the inhibitory effects of DID over actin assembly. This leads to activation of F-actin assembly and microtubule stabilization (Alberts, 2001; Palazzo *et al*., 2001; Wallar *et al*., 2006). (**b**) Spontaneous actin assembly progresses from monomers to actin dimers and trimers; in the absence of assembly factors, these quickly dissociate. FH2 domains, comprised of dimers linked by flexible tethers, bind to and stabilize actin dimer intermediates. Formins processively elongate filaments by creating an environment at the barbed (+) end that favors monomer addition (Otomo *et al*., 2005). The crystal structures (Xu *et al*., 2004; Otomo *et al*., 2005) of yeast and mammalian formins revealed unique 'lasso and post'-like dimers between FH2 domains. This so-called 'tethered dimer' allows for a dynamic association with the barbed end of growing filaments.

One mechanism of actin remodeling in response to external stimuli includes Rho GTPase signaling through their mDia formin effectors (Wallar and Alberts, 2003). mDia formins remodel the actin cytoskeleton through binding of a variety of different Rho GTPases (Figure 3), and the specificity of the actin-rich structure is dictated by the association of distinct mDia:Rho GTPase pairs. For instance, the interaction between either mDia1 or mDia2 and activated RhoB is integral to early endosomal trafficking (Wallar *et al*., 2007), although the interaction between mDia2 and activated Cdc42 promotes filopodia formation (Peng *et al*., 2003). mDia formins participate in changes in cell morphology previously thought to depend largely on activated Arp2/3. These processes include the dynamic actin remodeling underlying filopodia/microspike (Tominaga *et al*., 2002; Peng *et al*., 2003) and neurite formation (Dent *et al*., 2007), phagocytosis (Colucci-Guyon *et al*., 2005), vesicle trafficking (Fernandez-Borja *et al*., 2005; Wallar *et al*., 2007) and lamella/lamellipodial dynamics (Eisenmann *et al*., 2007; Gupton *et al*., 2007; Yang *et al*., 2007).

Evidence for mDia1 in tumor suppression

Both mDia1 and the related mDia2 directly bind to RhoB and act as effectors for RhoB signaling (Fernandez-Borja *et al*., 2005; Wallar *et al*., 2007). RhoB has a critical role in apoptotic responses to DNA damage, and the GTPase was shown to be a target of farnesyltransferase inhibitors (FTIs) (Lebowitz *et al*., 1997; Prendergast, 2001a, b; Adini *et al*., 2003). RhoB, like other Ras family members, is post-translationally modified on a C-terminal motif (CAAX) by farnesylation. In cells treated with FTIs, RhoB shifts to become geranyl-geranylated. This change in post-translational modification leads to enhanced activation of serum-response factor (SRF)-mediated gene expression and elevated sensitivity to DNA-damaging agents such as doxorubicin (Lebowitz *et al*., 1997; Lebowitz and Prendergast, 1998; Du *et al*., 1999). Although FTIs are in active clinical development, no mechanism accounting for how they trigger programmed cell death has yet been identified (Basso *et al*., 2006). Interestingly, a recent study found that expression of a dominant-negative version of mDia1 not only enhanced tumorigenesis of Ras-transformed mouse embryonic fibroblasts, but it also impeded the ability of FTIs to suppress tumor growth (Kamasani *et al*., 2007). This result indicates an important role for mDia1 in the FTI response and suggests a functional relationship between RhoB and mDia1 in tumor suppression. These observations also point to a potential mechanism for how FTIs control tumor cell growth and supports exploration into the clinical use of FTIs in the control of MPN and/or MDS, an idea that has received some attention (Cortes *et al*., 2002; Kurzrock, 2002; Kurzrock *et al*., 2002; Huang *et al*., 2003; Kotsianidis *et al*., 2008).

Potential mouse models of (del)5 or 5q– MDS

The effects of knocking out various murine equivalents of 5q genes within the hematopoietic compartment have been extensively documented; those genes targeted for knockout include, *EGR1*, and other 5q genes lying outside of the conventional 5q– CDRs, such as *APC* and nucleophosmin-1 (*NPM1*). The resulting phenotypes are compared within Table 1 and described briefly below.

Egr1-targeted mice

Egr1 has central roles in the proliferation and localization of HSCs (Min *et al*., 2008). In the context of myeloid function, Min *et al*. (Min *et al*., 2008) found that $EGR1^{-/-}$ mice have enhanced mobilization of HSCs into the bloodstream. It was not clear whether the phenotype was due to hyperproliferation that would potentially cause the excess progenitors (stem cells) to outstrip the capacity of the bone marrow to house them. A second study, indicating Egr1 as a tumor suppressor, determined that a subset of mice haploinsufficient for *Egr1* develop myelodysplastic features. To test the hypothesis that murine *Egr1* had a role in tumor suppression, Joslin *et al*. (Joslin *et al*., 2007) exposed both $Egr1^{+/-}$ and $Egr1^{-/-}$ mice to *N*-ethyl-nitrosourea (ENU). Although neither $Egr1^{+/-}$ nor $Egr1^{-/-}$ mice had any outright myeloproliferative defects without ENU treatment, both types of mice developed myeloproliferative defects at an increased rate with a shorter latency period than wild-type mice treated with ENU. The effects (summarized in Table 1) included an elevated level of white blood cells, anemia and thrombocytopenia. Although this points to a tumor-suppressor role for this transcription factor, *Egr1* haploinsufficiency alone does not seem to be sufficient to trigger myeloproliferative disorders in mice.

Table 1 The effects of knocking out murine equivalents of 5q genes

Mouse phenotype	Drf1	Egr1	Apc	Npm1
Elevated WBC	+	+	−	+
Lymphopenia	+	−	+	−
Monocytosis	+	+	−	+
Granulocytosis	+	+	−	+
Anemia	+	+	+	+
Thrombocytosis	+	−	−	−
Thrombocytopenia	−	+	+	+
Splenomegaly	+	+	−	+
Hepatomegaly	−	−	−	+
Extramedullary hematopoiesis	+	+	−	+
Loss of splenic organization	+	+	−	+
Myeloproliferative defects	+	+	−	+
Ineffective erythropoiesis in				
Bone marrow	+	+	+	+
Spleen	+	+	+	+

Abbreviations: *Egr1*, early growth response-1; *Npm1*, nucleophosmin-1.
Unique features:
Drf1, myeloproliferative defects are age dependent.
Egr1, increased rate of myeloproliferation with shorter latency; an absence of blasts.
Apc, depletion of hematopoietic stem cell and hematopoietic progenitor cell pools lead to immune failure.
Npm1, numerical and structural chromosomal abnormalities in genomic DNA; 75% myeloid malignancy, 25% B- and T-cell malignancy.

Mx1-cre/Flox-Apc mice

The gene encoding the actin-bundling protein, APC (5q22.2), lies outside the conventional CDR associated with (del)5 or 5q– subsets of MDS (Moseley *et al*., 2007). Ablating APC in developing myeloid progenitor cells in mice results in a failure of normal hematopoiesis due to an increase of apoptosis of HSC and HPC cells (Qian *et al*., 2008). Exhaustion of HSC and HPC cells leads to immune collapse and bone marrow failure. These mice show decreased levels of white blood cells and hemoglobin, are anemic and thrombocytopenic, and display defective erythrocytic and myeloid differentiation. Ineffective erythropoiesis is a result of differentiation arrest of late erythroblasts. Collectively, the severe hematopoietic defects observed in *Mx1*-cre/Flox-*Apc* mice suggest an important role for APC in the maintenance of the hematopoietic stem and progenitor compartments.

Npm1-targeted mice

Nucleophosmin is a nucleolar phosphoprotein having a role in centrosomal duplication and genomic stability in normal cells. Like APC, the gene encoding NPM1 lies outside the conventional (del)5 or 5q– CDRs. Mice heterozygous for NPM1 develop many features similar to human MDS (Sportoletti *et al*., 2008); 75% of such mice develop myeloid malignancies, whereas others develop B- and T-cell malignancies. The peripheral blood shows elevated levels of white blood cells and leukemic blasts, myeloid expansion and proliferation, and anemia and thrombocytopenia. Myeloid expansion and increased levels of leukemic blasts are seen in the bone marrow, spleen and liver. Splenomegaly is observed, with atypical lymphoid cells replacing normal spleen pulp.

Drf1-targeted/mDia1 knockout mice develop age-dependent myelodysplasia

On the basis of the potential functional role between RhoB and mDia1 in tumor suppression and its location in the 5q region associated with MDS, we targeted the murine *Drf1* gene for knockout (Watanabe *et al*., 1997; Peng *et al*., 2003). Our working hypothesis was that mice would develop myeloproliferative defects or myelodysplasia. On birth, $Drf1^{-/-}$ mice were developmentally and morphologically indistinguishable from their wild-type littermates, yet both $Drf1^{+/-}$ and $Drf1^{-/-}$ mice developed age-dependent myeloproliferative defects. The resulting phenotype (detailed in Table 1) included marked splenomegaly (attributed to hyperproliferation within the spleen) and hypercellular bone marrow (due to expansion of activated monocytes and macrophages). Analysis of the erythroid compartment showed both a significant increase in the percentage of splenic cells in the S phase and the expansion of erythroid precursors. Collectively, the overall phenotype of the *Drf1* knockout

mouse, specifically the marked increased proliferation of hematopoietic progenitors *in vivo*, supports a role for mDia1 as a tumor suppressor for (del)5 or 5q– MDS. The exact mechanism by which mDia1-mediated tumor suppression is mediated remains unclear. An answer may lie in the interrelated contribution(s) of neighboring genes within the 5q CDRs and the proteins they encode, namely, Egr1, α-catenin and RPS14, as described below.

Networking the 5q tumor-suppressor candidates: SRF as a sensor for actin dynamics

On loss of each candidate 5q gene, including *EGR1*, *RPS14*, *CTNNA1* and *DIAPH1*/*DRF1*, similar defects in hematopoiesis are observed; data from these studies suggest that defects in expression of one or more candidate genes mapping within the region of frequent deletion contributes to MDS development. We hypothesize that there is a common functional mechanism driving the progression toward MDS on loss of 5q. We suggest that the link binding the 5q candidates is SRF, which acts as an actin sensor to control the expression of other 5q genes, including *EGR1*. And we propose that the SRF sensor is controlled by two more 5q candidates: mDia1 and α-catenin.

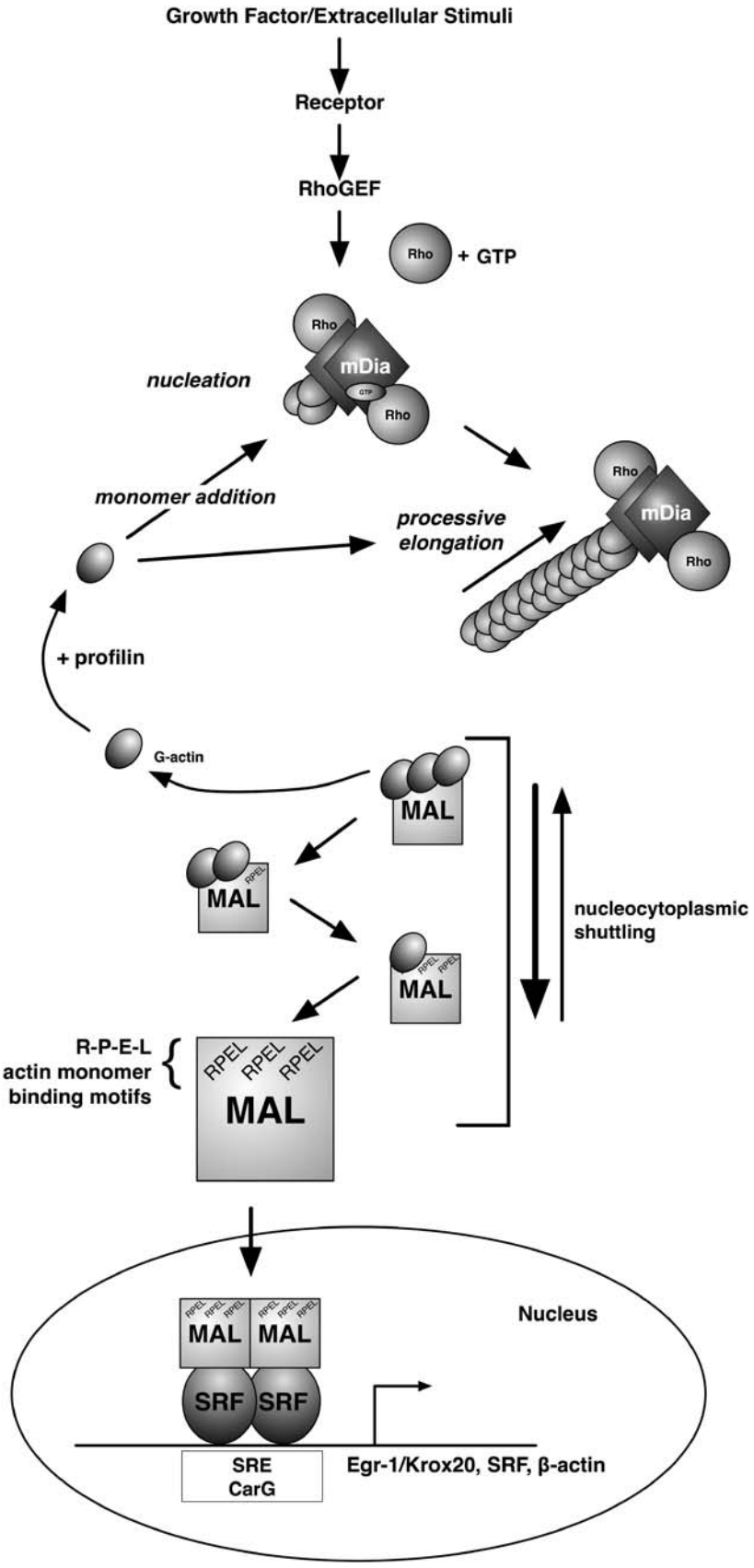
In addition to directly affecting the actin architecture through the nucleation, elongation, and (in some cases) bundling of actin filaments (Chhabra and Higgs, 2007), mDia proteins regulate the expression of cytoskeletal proteins by activating the transcriptional regulator SRF. SRF was initially characterized as a regulator of immediate-early gene expression caused by stimulation of cells with growth factors (Treisman, 1986, 1996; Hill and Treisman, 1995). Alone or in combination with other transcription factors, SRF has a central role in controlling transcriptional responses to growth factors and other external stimuli (Posern and Treisman, 2006). Over the past two decades, studies have shown that SRF regulates the expression of numerous genes associated with cytoskeletal remodeling, including the β-actin gene (Sotiropoulos *et al*., 1999; Posern and Treisman, 2006). Rho family members and their actin-nucleating effectors, including Rho-activated mDia1, are strong activators of SRF-mediated gene expression (Hill *et al*., 1995; Sahai *et al*., 1998; Sotiropoulos *et al*., 1999; Tominaga *et al*., 2000; Copeland and Treisman, 2002).

Furthermore, the 5q candidate α-catenin also modifies gene transcription through SRF. α-catenin was shown to activate a serum-response element reporter (a readout for SRF activity) (Merdek *et al*., 2008), potentially by acting downstream of Rho/ROCK or through a parallel, Rho-independent pathway. These findings were consistent with a role for the downstream anti-proliferation effects of α-catenin, as SRF is known to induce various genes associated with cell differentiation. The loss of α-catenin expression and subsequent defective growth control observed in many cancers may therefore be mediated through dysfunction of this novel α-catenin–SRF pathway.

Our proposed model is centered on the activity of SRF, and incorporates an actin sensor controlling the expression of other candidate 5q tumor-suppressor genes, including *EGR1* (Figure 4). How this actin sensor might function? The SRF coactivator MAL bears multiple *RPEL* motifs—so-called because of the amino-acid sequence on the protein that mediates actin binding—that bind directly to monomeric actin; as concentrations of G-actin decrease in the cytoplasm, occupancy of the RPEL motifs diminishes (Posern *et al*., 2004; Posern and Treisman, 2006; Guettler *et al*., 2008). Non-actin-bound MAL, which normally shuttles between the nucleus and the cytoplasm, occupies the nucleus and is free to activate SRF (Guettler *et al*., 2008).

Within the nucleus, SRF binds to the conserved serum response elements harbored in the promoters of numerous genes, including that of *EGR1*. SRF can, in fact, regulate the *EGR1* promoter at no less than five consensus serum-response element-/SRF-binding sites (Figure 5a) (Mora-Garcia and Sakamoto, 2000). Hence, through its dual activities of diminishing the cellular pools of G-actin in the process of nucleating and elongating F-actin filaments and of activating SRF, mDia1 potentially acts as part of a node for regulating the expression of *Egr1*. This mDia1-driven mechanism may act in parallel with α-catenin-mediated SRF activation. As Egr1 regulates the promoters of both *PTEN* and *p53*/*TP53* tumor-suppressor genes (Yu *et al*., 2007) (Figure 5a), we postulate a role for the mDia1/α-catenin-SRF-Egr1 node in promoting malignancy through the disruption of *PTEN* and *p53*/*TP53* expression (Figure 5b). To date, there are no data to suggest that inactivating mutations of SRF or loss of SRF expression coincides with malignancies. However, expression of a constitutively active form of SRF fused

Figure 4 Mammalian Diaphanous-related formin (mDia)-directed serum response factor (SRF) activation by changes in F-actin assembly. Growth factors or chemotactic stimuli propagate intracellular signals activating mDia1, in part, through binding to activated Rho GTPases. This subsequently allows for nucleation and processive elongation of non-branched actin filaments from a G-actin monomer pool. Diminishing concentration of actin monomer in the cytoplasm decreases the occupancy of RPEL motifs on the transcriptional coactivator MAL. In the absence of RPEL occupancy by G-actin, MAL translocates to the nucleus to bind and stimulate SRF (Posern *et al*., 2004; Posern and Treisman, 2006). In addition to other enhancer elements, the *EGR1* promoter contains five SRF-binding sites called serum-response elements (SREs). On docking to the SRE, MAL-bound SRF can promote the transcription of numerous genes including those for EGR1 as well as β-actin. mDia1 has the unique capacity to both diminish the cellular pools of G-actin in the process of nucleating and elongating F-actin filaments and activate SRF, thus amplifying the downstream readout of gene transcription.


Growth Factor/Extracellular Stimuli
Receptor
RhoGEF
Rho
+ GTP
Rho
mDia
GTP
Rho
nucleation
monomer addition
processive
elongation
Rho
mDia
Rho
+ profilin
G-actin
MAL
MAL
RPEL
MAL
RPEL
RPEL
nucleocytoplasmic
shuttling
R-P-E-L
actin monomer
binding motifs
RPEL
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SRF
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SRE
CarG
Egr-1/Krox20, SRF, β-actin

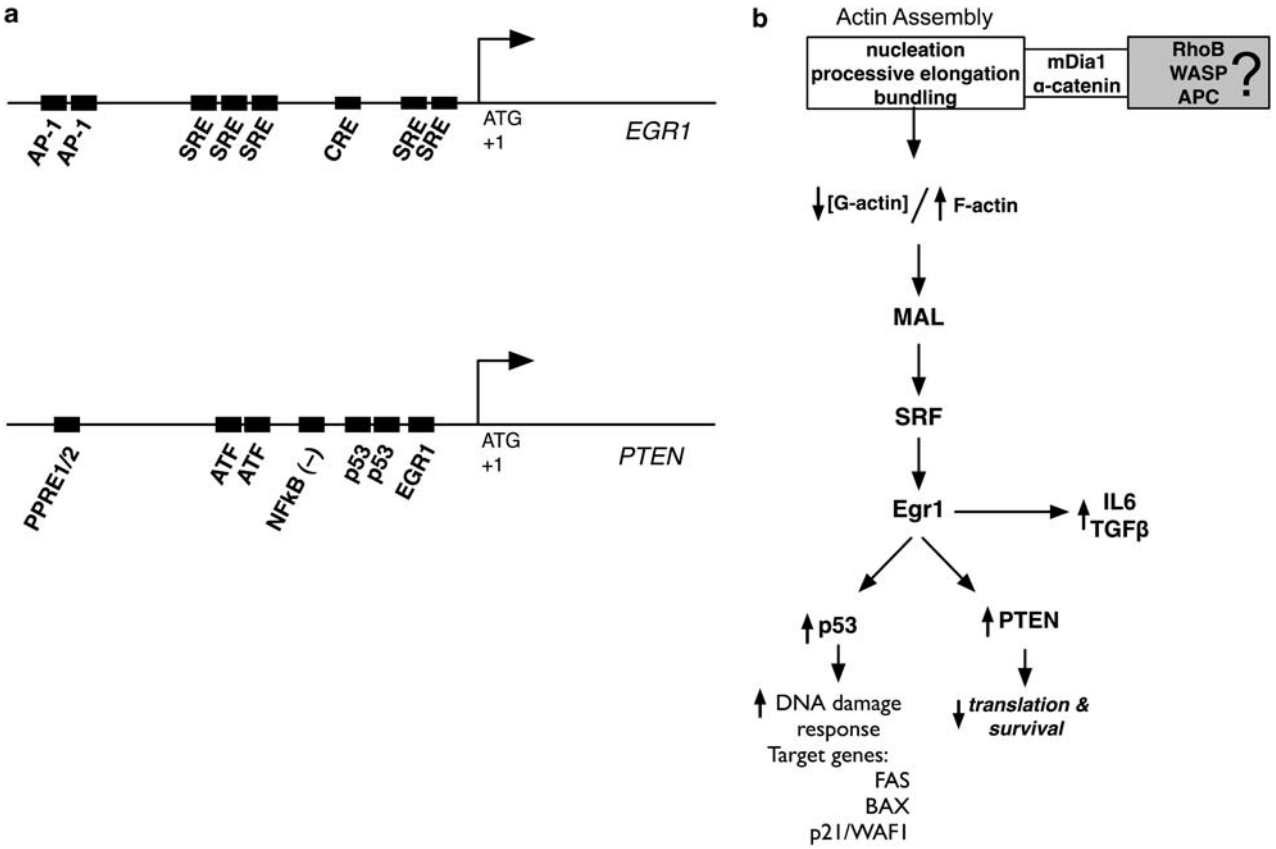

Figure 5 A model for an EGR1-dependent actin-sensing node in (del)5 or 5q– myelodysplastic syndromes that propagates p53/PTEN. (**a**) Schematic of the *EGR1* promoter, whose activity is regulated by at least five serum-response element (SRE) consensus sites. In turn, Egr1 protein can act as a transcriptional activator to enhance the promoter activity of the tumor suppressor PTEN. (**b**) By driving actin filament assembly and diminishing the cellular pools of G-actin, mammalian Diaphanous-related formin (mDia1) is a potent activator of serum response factor (SRF), which, in turn, induces the expression of Egr1. Egr1 can act as a transcriptional regulator, with target genes including *TGF-β*, *p53* and *PTEN*, thereby regulating cell proliferation and survival signaling in response to stress. Coupled with α-catenin-mediated SRF activation, multiple 5q candidates feed into this actin-sensing node, potentially propagating p53/PTEN signaling through Egr1. Furthermore, through its association with the phosphoinositide 3-kinase (PI3K) pathway, the 5q candidate, RPS14, can also amplify the p53/PTEN signal. Exquisite control of this actin-sensing mechanism centered on 5q candidate tumor-suppressor genes would be critical to controlling the proliferation and proper migration of hematopoietic progenitors and stem cells.

to a transcriptional activation domain from the viral VP16 protein suppressed the ability of activated/oncogenic Ras to transform cultured fibroblasts (Kim *et al*., 1994). Although this result was published roughly 15 years ago and was confirmed by several groups, the explanation for the result has never been adequate. Our model, which assigns tumor-suppressor roles to SRF and many of its 5q neighbors, deftly accounts for this important observation.

Concluding remarks

No clear molecular mechanism accounts for how, individually or together, 5q tumor-suppressor candidates either trigger MPNs or the myelodysplastic phenotype, or support the progression to malignancy. Candidate 5q tumor-suppressor genes mapped to conventional CDRs in the 5q31–33 regions include *RPS14*, *EGR1* and *CTNNA1*, and disruption of their expression leads to defects in hematopoiesis in mice. By driving actin filament assembly and bundling, respectively, mDia1 and α-catenin diminish cellular pools of G-actin to activate SRF, which in turn induces the expression of Egr1. Coupled with α-catenin-mediated SRF activation, multiple 5q candidates feed into this actin-sensing node, potentially affecting p53/PTEN signaling through EGR1. Furthermore, through its association with the PI3K pathway, the 5q candidate RPS14 can also amplify the p53/PTEN signal. Hence, multiple interdependent mechanisms exist within this node that, on disruption of one or more candidates, may lead to the progression towards malignancy in the (del)5 or 5q– subset of MDS.

An actin-dynamics sensing mechanism has the potential to control, or at least modulate, both the proliferation and proper migration of hematopoietic progenitors and stem cells. The mechanism could facilitate the cells' ability to make 'go' or 'grow' decisions as hematopoietic cells differentiate and migrate to new compartments while they progress through their differentiation program.

Intriguingly, APC (a 5q gene product) binds directly and bundles F-actin and thus has the potential to modulate SRF and Egr1-regulated gene expression. Likewise, defective function and/or expression of Wiskott-Aldrich syndrome protein, a canonical regulator of actin nucleation through the Arp2/3 complex and an activator of SRF, could release the growth-control machinery by affecting PTEN and p53 expression in the myeloid malignancies that arise in affected WAS patients. Thus, defects in an SRF-directed actin-dynamics sensing tumor-suppression mechanism may have an additional role in carcinogenesis in non-MDS tumors.

Conflict of interest

The authors declare no conflict of interest.

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