

C. Wang

Department of Oral Biology and Center for Molecular Biology of Oral Diseases, University of Illinois at Chicago, 801 South Paulina Street, RM530CB, m/c 860, Chicago, IL 60612, USA; chiayeng@uic.edu

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## ABSTRACT

Rhabdomyosarcoma (RMS) is a morphologically and clinically heterogeneous group of malignant tumors that resemble developing skeletal muscle and is the most common soft-tissue sarcoma in children and adolescents. The most prominent sites involve head and neck structures (~40%), genito-urinary tract (~25%), and extremities (~20%). Embryonal (ERMS) and alveolar (ARMS) are the two major RMS subtypes that are distinct in their morphology and genetic make-up. The prognosis for this cancer depends strongly on tumor size, location, staging, and child's age. In general, ERMS has a more favorable outcome, whereas the mortality rate remains high in patients with ARMS, because of its aggressive and metastatic nature. Over the past two decades, researchers have made concerted efforts to delineate genetic and epigenetic changes associated with RMS pathogenesis. These molecular signatures have presented golden opportunities to design targeted therapies for treating this aggressive cancer. This article highlights recent advances in understanding the molecular pathogenesis of RMS, and addresses promising research areas for further exploration.

**KEY WORDS:** pediatric cancer, sarcoma, muscle, genetic abnormalities, oncogenesis, fusion protein.

# Childhood Rhabdomyosarcoma: Recent Advances and Prospective Views

## INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common soft-tissue malignancy in the pediatric population. Current treatment for RMS relies on chemotherapy, with surgery and radiation as adjunct therapies. The cytotoxic actions of chemotherapeutic agents are not tumor-specific and are not effective in treating advanced and metastatic RMS. Since this cancer afflicts young patients, there are concerns of impairment in normal development and increased risks in developing secondary cancers as resulting from the long-term effects of the treatment. This article aims to (1) highlight recent advances in our understanding of RMS cell biology, which forms the critical basis for developing novel therapies that are more specific for tumor cells and less harmful to normal cells, and (2) offer prospective views on underexplored and promising research topics that could have further impact on this field.

## RHABDOMYOSARCOMA

The two most common rhabdomyosarcoma subtypes are embryonal (ERMS) and alveolar (ARMS). Clinically, they are distinguished by differences in histopathology, genetics, and clinical presentation and outcome (Saab *et al.*, 2011). ERMS and ARMS comprise ~60 to 70% and ~25 to 30% of US RMS cases, respectively (Ognjanovic *et al.*, 2009). Studies have not established an association of RMS with any environmental factor. ERMS most commonly presents in the head and neck region and the genito-urinary tract of children aged 1 to 14 yrs. ARMS occurs more frequently in the extremities and trunk region and is more prevalent among older children and adolescents. In general, RMS is more common in white than black children (3:1), and the male-to-female ratio is approximately 1.5:1.

## Histopathology

ERMS is composed of round or spindle-shaped cells and resembles embryonic muscle. ARMS displays a distinct alveolar architecture with aggregates of small round undifferentiated cells separated by dense hyalinized fibrous septa. Although ARMS cells express high levels of muscle differentiation markers (Dias *et al.*, 2000), these tumors lack mature muscle characteristics. Some solid ARMS variants lack this alveolar structure and may display overlapping histology with ERMS. Recent adoption of cytogenetic and molecular screening has greatly improved diagnosis.

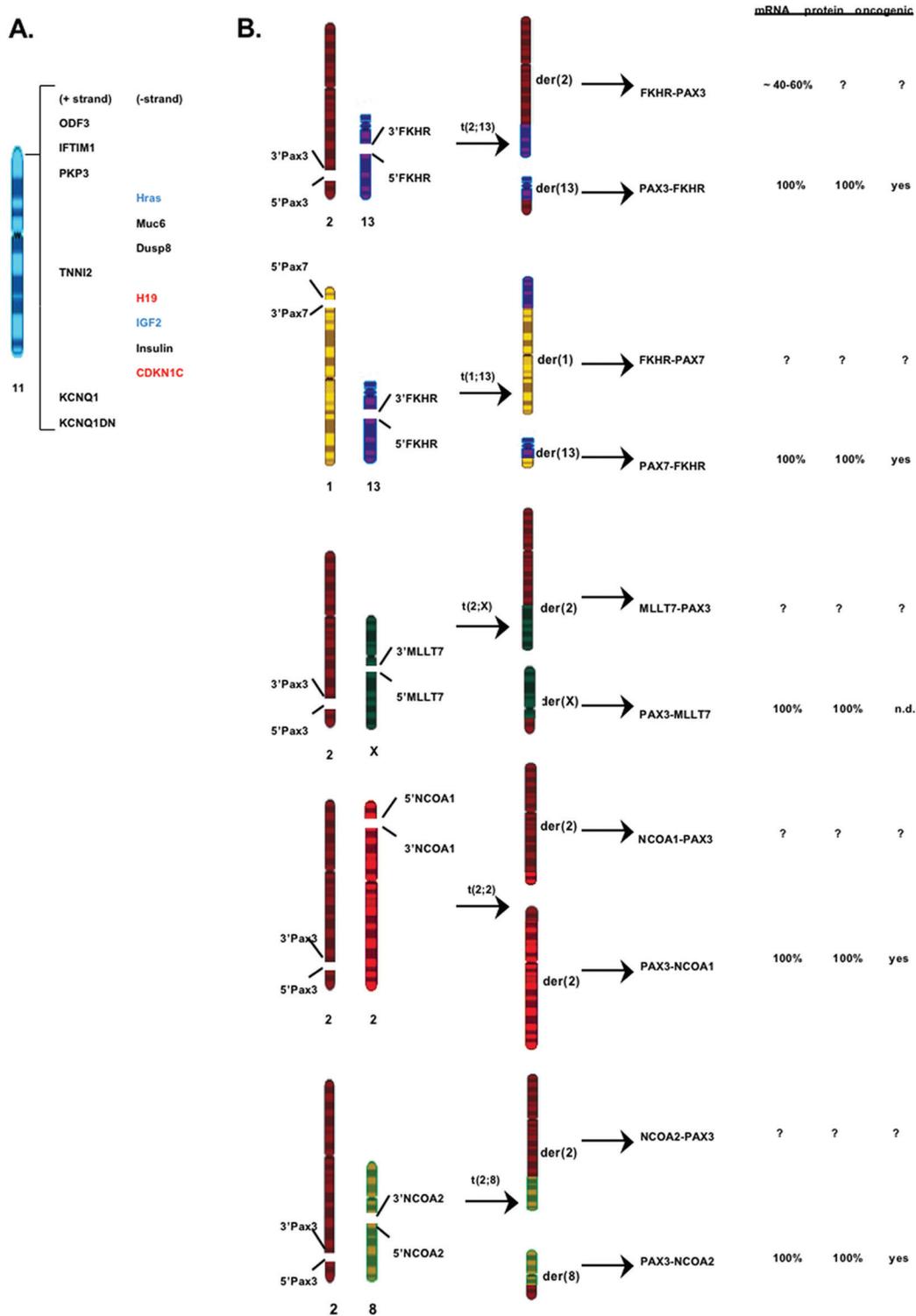
## Genetics

Several non-random chromosome alterations have been identified in RMS (Fig. 1). Loss of heterozygosity for chromosome 11p15.5 and chromosome gains (chr. 2, 8, 12, 13) are commonly detected in ERMS (Scrabble and Witte,

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**Figure 1.** Non-random chromosomal alterations found in association with RMS tumors. **(A)** ERMS-associated loss of heterozygosity in the p15.5 region of chromosome 11. Altered expression of some imprinted genes in ERMS is highlighted in red (down-regulation) and blue (up-regulation). **(B)** Reciprocal chromosomal translocations identified in ARMS tumors. The t(2;13) translocation is the most common gene re-arrangement. All translocations involve disruption of muscle-developmental PAX genes, PAX3 and PAX7. Each reciprocal translocation generates two fusion gene products, PAX-X and X-PAX, where X represents a member of the FOXO transcription factor family or a member of the nuclear co-activator NCOA family. Only the PAX-X fusion genes have been cloned and studied. FKHR-PAX3 is the only reciprocal gene whose transcript is detected in a fraction of t(2;13)-positive ARMS tumors and cell lines. Other X-PAX reciprocal genes have not been investigated.

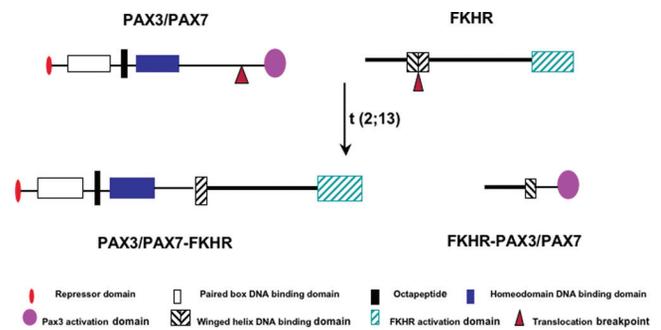
1989; Anderson *et al.*, 1999). The 11p15.5 region contains several imprinted genes from which the loss of one allele could inactivate the tumor suppressor gene (*e.g.*, H19, CDKN1C) or activate the proto-oncogene (*e.g.*, IGF2, HRAS) leading to cancer development. By contrast, recurrent reciprocal chromosomal translocations are observed in ARMS. A t(2;13) translocation is detected in ~70% of ARMS; a less common t(1;13) variant is found in ~10% of ARMS (Turc-Carel *et al.*, 1986; Wang-Wuu *et al.*, 1988). These translocations fuse *PAX3* (chr.2)/ *PAX7* (chr.1) with *FKHR* (*FOXO1A*, chr.13) to generate chimeric *PAX3/PAX7-FKHR* oncogenes (Barr *et al.*, 1993; Shapiro *et al.*, 1993). A rare t(2;X) involving fusion between *PAX3* and another FOXO family member, *MLL7* (*FOXO4*), has also been reported (Barr *et al.*, 2002). ARMS tumors that do not carry these typical PAX-FOXO translocations are referred to as “fusion-negative” ARMS (ARMSn) in the literature. Williamson *et al.* (2010) have reported a striking similarity between ARMSn and ERMS based on their clinical behaviors (*e.g.*, location, metastasis rate, treatment responses) and mRNA profiles. This suggests that ARMSn and ERMS arise from the same cellular lineage but undergo different developmental paths to produce distinct histological features. This is supported by recent findings that ARMSn and ERMS have distinct microRNA profiles (Gougelet *et al.*, 2011), and that some ARMSn harbor atypical translocations (Wachtel *et al.*, 2004; Sumegi *et al.*, 2010). A recurrent t(2;2) or t(2;8) translocation leading to the formation of respective *PAX3-NCOA1/NCOA2* fusions is detected in a subset of ARMSn tumors. The altered microRNA and gene re-arrangement profiles may explain why some ARMSn behave as aggressively as PAX-FOXO-positive ARMS. Given these new developments, it will be worthwhile to know if ERMS and ARMSn can be further clustered into subsets based on their mRNA profile, microRNA profile, translocation status, and clinical features. Such information can improve the diagnosis, prognosis, and treatment selection for the patients.

## Clinical Outcome

The overall survival rate for RMS patients has significantly improved over the past two decades. In patients with low-grade, localized ERMS, the five-year survival rate has reached >85%. This rate drops dramatically with increased age and more advanced tumor stages at diagnosis. There is also a striking difference in mortality between patients with metastatic *PAX3-FKHR* and *PAX7-FKHR* ARMS; the estimated five-year survival rate is 8% (*PAX3-FKHR*) as compared with 75% (*PAX7-FKHR*) (Sorensen *et al.*, 2002; Kazanowska *et al.*, 2007).

## PAX3-FKHR FUSION

Since the discovery of the RMS-associated chromosomal rearrangements, major progress has been made in the characterization of the fusion products. The prototype, *PAX3-FKHR*, has been the main research focus for the past two decades. These studies highlight the critical role of *PAX3-FKHR* in ARMS pathology and provide blueprints for investigating the newly emerged fusion gene products *PAX3-NCOA1/2*. Because *NCOA1/2* are transcriptional co-activators for nuclear hormone receptors instead of

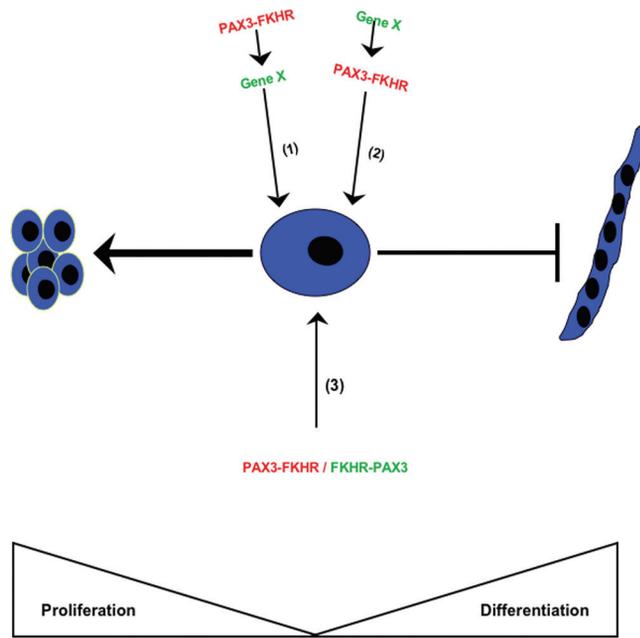


**Figure 2.** Diagrammatic illustration of *PAX3*, *FKHR*, *PAX3-FKHR*, and *FKHR-PAX3* protein structures. The translocation breaks the *PAX3* gene between the DNA-binding domains and activation domain, and breaks the *FKHR* gene within the *FKHR* DNA-binding domain. The repressor (R) domain represents the first 10-amino-acid sequence of the *PAX3* protein, which inhibits *PAX3* but not *PAX3-FKHR* transcription activity. The octapeptide sequence is thought to be involved in dimerization of *PAX3*, but whether it plays a similar role in *PAX3-FKHR* is not known. The balanced translocation results in expression of a 97-kDa *PAX3-FKHR* fusion protein and a putative 35-kDa *FKHR-PAX3* fusion protein.

transcription factors like *FKHR*, future studies are needed to compare the mechanisms and gene targets of *PAX3-NCOA1/2* with those of *PAX-FOXO* to see if different mechanisms are responsible for the heterogeneity of the ARMS subtype.

## Transcription Properties

*PAX3* is a paired-box transcription factor with roles in developmental and adult regenerative myogenesis (Buckingham and Relaix, 2007; Lagha *et al.*, 2008). *FKHR* is a wing-helix transcription factor with roles in cell growth, survival, and metabolic regulation (Birkenkamp and Coffey, 2003). The translocation breaks within intron-7 of the *PAX3* gene and intron-1 of the *FKHR* gene, leading to an in-frame *PAX3-FKHR* chimera (Fig. 2). Although the sequence-specific DNA-binding domains (paired- and homeo-) of *PAX3* are conserved in *PAX3-FKHR*, comparative expression profiles of ERMS and ARMS tumors, and of *PAX3/PAX3-FKHR*-expressing culture cells, suggest that these two proteins do not function equally (Khan *et al.*, 1999; Begum *et al.*, 2005; De Pitta *et al.*, 2006; Mercado *et al.*, 2008). Several key differences setting *PAX3-FKHR* apart from *PAX3* have been identified: *PAX3-FKHR* activates *PAX3*-dependent genes at a higher level as the result of the stronger activation domain of *FKHR* (Fredericks *et al.*, 1995), activates a unique set of genes not targeted by *PAX3* by uncoupling the interdependence of the two DNA-binding domains necessary for *PAX3* function (Epstein *et al.*, 1998; Zhang and Wang, 2006, 2011), and is not inactivated by several *PAX3* interacting inhibitory proteins (Wiggin *et al.*, 1998; Hollenbach *et al.*, 1999). The cumulative effects of these novel properties in *PAX3-FKHR* are further magnified through altered responses toward post-translational regulation. Phosphorylation destabilizes *PAX3* and controls the nuclear-cytoplasm localization of *FKHR*. While *PAX3-FKHR* retains many of the active phosphorylation sites, it is more resistant to degradation (Miller and Hollenbach, 2007) and resides constitutively in the nucleus (del Peso *et al.*,



**Figure 3.** Three putative mechanisms of chromosomal translocation initiating events in ARMS development. **(1)** ARMS forms as a result of *de novo* translocation leading to the formation of PAX3-FKHR oncoprotein that convert cells into a permissible state for acquiring additional mutations. **(2)** ARMS forms from cells with existing genetic mutation that predisposes to chromosomal translocation. **(3)** *De novo* translocation leading to the co-expression of PAX3-FKHR and FKHR-PAX3 is sufficient to initiate the ARMS phenotype. The non-translocation mutations shown in the first two models may be variable, contributing to the heterogeneity of ARMS.

1999). Together, these studies have identified novel mechanisms by which PAX3-FKHR transforms cells.

### Oncogenic Properties

Several lines of evidence support an oncogenic role of PAX3-FKHR in ARMS development. Ectopic expression of PAX3-FKHR in fibroblasts and myoblasts causes accelerated proliferation and anchorage-independent growth (Scheidler *et al.*, 1996; Lam *et al.*, 1999), and blocks terminal differentiation into multinucleated myotubes (Epstein *et al.*, 1995). Interestingly, expression of a PAX3-FKHR mutant with an impaired paired-DNA-binding domain in fibroblasts and myoblasts can induce cellular transformation and high-grade tumor formation similar to the wild-type PAX3-FKHR. Although myoblasts are not transformed by a PAX3-FKHR homeo-domain mutant, these cells form metastatic tumors in nude mice (Zhang *et al.*, 2009). These mutational studies highlight the biological significance of the gain-of-transcription target specificity in the oncogenic activity of the fusion protein. When introduced into ERMS cells, PAX3-FKHR produces morphological and proliferation rate changes associated with a higher transformation grade (Anderson *et al.*, 2001). Antisense knockdown of PAX3-FKHR expression reduces ARMS cell growth followed by apoptosis (Bernasconi *et al.*, 1996). However, establishing a direct role for PAX3-FKHR in the initiation of ARMS tumorigenesis in genetically engineered animals has not been as straightforward. Transgenic or knock-in

mice expressing PAX3-FKHR under the control of PAX3 promoter show developmental lethality without signs of muscle tumor formation (Anderson *et al.*, 2001; Lagutina *et al.*, 2002). The first PAX3-FKHR-driven ARMS animal model uses a conditional knock-in strategy, targeting the fusion gene expression in differentiated muscle cells (Keller *et al.*, 2004a). In this model, tumor incidence is extremely low (1/218) but is increased with additional mutation in the *p53* or *Ink4A/ARF* gene. This observation suggests that ARMS formation depends on cooperation between PAX3-FKHR and other genetic mutations (Fig. 3). It remains to be determined whether the cooperating event occurs before or after translocation during ARMS establishment. Collectively, the cell culture and animal studies indicate that persistent PAX3-FKHR expression is essential for the initiation and maintenance of ARMS.

### FKHR-PAX3 Fusion Gene: Just a Byproduct?

Reciprocal translocations can lead to the formation of two fusion genes. One fusion product is considered to be oncogenic, whereas the reciprocal product is often not detected or is detected in only a percentage of the tumors. All t(2;13)-positive ARMS express the oncogenic PAX3-FKHR protein. The reciprocal FKHR-PAX3 transcript is detected in ~40 to 60% of ARMS tumors tested, but the status of protein expression is unknown (Barr *et al.*, 1998; Frascella *et al.*, 1998). The inconsistent FKHR-PAX3 mRNA expression suggests that it may not have a role in ARMS. This could be a misconception, since primary tumors have already accumulated numerous secondary mutations at presentation. Analysis of recent data shows that some leukemia-associated reciprocal products are themselves not oncogenic but work in concert with the oncogenic partner in determining tumor pathology and lineage specificity, thus renewing interest in the role of other reciprocal products in human cancers (Rego and Pandolfi, 2002; Zheng *et al.*, 2009). In the absence of animal models that faithfully recapitulate the entire spectrum of human ARMS, a role for FKHR-PAX3 during tumor initiation cannot be ruled out (Fig. 3).

### DISRUPTED BALANCE BETWEEN GROWTH AND DIFFERENTIATION

Proliferation and differentiation are mutually exclusive events mediated by opposing cellular signals. In muscle, this balance is largely controlled through the actions of cell-cycle regulators and myogenic regulatory factors (MRFs). While cell-cycle and myogenesis pathways are mostly independently regulated, a coordinated interaction between proliferation and myogenic pathways is critical for normal development of mature muscle fibers. RMS cells exhibit aberrant regulation of several of these pathways, which could underlie the mechanistic basis for uncontrolled growth and failed differentiation.

### Growth Defects

RMS cells display many defects in cell-cycle checkpoints and growth factor signaling pathways, leading to accelerated proliferation, typically at the G1-S transition. Notable changes include elevated expression of positive cell-cycle regulators such as

cyclin D types (Zhang *et al.*, 2004), *cdk4* (Ragazzini *et al.*, 2004), and *Skp2* (Zhang and Wang, 2007), as well as repression of negative cell-cycle regulators such as cyclin inhibitors p16<sup>Ink4a</sup> (Iolascon *et al.*, 1996; Obana *et al.*, 2003), p21 (Moretti *et al.*, 2002), p27 (Zhang and Wang, 2003), and p57 (Roeb *et al.*, 2007). *Ink4A* and *cdk4* mutations occur more frequently in ARMS. Several mechanisms explain the direct effects of PAX3-FKHR on these cell-cycle regulators: PAX3-FKHR silences p16<sup>Ink4a</sup> expression by increasing methylation of the *Ink4A* promoter (Linardic *et al.*, 2007), up-regulates *Skp2* transcription through activating a novel E2F complex binding to the *Skp2* promoter (Zhang and Wang, 2007), and enhances degradation of ERG1 transcription factor involved in p57 gene activation (Roeb *et al.*, 2007). Several dysregulated growth factor signaling pathways further augment the cell-cycle regulator-mediated growth effects in RMS. The most frequently affected pathways include signaling controlled by IGF, FGF, HGF, and PDGF. Dysregulated FGF and HGF pathways are linked to increased invasion and metastasis (Taulli *et al.*, 2006; Taylor *et al.*, 2009). PAX3-FKHR is known to activate these pathways by transcriptional activation of the corresponding receptor genes, *IGFR1*, *FGFR4*, *c-met*, and *PDGFaR* (Epstein *et al.*, 1996, 1998; Ayalon *et al.*, 2001; Cao *et al.*, 2010). The ability of PAX3-FKHR to target both cell-cycle checkpoints and mitogenic signaling pathways contributes to ARMS aggressiveness.

### Differentiation Defects

Myogenesis occurs through an orderly cascade of events involving myogenic regulatory factor (MRF) family and the myocyte enhancer factor-2 (MEF2) family (Perry and Rudnick, 2000; Pownall *et al.*, 2002). The onset of myogenesis is controlled through a sequential activation of the MRFs, beginning with *MyoD/Myf5* (muscle specification factors) followed by *myogenin* (muscle differentiation factor), and finally by *Myf6* (muscle maturation factor). MEF2 factors work in concert with MRFs to stimulate transcription of muscle-specific structural genes. Although RMS cells express *MyoD*, *myogenin*, and *MEF2*, they fail to complete myogenesis under differentiation conditions. RMS-expressed MRFs and *MEF2* bind their cognate DNA sequences in *in vitro* binding assays, but they cannot activate reporter genes containing the same binding sites (Tapscott *et al.*, 1993). This implies that these factors are kept inactive. Recent studies suggest that increased FGF and TGF $\beta$ -signaling inhibits MRF and *MEF2* function. FGF induces *MEF2* phosphorylation that blocks *MEF2* DNA-binding activity (Li *et al.*, 1992). Although FGF level is not affected in RMS, over-expression of *FGFR4* may lead to increased FGF signaling and consequent inactivation of *MEF2* in RMS cells. *Myostatin* and TGF $\beta$ 1, members of the TGF $\beta$  superfamily, are over-expressed in RMS cells. Since HGF can induce *myostatin* expression in normal muscle cells (Yamada *et al.*, 2010), elevated *myostatin* in RMS may result from amplified signaling through the HGF receptor (*c-met*) that is frequently over-expressed in these cells. *Myostatin* and TGF $\beta$ 1 inhibit myogenesis by repressing *MyoD* and *MEF2* transcription function, respectively (Liu *et al.*, 2004; Yamada *et al.*, 2010). One proposed mechanism for these inhibitory effects of TGF $\beta$  members involves down-regulation of pro-myogenic

microRNAs, including miR-1, miR-206, and miR-29. These microRNAs negatively regulate *HDAC4*, a repressor of *MyoD* and *MEF2* transcription activities (Winbanks *et al.*, 2011).

### ETIOLOGY OF RHABDOMYOSARCOMA

The issue of RMS cellular origin remains controversial. Because RMS tumors express markers of developing skeletal muscle, it is thought that they arise from myogenic cells. Some tumors, however, develop in organs that lack a skeletal muscle component, thus raising the possibility that RMS could develop from non-muscle cells. Given the heterogeneity of RMS tumors and their variable clinical presentation, identification of cell-of-origin is important to understanding RMS biology as well as for improving patient prognosis and treatment. Current knowledge on RMS cellular origin comes from two general approaches: xenograft mouse models with genetically modified cells of defined lineages, and genetically engineered mice. Analysis of these data suggests that multiple cell origins are involved in RMS tumorigenesis (Fig. 4), and points to the important contributions of both cell type and specific genetic alteration in defining the RMS subtype development.

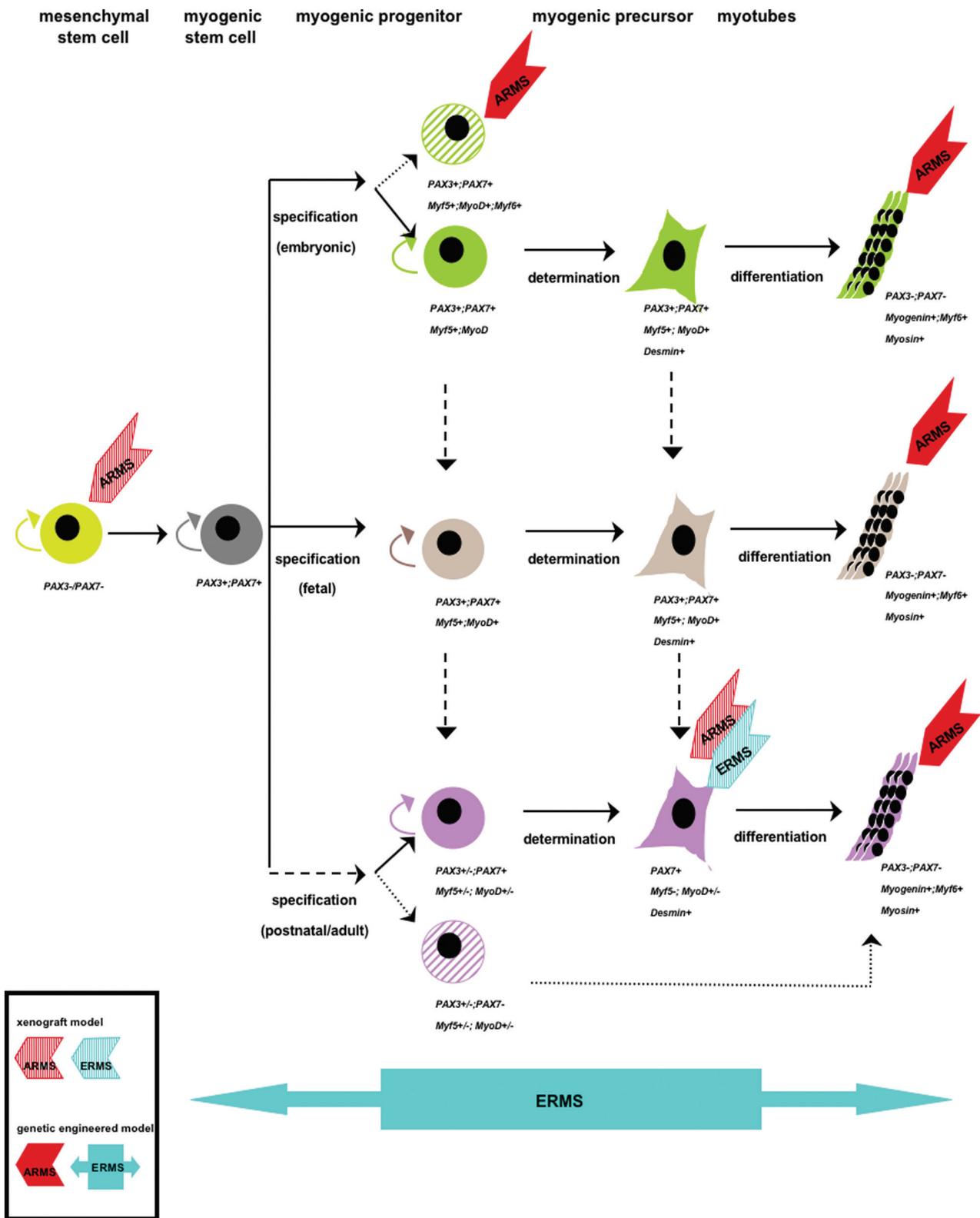
### Xenograft Models

Studies have shown that genetically manipulated satellite and MSC cultured cells can induce RMS tumor formation when implanted into immunodeficient mice. Linardic *et al.* (2005) have shown that concomitant expression of *T-antigen/Ras/hTERT* genes in post-natal human satellite cells gives rise to ERMS-like tumors. ARMS-like tumors can develop from PAX3-FKHR expressing MSC and satellite cells. Ren *et al.* (2008) have shown that MSC expressing PAX3-FKHR in the presence of T-antigen, H-Ras activation, and/or p53 mutation gives rise to ARMS. The finding that ARMS tumors can develop from non-myogenic MSC cells is significant, due to their widespread distribution throughout the body, and could account, in part, for tumor development at sites that lack skeletal muscle. Murine satellite C2 cells expressing PAX3-FKHR induce ARMS-like tumors in nude mice without bringing in additional genetic mutation (Zhang *et al.*, 2009). It is worth noting that C2 is a spontaneously immortalized line that likely has acquired unknown genetic modifications that cooperate with PAX3-FKHR for promoting ARMS tumors; however, they do not carry mutations known to cooperate with PAX3-FKHR in other experimental models (Keller *et al.*, 2004a; Ren *et al.*, 2008).

### Genetically Engineered Models

The major drawback of xenograft tumor models is the reliance on cell lines with unknown genetic modifications. Additionally, cell-line-induced tumors do not reflect the architectural complexity of *in vivo* tumors. Genetically engineered models with germ-line or conditional mutations are more likely to mirror the natural history of RMS development (see De Giovanni *et al.*, 2009, for review). This article focuses only on models that provide direct information on the cellular origin of RMS.

Using a conditional knock-in approach to control temporal- and lineage-specific PAX3-FKHR expression from the *PAX3*



**Figure 4.** Model of multiple cells-of-origin of RMS development based on xenograft and genetically engineered animal studies. The diagram depicts three general stages in muscle development: specification, where activation of MRFs commits cells to myogenic lineage (e.g., satellites); determination, where committed muscle cells lose self-renewing capacity (e.g., myoblasts); and differentiation, where cells acquire muscle-specific phenotype (e.g., myotubes) and muscle-specific gene expression (e.g., myogenin, Myf6, myosin). RMS heterogeneity may result from different muscle development stages when the initial oncogenic event occurs as well as from different cooperating primary and secondary genetic events involved in tumor initiation.

locus, Keller *et al.* (2004a,b) have shown that ARMS develops in mice only when PAX3-FKHR expression is activated in Myf6+ cells. Activation of PAX3-FKHR in PAX7+ cells that represent embryonic muscle stem cells and the majority of satellite cells in adult muscle reduces satellite cell number but does not induce ARMS. These findings are intriguing from two aspects:

(1) ARMS develop from Myf6+ cells that are thought to represent mature muscle fibers, yet these tumors lack muscle characteristics that are typically observed in human ARMS. The authors suggest that the ARMS tumors in the Myf6+ model could also arise from a sub-population of myogenic progenitor cells that transiently express Myf6+ but do not contribute to muscle development during embryogenesis. This conditional model cannot distinguish these two cell populations. Nevertheless, the concept of mature muscle as the origin of ARMS is supported by a *Drosophila* model, which has a much better characterized genetic program for myogenic lineage specification. In this model, the authors show that ARMS develops when PAX7-FKHR is conditionally induced in syncytial muscle fibers (Galindo *et al.*, 2006). An alternative explanation for the lack of muscular characteristics in the Myf6+-driven ARMS tumors is that PAX3-FKHR has the potential to de-differentiate mature muscle cells.

(2) The lack of ARMS in the PAX7+ cell lineage challenges the findings in the C2 xenograft model, because C2 cells are thought to resemble satellite cells. Keller's model produces ARMS at a low frequency unless *p53* or *INK4A/ARF* is mutated. One possible explanation is that other types of cooperating events are needed for PAX3-FKHR-induced ARMS in satellite cells, as previously mentioned. Another possibility is that only satellite cells with low PAX7 or capable of losing PAX7 expression can be coaxed into rhabdomyosarcomagenesis. It has been reported that satellite progenitor cells prepared from human muscle fibers contain both PAX7- and PAX7+ populations (Reimann *et al.*, 2004). Furthermore, PAX3-FKHR represses PAX7 expression in ERMS (Tomescu *et al.*, 2004) and C2 cells (C. Wang, unpublished observations); however, down-regulation of PAX7 by PAX3-FKHR is not observed in Keller's model. Although it is not clear why PAX7 expression is resistant to repression by PAX3-FKHR in Keller's model, the high level of PAX7 might offset the oncogenic potency of the fusion protein. More research is needed to clarify the identity and role of secondary mutations and cell lineage in ARMS initiation and development.

Rubin *et al.* (2011) have reported that ERMS tumors are formed in a series of *Ptch* (+/-) and/or *p53* (-/-) engineered mice with conditionally targeted expression in PAX3+ cells (pre- and post-natal myogenic progenitor), PAX7+ cells (satellite), or Myf6+ cells (mature muscle). All lineages in *Ptch* (+/-)/*p53* (-/-) mice develop mesenchymal tumors, of which various percentages are of the ERMS type. Strikingly, mice with the *p53* mutation in Myf6+ mature muscle cells are 100% ERMS. The authors suggest that the precise pairing of cell lineage with specific mutations is key to determining the type of sarcoma that develops. The degree of differentiation observed in these models seems to correlate with the targeted cell lineages, with PAX7+ satellite cell-derived tumors displaying the least myogenic differentiation and Myf6+ cell-derived tumors showing the most myogenic differentiation. The various extents of myogenesis in

the mouse ERMS correlate well with the differentiation spectrum observed in human ERMS. The high degree of myogenesis in Myf6+-derived ERMS is in stark contrast to the lack of myogenesis in Myf6--derived ARMS. The difference might reflect the different Myf6+ cell populations being targeted for ERMS vs. ARMS. Alternatively, the degree of myogenesis in the RMS may depend on the specific combination of genetic mutations.

## TARGETED CANCER THERAPY

Several target-specific compounds have been identified that are effective in suppressing the growth and tumorigenesis of RMS cells. These compounds include inhibitors for receptor tyrosine kinases (Croce and Linardic, 2011), intracellular signaling molecules (Martins *et al.*, 2011), and angiogenic factors (Bid and Houghton, 2011). Many of these drugs and analogs are in clinical trials. Because PAX3-FKHR is specific for ARMS, investigators have focused on developing reagents that directly alter its expression or function. These reagents include siRNAs (Bernasconi *et al.*, 1996), transcription repressors (Fredericks *et al.*, 2001), vaccines against peptide spanning the fusion region (Rodeberg *et al.*, 2005), and kinase inhibitors (Zeng *et al.*, 2010). Readers are referred to a comprehensive review on these topics for more information (Wachtel and Schafer, 2010).

This article focuses on the therapeutic value of "differentiation therapy" for treating RMS. Differentiation therapy aims to suppress tumor behavior by forcing cancer cells to terminally differentiate with a concomitant reduction in proliferation. There is evidence that RMS cells are susceptible to such therapeutic interventions. For example, some RMS cells differentiate upon treatment with retinoic acid (Crouch and Helman, 1991) or TPA (Aguanno *et al.*, 1990; Bouche *et al.*, 1993). Introduction of a constitutively active MKK6 induces myogenesis in RMS cells by reactivating the p38 MAPK that is normally required for muscle differentiation but is impaired in RMS cells (Puri *et al.*, 2000). However, these approaches work only on selected RMS cell lines, suggesting that the myogenic defects in RMS are heterogeneous, involving multiple, most likely parallel, pathways. Since RMS cells can be coaxed into differentiation, there must be common downstream effectors in the various defective pathways, which are susceptible for reactivation in all RMS cell types. Indeed, introduction of late-stage effectors such as Myf6 (Sirri *et al.*, 2003) and microRNAs (Wang *et al.*, 2008; Taulli *et al.*, 2009; Yan *et al.*, 2009; Rao *et al.*, 2010) restores myogenesis in many RMS cell lines. Re-expression of miR-29 and miR-1/206 in RMS cells also blocks tumor formation in mice. Additionally, Taulli *et al.* (2009) showed that activation of miR1/206 in xenograft tumors attenuates further tumor expansion.

One of the challenges of chemical-based therapies is the risk that cancer cells will develop drug resistance or activate alternative pathways to overcome the drug effects. The high frequency of overactive IGF/IGFR signaling in RMS has sparked interest in testing IGF inhibitors in clinical trials. However, analysis of recent data shows that RMS cells treated with IGF inhibitors become resistant through up-regulation of EGFR/Her1 or Her2/Neu receptor (Huang *et al.*, 2009; Abraham *et al.*, 2011). Since microRNAs occur naturally in cells and target multiple genes and pathways, they may represent unique opportunities for

therapeutic intervention that requires lower dosage with minimal side effects. MicroRNA-related research in RMS is still in its infancy. Recent profiling studies comparing microRNA expression patterns in RMS subtypes and normal skeletal muscle have expanded the number of microRNA candidates that can be further explored (Wei *et al.*, 2009; Sarver *et al.*, 2010).

## CONCLUDING REMARKS

There has been tremendous progress made in the understanding of RMS biology since the identification of associated genetic defects. Considering the high degree of heterogeneity in RMS, there remain many aspects of RMS pathogenesis in need of clarification, such as (1) developing additional genetically relevant pre-clinical animal models that reflect the diverse RMS groups, (2) establishing the origin and evolution of different RMS phenotypes, (3) defining the spectrum of cooperating genetic and molecular events that drive RMS initiation and progression, and (4) determining if chromosomal translocation is the first step in the transformation of MSC or myogenic cells to cancerous ARMS cells. This information is critical for assessing the heterogeneity of clinical responses, and for designing the most effective molecular targeted therapies for RMS treatment.

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