Histol Histopathol (2012) 27: 13-22
DOI: 10.14670/HH-27.13

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Advances in pediatric rhabdomyosarcoma characterization and disease model development

Dennis O' Brien, Aishwarya G. Jacob, Stephen J. Qualman and Dawn S. Chandler
The Center for Childhood Cancer, Columbus Children's Research Institute
and the Department of Pediatrics, The Ohio State University, Columbus, Ohio, USA

Summary. Rhabdomyosarcoma (RMS), a form of soft tissue sarcoma, is one of the most common pediatric malignancies. A complex disease with at least three different subtypes, it is characterized by perturbations in a number of signaling pathways and genetic abnormalities. Extensive clinical studies have helped classify these tumors into high and low risk groups to facilitate different treatment regimens. Research into the etiology of the disease has helped uncover numerous potential therapeutic intervention points which can be tested on various animal models of RMS; both genetically modified models and tumor Xenograft models. Taken together, there has been a marked increase in the survival rate of RMS patients but the highly invasive, metastatic forms of the disease continue to baffle researchers. This review aims to highlight and summarize some of the most important developments in characterization and *in vivo* model generation for RMS research, in the last few decades.

Key words: RMS subtypes, Sarcoma, Mouse models

Introduction

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood, with approximately 250 children being diagnosed every year in the United States. Thanks to successive treatment protocols from the Intergroup Rhabdomyosarcoma Study Group (IRSG), a large majority of children with localized disease are now able to be cured of their disease (70% vs. 25% in the 1970's) (Meza et al., 2006). This is, in large part, due to the discovery of different risk-factors and the tailoring of treatment based upon the risk of treatment failure. For instance, evaluation of the results of the early IRSG treatment protocols revealed that the alveolar subtype (ARMS) was associated with a poorer outcome than the embryonal subtype (ERMS) in children with localized disease (Gaiger et al., 1981). This led to more intensive chemotherapy for children with localized ARMS in later treatment protocols. Newer risk-factors, based on histologic findings and molecular characterizations, are helping to further distinguish patients who are at a higher risk of treatment failure (and should therefore be treated with more aggressive therapy). Embryonal tumors with areas of anaplasia seem to behave more aggressively than other embryonal variants. Alveolar tumors, generally the more aggressive subtype, are known to be associated with two chromosomal translocations (t(2;13) and t(1;13)), which result in the creation of the abnormal transcription factors PAX3-FKHR and PAX7-FKHR (also known as PAX3-FOXO1 and PAX7-FOXO1), respectively. It is now clear that these different variants of ARMS not only have different prognoses, but also have different metastatic profiles. Aberrations in the p53 pathway, caused by p53 mutations or abnormal expression of p53 regulators, such as MDM2, have been found in rhabdomyosarcomas. MDM2 has been shown to be over-expressed in a variety of soft-tissue sarcomas, including RMS (Keleti et al., 1996). Expression of alternatively-spliced variants of *MDM2* (*MDM2-alt*) have been correlated with advanced disease in RMS as well (Bartel et al., 2001a). Similar findings have been reported with the related protein MDM4. These molecular characterizations may help to further define patients who are at risk for treatment failure, may suggest methods of pathogenesis of these tumors, and eventually lead to the development of targeted therapies in the treatment of this disease. This is especially needed for children who present with metastatic disease, in

Offprint requests to: Dawn S. Chandler, Ph.D., Center for Childhood Cancer, WA5023, Children's Research Institute, 700 Children's Drive, Columbus, OH 43205, Ohio, USA. e-mail: chandler.135@osu.edu

whom the prognosis remains poor, despite increasing intensive chemotherapy regimens (Breneman et al., 2003).

Background

Rhabdomyosarcomas are soft tissue sarcomas that are thought to arise from primitive mesenchymal cells which show evidence of skeletal muscle differentiation. A majority of these tumors are found in children under the age of 10 years, and with a slight male predominance (1.5:1) (Ruymann and Grovas, 2000). The site of occurrence varies with age, with tumors of the genitourinary system, bladder, and prostate being more common in children <5 years of age. Tumors of the head and neck are found frequently in children aged 5-9 years, and tumors of the extremities, trunk, and paratesticular region being more common in older children and adolescents (Ruymann and Grovas, 2000). Tumors usually present either as a painless mass or as the result of the tumor displacing a vital structure (i.e., the eye) or interfering with that structure's normal function.

Prior to the use of chemotherapy, treatment consisted of surgical resection with or without radiation to the primary site. Most children would eventually develop metastases and succumb to the disease. Since the 1970's, the Intergroup Rhabdomyosarcoma Study Group (IRSG) has developed successive treatment protocols for children and adolescents with rhabdomyosarcoma using a combination of surgery, chemotherapy, and radiation therapy. These treatment protocols tailored the intensity of chemotherapy based on early risk factors (site of disease, presence of metastases). As the tailored therapy regimens have improved overall survival, they have also changed the risk factors associated with treatment failure. Patients currently are placed into risk categories based on well-known risk factors such as tumor subtype, staging (tumor site, size, regional lymph node involvement, and metastases), and clinical grouping (completely resected disease vs. incomplete, local disease vs. regional spread vs. metastases). Localized ERMS at favorable sites or completely resected ERMS at unfavorable sites are considered "low-risk". Incompletely resected ERMS at unfavorable sites and all non-metastatic ARMS tumors are considered "intermediate-risk", and all metastatic RMS tumors are considered "high-risk". These molecular characterizations, therefore, will help determine future risk-group stratification and therefore the type and intensity of therapy given.

With the exception of certain chemotherapeutic agents, ionizing radiation, and parental recreational drug use, there are no known environmental triggers in the development of RMS (Ruymann and Grovas, 2000). There are, however, several inherited conditions with a strong association with the development of RMS. Children with neurofibromatosis type-1, a neuro-cutaneous disorder, have an increased incidence of several tumor types, including rhabdomyosarcomas. Approximately 1.5-6% of those with neurofibromatosis type-1 will develop RMS (Sung et al., 2004). Rhabdomyosarcoma is also seen in the Li-Fraumeni Syndrome, an autosomal dominant disorder involving a germline mutation of the p53 tumor suppressor gene (Ruymann and Grovas, 2000). Families affected with the Li-Fraumeni syndrome have a high incidence of soft tissue sarcomas, adrenocortical carcinoma, and breast cancer. The Beckwith-Wiedemann overgrowth syndrome is associated with gene abnormalities on chromosome 11p15. This locus is where the gene for insulin-like growth factor II (IGF-II) is found. The Beckwith-Wiedemann Syndrome is most readily associated with Wilms' tumor (nephroblastoma), but these children also have an increased risk of other embryonal tumors (including rhabdomyosarcomas) (Matsumoto et al., 1994; Smith et al., 2001).

Histology of rhabdomyosarcoma

A revision of the new classification system introduced in the 1990's, the International Classification of Rhabdomyosarcoma (ICR), divided rhabdomyosarcomas into embryonal (with botryoid and spindle cell variants), alveolar (and solid variant), and anaplastic subtypes. The malignant cell of rhabdomyosarcomas is termed the rhabdomyoblast (Qualman et al., 1998). The classification of RMS into its various subtypes not only depends on the appearance of the cellular background but also on the appearance of the cell itself. The ERMS subtype (approximately 50% of all cases) is composed of sheets of rhabdomyoblasts that have irregularly distributed dense chromatin (Fig. 1A) (Bennicelli et al., 1995). Overall, children with ERMS have a 5-year survival of 66%. The botryoid variant ERMS is less common (around 6% of all cases) and has a classic gross morphologic appearance (resembling a cluster of grapes). Microscopically, it is defined by a cambium, or layer of rhabdomyoblasts, beneath an intact epithelial layer (Fig. 1B) (Qualman et al., 1998). Children with this type of tumor have an excellent 5-year survival rate of 95% (Qualman et al., 1998). A more recent ERMS variant has been described, the spindle cell variant. As the name implies, it has a spindled appearance under microscopic examination (Fig. 1C). The rhabdomyoblasts show varying degrees of myogenic differentiation. It is found mainly in the paratesticular region, and is also associated with a superior 5-year survival (88%).

Though alveolar tumors are not as common as embryonal tumors, they account for the majority of disease-related deaths. The alveolar subtype derived its name from the histologic similarity to lung alveoli. This is caused by segmentation of rhabdomyoblasts into clusters and the subsequent death and loss of the centrally located cells, resulting in cleft-like spaces lined with tumor cells (Fig. 1D) (Gaiger et al., 1981; Qualman et al., 1998). The rhabdomyoblasts have nuclei with coarse chromatin and uniform, popcorn configuration nuclei, and generally have less myogenic differentiation

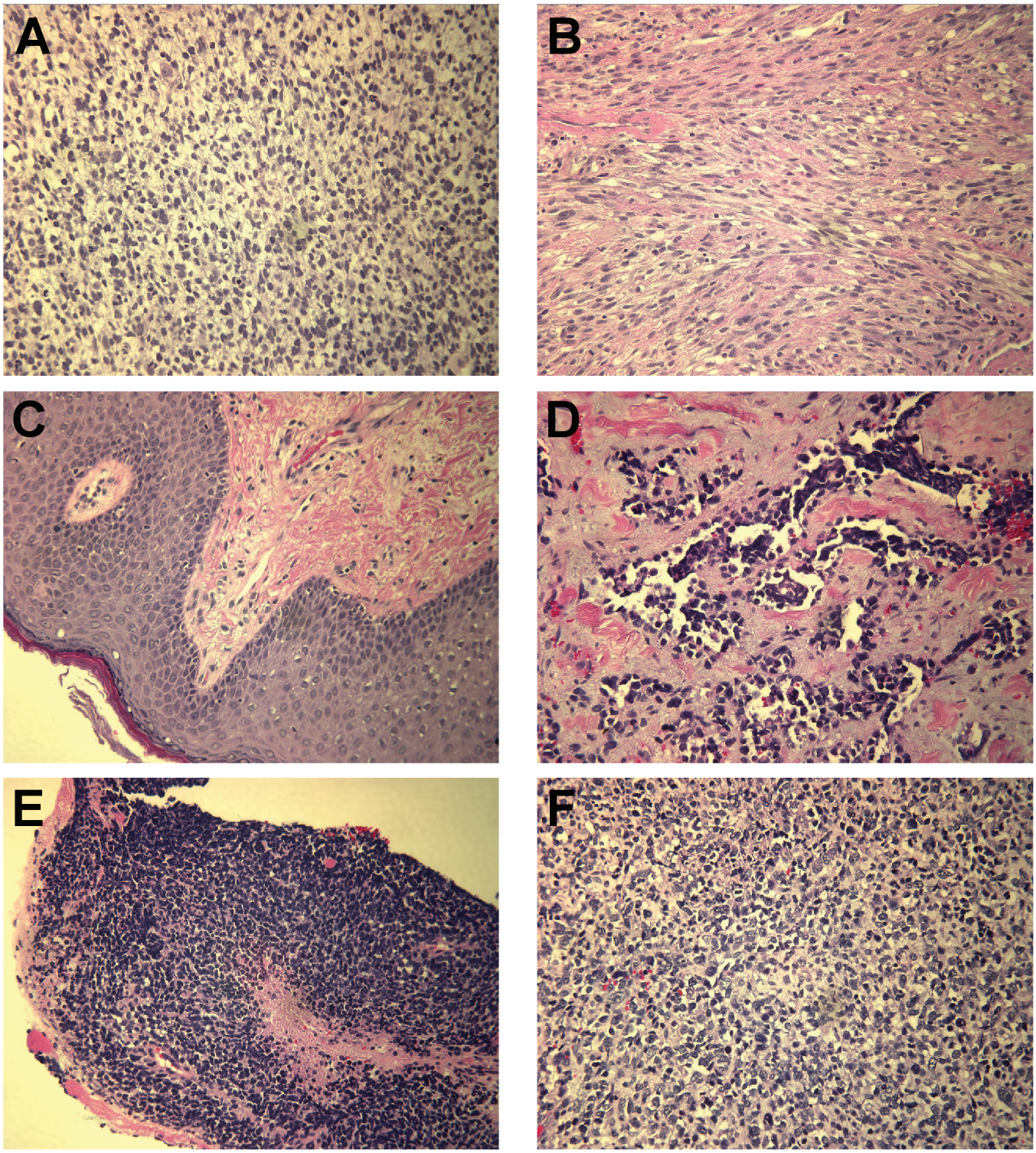

Fig. 1. Typical light microscopic appearance of pediatric rhabdomyosarcoma subtypes. **A.** Embryonal RMS (NOS). **B.** Spindle-cell variant of embryonal RMS. **C.** Botryoid variant of embryonal RMS. **D.** Alveolar RMS. **E.** Solid variant of alveolar RMS. **F.** Anaplastic RMS. Haematoxylin and eosin, x 100

than their embryonal counterparts. These tumors are most often seen in the extremities and trunk, and are more common in older children and adolescents. A solid variant of the alveolar subtype has also been described.

The revision of the International Classification of Rhabdomyosarcoma (ICR) describes another subtype of rhabdomyosarcoma, anaplastic RMS (Qualman et al., 1998). In the past this subtype was also known as the pleomorphic subtype. The rhabdomyoblasts are defined by large, lobulated, and hyperchromatic nuclei as well as atypical mitoses (Fig. 1E). Focal or diffuse anaplasia can be seen in both ERMS and ARMS in approximately 13% of cases. This anaplastic hallmark has been shown to be an independent prognostic factor (Qualman et al., 2008). The poorer survival in patients with anaplastic ERMS may help to explain the difference in survival between the botryoid and spindle-cell ERMS variants and conventional ERMS, which previously included anaplastic ERMS. Despite the decreased survival experienced by patients with anaplastic RMS, there are, at this time, no new therapeutic agents or regimens to help improve the prognosis.

The purely histologic diagnosis has been supplemented clinically by newer, molecular diagnostic techniques (especially for ARMS). Every tumor submitted to the Children's Oncology Group is currently undergoing FISH and/or RT-PCR for translocation detection. This helps to clarify the diagnosis in cases where sampling error during biopsy or incomplete resection could cause an ARMS to be called ERMS based on histology. The use of molecular biology will better define these tumors and their subsequent biological activity.

Molecular findings in RMS

A purely histologic classification of rhabdomyosarcomas, as both a diagnostic and prognostic tool, is being supplemented by a molecular characterization of these tumors. This molecular characterization will not only help to define tumors according to risk of treatment failure, but will also help to uncover the mechanisms involved in the development of rhabdomyosarcomas, and possibly lead to novel therapeutic options.

Chromosomal aberrations are the most well known molecular abnormalities in RMS. In particular, alveolar RMS is strongly associated with two chromosomal rearrangements. The t(2;13)(q35;q14) and the less-common t(1;13)(p36;q14) account for approximately two-thirds of alveolar RMS tumors (approximately one-third have no translocation). The t(2;13) translocation results in the fusion of the gene encoding the DNA binding domain of *PAX3* with the gene encoding the transcriptional activation domain of FKHR (FOXO1) on chromosome 13. *PAX3* is thought to be responsible for the migration of myogenic cells to the limb buds during early embryonic development (Daston et al., 1996). The resultant aberrant transcription factor PAX3-FOXO1 is a more potent transcriptional activator than wild-type PAX3 (Bennicelli et al., 1995), although the fusion gene itself is rarely amplified. Several transcriptional targets of the PAX3-FOXO1 transcription factor have been studied. The anti-apoptotic protein BCL-XL was shown to be up-regulated in the presence of PAX3-FOXO1 (Margue et al., 2000). PAX3-FOXO1 has also been shown to inhibit myogenic differentiation and block normal PAX3 function (Xia et al., 2002). Other genes involved in myogenesis, such as transcription factors (*MYOD, MYOG, SIX1*) and insulin-like growth factor-II (IGF-II), are also up-regulated by PAX3-FOXO1 (Xia et al., 2002). These findings may help explain why alveolar tumors are more aggressive and frequently metastasize. The t(1;13) translocation likewise results in an aberrant transcription factor, in this case PAX7-FOXO1. *PAX7*, while not believed to be involved in the migration of myogenic cells, is involved in myogenic differentiation. Although less common than t(2;13) in alveolar RMS, some studies have suggested that patients with the t(1;13) translocation tend to have a better prognosis (though still poor) than those with t(2;13) (Kelly et al., 1997; Sorensen et al., 2002). This data must be interpreted with caution, however, as the number of patients with the t(1;13) translocation is small.

In embryonal tumors, the most common chromosomal aberration is allele loss at chromosome 11p15.5. In addition to embryonal RMS, other pediatric embryonal tumors, such as Wilms' tumor and hepatoblastoma, also display gene disruption at this locus. Disruption can be caused by loss of heterozygosity (LOH) or loss of imprinting (LOI). As stated earlier, the Beckwith-Wiedemann syndrome is associated with gene alterations at this site. While there are numerous genes within this region, most of the focus in this region involves insulin-like growth factor II (IGF-II). IGF-II has been found to be overexpressed in rhabdomyosarcoma tumors (Minniti et al., 1994). Other candidate tumor suppressor genes, such as *H19* and p57 (CDKN1C), have also been localized to 11p15 (Xia et al., 2002).

In both embryonal tumors with anaplasia and alveolar tumors, genomic amplification has been shown at similar frequencies (Bridge et al., 2002). Amplification of the locus of the insulin-like growth factor type I receptor gene (*IGF1R*) has been implicated in a subset of these tumors (Makawita et al., 2009).

The p53 pathway has been implicated in the development of rhabdomyosarcomas. Individuals with the Li-Fraumeni syndrome (who have inherited germ-line p53 mutations) are at an increased risk of developing rhabdomyosarcomas, suggesting an association between mutation of p53 and the subsequent development of RMS. p53 mutations (both germ-line and somatic) have also been found in cases of sporadic RMS. While mutations of p53 are found in many human cancers, reported rates of p53 mutations in sporadic cases of RMS vary considerably (Taylor et al., 2000).

In addition to mutations in p53 itself, dysfunction and/or dysregulation of the p53 pathway may also play a

role in the development of tumorigenesis. MDM2, a negative regulator of p53, is being investigated for its potential role in the development of rhabdomyosarcomas. *MDM2* is itself induced by p53 expression, and plays two roles in p53's regulation. First, the MDM2 protein directly binds to p53, blocking its transactivational activity. Secondly, MDM2 acts as an E3-ubiquitin ligase, mediating p53's translocation to the cytoplasm and subsequent degradation. Therefore, amplification in either MDM2 gene expression or protein function could result in decreased p53 activity and tumorigenesis. MDM2 may also have p53-independent oncogenic properties that are still being elucidated. Indeed, *MDM2* has been found to be amplified in adult soft tissue sarcomas (approximately 30%) as well as other adult tumors, and have been correlated with a poorer prognosis in adult soft tissue sarcomas (Bartel et al., 2001b). In pediatric rhabdomyosarcomas, however, *MDM2* has been found to be amplified in only a minority of tumor samples (10-17%) (Taylor et al., 2000; Takahashi et al., 2004), and protein expression was elevated in a similar number of cases (Takahashi et al., 2004). This suggests that MDM2 gene amplification and elevated protein expression by themselves may not be a major contributor to tumorigenesis in pediatric RMS.

Besides gene amplification, another means of altering MDM2 expression and function involves pre-mRNA splicing. Many of these alternatively-spliced variants have been described in the literature, and most lack the p53 binding sequence found in full-length MDM2. They appear to function, at least in part, by directly binding to and inhibiting full-length MDM2 (Evans et al., 2001; Chandler et al., 2006). Their presence would therefore suggest activation of the p53 pathway. However, some alternatively-spliced forms of MDM2 have been shown to have oncogenic properties in transgenic mice, and so the mechanism of contribution of these alternatively-spliced variants of MDM2 to tumorigenesis remains unknown (Steinman et al., 2004). Alternatively-spliced variants of MDM2 have been described in adult soft tissue sarcomas, but have not been shown to correlate with prognosis (Bartel et al., 2001b). Reports have described expression of many different alternatively-spliced MDM2 variants in pediatric RMS cell lines and tumors at a high frequency (75% and 82%, respectively) (Bartel et al., 2001a). This rate is much higher than have been described for either p53 mutation or MDM2 amplification in pediatric RMS.

MDM4, a protein related to MDM2, is another regulator of p53. Like MDM2, MDM4 can bind directly to p53 and block its transactivational activity. Unlike MDM2, MDM4 is not known to possess an E3-ubiquitin ligase activity. The contribution of MDM4 expression to tumorigenesis remains unclear. Besides inactivating p53, MDM4 is thought to have several interactions with MDM2. These include stabilizing MDM2 protein as well as stimulating MDM2-mediated p53 degradation (Stad et al., 2001; Linares et al., 2003). The MDM4 gene was found to be overexpressed in 17% of soft tissue sarcomas and was associated with a poor prognosis (Bartel et al., 2005). Alternatively-spliced variants of MDM4 have also been described (Giglio et al., 2005; Prodosmo et al., 2008). Some of these splice variants (MDM4-E) can directly bind and inhibit p53 and have been reported as significant prognostic markers in soft tissue sarcomas (Bartel et al., 2005).

Other tumor suppressor pathways may play a role in tumorigenesis in rhabdomyosarcomas (Table 1). The RB tumor suppressor gene is another target of interest in rhabdomyosarcomas. RB serves in the regulation of myogenic differentiation (Cam et al., 2006). No RMS tumors have been found with mutations in the *RB* gene, though amplifications and mutations in the regulators of *RB* have been discovered. p63 and p73 regulate the level of RB protein through phosphorylation via the cyclin-dependent kinase inhibitor p57 (CDKN1C). An isoform of p73, termed DNp73, inhibits both p63 and p73, thus preventing dephosphorylation of the RB protein and preventing the cell from exiting the cell cycle (Cam et al., 2006). DNp73 was found to be overexpressed in RMS tumor samples (Cam et al., 2006). The gene for p57 is located on chromosome 11p15, and is usually expressed from the maternal allele – the allele that is often lost in embryonal RMS (Matsuoka et al., 1996). Other cyclin-dependent kinase proteins also inhibit RB through phosphorylation. *CDK4* amplification has been described in RMS cell lines (Khatib et al., 1993). *CDK4* has the same gene locus (chromosome 12q13-15) as *MDM2*, although they are not necessarily amplified together (Ragazzini et al., 2004). Homozygous deletion of *CDKN2A,* a gene which encodes an inhibitor of CDK4, was found in RMS tumors and cell lines (Iolascon et al., 1996). Mutations of the Ras family of membrane-bound proteins, have been described in a small number of embryonal RMS tumors, at a frequency of approximately 20% (Xia et al., 2002). These proteins are involved in cellular transduction and signaling in various growth and differentiation pathways. The *MYCN* oncogene has been implicated in some alveolar RMS tumors. Amplification of *MYCN* was found exclusively in alveolar RMS subtypes, and the presence of amplification correlated with a poorer prognosis (Hachitanda et al., 1998).

Durbin et al. have described a unique molecular connection between ERMS and ARMS through Integrin Linked Kinase (ILK), which acts as a tumor suppressor in ERMS but as an oncogene in high grade ERMS, and in all ARMS tumors. Furthermore, ILK is sensitive to the expression of Pax3/FOXO1 fusion transcription factor, which is a common translocation in ARMS. Their study showed that Pax3/FOXO1 expression in ERMS is capable of converting the tumor suppressive ILK into a growth promoting factor by its impact on JNK1-c-jun signaling pathways (Durbin et al., 2009).

Rhabdomyosarcomas represent a very heterogeneous group of pediatric cancers. It is clear that their ability to grow, metastasize, and escape normal treatment methods

differ depending on their biologic properties – not all of which are understood at the present time. The implications for the further molecular characterization of pediatric rhabdomyosarcomas are several. The first benefit will be the ability to more accurately define patients within the current risk-stratification scheme and determine those who are at a higher or lower risk of treatment failure. Secondly, molecular characterization will help to find new therapeutic targets, especially in those patients for whom conventional chemotherapy has proven ineffective.

Animal models of RMS

RMS, though a common type of childhood cancer, is still poorly understood and there is the need to develop a reliable animal model to understand the etiology of this disease and to enable therapeutic testing. Over the years, mouse, fly and fish models have been generated that mimic human alveolar RMS and/or embryonal RMS and pleomorphic RMS. The important transgenic mouse RMS models are outlined in Table 2 in detail. While some of these models tried to recapitulate the chromosomal aberrations that characterize RMS conditions, others tried to identify the crucial signaling pathways whose deregulation can lead to formation of any of the three types of RMS.

>70% of the alveolar RMS tumors are characterized by translocation events that lead to the generation of a potent transcriptional activator Pax/FOXO1 fusion protein. Studies that mimicked the t(2;13) Pax3/FOXO1 fusion event, have reported a scenario in which the Pax3/FOXO1 fusion protein expression alone did not induce tumorigenesis though it acted as a toxic, dominant negative protein to Pax3 on ubiquitous expression during embryonic development leading to severe developmental defects (Anderson et al., 2001; Lagutina et al., 2002; Relaix et al., 2003). Hence the need arose for a tissue specific, conditional expression of the PAX/FOXO1 fusion proteins, to understand their *in vivo* role in tumorigenesis. A conditional PAX3/FOXO1 knock in model was developed, that faithfully recapitulates the translocation t(2;13) in a Cre Recombinase dependent manner in post-natal terminally differentiating myofibers and formed ARMS tumors at a very low penetrance and long latency specifically from cells expressing the Myf6 Cre in skeletal muscle (Keller et al., 2004b). Furthermore, in a parallel study, Keller et al used the same mouse model to express the PAX3/FOXO1 fusion protein ubiquitously (RajCre) confirming the toxic, embryonic lethal effects of its expression (Keller et al., 2004a). This study also showed that expression of the PAX3/FOXO1 fusion protein in muscle satellite cell pools did not result in tumor formation. These studies together lent support to the theory that post natal differentiated myoblasts or terminally differentiating myofibers are the cells of origin in ARMS tumors and that these translocations are unlikely to be germline mutations (Keller et al., 2004a,b). Similarly a drosophila model expressing PAX7/FOXO1 t(1;13) in differentiated fly muscle led to "dedifferentiation" and formation of discrete mono-nuclear cells from synctial myofibers, providing more support for the possibility of a differentiated myoblast

Table 1. Common molecular pathway abnormalities of rhabdomyosarcomas.

RMS subtype	Molecular Pathway	Gene	Abnormality	Frequency	Reference(s)
Embryonal	p53	TP53	Germline mutation	10%	Diller et al., 1995
			Mutation in tumor	15%	Felix et al., 1992; Leuschner et al., 2003; Takahashi et al., 2004
		MDM2	Gene amplification	9%	Taylor et al., 2000
			Protein overexpression	10%	Leuschner et al., 2003; Takahashi et al., 2004
			Alternative splicing	82%*	Bartel et al., 2001a
		ARF	Deletion	33%	Iolascon et al., 1996
	RB	CDKN2A/B	Deletion	33%	Iolascon et al., 1996
		ΔNp73	Overexpression	86%*	Cam et al., 2006
	RAS		Mutation	19%	Stratton et al., 1989; Yoo and Robinson, 1999
	MYCN		Amplification	16%	Williamson et al., 2005
	IGF	IGF2	Protein overexpression	94%	Makawita et al., 2009
	FGF	GPC5	Amplification	12%	Williamson et al., 2007
Alveolar	p53	TP53	Germline mutation	8%	Diller et al., 1995
			Mutation in tumor	9%	Felix et al., 1992; Leuschner et al., 2003
		MDM2	Gene amplification	20%	Taylor et al., 2000
			Protein overexpression	7%	Leuschner et al., 2003; Takahashi et al., 2004
			Alternative splicing	82%*	Bartel et al., 2001
		ARF	Deletion	17%	Iolascon et al., 1996
	RB	CDKN2A/B	Deletion	17%	Iolascon et al., 1996
	MYCN		Amplification	32%	Dias et al., 1990; Driman et al., 1994; Hachitanda et al., 1998
	IGF	IGF2	Protein overexpression	25%	Makawita et al., 2009
	FGF	GPC5	Amplification	16%	Williamson et al., 2007
Anaplastic	p53	TP53	Mutation in tumor	50%	Takahashi et al., 2004

*: No discrimination between ARMS & ERMS

being the cell of origin for alveolar RMS; a question that had been puzzling researchers for decades (Galindo et al., 2006). These models also showed that perturbations in critical signaling pathways like p53, Ras are modifiers of the disease that work in cooperation with the fusion proteins generated by the translocations to form alveolar RMS. This cooperativity between these perturbed signaling pathways and the fusion proteins seemed to be essential as these models generated alveolar RMS tumors only in p53 null, ARF null or Ras mutated backgrounds suggesting that the translocation events are not sufficient to induce tumorigenesis. To date, the model developed by Keller et al. (2004a,b) expressing the PAX3/FOXO1 fusion protein in terminally differentiating myoblasts, remains the only convincing translocation-positive ARMS tumor model despite its low penetrance and slow tumor onset unless supplemented by p53 or ARF knockout. Indeed, it has been shown that these mouse models do exhibit an expression profile analogous to human ARMS to affirm the potential use of this mouse for therapeutic testing (Nishijo et al., 2009).

On the other hand, embryonal RMS and pleomorphic RMS tumors show perturbations in various tumor pathways but are translocation negative. Translocation-negative mouse models showing mutations in p53, Ras, ARF null alleles, Fos null alleles, constitutive activation of signaling pathways and oncogenes, all showed formation of spontaneous, relatively quick onset, embryonal or pleomorphic RMS with synergistic effects when two or more of these pathways were inactivated simultaneously. These mice are therefore, good model systems to study embryonal, pleomorphic and translocation negative alveolar RMS.

The question about the cells of origin of ERMS was answered by a zebrafish model expressing mutant Ras, which identified muscle satellite like cells as the cells of origin for ERMS (Langenau et al., 2007). Furthermore, an analysis of the tumors from these models showed enrichment of cells expressing high levels of mutant proteins and deregulated pathways providing an idea about the cells of origin of these tumors. These studies, therefore, enabled an understanding of the cross talk occurring between different oncogenic and tumor-

Table 2. Genetically modified mouse models for RMS.

Cancer Type	Genetic Manipulation	Phenotype and Penetrance	Reference
Cardiac RMS	Expression of SV40 Tumor antigen under SM22α promoter in smooth muscles: vascular, GI Tract, genitourinary systems and transiently in embryonic, murine heart.	80% of mice show cardiac tumors within 12 weeks. Increased cell density, DNA content in transgene expressing cardiac tumors compared to transgene expressing and non expressing normal tissues.	Kobbert et al., 2008
Embryonal RMS	HGF/SF transgenic mice on INK4a/ARF$^{-/-}$ background	Almost 100% INK4a/ARF null mice expressing HGF/SF showed highly invasive, multicentric ERMS with appearance of skeletal muscle cells at ectopic sites at 3.3 months. HGF/SF expression changes ARF$^{-/-}$ tumor spectrum.	Sharp et al., 2002
Embryonal RMS	p53$^{-/-}$ Fos$^{-/-}$	ERMS of the head and neck in 93% of double negative mice at 25 weeks. Significantly lower tumor incidence in p53 or Fos heterozygote nulls. Indicative of cross talk between p53 and Fos pathways	Fleischmann et al., 2003
Embryonal RMS	Her2/neu oncogene activation in BALB p53$^{+/-}$ background	Spontaneous rhabdomyosarcomas in genitourinary region in100% male mice; onset at 11-21 weeks.	Nanni et al., 2003
Embryonal/Alveolar RMS	Spontaneous mutation in X23 of dystrophin gene creating premature stop codon	6.4 to 9% mice affected by rhabdomyosarcomas; onset 16-20 months	Chamberlain et al., 2007; Fernandez et al., 2010
Embryonal RMS	SGCA$^{-/-}$	Axial ERMS from skeletal muscles of 5% SGCA$^{-/-}$ mice, onset >12 months.	Fernandez et al., 2010
Alveolar RMS	Conditional fusion PAX3:FOXO1 controlled by Myf6 Cre in differentiated skeletal muscle fibers	Low penetrance 1/228 mice heterozygote for conditional fusion allele at 383 days. 40% penetrance at 90 days when fusion allele was homozygous in p53 null and 28% when fusion allele was homozygous in ARF null backgrounds.	Keller et al., 2004b
Multifocal RMS	Gorlin syndrome associated Ptch1$^{+/-}$ background with Tamoxifen inducible Cre mediated SmoM2 (mutant Smoothened causing constitutive Hedgehog activation) expression	100% multifocal rhabdomyosarcomas; tumors arise at 5 weeks following Tamoxifen administration or at 9 weeks due to sporadic SmoM2 expression	Mao et al., 2006
Pleomorphic RMS	Conditional KRasG12V knockin somatic mutation in p53 null or heterozygote null background	100% tumors in p53$^{-/-}$ 10 weeks after Cre electroporation and 40% tumors in p53$^{+/-}$ background at a slower rate	Tsumura et al., 2006
Pleomorphic RMS	KRasG12V expression in a) p53$^{+/fl}$, b)p53$^{fl/fl}$, c) p53R172H/fl , d) p53$^{R172H/+}$ mice controlled by AhCre	a) KRasG12V p53$^{+/fl}$: 6% at 112 days; b) KRasG12V p53$^{fl/fl}$: 94% at 48 days; c) KRasG12V p53$^{R172H/fl}$:100% at 51 days; d) KRasG12V p53$^{R172H/+}$: 88% at 62 days	Doyle et al., 2010

suppressive pathways, which lead to RMS formation and also provided an idea of the nature of the cells giving rise to RMS.

RMS is a pediatric disease that is observed in children below ten years of age. Interestingly, old age related, low penetrance ARMS and ERMS formation was observed in dystrophin and sarcoglycan deficient mice respectively (Chamberlain et al., 2007; Langenau et al., 2007; Fernandez et al., 2010) which model muscular dystrophy. This opens up another alley of investigation to understand the pathways involved in the generation of RMS.

The need for a genetically engineered animal model that perfectly satisfies all criteria for RMS development is far from over and better model systems remain to be established that will fully recapitulate the disease conditions and enable therapeutic testing. In the absence of available genetically manipulated models, mouse xenograft models using human RMS tumors are frequently used to test therapeutic modalities. To this end, the National Cancer Institute has supported a consortium referred to as the Pediatric Preclinical Testing Program, which has established xenografts and cell lines from pediatric cancers, including seven rhabdomyosarcomas (Houghton et al., 2007). These models have been extensively characterized at the molecular level using Affymetrix gene profiling. When comparing the rhabdomyosarcoma xenograft models to clinical samples, the models were shown to have similar copy number alterations and expression profiles indicating their promise for a representative response to therapeutics (Neale et al., 2008). Since its inception, the PPTP has utilized the xenograft animal panel to test numerous drugs for efficacy in treatment of RMS (Houghton et al., 2008, 2010; Kolb et al., 2011).

The last few years have seen remarkable progress in field of RMS study with the development of better diagnostic and prognostic tools. The potential of tailored therapeutics is becoming more apparent as the different molecular mechanisms directing the nature and progress of this disease are being uncovered. Model systems to test the efficacy of various therapies are emerging that can be used to specify treatment options for patients with perturbations in specific molecular pathways or genetic aberrations. Despite all this, the fight against RMS is far from over and extensive collaborative efforts are necessary between clinicians and researchers to bring about an end to rhabdomyosarcoma.

Acknowledgements. This work was supported in part by funds from Alex's Lemonade Stand Foundation, The Sarcoma Foundation of America (SFA), and the National Institutes of Health National Cancer Institute (1R01CA133571) to DSC. We thank Julie Moore and the Cooperative Human Tissue Network (CHTN) for tumor samples and Dr. Sue Hammond for the histology review.

References

Anderson M.J., Shelton G.D., Cavenee W.K. and Arden K.C. (2001). Embryonic expression of the tumor-associated PAX3-FKHR fusion protein interferes with the developmental functions of Pax3. Proc. Natl. Acad. Sci. USA 98, 1589-1594.

Bartel F., Taylor A.C., Taubert H. and Harris L.C. (2001a). Novel mdm2 splice variants identified in pediatric rhabdomyosarcoma tumors and cell lines. Oncol. Res. 12, 451-457.

Bartel F., Meye A., Wurl P., Kappler M., Bache M., Lautenschlager C., Grunbaum U., Schmidt H. and Taubert H. (2001b). Amplification of the MDM2 gene, but not expression of splice variants of MDM2 MRNA, is associated with prognosis in soft tissue sarcoma. Int. J. Cancer 95, 168-175.

Bartel F., Schulz J., Bohnke A., Blumke K., Kappler M., Bache M., Schmidt H., Wurl P., Taubert H. and Hauptmann S. (2005). Significance of HDMX-S (or MDM4) mRNA splice variant overexpression and HDMX gene amplification on primary soft tissue sarcoma prognosis. Int. J. Cancer 117, 469-475.

Bennicelli J.L., Fredericks W.J., Wilson R.B., Rauscher F.J. 3rd and Barr F.G. (1995). Wild type PAX3 protein and the PAX3-FKHR fusion protein of alveolar rhabdomyosarcoma contain potent, structurally distinct transcriptional activation domains. Oncogene 11, 119-130.

Breneman J.C., Lyden E., Pappo A.S., Link M.P., Anderson J.R., Parham D.M., Qualman S.J., Wharam M.D., Donaldson S.S., Maurer H.M., Meyer W.H., Baker K.S., Paidas C.N. and Crist W.M. (2003). Prognostic factors and clinical outcomes in children and adolescents with metastatic rhabdomyosarcoma--a report from the Intergroup Rhabdomyosarcoma Study IV. J. Clin. Oncol. 21, 78-84.

Bridge J.A., Liu J., Qualman S.J., Suijkerbuijk R., Wenger G., Zhang J., Wan X., Baker K.S., Sorensen P. and Barr F.G. (2002). Genomic gains and losses are similar in genetic and histologic subsets of rhabdomyosarcoma, whereas amplification predominates in embryonal with anaplasia and alveolar subtypes. Genes Chromosomes Cancer 33, 310-321.

Cam H., Griesmann H., Beitzinger M., Hofmann L., Beinoraviciute-Kellner R., Sauer M., Huttinger-Kirchhof N., Oswald C., Friedl P., Gattenlohner S., Burek C., Rosenwald A. and Stiewe T. (2006). p53 family members in myogenic differentiation and rhabdomyosarcoma development. Cancer Cell 10, 281-293.

Chamberlain J.S., Metzger J., Reyes M., Townsend D. and Faulkner J.A. (2007). Dystrophin-deficient mdx mice display a reduced life span and are susceptible to spontaneous rhabdomyosarcoma. FASEB J. 21, 2195-2204.

Chandler D.S., Singh R.K., Caldwell L.C., Bitler J.L. and Lozano G. (2006). Genotoxic stress induces coordinately regulated alternative splicing of the p53 modulators MDM2 and MDM4. Cancer Res. 66, 9502-9508.

Daston G., Lamar E., Olivier M. and Goulding M. (1996). Pax-3 is necessary for migration but not differentiation of limb muscle precursors in the mouse. Development 122, 1017-1027.

Dias P., Kumar P., Marsden H.B., Gattamaneni H.R., Heighway J. and Kumar S. (1990). N-myc gene is amplified in alveolar rhabdomyosarcomas (RMS) but not in embryonal RMS. Int. J. Cancer 45, 593-596.

Diller L., Sexsmith E., Gottlieb A., Li F.P. and Malkin D. (1995). Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma. J. Clin. Invest. 95, 1606-1611.

Doyle B., Morton J.P., Delaney D.W., Ridgway R.A., Wilkins J.A. and Sansom O.J. (2010). p53 mutation and loss have different effects on tumourigenesis in a novel mouse model of pleomorphic rhabdomyosarcoma. J. Pathol. 222, 129-137.

Driman D., Thorner P.S., Greenberg M.L., Chilton-MacNeill S. and Squire J. (1994). Mycn gene amplification in rhabdomyosarcoma. Cancer 73, 2231-2237.

Durbin A.D., Somers G.R., Forrester M., Pienkowska M., Hannigan G.E. and Malkin D. (2009). JNK1 determines the oncogenic or tumor-suppressive activity of the integrin-linked kinase in human rhabdomyosarcoma. J. Clin. Invest. 119, 1558-1570.

Evans S.C., Viswanathan M., Grier J.D., Narayana M., El-Naggar A.K. and Lozano G. (2001). An alternatively spliced HDM2 product increases p53 activity by inhibiting HDM2. Oncogene 20, 4041-4049.

Felix C.A., Kappel C.C., Mitsudomi T., Nau M.M., Tsokos M., Crouch G.D., Nisen P.D., Winick N.J. and Helman L.J. (1992). Frequency and diversity of p53 mutations in childhood rhabdomyosarcoma. Cancer Res. 52, 2243-2247.

Fernandez K., Serinagaoglu Y., Hammond S., Martin L.T. and Martin P.T. (2010). Mice lacking dystrophin or alpha sarcoglycan spontaneously develop embryonal rhabdomyosarcoma with cancer-associated p53 mutations and alternatively spliced or mutant Mdm2 transcripts. Am. J. Pathol. 176, 416-434.

Fleischmann A., Jochum W., Eferl R., Witowsky J. and Wagner E.F. (2003). Rhabdomyosarcoma development in mice lacking Trp53 and Fos: Tumor suppression by the Fos protooncogene. Cancer Cell 4, 477-482.

Gaiger A.M., Soule E.H. and Newton W.A. Jr. (1981). Pathology of rhabdomyosarcoma: Experience of the Intergroup Rhabdomyosarcoma Study, 1972-78. Natl. Cancer. Inst. Monogr. 19-27.

Galindo R.L., Allport J.A. and Olson E.N. (2006). A Drosophila model of the rhabdomyosarcoma initiator PAX7-FKHR. Proc. Natl. Acad. Sci. USA 103, 13439-13444.

Giglio S., Mancini F., Gentiletti F., Sparaco G., Felicioni L., Barassi F., Martella C., Prodosmo A., Iacovelli S., Buttitta F., Farsetti A., Soddu S., Marchetti A., Sacchi A., Pontecorvi A. and Moretti F. (2005). Identification of an aberrantly spliced form of HDMX in human tumors: A new mechanism for HDM2 stabilization. Cancer Res. 65, 9687-9694.

Hachitanda Y., Toyoshima S., Akazawa K. and Tsuneyoshi M. (1998). N-myc gene amplification in rhabdomyosarcoma detected by fluorescence *in situ* hybridization: Its correlation with histologic features. Mod. Pathol. 11, 1222-1227.

Houghton P.J., Morton C.L., Tucker C., Payne D., Favours E., Cole C., Gorlick R., Kolb E.A., Zhang W., Lock R., Carol H., Tajbakhsh M., Reynolds C.P., Maris J.M., Courtright J., Keir S.T., Friedman H.S., Stopford C., Zeidner J., Wu J., Liu T., Billups C.A., Khan J., Ansher S., Zhang J. and Smith M.A. (2007). The pediatric preclinical testing program: Description of models and early testing results. Pediatr. Blood Cancer 49, 928-940.

Houghton P.J., Morton C.L., Kolb E.A., Gorlick R., Lock R., Carol H., Reynolds C.P., Maris J.M., Keir S.T., Billups C.A. and Smith M.A. (2008). Initial testing (stage 1) of the mTOR inhibitor rapamycin by the pediatric preclinical testing program. Pediatr. Blood Cancer 50, 799-805.

Houghton P.J., Morton C.L., Gorlick R., Kolb E.A., Keir S.T., Reynolds C.P., Kang M.H., Maris J.M., Wu J. and Smith M.A. (2010). Initial testing of a monoclonal antibody (IMC-A12) against IGF-1R by the Pediatric Preclinical Testing Program. Pediatr. Blood Cancer 54, 921-926.

Iolascon A., Faienza M.F., Coppola B., Rosolen A., Basso G., Della Ragione F. and Schettini F. (1996). Analysis of cyclin-dependent kinase inhibitor genes (CDKN2A, CDKN2B, and CDKN2C) in childhood rhabdomyosarcoma. Genes Chromosomes Cancer 15, 217-222.

Keleti J., Quezado M.M., Abaza M.M., Raffeld M. and Tsokos M. (1996). The MDM2 oncoprotein is overexpressed in rhabdomyosarcoma cell lines and stabilizes wild-type p53 protein. Am. J. Pathol. 149, 143-151.

Keller C., Hansen M.S., Coffin C.M. and Capecchi M.R. (2004a). Pax3:Fkhr interferes with embryonic Pax3 and Pax7 function: implications for alveolar rhabdomyosarcoma cell of origin. Genes Dev. 18, 2608-2613.

Keller C., Arenkiel B.R., Coffin C.M., El-Bardeesy N., DePinho R.A. and Capecchi M.R. (2004b). Alveolar rhabdomyosarcomas in conditional Pax3:Fkhr mice: Cooperativity of Ink4a/ARF and Trp53 loss of function. Genes Dev. 18, 2614-2626.

Kelly K.M., Womer R.B., Sorensen P.H., Xiong Q.B. and Barr F.G. (1997). Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. J. Clin. Oncol. 15, 1831-1836.

Khatib Z.A., Matsushime H., Valentine M., Shapiro D.N., Sherr C.J. and Look A.T. (1993). Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. Cancer Res. 53, 5535-5541.

Kobbert C., Mollmann C., Schafers M., Hermann S., Baba H.A., Hoffmeier A., Breithardt G., Scheld H.H., Weissen-Plenz G. and Sindermann J.R. (2008). Transgenic model of cardiac rhabdomyosarcoma formation. J. Thorac. Cardiovasc. Surg. 136, 1178-1186.

Kolb E.A., Gorlick R., Lock R., Carol H., Morton C.L., Keir S.T., Reynolds C.P., Kang M.H., Maris J.M., Billups C., Smith M.A. and Houghton P.J. (2011). Initial testing (stage 1) of the IGF-1 receptor inhibitor BMS-754807 by the pediatric preclinical testing program. Pediatr. Blood Cancer 56, 595-603.

Lagutina I., Conway S.J., Sublett J. and Grosveld G.C. (2002). Pax3-FKHR knock-in mice show developmental aberrations but do not develop tumors. Mol. Cell. Biol. 22, 7204-7216.

Langenau D.M., Keefe M.D., Storer N.Y., Guyon J.R., Kutok J.L., Le X., Goessling W., Neuberg D.S., Kunkel L.M. and Zon L.I. (2007). Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev. 21, 1382-1395.

Leuschner I., Langhans I., Schmitz R., Harms D., Mattke A. and Treuner J. (2003). p53 and mdm-2 expression in rhabdomyosarcoma of childhood and adolescence: Clinicopathologic study by the Kiel Pediatric Tumor Registry and the German Cooperative Soft Tissue Sarcoma Study. Pediatr. Dev. Pathol. 6, 128-136.

Linares L.K., Hengstermann A., Ciechanover A., Muller S. and Scheffner M. (2003). Hdmx stimulates Hdm2-mediated ubiquitination and degradation of p53. Proc. Natl. Acad. Sci. USA 100, 12009-12014.

Makawita S., Ho M., Durbin A.D., Thorner P.S., Malkin D. and Somers G.R. (2009). Expression of insulin-like growth factor pathway proteins in rhabdomyosarcoma: IGF-2 expression is associated with translocation-negative tumors. Pediatr. Dev. Pathol. 12, 127-135.

Mao J., Ligon K.L., Rakhlin E.Y., Thayer S.P., Bronson R.T., Rowitch D. and McMahon A.P. (2006). A novel somatic mouse model to survey tumorigenic potential applied to the hedgehog pathway. Cancer Res. 66, 10171-10178.

Margue C.M., Bernasconi M., Barr F.G. and Schafer B.W. (2000). Transcriptional modulation of the anti-apoptotic protein BCL-XL by the paired box transcription factors PAX3 and PAX3/FKHR. Oncogene 19, 2921-2929.

Matsumoto T., Kinoshita E., Maeda H., Niikawa N., Kurosaki N., Harada N., Yun K., Sawai T., Aoki S., Kondoh T. and Tsuji Y. (1994). Molecular analysis of a patient with Beckwith-Wiedemann syndrome,

rhabdomyosarcoma and renal cell carcinoma. Jpn. J. Hum. Genet. 39, 225-234.

Matsuoka S., Thompson J.S., Edwards M.C., Bartletta J.M., Grundy P., Kalikin L.M., Harper J.W., Elledge S.J. and Feinberg A.P. (1996). Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor, p57KIP2, on chromosome 11p15. Proc. Natl. Acad. Sci. USA 93, 3026-3030.

Meza J.L., Anderson J., Pappo A.S. and Meyer W.H. (2006). Analysis of prognostic factors in patients with nonmetastatic rhabdomyosarcoma treated on intergroup rhabdomyosarcoma studies III and IV: The Children's Oncology Group. J. Clin. Oncol. 24, 3844-3851.

Minniti C.P., Tsokos M., Newton W.A. Jr. and Helman L.J. (1994). Specific expression of insulin-like growth factor-II in rhabdomyosarcoma tumor cells. Am. J. Clin. Pathol. 101, 198-203.

Nanni P., Nicoletti G., De Giovanni C., Croci S., Astolfi A., Landuzzi L., Di Carlo E., Iezzi M., Musiani P. and Lollini P.L. (2003). Development of rhabdomyosarcoma in HER-2/neu transgenic p53 mutant mice. Cancer Res. 63, 2728-2732.

Neale G., Su X., Morton C.L., Phelps D., Gorlick R., Lock R.B., Reynolds C.P., Maris J.M., Friedman H.S., Dome J., Khoury J., Triche T.J., Seeger R.C., Gilbertson R., Khan J., Smith M.A. and Houghton P.J. (2008). Molecular characterization of the pediatric preclinical testing panel. Clin. Cancer Res. 14, 4572-4583.

Nishijo K., Chen Q.R., Zhang L., McCleish A.T., Rodriguez A., Cho M.J., Prajapati S.I., Gelfond J.A., Chisholm G.B., Michalek J.E., Aronow B.J., Barr F.G., Randall R.L., Ladanyi M., Qualman S.J., Rubin B.P., LeGallo R.D., Wang C., Khan J. and Keller C. (2009). Credentialing a preclinical mouse model of alveolar rhabdomyosarcoma. Cancer Res. 69, 2902-2911.

Prodosmo A., Giglio S., Moretti S., Mancini F., Barbi F., Avenia N., Di Conza G., Schunemann H.J., Pistola L., Ludovini V., Sacchi A., Pontecorvi A., Puxeddu E. and Moretti F. (2008). Analysis of human MDM4 variants in papillary thyroid carcinomas reveals new potential markers of cancer properties. J. Mol. Med. 86, 585-96.

Qualman S.J., Coffin C.M., Newton W.A., Hojo H., Triche T.J., Parham D.M. and Crist W.M. (1998). Intergroup Rhabdomyosarcoma Study: Update for pathologists. Pediatr. Dev. Pathol. 1, 550-561.

Qualman S., Lynch J., Bridge J., Parham D., Teot L., Meyer W. and Pappo A. (2008). Prevalence and clinical impact of anaplasia in childhood rhabdomyosarcoma : A report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group. Cancer 113, 3242-3247.

Ragazzini P., Gamberi G., Pazzaglia L., Serra M., Magagnoli G., Ponticelli F., Ferrari C., Ghinelli C., Alberghini M., Bertoni F., Picci P. and Benassi M.S. (2004). Amplification of CDK4, MDM2, SAS and GLI genes in leiomyosarcoma, alveolar and embryonal rhabdomyosarcoma. Histol. Histopathol. 19, 401-411.

Relaix F., Polimeni M., Rocancourt D., Ponzetto C., Schafer B.W. and Buckingham M. (2003). The transcriptional activator PAX3-FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Met signaling in vivo. Genes Dev. 17, 2950-2965.

Ruymann F.B. and Grovas A.C. (2000). Progress in the diagnosis and treatment of rhabdomyosarcoma and related soft tissue sarcomas. Cancer Invest. 18, 223-241.

Sharp R., Recio J.A., Jhappan C., Otsuka T., Liu S., Yu Y., Liu W., Anver M., Navid F., Helman L.J., DePinho R.A. and Merlino G. (2002). Synergism between INK4a/ARF inactivation and aberrant HGF/SF signaling in rhabdomyosarcomagenesis. Nat. Med. 8, 1276-1280.

Smith A.C., Squire J.A., Thorner P., Zielenska M., Shuman C., Grant R., Chitayat D., Nishikawa J.L. and Weksberg R. (2001). Association of alveolar rhabdomyosarcoma with the Beckwith-Wiedemann syndrome. Pediatr. Dev. Pathol. 4, 550-558.

Sorensen P.H., Lynch J.C., Qualman S.J., Tirabosco R., Lim J.F., Maurer H.M., Bridge J.A., Crist W.M., Triche T.J. and Barr F.G. (2002). PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: A report from the children's oncology group. J. Clin. Oncol. 20, 2672-2679.

Stad R., Little N.A., Xirodimas D.P., Frenk R., van der Eb A.J., Lane D.P., Saville M.K. and Jochemsen A.G. (2001). Mdmx stabilizes p53 and Mdm2 via two distinct mechanisms. EMBO Rep. 2, 1029-1034.

Steinman H.A., Burstein E., Lengner C., Gosselin J., Pihan G., Duckett C.S. and Jones S.N. (2004). An alternative splice form of Mdm2 induces p53-independent cell growth and tumorigenesis. J. Biol. Chem. 279, 4877-4886.

Stratton M.R., Fisher C., Gusterson B.A. and Cooper C.S. (1989). Detection of point mutations in N-ras and K-ras genes of human embryonal rhabdomyosarcomas using oligonucleotide probes and the polymerase chain reaction. Cancer Res. 49, 6324-6327.

Sung L., Anderson J.R., Arndt C., Raney R.B., Meyer W.H. and Pappo A.S. (2004). Neurofibromatosis in children with Rhabdomyosarcoma: A report from the Intergroup Rhabdomyosarcoma study IV. J. Pediatr. 144, 666-668.

Takahashi Y., Oda Y., Kawaguchi K., Tamiya S., Yamamoto H., Suita S. and Tsuneyoshi M. (2004). Altered expression and molecular abnormalities of cell-cycle-regulatory proteins in rhabdomyosarcoma. Mod. Pathol. 17, 660-669.

Taylor A.C., Shu L., Danks M.K., Poquette C.A., Shetty S., Thayer M.J., Houghton P.J. and Harris L.C. (2000). P53 mutation and MDM2 amplification frequency in pediatric rhabdomyosarcoma tumors and cell lines. Med. Pediatr. Oncol. 35, 96-103.

Tsumura H., Yoshida T., Saito H., Imanaka-Yoshida K. and Suzuki N. (2006). Cooperation of oncogenic k-ras and p53 deficiency in pleomorphic rhabdomyosarcoma development in adult mice. Oncogene 25, 7673-7679.

Williamson D., Lu Y.J., Gordon T., Sciot R., Kelsey A., Fisher C., Poremba C., Anderson J., Pritchard-Jones K. and Shipley J. (2005). Relationship between MYCN copy number and expression in rhabdomyosarcomas and correlation with adverse prognosis in the alveolar subtype. J. Clin. Oncol. 23, 880-888.

Williamson D., Selfe J., Gordon T., Lu Y.J., Pritchard-Jones K., Murai K., Jones P., Workman P. and Shipley J. (2007). Role for amplification and expression of glypican-5 in rhabdomyosarcoma. Cancer Res. 67, 57-65.

Xia S.J., Pressey J.G. and Barr F.G. (2002). Molecular pathogenesis of rhabdomyosarcoma. Cancer Biol. Ther. 1, 97-104.

Yoo J. and Robinson R.A. (1999). H-ras and K-ras mutations in soft tissue sarcoma: Comparative studies of sarcomas from Korean and American patients. Cancer 86, 58-63.

Accepted July 15, 2011