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Review

DNA repair systems in rhabdomyosarcoma

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Summary. Rhabdomyosarcoma (RMS) represents the most common soft tissue sarcoma in children and adolescent population. There are two major histological subtypes, embryonal (ERMS) and alveolar (ARMS), differing in cytogenetic and morphological features. RMS pathogenesis remains controversial and several cellular mechanisms and pathways have been implicated. Application of intense chemo- and radiotherapy improves survival rates for RMS patients, but significant efficacy has not been proved as DNA damage induced-resistance frequently occurs. The present review is aimed at summarizing the current evidence on DNA repair systems, implications in RMS development, focusing on gene expression alterations and point mutations of genes encoding for DNA repair enzymes. Understanding of DNA repair systems involvement in RMS pathogenesis could diversify RMS patients and provide novel individualized therapeutic targets.

Key words: Rhabdomyosarcoma, DNA repair, Drug resistance

Introduction

Rhabdomyosarcoma (RMS) is one of the most common soft tissue tumors in children and adolescent population, representing 4% of all childhood malignancies (Sultan et al., 2009, Wolden and Alektiar, 2010). The most favorable RMS primary site is head and neck (30%), extremity (20%) and genitourinary (15%) (Slater and Shipley, 2007). Histopathologically, RMS can be divided into two major subtypes: embryonal (ERMS) and alveolar rhabdomyosarcoma (ARMS). Botryoid (BRMS) and pleomorphic rhabdomyosarcoma (PMS) are typically observed in adults, being usually correlated with poor prognosis (Arndt and Crist, 1999; Hayes-Jordan and Andrassy, 2009). ERMS comprises 50-60% of all RMS cases and typically manifests a

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Abbreviations. All-trans-retinoic acid (ATRA), alveolar rhabdomyosarcoma (ARMS), apurinic/apyrimidinic endonuclease (APE), apurinic/apyrimidinic site (AP), ataxia telengiectasia mutated (ATM), ataxia telengiectasia related (ATR), base excision repair (BER), botryoid rhabdomyosarcoma (BRMS), breast cancer (BRCA), café-au-lait spots (CLS), chloroethylnitrosourea (CENU), chloroethylnitrosoureas (MeCCNU), comparative genomic hybridization analysis (CGH), constitutional mismatch-repair-deficiency syndrome (CMMR-D), DNA dependent protein kinase catalytic subunit (DNA-PKcs), double-strand break (DSB), embryonal rhabdomyosarcoma (ERMS), flap endonuclease 1 (FEN1), forkhead transcription factor (FKHR/FOXO1), fractional allelic loss (FAL), global genome nucleotide excision repair (GG-NER), heterodublex DNA (hDNA), histone deacetylase inhibitors (HDACIs), homologous recombination (HR) human MutL homolog (MLH), human MutS homolog (MSH), insulin-like growth factor 2 (IGF2), International Agency for Research on Cancer (IARC), ionizing radiation (IR), Li-Fraumeni syndrome (LFS), long patch BER (LP-BER), loss of heterozygosity (LOH), microhomology-mediated end-joining (MMEJ), microsatellite instability (MIN), mismatch repair (MMR), mono-3-alkyladenine (A-M-OH), mono-7-alkylguanine (G-M-OH), neurofibromatosis type 1 (NF1), Nijmegen breakage syndrome (NBS), non homologous end-joining (NHEJ), nuclear receptor coactivator (NCOA1), nucleotide excision repair (NER), O^6-Alkylguanine DNA alkyltransferase (AGT), O^6-benzylguanine (O^6-BG), O^6-methylguanine (O^6-MeG), O^6-methylguanine methyltransferase (O^6-MGMT), patched (PTCH1), pleomorphic rhabdomyosarcoma (PMS), poly(ADP-ribose) polymerase (PARP), proliferating cell nuclear antigen (PCNA), reactive oxygen species (ROS), recombination repair (RR) replication error repair (RER), replication factor A (RPA), rhabdomyosarcoma (RMS), short patch BER (SP-BER), single-strand break (SSB), smoothened (Smo), sonic hedgehog (Shh), Surveillance, Epidemiology, and End Results Program (SEER), transcription coupled nucleotide excision repair (TC-NER), transcription factor IIH (TFIIH), tumor initiating cells (TICs), ultraviolet light (UV), xeroderma pigmentosum (XP), XPA-binding protein 2 (XAB2), X-ray cross complementing (XRCC).

favorable outcome, while 20-30% of RMS is the more aggressive ARMS subtype that is associated with frequent metastasis at the time of initial diagnosis (Stevens, 2005).

RMS patients are classified into risk stratification schemes (high, intermediate and low risk), based on histological type, favorable versus unfavorable primary site, tumor size, metastatic disease and the extent of surgical resection (Meza et al., 2006). Standard therapy includes surgery, systemic chemotherapy with different drugs depending on the risk stratification and ionizing radiation. Multimodality therapy results in five-year survival rates of less than 30%, 70% and 90% for the high, intermediate and low risk groups, respectively (Christ et al., 2001; Oberlin et al., 2008; Arndt et al., 2009).

RMS initiating cell remains controversial. Its presence in anatomical sites where muscle or diffuse bone marrow do not normally exist, supports its provenance from mesenchymal progenitor or stem cells with the capacity to differentiate into skeletal muscles. However, RMS frequently arises directly within skeletal muscles, indicating a direct skeletal muscle emanation (Saab et al., 2011).

Extensive cytogenetic studies displayed that ERMS is generally associated with loss of heterozygosity (LOH) for multiple closely linked loci at chromosomal 11p15.5 (Barr et al., 1995; Bridge et al., 2000). LOH at this site leads to loss of imprinting at this genomic locus with secondary inactivation of one or more tumor-suppressor gene(s) and/or activation of oncogenes (Scrable et al., 1989). Either mutations and/or amplifications of oncogenes and tumor suppressor genes (including p53, N-ras, N-myc, FGFR4 etc.) have frequently been observed, although their role in this specific tumor pathogenesis remains largely unclear (Huh and Skapek, 2010). Located in 11p15.5 chromosome genes $p57^{Kip2}$ and insulin-like growth factor 2 (IGF2) are of great interest, as they are associated with normal developmental program arrest, cell proliferation and enhanced muscle gene expression, respectively (De Giovanni et al., 2009; Saab et al., 2011).

Eighty percent (80%) of ARMS display a characteristic chromosomal translocation resulting in fusion of the transactivation domain of the forkhead transcription factor (FKHR/FOXO1), located on the long arm of chromosome 13 and homeo DNA binding domain of PAX3, or less commonly PAX7 transcription factors, located on the long arm of chromosome 2 [t(2; 13) (q35; q14)] and 1 [t(1; 13)(p36; q14)], respectively (Bennicelli et al., 1995). A fusion gene with PAX3 and the nuclear receptor coactivator (NCOA1) has recently been reported, but the prognostic value for this rare variant remains unclear (Sumegi et al., 2010).

Fusion genes yield much more potent transcription factor than any protein (Fredericks et al., 1995). Fusion genes can by themselves be overexpressed by distinct mechanisms, as PAX3-FKHR overexpression results from increased gene copy number, while PAX7-FKHR overexpression is due to fusion gene amplification (Charytonowicz et al., 2009). However, the exact mechanisms through which chimeric fusion proteins contribute to pathogenesis of RMS still remain unclear.

Chromosomal rearrangements, as well as base substitutions, insertions or deletions of small or large DNA segments, gene amplification and epigenetic silencing are frequent findings of the cancer genome. DNA repair is critical for genome stability and integrity maintenance by intercepting the aforementioned genetic damage caused by exogenous or endogenous mechanisms (Jackson and Bartek, 2009; Stratton et al., 2009).

Normal cells' genome is in a dynamic equilibrium, representing a balance between processes that generate mutations and maintain the normal nucleotide sequence. Thus, defects in DNA repair contribute to a "mutator phenotype" and promote carcinogenesis, while adequate DNA repair, especially in tumor initiating cells (TICs) results in chemo- and radio-resistance (Loeb et al., 2003; Helleday et al., 2008; Mathews et al., 2011). These controversial aspects could be combined according to "synthetic lethality", by impairment of different components of the DNA repair system in order to accomplish genome instability and cancer cells death (Farmer et al., 2005; Martin et al., 2008).

In view of the above consideration, the purpose of this review is to summarize the data available so far on the implications of the DNA repair system to rhabdomyosarcomatogenesis with possible treatment options for RMS patients.

DNA repair systems

In mammals, DNA lesions are repaired either directly without affecting DNA structure or indirectly by DNA phosphodiester backbone cleavage. Direct repair includes repair during replication, as well as enzymatic repair. Indirect repair comprises excision repair system [base excision repair (BER) and nucleotide excision repair (NER)], mismatch repair (MMR) and recombination repair (RR) [Homologous recombination (HR), Non-homologous end-joining (NHEJ) and Microhomology-mediated end-joining (MMEJ)] (Lindahl and Wood, 1999; Hoeijmakers, 2001; Gatzidou et al., 2010). Inherited or acquired deficiencies in any system may be involved in the onset of tumorigenesis. The DNA repair systems and their basic categories are depicted in Fig. 1.

Direct repair

Direct repair mechanisms

Direct enzymatic DNA repair represents the simplest repair system in which damaged bases are enzymatically reversed without affecting DNA structure (Hoeijmakers, 2001). O^6-methylguanine-DNA methyltransferase

(MGMT), also known as O^6-Alkylguanine DNA alkyltransferase (AGT), is a direct repair enzyme which removes alkyl adducts caused by endogenous and environmental alkylating agents from the O^6-position of guanine (Pegg, 2001). During replication, O^6-methylguanine (O^6-MeG) mispairs with thymine (T), resulting in G:C to A:T transitions. O^6-alkylguanine DNA adducts crosslinks with the opposite cytosine residues, blocking DNA replication (Esteller and Herman, 2004). MGMT repairs these lesions in a one-step irreversible reaction, occurring in its active centre by the transfer of the methyl or chloroethyl group from the O^6 position of guanine to a cysteine residue (Cys145). Alkylated-inactivated MGMT then detaches from DNA in order to be degraded by ubiquitination (Srivenugopal et al., 1996). The stoichiometry of the repair reaction indicates that cells' repair capacity is directly related to MGMT molecule number (Esteller and Herman, 2004; Kaina et al., 2010). During DNA replication O^6-MeG mispairs are repaired by the MMR system (Kaina et al., 2010).

MGMT expression has been shown to depend on the methylation status of the MGMT promoter (Esteller and Herman, 1999). Hypermethylation of MGMT CpG islands is associated with transcription loss and decreased protein expression (Harris et al., 1991). Moreover, in cell lines it has been correlated with a lack of MGMT activity and thus defective DNA repair (Harris et al., 1991).

Methylating (streptozotocine, procarbazine, dacarbacine, temozolomide) and chloroethylating (ACNU, BCNU, CCNU, MeCCNU, HeCCNU, estramustine, fotenustine) agents are widely used as chemotherapeutics. These agents exert their effects by reacting with nucleophilic sites on DNA, one of the most important of which is the O^6 position of guanine. MGMT repairs these lesions and reverses the cytotoxic activity of the methylating and chloroethylating agents, reducing their antitumor effects, which may lead to resistance to cancer therapy. In order to increase response to chemotherapeutics, MGMT inhibitors have been developed. In this context, O^6-benzylguanine (O^6-BG) has been recently designed to inactivate MGMT by acting as an alternate substrate (Kaina et al., 2010).

Direct base repair and rhabdomyosarcoma

For pediatric RMS patients, chemotherapy with alkylating agents represents the major treatment option, although significant efficacy has not been proved (Chang et al., 1976; Creagan et al., 1976). Several studies indicate that chemotherapy resistance could be mediated by MGMT.

MGMT activity was measured in cell lines (from primary tumors Rh12, Rh18 and Rh39, bone marrow Rh30 and Rh35 and metastatic mass Rh28) established as xenografts, after administration of the antineoplastic agent chloroethylnitrosoureas (MeCCNU). Rh28 RMS xenografts showed high sensitivity, with 84% (21 tumors out of 25) complete tumor regression and 16% (4 tumors out of 25) with >50% regression. Rh30, Rh35, and Rh39 tumor lines had a high proportion of partial responses, but no regression, while Rh35 xenografts showed rapid tumor regrowth. Rh12 and Rh18 tumors were refractory to MeCCNU, manifesting only slight growth inhibition and possessed the highest MGMT activity. Rh30, Rh35, and Rh39 with intermediate responses, had intermediate levels of MGMT activity, while the most sensitive metastatic Rh28 lacked MGMT activity. Thus, MGMT activity was considered as a prognostic marker in discriminating cases with drug-sensitive and resistant tumors and sequential patients' response to MeCCNU (Brent et al., 1985). In two cell lines derived from these xenografts (MeCCNU resistance Rh18 and the MeCCNU sensitive Rh28), DNA interstrand cross-link formation and chloroethylnitrosourea (CENU) sensitivity were also examined. Cultured cells retained the same responsiveness and MGMT activity pattern that characterized the parental xenograft lines. More DNA interstrand cross-links were detected in Rh28 cells than in the Rh18; however, a relatively large number of DNA single-strand breaks in Rh18 cells compared to Rh28 was noted. Thus, the amount of MGMT activity within the tumor cells was inversely correlated with CENU-induced cytotoxicity and DNA-interstrand cross-link formation. Paradoxically, a large number of DNA single-

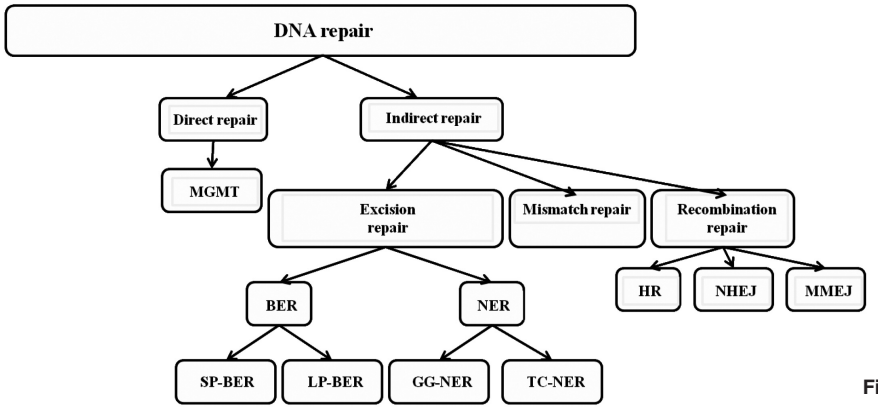

Fig. 1. DNA repair systems

strand breaks was noted in resistant cells and was ascribed to cellular differences of intermediates in excision repair or to high doses of CENUs (Smith and Brent, 1989). The RMS xenograft (Rh30, Rh30c, Rh28, Rh28c, Rh18, Rh66 and Rh12) sensitivity to the methylating agent temozolomide *in vivo* was also correlated with MGMT activity. Tumors displaying high (Rh30, Rh30c) or intermediate (Rh28) sensitivity to temozolomide had undetectable MGMT and detectable MMR protein levels. Low sensitive tumors fall into two groups: those with high MGMT and detectable expression levels of MMR proteins (Rh18, Rh66, Rh12) and those with undetectable MGMT and MMR (Rh28c) levels. According to this study, MGMT proved the major mechanism of resistance towards temozolomide. Thus, MGMT activity was considered to predict RMS response in methylating agents, although methylation adducts other than O^6-MeG exist and other cellular resistance mechanisms or extracellular factors may interfere with the course of therapy (Middlemas et al., 2000). The RMS mechanism of resistance diversified when temozolomide was combined with irinotecan, [CPT-11, a camptothecin prodrug activated by carboxylesterases to the active topoisomeraseI poison SN-38] in RMS xenografts with proficient MGMT and MMR (Rh12), proficient MGMT and deficient MMR (Rh18) and vice versa (Rh30). The combination of the two drugs induced superior tumor responses than either agent alone, against tumors that were either MGMT proficient or MMR deficient and irrespective of either p53 genotype or functional status. These data suggested that the interaction between CPT-11 and temozolomide might be, in part, independent of O^6-MeG. In addition to O^6-guanine, temozolomide and alkylating agents react with N^7-guanine or N^3-adenine. Such modifications may facilitate topoisomeraseI recruitment to DNA and increase drug effectiveness (Houghton et al., 2000). This notion was further supported by another study, in which RD RMS cells were treated with melphalan, whose main sites of reaction are N^7-guanine and N^3-adenine. The principal products were the alkylated purines mono-7-alkylguanine (G-M-OH), mono-3-alkyladenine (A-M-OH) and the cross-linked products G-M-G and A-M-G. The adenine adduct A-M-OH was lost faster than the major melphalan-guanine adduct G-M-OH, but it was not elucidated whether this was due to cell growth or enzyme-mediated removal process (Osborne et al., 1995). High MGMT activity was also verified in human tumor samples (29 of primary origin and 1 metastatic) with no significant difference between ARMS and ERMS. Twenty-one out of 25 (84%) RMS samples demonstrated high MGMT levels (10 out of 10 ERMS and 11 out of 15 ARMS), while 4 ARMS samples displayed moderate levels. High MGMT activity was correlated with unfavorable tumor anatomic location without reaching statistical significance. Consistent with the high MGMT activity, MGMT promoter hypermethylation was absent in RMS samples. Only in one ARMS case was MGMT hypermethylated, but MGMT activity was not obtained for this sample (Yeager et al., 2003). Lack of MGMT promoter hypermethylation in RMS primary tumors and cell lines was also reported by Harada et al. In their study, MGMT promoter hypermethylation was observed only in one out of 18 primary tumors (6%) and in one (SJRH30) out of 5 cell lines (20%) (Harada et al., 2002). MGMT mediated resistance due to increased expression and activity levels could be overcome by combining chloroethylating or methylating agents with O^6-BG. However, a detailed *in vitro* mutagenesis study of a large domain of the conservative binding pocket for the substrate base of MGMT, which contained the residues from 150 to 173, revealed numerous amino acid substitutions, which affected the ability of human MGMT to accept O^6-BG at the active site. These mutations did not impair the ability of MGMT to repair alkylated DNA and predicted that the O^6-BG-resistant MGMT mutants may be a significant setback for the clinical development of this drug. In fact, a glycine (GGC) to cysteine (TGC) substitution at codon 156 and a lysine (AAG) to threonine (ACG) substitution at codon 165 caused the most abundant increase in O^6-BG resistance (Xu-Welliver and Pegg, 2000).

A subline of the human RMS-derived continuous cell line RD resistance to BCNU (TE-671 BR) displayed high MGMT activity compared to the parental cell line and both were totally depleted when exposed to O^6-BG. TE-671 resistance to BCNU plus O^6-BG (TE-671 OBR) demonstrated enhanced survival and measurable MGMT activity, even after treatment with extremely high O^6-BG concentrations. This constant MGMT activity was ascribed to a glycine (GGC) to cysteine (TGC) mutation at codon 156 (Bacolod et al., 2002). Genomic DNA sequence revealed lack of heterozygosity in MGMT mutations in TE-671 OBR cells. These data confirmed the presence of a single MGMT allele and explained the observed lack of MGMT promoter hypermethylation (Bacolod et al., 2004). In contrast, TE-671 xenograft resistance to melphalan (TE-671 MR) showed additional resistance to chlorambucil, cyclophosphamide, ifosfamide, Thio-TEPA, cisplatin, and mitomycin. BCNU, which was inactive against TE-671, was similarly inactive against TE-671 MR. TE-671 MR was cross-resistant to bleomycin, retained sensitivity to actinomycin D, and demonstrated collateral sensitivity to VP-16. The observed resistance to these drugs was considered not to be mediated by MGMT, as TE-671 MR and the parental line had virtually identical increased enzyme levels (Lilley et al., 1991).

MGMT differential expression between normal and cancer cells, as well as its subcellular localization was suggested to be used as a prognostic marker of response to therapy, being obtained by immunohistochemical analysis. In this aspect, in situ immunohistochemistry in pediatric RMS xenografts using an anti-human specific antibody showed specificity and sensitivity, as two

MGMT-deficient xenograft tumor lines were negative, whereas three MGMT-expressing lines exhibited nuclear staining. Thus, in situ discrimination of drug-sensitive MGMT-deficient and drug-resistant MGMT-proficient tumors was considered to predict response to therapy (Brent et al., 1993).

Indirect repair

Excision repair systems

Base excision repair (BER)

BER represents the primary DNA repair pathway for lesions emerging from cellular metabolism, including methylation, alkylation, oxidation, deamination and hydroxylation. These lesions evoke base alterations without distorting DNA helix. BER also repairs DNA SSB (Lindah, 2000; Krokan et al., 2000; Hoeijmakers, 2001; Hegde et al., 2008). The initiating event is damage recognition by a specific DNA glycosylase, which catalyses the excision of an N-glycosidic bond, releasing the damaged base and creating an apurimidinic/apurinic (AP) site. The DNA backbone is cleaved by either an AP endonuclease that creates a single-stranded DNA nick 5' to the AP site, via a hydrolytic reaction, or an AP lyase - an activity present in some glycosylases, which creates a nick 3' to the AP site via ß or ß/δ elimination (Memisoglu and Samson, 2000; Robertson et al., 2009). BER may proceed by two distinct pathways: the "short patch" (SP-BER) or the long patch (LP-BER) pathway. In SP-BER a single base replacement is performed by DNA polymerase b (Polb), DNA ligase III (Lig III) and X-ray cross-complementing protein 1 (XRCC1), which acts as a scaffold for recruiting. In LP-BER DNA multiple nucleotides (2-8) are synthesized by DNA Polß or DNA Polδ/ε, flap endonuclease 1 (FEN1), proliferating cell nuclear antigen (PCNA) and DNA Lig I. XRCC1 and Poly(ADP-ribose) polymerase (PARP) serve as SSB sensors, leading to BER activation (Lindahl and Wool, 1999; Hoeijmakers, 2001; Hegde et al., 2008).

BER and rhabdomyosarcoma

BER as the major DNA repair system against damage resulting from cellular metabolism can reverse the cytotoxic effects of alkylating agents used as antineoplastics, with subsequent tumor progression and death. BER proteins have been implicated in RMS mechanism of resistance to chemotherapeutic agents. PARP1 activity has been inversely correlated with degree of tumor cell differentiation (Miknyoczki et al., 2003). Several studies have shown the ability of PARP-1 inhibitors to increase the *in vitro* and *in vivo* antitumor effects of radiation or chemotherapy in resistant tumor cells (Calabrese et al., 2004).

The effects of PARP-1 inhibitor CEP-8983 (4-O-methoxy-carbazole and its prodrug, CEP-9722) in sensitizing temozolomide resistant, MMR deficient Rh18 RMS cell line have been evaluated. Cell cycle analysis by flow cytometry demonstrated cell cycle arrest, with 32% cell accumulation in the G2-M phase (compared with control 20% accumulation), within 24h of temozolomide exposure. Incubation of temozolomide with CEP-8983 resulted in an increase in the fraction of RH18 cells accumulated at G2-M at the 24h time point (60%) compared with temozolomide alone, which was maintained up to 72h (53%). Maximal G2-M accumulation was observed at the 40h time point (74%) with the magnitude of cells accumulating at G2-M decreasing at each subsequent time point. CEP-8983 exposure alone had no significant effects on the cell cycle. Apoptosis induction evoked a 10-20 fold increase of RH18 cells undergoing apoptosis, compared with control over a 48h time period. Incubation of CEP-8983 after temozolomide exposure resulted in a further 4 to 5 fold increase in apoptosis, while CEP-8983 alone had minimal effects. Thus, inhibition of PARP activity by CEP-8983 resulted in increased genomic instability and accumulation of damaged cells at the G2-M phase of the cell cycle, for DNA damage repair or apoptosis induction. PARP activation is an ATP-depleting process and cells with DNA damage cannot undergo apoptosis or necrosis due to low ATP levels. Therefore, inhibition of PARP in combination with irreparable or substantial

Table 1. Altered gene expression of proteins of DNA repair systems reported in rhabdomyosarcoma.

DNA repair system	Genes upregulated	Genes downregulated	Reference
Direct repair			
	MGMT		Brent et al. 1985
	MGMT		Smith and Brent 1989
	MGMT		Middlemas et al. 2000
	MGMT*		Yeager et al. 2003
	MGMT		Harada et al. 2002
	MGMT*		Bacolod et al. 2004
	MGMT		Lilley et al. 1991
	MGMT		
	MGMT		Brent et al. 1993
BER			
	PARP-1		Miknyoczki et al. 2007
	APE/ref1		Thomson et al. 2001
NER			
	XAB2		Ohnuma-Ishikawa et al. 2007
MMR			
		MMR proteins (LOH,MIN)	Visser et al. 1996
		MSH2	den Bakker et al. 2003
		PMS1	Kratz et al. 2009
		MSH2/MLH1	Middlemas et al. 2000
Recombination repair			
		Rad51	Hahn et al. 2004
		ATM	Zhang et al. 2003
	p53/p63/p73		Cam et al. 2006
	Rad51/Ku80		Blattmann et al. 2010

*: absence of promoter hypermethylation.

DNA damage was considered to conserve cellular energy levels, allowing tumor cells to undergo apoptosis in response to DNA-damaging agents and thereby eliminating local toxic effects or immune responses associated with necrosis (Miknyoczki et al., 2007). Apurinic/apyrimidinic endonuclease APE/ref1 is proposed as the rate limiting protein in BER and also functions as a reduction-oxidation (redox) factor, maintaining certain transcription factors in an active reduced state (Ramana et al., 1998). Thus, it enhances DNA-binding activity of Fos, Jun (AP-1 protein), NFκB, Myb, members of the ATF/CREB family, PAX-8, HIF-1α (hypoxia-inducible factor) and p53 (Thomson et al., 2003). In this context, APE/ref1 was evaluated immunohistochemically by Thomson et al., in RMS samples (15 ERMS and 16 ARMS). Metastatic and nonmetastatic ERMS displayed high nuclear expression of APE/ref1 [88% (7 out of 8) and 85% (6 out of 7) respectively]. In contrast, both metastatic and nonmetastatic ARMS presented low APE/ref1 protein levels [67% (4 out of 6) and 70% (7out of 10), respectively]. High nuclear expression level was observed with no difference between localized and metastatic RMS cases. These results were statistically significant, despite the small sample size of the study, supporting evidence for an association between tumor type and APE/ref1 expression. Low expression levels of APE/ref1 in ARMS were associated with tumor aggressive biologic behavior and the redox activity of APE/ref1. It was proposed that this might be secondary to the fusion transcripts, PAX3/FKHR or PAX7/FKHR, which cause pathological up-regulation of transcriptional activity and APE/ref1 down-regulation through a negative feedback mechanism (Thomson et al., 2001). This hypothesis could explain ARMS chemoresistance in agents such as daunomycin, which undergoes bioactivation via an APE/ref1-dependent mechanism, as low levels of APE/ref1 render these chemotherapies less effective in ARMS treatment (Prieto-Alamo and Laval, 1999).

Nucleotide excision repair (NER)

NER eliminates a wide range of helix-distorting DNA lesions caused by ultraviolet light (UV), irradiation and chemical mutagens (Hoeijmakers, 2001; Liu et al., 2010). NER is divided into two sub-pathways, which differ in damage recognition factors: the transcription independent pathway referred to as global genome repair (GG-NER) and the transcription dependent pathway termed transcription-coupled repair (TCR-NER). GG-NER removes lesions throughout the entire genome, while TCR-NER is responsible for repairing DNA lesions in transcribed strands of expressed genes (Peterson and Cote, 2004). GG-NER is initiated by the damage recognition heterodimer XPC-hHR23b, which in a subset of lesions is facilitated by the XPE complex (DDB-p48 heterodimer). TC-NER is triggered upon blockage of RNA polymeraseII translocation at a DNA damage site (Volker et al., 2001; Liu et al., 2010). The subsequent steps are common at both sub-pathways and are carried out by a set of NER factors. TFIIH transcription factor is recruited and unwinds DNA with 3'-5' and 5'-3' ATP dependent helicase activity of its subunits XPB and XPD, respectively (Hoeijmakers, 2001; Mitchell et al., 2003; Dip et al., 2004; Liu et al., 2010). The XPA-RPA (Replication Factor A) complex verifies the authenticity of the DNA lesions and stabilizes the open bubble structures or DNA helicases. Structure-specific endonucleases, ERCC1-XPF and XPG generate 5' and 3' incisions respectively, leading to the release of a 24-32 oligonucleotide. The single-stranded gap is subsequently filled by DNA polymerase d/e in the presence of PCNA and RPC, and the nick is sealed by DNA ligaseI (Dip et al., 2004, Liu et al., 2010; Wood, 2010).

NER and rhabdomyosarcoma

As mentioned above chemotherapy is often insufficient in RMS patients. Thus, differentiation induced anticancer therapies could represent an alternative therapeutic strategy (Altucci and Gronemeyer, 2001). Retinoids and their metabolites, such as all-trans-retinoic acid (ATRA), which are natural and synthetic derivatives of vitamin A, are frequently used for this purpose (Hong and Sporn, 1997). Despite their effectiveness, clinical resistance is also frequent for these agents (Freemantle et al., 2003). In MM-1-19-P ATRA-sensitive human RMS cell line, overexpression of exogenous XPA-binding protein 2 (XAB2) inhibited ATRA-mediated cellular differentiation. In the presence of XAB2, pharmacologic concentrations of ATRA were required in order to disrupt the repressor complex containing HDAC3 and XAB2. However, ATRA treatment combined with knockdown of XAB2 by siRNA allowed dissociation and recruitment of coactivator complexes, leading to target gene activation and differentiation (Ohnuma-Ishikawa et al., 2007). Treatment of Rh30 (ARMS) and JR-1 (ERMS) with ATRA reduced cell proliferation and promoted muscle differentiation, with Myosin Heavy chain (MyHC) expression. On the contrary, ATRA enhanced Myogenin expression in mouse C2C12 myoblasts, but it did not induce MyHC expression and cell cycle arrest. RMS xenografts bearing minimal residual disease treated with ATRA relapsed and the recurrences expressed muscle late differentiation markers (MyHC). Thus, ATRA was not efficient as a single agent therapy, but as it discerned muscle differentiation without cell cycle withdraw, it was suggested to be combined with agents engaging the terminal differentiation program (Al-Tahan et al., 2012).

Mismatch repair system

MMR maintains genomic stability by repairing mismatched bases and insertion/deletion (ID) loops arising from misincorporation or strand slippage in

repetitive sequences, as well as mismatches in heterodublex DNA (hDNA) created as a result of sequence heterologies during recombination (Evans and Alani, 2000; Hoeijmakers, 2001). Human MMR proteins have been identified based on their homology to *E. coli* MMR proteins. These include the homologues to bacterial MutS MSH2 (human MutS homolog 2), MSH3 and MSH6, and the MutL homologues MLH1, MLH3, PMS1 and PMS2. MutH homologues appear to be absent. MSH proteins form two heterodimeric complexes, hMutSα and hMutSß, with ATPase activity and a crucial role in mismatch recognition and repair initiation (Li, 2008). hMutSα consists of hMSH2 and hMSH6 and preferentially recognizes base-base mismatches and small ID mispairs (1-2 nucleotides). hMutSß is composed of hMSH2 and hMSH3 and identifies bigger ID mispairs (2-16 nucleotides) (Harfe et al., 2000; Hoeijmakers, 2001). MLH1 hetero-dimerizes with PMS2, PMS1 and MLH3 to form the heterodimeric complexes hMutLα, hMutLß and hMutLγ respectively. hMutLα regulates termination of mismatch-provoked excision and possesses a PCNA/RFC-dependent endonuclease activity. hMutLγ is implicated in meiosis, while the hMutLß biological role remains unidentified (Li et al., 2008). After DNA strand discrimination MutLα interacts with the MSH complexes to activate mismatch excision and DNA resynthesis factors, such as RPA, Exo1, RFC, PCNA, HMGB1, polδ and DNA ligase1 (Harfe et al., 2000; Hoeijmakers, 2001). hMutSα and hMutLα have been implicated in a signaling cascade from DNA damage to cell cycle arrest and/or apoptosis, which could partially explain the observed drug resistance in MMR-deficient cells (Li, 2008). Thus, for maximum sensitivity to anticancer therapy, MGMT deficiency and high MMR activity is required (Liu et al., 1996).

MMR and rhabdomyosarcoma

MMR deficiencies have been associated with the mutator phenotype hypothesis. According to this hypothesis, a malignant tumor cell phenotype arises from mutations in genes maintaining normal DNA sequence, with diverse manifestations, such as point mutations, microsatellite instability (MIN) and LOH (Loeb et al., 2003). MIN and LOH represent different mechanisms of tumorigenesis, as MIN evokes defective replication error repair (RER), while LOH is characterized by loss of tumor suppressor genes through fractional allelic loss (FAL). The occurrence of RERs and the relation of RERs with FAL in RMS were analyzed with 57 microsatellite markers, covering all autosomes, in 32 primary RMS (26 EMRS, 6 ARMS). Eight relapses and 3 metastases of 10 primary ERMS and 1 relapse and 6 metastases of 3 primary ARMS were also included in the analysis. ARMS showed remarkably lower FAL than ERMS. 53,8% (14 of 26) ERMS and only 16,7% (1 of 6) ARMS tumors were RER positive. The difference in the mean FAL in the RER-positive and -negative ERMS was statistically significant, as well as the difference in FAL between RER-positive and -negative in RMS. No significant difference between LOH pattern in RER-positive and -negative tumors was found in chromosomal position of genes of the MMR system. Only 22 of the 57 microsatellites tested demonstrated instability. Some loci (CFTR, D12S62, D14S51, CYP19, D9S66) were frequently affected, supporting evidence that in RMS only a subset of microsatellite repeat loci is affected and is therefore susceptible to RERs, whereas others are not. These locus-specific RERs were not found in other types of tumors, so it was suggested that they may not represent a general phenomenon in malignancy. The RER pattern between the primary tumor and either relapse or metastases of the same patient revealed many differences, in contrast with minor differences observed in the LOH pattern. There were RERs in the relapse, absent from primary tumor, indicating its generation after the occurrence of the metastasis from the primary site. Moreover, RERs were detected only in the primary tumor, which had to be generated after the formation of metastases. Based on these data it was suggested that

Table 2. Point mutations of genes encoding proteins of DNA repair systems associated with rhabdomyosarcoma.

DNA repair system	Gene	Mutation	Correlation with RMS	Reference
Direct repair				
	MGMT	Poin mutation cod156:GGC-TGC	Increased activity/Drug resistance	Xu-Welliver et al., 2000
	MGMT	Poin mutation cod156:GGC-TGC	Increased activity/Drug resistance	Bacolod et al., 2002
MMR				
	PMS2	Point mutation p.Cys73X	MIN	Kratz et al., 2009
Recombination repair				
	NBS1	657-661delACAAA/1142delC	RMS pathogenesis	The International Nijmegen Breakage Syndrome Study Group, 2000
	NBS1	chromosome rearrangements at Chr. 7/14	RMS pathogenesis	Der Kaloustian et al., 1996
	NBS1	657del5	RMS pathogenesis	Tekin et al., 2002
	NBS1	698del4	RMS pathogenesis	Meyer et al., 2004
	p53	c.818G>A, p.R273H	RMS pathogenesis	Ognjanovic et al., 2012
	p53	c.818G>A, p.R273H	RMS pathogenesis	Sugawara et al., 2011

MIN may not be a prerequisite for the development of either RMS or metastases and that the development of RER in RMS may be a late constant phenomenon. Furthermore, the strong correlation of high FAL with RER positivity in ERMS supported evidence that RERs may be a secondary phenomenon, possibly due to the loss of genes involved in MMR, which could be located near unidentified tumor suppressor genes involved in RMS, and may be selectively lost in RMS. Thus, ERMS with high FAL may accumulate more mutations than their counterparts with low FAL, and the "mutator phenotype" may occur only in tumor cells that already present increased genome instability. Thus, if coincidental loss of repair genes is true, RMS will represent a tumor that only after the initial steps of malignant transformation has acquired a defective MMR system (Visser et al., 1996). This hypothesis was further supported by other studies reporting that MIN was absent in primary and present in secondary RMS tumors. A germ-line mutation of the MMR gene MSH-2 and MIN was reported in one case of pleomorphic RMS, following the diagnosis of HNPCC and duodenal cancer at an early age. RMS was ascribed to mutations in target genes caused by RERs, induced by loss of the remaining MSH-2 allele in a mesenchymal stem cell, and proposed that sarcomas may occasionally present as part of the HNPCC phenotype (Den Bakker et al., 2003). Two RMS cases from families harboring biallelic germline mutations of MMR genes have been reported (Kratz et al., 2009). Constitutive biallelic inactivation of one of the MMR genes causes a recessive childhood cancer syndrome characterized by early-onset malignancies, café-au-lait spots (CLS) and/or other signs of neurofibromatosis type 1 (NF1). Kratz et al. referred to this syndrome as constitutional mismatch-repair-deficiency (CMMR-D) syndrome. In one case diagnosed with ERMS followed by colon adenocarcinoma, a germline mutation analysis and PMS2 analysis revealed a novel homozygous mutation, c.[219T?A]+[219T?A], leading to a premature stop codon (p.Cys73X) in exon 3 of the PMS2 gene. Both parents were heterozygous carriers of the mutant PMS2 allele (Kratz et al., 2009). In the other case, the patient had four synchronous adenocarcinomas of the rectum, sigmoid, transverse colon and caecum. Immunohistochemical analysis in both normal and tumor tissues showed complete lack of PMS2 expression. MIN analysis of BAT25 and BAT26 in two representative tumors showed instability at BAT25 in one tumor and stability in the other, whereas BAT26 was stable in both tumors. The sister of the patient was diagnosed with anaplastic astrocytoma and after a few months had an undifferentiated sarcoma, which was classified as having ERMS-like features. Complete lack of PMS2 expression in the astrocytoma and the sarcoma, as well as in normal tissue of the patient was also shown. Collectively, such data suggested underlying biallelic PMS2 germline mutations in these patients. No reliable germline PMS2 analysis was possible in order to further confirm the suspected biallelic PMS2 germline mutations in both sisters (Kratz et al., 2009). As increased incidence of RMS has been associated with NF1 (Sung et al., 2004), Kratz et al. suggested that some RMS patients with clinical (mis)diagnosis of NF1 actually have CMMR-D syndrome because of the clinical overlap between CMMR-D syndrome and NF1. These authors also proposed careful examination of the family history, analysis of the tumor(s) for loss of one of the MMR proteins and MIN and subsequent mutation analysis in order to establish a definitive diagnosis of the underlying disorder in patients with RMS and signs of NF1 (Kratz et al., 2009). In another study, the vast majority of RMS cases was not associated with familial syndromes, being probably the result of interactions of polymorphic susceptibility genes with a variety of environmental factors (Ponder, 2001; Hunter and Williams, 2002). Besides NF1 a small proportion of RMSs cases were related with distinct inherited cancer syndromes such as Li-Fraumeni (LFS) and Gorlin syndromes, which were caused by germline mutations in the tumor-suppressor genes TP53 and Patched (PTCH1), respectively (Slater and Shipley, 2007). Patched-1 (Ptc1) receptor binds Sonic hedgehog (Shh) and activates Shh signaling pathway. In the absence of Shh, Ptc1 suppress Smoothened (Smo). Shh binding to Ptc1 relieves the inhibition of Smo, culminating in the activation of one or more of the Gli transcription factors that regulate the expression of downstream targets (Altaba et al., 2002). In this aspect, deregulations of the Shh/Ptc1 signaling pathway were detected at a substantial percentage in sporadic RMS (Ragazzini et al., 2004). In fact, the $Ptch1^{neO67/+}$ mouse model of Gorlin syndrome was used in order to identify loci involved in susceptibility to RMS. A single locus on chromosome 2, Parms1, between markers D2Mit37 and D2Mit102, was identified as harboring a RMS susceptibility modifier locus. Parms1 spans approximately 7.5 cM on mouse chromosome 2 and includes the DNA repair genes PMS1 and Rad51. No systematic differences in morphology or differentiation between tumors from homozygous and heterozygous mice were noticed, suggesting that Parms1 locus may be involved in the early stages of tumor induction, rather than in later stages. The mouse locus between markers D2Mit37 and D2Mit102 shows conserved synteny to human chromosomes 2q31-q33, 11p11-p13, and 15q14- q15 (Hahn et al., 2004). These chromosomal regions were associated with RMS development in several studies. In 12 ERMS samples, Comparative genomic hybridization analysis (CGH) showed strong gains of chromosomes or chromosomal regions 2,7,8,11,12,13q21, and 20, and losses of 1p35-36.3, 6, 9q22, 14q21-32 and 17 (Bridge et al., 2000). With the same methodology (CGH), Li et al. reported genetic imbalances on chromosome 2 in 25 RMS (10 ARMS, 12 ERMS, 3 PRMS) cases. In ERMS, gains were observed in chromosome arms 7p, 9q (6/12), 2p,18q(5/12) and 1p,8q (4/12) and losses on chromosome 11p (5/12). In ARMS, the most frequently

occurring chromosomal gains and losses were 12q (7/10) and 3p,6p (5/10) respectively. No consistent patterns were seen in gains and losses in PRMS (Li et al., 2009). In accordance with the aforementioned studies, Paulsen et al. demonstrated by high resolution array CGH in 26 ERMS, gains of chromosomes 2, 11, 12, 13, 19, and 20 and losses of chromosomes 6, 9, 10, 14, 15, 16, and 18 (Paulson et al., 2011). MMR deficiencies were also associated with RMS chemoresistance. In the aforementioned study by Middlemas et al., the *in vivo* sensitivity of the RMS xenografts (Rh30, Rh30c, Rh28, Rh28c, Rh18, Rh66 and Rh12) to the methylating agent temozolomide was correlated with MGMT and MSH-2 and MLH-1 expression. Tumors displaying high (Rh30, Rh30c) or intermediate (Rh28) sensitivity to temozolomide had detectable MSH-2 and MLH-1 protein levels. Low sensitive tumors had either high MGMT and detectable expression levels of MSH-2 and MLH-1 (Rh18, Rh66, Rh12) or undetectable MGMT and MLH-1 (Rh28c). MGMT-deficient Rh30 and Rh30c tumors had marginal levels of MMR proteins, although Rh30c tumors treated with low temozolomide doses (28 mg/kg) regressed completely with no regrowth. On the contrary, MGMT-deficient Rh28 and Rh28c, with undetectable levels of MLH1 showed intermediate or low sensitivity and Rh12 tumors with high MGMT and detectable MMR protein levels didn't regress, even at the highest tolerated dose level (66 mg/kg). Thus, MGMT deficient tumors may escape its effects, by virtue of defective MMR. Thus, MGMT and MMR activity was considered to predict RMS response in methylating agents (Middlemas et al., 2000).

Recombination repair system

Recombination repair corrects DNA double-strand breaks (DSBs), as well as interstrand crosslinks (Hoeijmakers, 2001). DSBs are naturally generated by reactive oxygen species (ROS), V(J)D recombination, class-switch recombination and meiosis. DSBs are also produced by DNA damaging agents such as ionizing radiation (IR), UV and chemical agents, including cancer chemotherapeutics (Khanna and Jackson, 2001). DSBs in euchromatic DNA are readily repaired by NHEJ. The Ku70-Ku86 complex recognizes DSBs and binds each DNA broken end. Ku-DNA complex interacts with the nuclease Artemis/DNA-dependent protein kinase (DNA-PKcs) complex, the polymerases Polμ and λ and ligase XRF-XRCC4-ligase4 complex and triggers the iterative process of each DNA end. Ku-DNA complex can recruit the nuclease, polymerase and ligase activities in any order. A less well characterized Ku-independent NHEJ subpathway, MMEJ, results in sequence deletions (Lieber, 2008, 2010). NHEJ is the major pathway for DSB repair as it functions throughout the cell cycle and does not require a homologous chromosome. NHEJ distinct for its imprecision, as it rejoins DNA ends by random nucleotide addition or resection (Lieber, 2010). DSB repair in heterochromatic DNA is initiated with recruitment and activation of the ataxia telengiectasia mutated (ATM) and ataxia telengiectasia related (ATR) proteins, which have multiple downstream targets, involved in cell cycle regulation (through activation of p53) and DNA repair (Thompson and Schild, 2002; Shrivastav et al., 2008). Repair may then proceed by two principal pathways, the error free HR during S and G2 phase of cell cycle when sister chromatid are directly adjacent, and the error prone NHEJ during G1 phase (Helleday, 2010). In HR repair system ssDNA are generated by MRN complex (MRE11-RAD50-NBS1) and serve as templates for long-range DNA end resection by BLM or EXO1. The exposed ssDNA are coated by RPA, which recruits the Rad52 epistasis group of proteins (Rad52, Rad55, Rad57, Rad59, Rad54, Rdh54) to enable Rad51 filament formation, assisted by the breast-cancer susceptibility proteins BRCA1 and BRCA2. Rad51nucleoprotein filament finds a DNA homology and forms a joint structure. DNA repair results in double Holliday junction formation, which are resolved by BLAP75 (BLM-TOP3α-RMI1) complex (San Filippo et al., 2008; Bernstein and Rothstein, 2009).

Recombination repair system and rhabdomyosarcoma

ATM kinase is critical in the DNA damage signaling pathway. The immunohistochemical characteristics of ATM protein in 17 clinical RMS cases were recently examined. ATM was ubiquitous, but a significantly high number of RMS cases (7 out of 17, 41%) lacked the proper ATM gene product, with no differences in the staining pattern between RMS subtypes. ATM protein was predominantly located in the cytoplasm, in contrast with the nuclear localization noted in RD cell line, indicating different roles of this protein both *in vivo* and *in vitro*. At transcriptional level, ATMs mRNA in Rh28 and RD cell lines was identical with control fibroblasts. ARMS Rh30 cells, which harbor a p53 mutation, presented three separate deletions/mutations, encoding a smaller form of the ATM protein. Differences in ATM mRNA translation were also observed, as all RMS cell lines were found to express an aberrant form of ATM protein, significantly smaller than control normal fibroblasts. Additionally, Rh30 cells presented lower protein levels compared to Rh28 and RD. Collectively, such data supported evidence for a possible contribution of the mutated ATM to pathogenesis of RMS (Zhang et al., 2003). A strong argument supporting this perspective was that the ATM gene was large, spanning about 150 kbs of genomic DNA, located on chromosome 11q22-23, and ERMS was strongly associated with LOH for multiple closely linked loci at chromosomal 11p15.5. In accordance with the aforementioned study, the RD cell line showed an abnormally high frequency of chromatid breaks (112) and gaps (94) after x-irradiation, but addition of isochromosome 11 (with no short arm) resulted in a significant decrease of chromatid breaks (16 and 22) and gaps (14 and 19), associated with efficient

DNA repair and indicating a reversible DNA repair deficiency. Restoration of efficient DNA repair was associated with partial tumor suppression, as both *in vitro* and *in vivo* cells grew more slowly. Thus, chromosome 11 was suggested to contain a single or a cluster of DNA repair genes, and addition of chromosome 11 may complement expression of the specific gene or genes mutated or lacking (Parshad et al., 1992). Alternatively, the repair deficiency may result from inaccessibility of repair enzymes to damaged sites due to abnormal chromatin conformation, and chromosome 11 may supply a chromatin accessibility factor (Hanawalt, 1989). In another study, introduction of a normal chromosome 11 (microcell hybridization) in RD cells also entailed loss of tumor suppressor function. The authors cited evidence that 11p15.5 region contained a gene that affected cellular proliferation, without completely suppressing tumorigenicity, and a second gene that retarded growth of tumor cells. Thus, it was suggested that loss of the growth suppressor gene on chromosome 11p may participate in an early step in RMS development (Loh et al., 1992).

Other Recombination Repair proteins implicated in RMS are the MNR complex components. NBS-1 is located on chromosome 8q21 and encodes the DNA double strand break repair protein nibrin. Defects in NBS-1 gene result in Nijmegen breakage syndrome (NBS), a rare autosomal recessively transmitted chromosomal instability disorder, belonging to DNA repair related disorders. NBS patients have predisposition to malignancy, as 40% develop cancer before the age of 21 years (Der Burgt et al., 1996; The International Nijmegen Breakage Syndrome Study Group, 2000). The domains found in nibrin and the NBS phenotype suggest that NBS is caused by defective responses to DNA double strand breaks (Varon et al., 1998; Matsuura et al., 1998). Eight different mutations causing NBS have been reported in the NBS1 gene, though more than 90% of patients have been found to carry a 5-bp deletion (657del5) in exon 6 of NBS1 (Chrzanowska et al., 2002). No specific genotype-phenotype relation was established for NBS patients, as specific mutations did not lead to specific clinical features. The International Nijmegen Breakage Syndrome Study Group conducted a computer aided literature search to obtain data on patients with NBS syndrome. Of 55 patients included in the NBS registry, one Canadian male, who harbored a distinct truncating deletion, 657-661delACAAA/1142delC, in NBS1 gene developed RMS (The International Nijmegen Breakage Syndrome Study Group, 2000). A few more cases of RMS in children with NBS have also been reported. The first reported case of NBS patient developing RMS was a premature born boy with growth retardation and several episodes of respiratory and mucocutaneous infections. At the age of four he was diagnosed with ARMS and displayed the characteristic for NBS chromosome rearrangements, including translocations, deletions, inversions, and rearrangements involving chromosome 7 and/or 14 known at that time (Der-Kaloustian et al., 1996). Moreover, a patient with severe growth retardation was diagnosed with perianal ERMS at 15 months of age and a tumor recurrence in the same region five years later. Molecular analysis of NBS1 gene revealed homozygosity for a 5-bp deletion (657del5) in exon 6, whereas his parents were heterozygous. Their first child was a male born with a very low birth weight, anal atresia, syndactyly of the second and third fingers and toes and died on the 25th day of life following surgery for anal atresia. Although molecular analysis was not conducted for the first child it was almost certain that he had the same homozygous deletion (Tekin et al., 2002). Another ARMS patient, negative for the PAX3-FKHR and PAX7-FKHR fusion gene transcripts and normal karyotype, showed extreme toxicity during chemotherapy. Three years later repeat karyotyping revealed abnormalities involving chromosomes 7 and 14, which are characteristic for NBS. Molecular analysis of the NBS1 gene revealed homozygosity for a 4-base pair deletion at position 698→701 of exon 6 of the NBS1 gene, (698del4) (Meyer et al., 2004). In this aspect, it should be noted that DNA damage results in activation of p53 and cell cycle arrest to allow DNA repair or apoptosis induction. p53 is also activated during differentiate processes such as hematopoiesis, spermatogenesis and myogenesis (Almog and Rotter, 1997). Ognjanovic et al. analyzed the International Agency for Research on Cancer (IARC) TP53 database and correlated with the Surveillance, Epidemiology, and End Results Program (SEER) database and reported that 16.5% of carriers of inherited TP53 mutations develop RMS, with increased risk at age <3 years. The most frequent mutation among RMS patients was the missense c.818G>A, p.R273H in the DNA-binding domain (Ognjanovic et al., 2012). A heterozygous p.R273H mutation was also detected in a ERMS patient who fulfilled the clinical criteria for LFS. However, a second tumor in another anatomic site harbored homozygous p.R273H mutation and 5q11.2 and 11q22.1 amplicons, suggesting that the second sarcoma was a new primary tumor (Sugawara et al., 2011).

p63 and p73 are members of the p53 family and regulate an overlapping set of target genes (Kaghad et al., 1997; Yang et al., 1998). In 21 ex vivo RMS specimens (12 ERMS and 9 ARMS) p73 was overexpressed in the majority of the samples and was correlated with compromised p73 activity. High TAp73 levels were detected in 85.7% of patients and were associated with overexpression of DNp73 generated by either alternative splicing of exon 2 (DN^{AS}) or alternative promoter usage (DN^{AP}). Immunohisto-chemical analysis indicated that RB and p57 were inversely regulated, while depletion of p73 in Rh30 cells resulted in a strong increase in p57 levels associated with cell cycle arrest. Based on these data, it was suggested that all p53 family members may be involved in regulating the process of muscle differentiation. p53 is required to induce transcription of the RB gene, whereas

p63 and p73 control expression of p57, which maintains RB in an active hypophosphorylated state. Ablation of all p53 family members functions blocks myogenic differentiation and enables cooperating oncogenes to transform myoblasts. Thus, these data may explain the high frequency of p53 pathway alterations in RMS (Cam et al., 2006).

Several anticancer drugs and radiotherapy cause toxic DSBs and it is generally accepted that the ability of cancer cells to repair such DSBs in?uences the therapeutic outcome. RMS radiotherapy could be enhanced by agents subscribing radiosensitization without impairing normal cells, such as histone deacetylase inhibitors (HDACIs). The capacity of the HDACI, suberoylanilide hydroxamic acid (SAHA) to modulate radiation response, by enhancing radiation-induced cell death in RMS cell lines (RD and A-204) has recently been evaluated (Blattmann et al., 2010). Pretreatment with SAHA reduced clonogenic survival (1-to2-log decrease) after XRT exposure and enhanced radiosensitivity in RMS cell lines, but not in normal ?broblasts. SAHA did not affect the radiation-induced cell cycle block and provoked marginal induction of apoptosis. SAHA pretreatment enhanced acetylation of the DSB sensor histone H3 and reduced expression of Rad51 and Ku80 proteins, thus increased radiosensitivity, post-radiation exposure. SAHA induced apoptosis and reduced Rad51 and Ku80 levels could represent a radiosensitizing mechanism by regulating the transcription rate of genes involved in DNA damage repair (Blattmann et al., 2010).

Conclusions

Several studies provided accumulating data indicating that RMS is sporadically associated with familial syndromes, characterized by germline mutations of DNA repair genes. RMS probably results from interactions of polymorphic susceptibility genes with a variety of environmental factors. DNA repair gene alterations in RMS represent a secondary phenomenon, after the initial steps of malignant transformation and reflect advantageous evolutionary alterations of the cancer genome. These modifications in DNA repair enzymes expression or activity, due to mutations or promoter methylation, may lead to resistance to chemo- and radiotherapy in RMS tumor cells. Defective DNA repair mechanisms caused by either point mutations or LOH in RMS cases associated with familiar syndromes could also act synergistically with other predisposing factors and accrue rhabdomyosarcomatogenesis. MMR proteins, ATM, NBS1 and p53 gene status must be established in order to pursue a more individualized approach to disease progression and therapeutic management. MGMT and MMR protein activity and expression levels seem to be crucial for efficient treatment and could be used as predictor indices for therapy outcome in the majority of RMS patients. Additionally, in cases with pure response to therapy, alteration in BER proteins could be considered as possible causes of increased resistance. RMS represents an individualized tumor and, according to each patient genetic profile downregulation of activated DNA repair enzymes or upregulation of DNA repair deficiencies, could provide novel targets for treatment in combination with standard anti-cancer therapy.

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