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Genetics of Bladder Malignant Tumors in Childhood

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Abstract: Bladder masses are represented by either benign or malignant entities. Malignant bladder tumors are frequent causes of disease and death in western countries. However, in children they are less common. Additionally, different features are found in childhood, in which non epithelial tumors are more common than epithelial ones. Rhabdomyosarcoma is the most common pediatric bladder tumor, but many other types of lesions may be found, such as malignant rhabdoid tumor (MRT), inflammatory myofibroblastic tumor and neuroblastoma. Other rarer tumors described in literature include urothelial carcinoma and other epithelial neoplasms. Rhabdomyosarcoma is associated to a variety of genetic syndromes and many genes are involved in tumor development. PAX3-FKHR and PAX7-FKHR (P-F) fusion state has important implications in the pathogenesis and biology of RMS, and different genes alterations are involved in the pathogenesis of P-F negative and embryonal RMS, which are the subsets of tumors most frequently affecting the bladder. These genes include p53, MEF2, MYOG, Ptch1, Gli1, Gli3, Myf5, MyoD1, NF1, NRAS, KRAS, HRAS, FGFR4, PIK3CA, CTNNB1, FBXW7, IGF1R, PDGFRA, ERBB2/4, MET, BCOR. Malignant rhabdoid tumor (MRT) usually shows SMARCB1/INI1 alterations. Anaplastic lymphoma kinase (ALK) gene translocations are the most frequently associated alterations in inflammatory myofibroblastic tumor (IMT). Few genes alterations in urothelial neoplasms have been reported in the paediatric population, which are mainly related to deletion of p16/lnk4, overexpression of CK20 and overexpression of p53. Here, we reviewed available literature to identify genes associated to bladder malignancies in children and discussed their possible relationships with these tumors.

Keywords: RMS, Urothelial neoplasms, IMT, Pediatric age, Malignancy, Cancer genetics.

1. INTRODUCTION

Bladder tumors include a variety of benign and malignant entities and, although not uncommon in adults, they are rare in children. The histologic types of tumors seen in the pediatric population differ from those seen in adults. Nonepithelial tumors are more common than epithelial ones and, although rhabdomyosarcoma is the most common pediatric bladder tumor, many other benign, malignant, and reactive lesions can be encountered [1, 2].

Soft tissue tumors are more commonly seen in pediatric patients, with rhabdomyosarcoma being the most common malignancy in this location. Other non epithelial malignant tumors of the bladder, such as malignant rhabdoid tumor (MRT) and inflammatory myofibroblastic tumor are even more rarely observed and some others, as neuroblastoma, have exceptionally been reported [3-5]. Urothelial carcinoma and other epithelial neoplasms of the urinary bladder are rare in children and young adults. In this age group they appear to exhibit unique clinicopathologic features, with low-grade morphology and decreased tendency to recurrence and progression [4]. Transitional cell carcinoma of the bladder (TCCB) [6] and squamous cell carcinoma [7] are rarely seen in children.

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Due to their rarity, malignant tumors of the bladder in children may present challenging diagnostic and therapeutic aspects. Clinical behavior of these tumors may vary greatly from good to very poor prognosis and classification in many cases cannot rely only on pure histologic criteria. Increasing knowledge on genetic features of different pediatric malignancies is achieving a better understanding of the nature of many entities and identification of subsets of tumors, in order to identify more targeted therapeutic strategies [8, 9].

We reviewed the literature focusing on achievements in genetics of most frequent pediatric malignancies of the bladder, relevant to improvement of classification, prognosis and treatment.

2. RHABDOMYOSARCOMA

Rhabdomyosarcoma is a malignant tumor arising from primitive totipotential embryonal mesenchyme. It is the most common sarcoma of childhood [10]. The most common location is the head and neck, followed by the genitourinary tract. Most commonly affected sites in the genitourinary tract are bladder, prostate, vagina, uterus, vulva, paratesticular regions and, rarely, kidney, ureter, ovary, fallopian tube. The incidence of genitourinary rhabdomyosarcoma (GURMS) ranges from 0.5 to 0.7 cases per million children under 15 years of age. Most cases present within the first decade of life, with two specific age peaks occurring between 2 and 6 and 15 and 19 years of age. It frequently arises in the bladder/prostate (BP), accounting for 5% of all RMS. It is the

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most common bladder neoplasm in children under 10 years of age and the median age of children presenting with B/P RMS is 5 years (10). Male to female ratio is (1.4:1) and it is more often found in Caucasians than African-Americans (2.3:1) [11, 12].

Classification of the Intergroup Rhabdomyosarcoma Study Group (IRSG) recognizes three major histologic groups of prognostic significance. (1) Embryonal histology (E-RMS), with favorable prognosis, accounts for 90% of genitourinary localization and most bladder RMS are of embryonal subtype. (2) Botryoid histology, common in bladder and vaginal RMS, which is a variant of E-RMS. (3) Alveolar histology (A-RMS) accounts for the remaining 10% of GURMS and is associated with the worst outcomes [3]. Beyond basic histologic classification, the pathologic diagnosis of RMS is complimented by immunohistochemistry (IHC) and cytogenetics.

Children with bladder RMS are usually under 4 years of age and may present with hematuria, urinary obstruction and, rarely, extrusion of tumor tissue. RMS of the bladder in children tends to remain localized. Deep extension and metastasis to regional lymph nodes and to distant sites is a relatively late occurrence [13].

The increased incidence in siblings of children with central nervous system tumors and adrenocortical carcinoma and the familial aggregations of RMS with other sarcomas, breast tumors, and brain neoplasms are suggestive of a possible genetic factor [12].

Evidence suggests a hereditary basis for RMS. In as many as 32% of cases, Rhabdomyosarcoma is associated with a number of genetic syndromes, including Li-Fraumeni syndrome, neurofibromatosis type 1 and 2, Costello Syndrome, Gorlin's basal cell nevus syndrome, Rubinstein-Taybi syndrome, trisomy 21 syndrome, Beckwith-Wiedemann syndrome and the fetal alcohol syndrome [10, 14].

Individuals with rhabdomyosarcoma also have an increased incidence of genitourinary anomalies similar to the defects seen in Wilm's tumor.

Rhabdomyosarcoma results from dysregulation of the skeletal myogenesis mechanisms. The activation of muscle-specific gene expression is dependent on a cooperative function of the MyoD family and the MADS protein, MEF2. The steroid receptor coactivator SRC-2/GRIP-1/TIF-2, acting as a cofactor for the transcription factor, MEF2, is necessary for skeletal muscle differentiation. SRC-2, belonging to the SRC family of transcriptional coactivators/cofactors, is essentially localized in the nucleus of proliferating myoblasts; MEF2 is primarily expressed in the nucleus,. GRIP-1 and MEF2 are coexpressed in the nucleus during skeletal muscle differentiation, consistent with the direct interaction of these proteins. Rhabdomyosarcoma cells derived from malignant skeletal muscle tumors have been proposed to be deficient in cofactors and display aberrant SRC localization and expression, which may underlie certain features of the RMS phenotype [15].

Genetic studies specifically referring to bladder RMS are lacking, due to the small number of cases in each reported series. Data on bladder RMS can only be extracted from studies on large series of RMS arising in different sites. Most of the available literature on bladder RMS shows its association with embryonal variants [16-18]. Within 65 specimens of bladder and vagina RMS, Leuschner *et al.* found 51 urinary bladder RMS and 14 vaginal RMS. Histology showed 31 "classical" embryonal RMS, 26 embryonal RMS of botryoid subtype, 3 embryonal RMS of spindle cell subtype, and 5 alveolar RMS [19]. Martinelli and collaborators investigated the biological and clinical relevance of mutations with perturbing consequences on RAS signaling in 31 embryonal RMS, among which 7 cases of bladder localization were observed [16]. Of 33 cases of RMS reported by Diller and coworkers, bladder was involved in 5, all showing embryonal histologic features [20]. In a series of 32 RMS, Tonin and colleagues found 2 bladder RMS, both of embryonal type [21]. Similarly, in a study on fresh tissue sections from 49 tumors, Dias and collaborators reported 2 RMS of the bladder, both showing embryonal characterization [22].

Therefore, since most of bladder RMS are embryonal or an embryonal variant, such as botryoides RMS, further studies on bladder RMS should focus on genetic characterization of E-RMS. Moreover, multicentric studies are needed to provide investigation on large series of patients affected by bladder RMS.

Anderson and coworkers analysed a group of 91 Rhabdomyosarcomas for the presence of the *PAX3-FKHR* and *PAX7-FKHR* gene fusions and correlated these results with tumour phenotype and clinical outcome, suggesting a prognostic significance of t(1;13)/PAX7-FKHR, t(2;13)/PAX3-FKHR and alveolar histology. In this study, 5 RMSs of the bladder are reported, included in a group of 46 tumors lacking PAX3-FKHR translocations. This finding gives further evidence that bladder localisation of RMS is predominantly associated with embryonal or at least *PAX3-FKHR* negative tumors [22].

25% to 45% A-RMS cases lack known P-F fusions. E-RMS do not demonstrate recurrent chromosomal translocations (i.e., P-F-negative), but show greater genomic instability (manifested as highly variable karyotypes) and recurring allelic imbalances such as loss of heterozygosity (LOH) at chromosome 11p15.5-15.16.

Despite improvements in morphological and cytological examination, as many as one third of the patients could be incorrectly assigned to treatment protocols because of inconsistency and uncertainty as determined by institutional pathology diagnosis [18]. Genomic analysis of human tumor specimens has an increasingly significant impact on the field of tumor pathology [23, 24], redefining tumor classes based on molecular features [25, 26] and identifying new subclasses previously unrecognized by conventional histology or cytogenetics.

Clinical behavior and prognosis of RMS vary with histological type. Although distinction between alveolar and embryonal types accounts for most of the different clinical features of RMS, genetic alterations allow further differentiation and have significant diagnostic and therapeutic implications. Most A-RMS show reciprocal chromosomal translocations resulting in the expression of the chimeric transcription factors, PAX3-FOXO1 or PAX7-FOXO1 (henceforth, P-F or PAXFKHR), while E-RMS and a subset of A-RMS

are gene fusion-negative and harbor different genetic characteristics (8). This is also summarized in a comprehensive review by Shren and collaborators [27].

These studies identified molecularly defined classes, highly reproducible and objectively defined, thus providing several advantages compared to current histopathological classification schemes, including prognostically relevant information, useful in optimizing risk-adapted therapy. A molecular-based classification may allow better characterization of molecularly distinct subgroups of RMS and might be more relevant toward directing subtype-specific therapeutics compared with historically defined classes based on histology. Perhaps, a hybrid model combining histopathological and molecular-based class (i.e., P-F expression status) will be the most useful to clinicians and their patients [28].

2.1. Genes in RMS

Many genes have been implicated in RMS pathogenesis and characterization. Multiple genes were found to be recurrently altered in P-F negative RMS, including NRAS, KRAS, HRAS, FGFR4, PIK3CA, CTNNB1, FBXW7, IGF1R, PDGFRA, ERBB2/4, MET, BCOR and the receptor tyrosine kinase/RAS/PIK3CA axis [27].

Also important for the characterization of RMS are *PAX3-FKHR* and *PAX7-FKHR* gene fusions, known to be found in a subset of RMS.

The role for wild-type Pax3 and Pax7 in neurogenesis during early development is well-established [29]. PAX-FKHR fusion gene expression in RMS cells may aberrantly activate neurogenesis transcription normally silent in the myogenic lineage, in which tumor cells show muscle phenotype accompanied by the expression of genes characteristic of neurogenic cell lineages. Genes seemingly repressed in P-F RMS, with increased expression in P-F-negative tumors, were overrepresented by genes localized to chromosome 8q. This correlates with the increased incidence of 8q LOH (specifically 8q24) in P-F-negative tumors, supporting the hypothesis that deregulation of gene expression in chromosome 8 is a consequence of copy number change specific to E-RMS [28, 30, 31]. These tumors can be distinguished from undifferentiated and NRSTS histology tumors by expression of muscle differentiation and chromosome 11 gene expression signatures, and by increased LOH of chromosome 11. Although most LOH in tumors is thought to arise sporadically, recurrent regions of LOH are described in E-RMS, such as that of chromosome 11p15.5 [32, 33]. A potential consequence of LOH is gene dosage imbalance, in which loss of one allele can cause overexpression from the retained allele (dosage compensation) [34] or allele-specific amplification [35]. This phenomenon may explain why several chromosomal regions were overrepresented in specific gene expression signatures even though there was also increased frequency of LOH in the same tumor class. The most striking example is the enrichment of genes localized to chromosome 11 in the chromosome 11 expression signature, coupled with the predominance of LOH along chromosome 11 in P-F-negative RMS but not UDS/NRSTS.

Spindle cell or botryoid E-RMS variant tumors did not show a distinct expression signature and are recognized as highly myogenic, well differentiated tumors with superior expected outcomes for patients [36]. However, these variants could not be distinguished on a molecular level from a subset of other P-F-negative RMS tumors (including, surprisingly, P-F-negative alveolar histology). These tumors are classified as the well differentiated (WD) RMS subclass in consequence of elevated expression of a characteristic muscle differentiation gene signature. These can be distinguished from the moderately differentiated, or MD RMS subclass, which had intermediate expression levels of this signature, and the undifferentiated and nonrhabdomyosarcoma (UDS/NRSTS) molecular class, where this expression signature was not detected.

2.1.1. PAX3-FKHR and PAX7-FKHR Fusion

Many studies characterized *PAX3-FKHR* and *PAX7-FKHR* gene fusions to determine whether a new molecular-based classification scheme might be more suitable than conventional histological methods to define RMS. Comparisons of *PAX3*/*7–FOXO1* fusion positive RMS (P-F RMS) to fusion negative RMS (P-F-negative RMS) showed little distinction between P-F negative A-RMS and E-RMS gene expression profiles, but these tumors are clearly distinct from P-F-positive tumors, suggesting a more appropriate definition of tumors both by histological appearance (alveolar, embryonal, and the embryonal variants) and by fusion status (P-F-positive and P-F-negative). Others have similarly reported on comparative genomic hybridization data of *PAX-FKHR* and P-F-negative A-RMS, in which the latter share similar chromosomal gains with E-RMS tumors but not with P-F-positive A-RMS [28].

A comprehensive characterization for the genomic alterations in RMS showed similar findings and confirmed that the presence or absence of a *PAX3*/*7–FOXO1* gene fusion is a crucial prognostic indicator in this disease. In this study the importance of the *PAX* gene fusion as the dominant driver in this subtype is also underscored, suggesting that it alters a host of downstream targets through its transcriptional reprogramming. The authors also note that PAX3–FOXO1 by itself cannot cause rhabdomyosarcoma and that a coexisting genetic lesion is necessary. Cooperating lesions, experimentally validated in a mouse model of alveolar rhabdomyosarcoma, include *TP53* and *Ink 4a*/*ARF* loss. The most common cooperating events are genetic amplification (such as *MYCN*, *CDK4*, and *MIR-17-92*) or deletion (*CDKN2A*, LOH of Chr11p15.5), while fusion-negative tumors seem to have accumulated a higher degree of aneuploidy and mutational burden at the time of clinical presentation [27].

2.1.2. FGFR

The fibroblast growth factor receptors consist of a cellular ligand domain composed of three immunoglobulin-like domains, a single transmembrane helix domain, and an intracellular domain with tyrosine kinase activity. These receptors bind fibroblast growth factors, members of the largest family of growth factor ligands, comprising 22 members [37].

Fibroblast growth factor receptor (FGFR) signaling has been found the most significantly altered pathway and mutations in this pathway, including recurrent mutations of *FGFR4,* were found in 88% of P-F Negative samples (22 of

25 tumors) that were analyzed by whole-genome sequencing (WGS) [27].

2.1.3. MET

MET proto-oncogene is located in the 7q31 locus of chromosome 7. It encodes the MET protein, which possesses tyrosine kinase activity [38]. It is a membrane receptor that is essential for embryonic development and wound healing, inducing several biological responses collectively resulting in a program known as invasive growth. Normally, expression of MET allows stem cells and progenitor cells to grow invasively in order to generate new tissues in an embryo or regenerate damaged tissues in an adult. MET is deregulated in many types of human malignancies and various mutations in the MET gene are associated with papillary renal carcinoma [39].

MET is among several genes found altered in P-F Negative tumors, which are known downstream targets of PAX3 and PAX3–FOXO1 [27].

2.1.4. CCND2

CCND2 gene encodes the G1/S-specific cyclin-D2 protein, belonging to the cyclin family. Cyclins function as regulators of cyclin-dependent kinases. Different cyclins contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, has been shown to be involved in the phosphorylation of tumor suppressor protein Rb [40].

Multiple genes, including *CCND2,* that were found to be mutated in PFN tumors, were among top upregulated genes when PAX3–FOXO1 was expressed [27].

2.1.5. IGF

Several studies have investigated the role of development genes in the sarcomatogenesis and many of them have shown a significant involvement of the IGF pathway in the pathogenesis of RMS [24-26].

The decline in the expression of growth-promoting imprinted genes, including IGF2, is involved in normal postnatal slowing of somatic growth. Rezvani and collaborators hypothesized that persistent rapid proliferation in embryonal cancers may be caused in part by abnormal persistent high expression of growth-promoting imprinted genes [41]. Analysis of microarray data by these authors showed elevated expression of MEST, PLAGL1, PEG3, DLK1, and IGF2 in various embryonal cancers, especially rhabdomyosarcoma, as compared to nonembryonal cancers and normal tissues. This study also showed that siRNA-mediated knockdown of MEST, PLAGL1, PEG3, and IGF2 expression inhibited proliferation in Rh30 rhabdomyosarcoma cells. Their findings suggest that failure of normal postnatal downregulation of growth-promoting imprinted genes in some embryonal cancers, particularly rhabdomyosarcoma, contributes to the persistent rapid proliferation of tumor cells and, more generally, that failure of the mechanisms responsible for normal somatic growth deceleration can promote tumorigenesis [41]. In order to explore the natural history of rhabdomyosarcoma, Ianzano and coworkers studied male mice carrying a heterozygous deletion of p53 and an activated HER-2/neu transgene (BALB-p53Neu mice), that develop urethral rhabdomyosarcomas. The insulin-like growth factor 2 (IGF2) was among genes prominently upregulated in preneoplastic tissue, and more highly expressed [42]. De Souza and colleagues investigated the IGF2 gene expression in rhabdomyosarcoma samples. Gene expression was quantified by polymerase chain reaction-reverse transcription and related with clinic pathological parameters. Of the 25 samples, nine (36%) were A-RMS and 16 (64%) were E-RMS. The authors observed IGF2 overexpression in 80% of samples and could indicate an important role of this pathway in RMS biology [24] In another study on RMS, Ewing's sarcoma and synovial sarcoma, the same authors confirmed a high IGF2 gene expression in 29 RMS tumor samples [43].

2.1.6. p53

p53 is a protein encoded by TP53 gene in humans, and it is a tumor suppressor gene. TP53 is located on the short arm of chromosome 17. It plays an important role in apoptosis, in the inhibition of angiogenesis and in genomic stability. Loss of p53 causes the joining of short telomeres that generate chromosomal end to end fusions. These fused chromosomes result in cycles of chromosome fusion-bridge-breakage cycle which can lead to different forms of cancer, especially in epithelial tissues. It can contribute to the development, life expectancy, and protection against cancer development.

Germline mutations of the TP53 are linked to Li–Fraumeni syndrome, an autosomal dominant hereditary disorder characterized by cancer predisposition and also known as the sarcoma, breast, leukaemia and adrenal gland (SBLA) syndrome. It was named after Frederick Pei Li and Joseph F. Fraumeni, Jr., who first recognized the syndrome after reviewing the medical records and death certificates of 648 childhood rhabdomyosarcoma patients [44].

The mutations can be inherited, or can arise de novo early in embryogenesis, or in one of the parent's germ cells.

Most individuals with Li–Fraumeni syndrome are heterozygous for a mutant TP53 gene, and some p53 mutants can inhibit the function of the wild-type p53 in a dominant negative manner. Mutated p53 proteins are typically more stable than wild-type, and can inhibit the activity of the wild-type protein in suppressing cell proliferation and in inducing cell cycle arrest. Due to the mutant p53 being able to inhibit some wild-type p53, damaged cells are at an even greater susceptibility to proliferate and become transformed, resulting in cancer [45].

Normal and tumor genotypes were analyzed at loci on chromosome 17p and the structural integrity of the p53 locus was analyzed for 241 patients, of which 31 were affected by rhabdomyosarcoma [46]. Loss of heterozygosity was detected in 7 of 31 tumors examined. Structural alterations of the p53 locus were identified in 5 tumors. Three classes of alteration were identified, in which complete deletion of both alleles of the p53 locus, loss of heterozygosity at loci on chromosome 17 with a diminution of copy number of the p53 locus and no loss of heterozygosity on chromosome 17 were detected. However, in each of these classes densitometric analysis showed dosage equivalence of loci on the short and long arms of chromosome 17 and approximately half the p53 locus copy number. In summary, this study showed fre-

quent inactivation of p53 in rhabdomyosarcomas. Of the 31 rhabdomyosarcomas examined, 14 showed defects in the structure or expression of the p53 gene, loss of heterozygosity for loci in the vicinity of p53, or failed to express p53 transcript or protein as detectable by Western blot analysis. Moreover, the identification of homozygous deletions of the p53 locus implies that truly recessive mechanisms of p53 inactivation can contribute to neoplastic growth *in vivo* and the authors suggest that the attenuation of normal p53 function has an important role in human neoplasia rather than the genetic mechanism by which this is accomplished. The alteration of p53 in tumors for which other, primary mutations have been defined implies that p53 plays a progressional role in tumorigenesis [46].

Early studies of RMS tumor samples revealed a high frequency of alterations at the p53 tumor suppressor locus [46, 47].

Leuschner and coworkers studied 150 cases of RMS in order to correlate p53 with histological subtype, mitotic count, and various clinical parameters. The results did not show differences between embryonal and alveolar RMS. Since tumors of patients with metastatic embryonal RMS showed significantly higher levels of p53 protein than non-metastatic tumors, these authors suggested a role of p53 protein in metastatic embryonal RMS [48].

Diller and collaborators provided genetic evidence that RMS, in a subset of patients, is associated with the carrier state of a constitutionally altered allele of the p53 tumor suppressor gene. These authors detected heterozygous constitutional mutations in 3/33 patients with sporadic rhabdomyosarcoma. Two of these missense mutations are located in exon 7 and one in exon 8 of the p53 gene. The presence of a constitutional p53 mutation was associated with young age at diagnosis, while it did not correlate with tumor histology, stage, or site. 3/13 children under the age of 3 years at diagnosis carried mutations, whereas none of 20 children over 3 years of age at diagnosis harbored a detectable constitutional mutation. Although these results in children with RMS corroborate previous findings suggesting that the mutant p53 carrier state may predispose individuals to malignancy at an early age, none of the 5 patients with bladder RMS carried a constitutional p53 mutation [20].

2.1.7. MyoD1Fr

MyoD is a protein belonging to the family of myogenic regulatory factors (MyoD, Myf5, myogenin, Myf6). Myogenin and MyoD1 are expressed early in skeletal muscle differentiation and play a role in repair of damaged tissue. MyoD is inhibited by CDKs. Myogenin and MyoD1 are considered sensitive and specific markers for RMS. Expression of myogenin and MyoD1 in rhabdomyosarcoma subtypes has been assessed by Cessna and colleagues in archival tissue from 32 RMS. Samples were stained for myogenin and MyoD1 with standard immunohistochemical techniques. All RMSs expressed myogenin. Alveolar RMSs showed strong nuclear staining, Embryonal RMSs were more variable in myogenin staining pattern and intensity. Because the extent of myogenin expression in RMS is much greater than in non-RMS, it is a very useful marker when interpreted in the context of other clinicopathologic data [22, 49].

MyoD and myogenin are markers of RMS and, although distinction between subsets of tumor types cannot rely on their expression, few reports confirm their detection not only in E-RMS, but also in bladder localization. Tonin and coworkers investigated the expression of various genes, among which the myogenic regulatory genes MyoD, myogenin, MRF4, and Myf5, in embryonal and alveolar subtypes. The data showed that each of the RMS tumors tested, regardless of histological features, expressed MyoD1 and MRF4 transcripts. Expression of the myogenin gene was detectable in all alveolar RMS (n=8), while myogenin transcripts were expressed in 5/8 embryonal RMS. Trace levels of Myf5 transcripts were visible in all alveolar RMS and 7/8 embryonal RMS. In this series, two cases of bladder localization were observed, both showing expression of MyoD1 MRF4 and Myf5, while myogenin expression was detected in only one [21].

Although MyoD1 and myogenin are not distinctive of tumor subsets, Kohsaka and collaborators discovered a recurrent somatic point mutation (Leu122Arg) in the myogenic transcription factor, MYOD1, in a distinctive subset of ERMS with poor outcomes [50].

2.1.8. Patched1 (Hedgehog Pathway)

Ptch1 is a member of the patched family of genes and it is encoded by the *ptch1* gene. Ptch1 is the receptor for a molecule responsible of the formation of embryonic structures and tumorigenesis, called sonic hedgehog (Shh). *Ptch1* gene contains 23 exons and it is localized on chromosome 9q 22.3. Mutations of this gene are associated with transitional cell carcinoma of the bladder, esophageal squamous cell carcinoma, Gorlin's syndrome, and nevoid basal cell carcinoma syndrome.

The hedgehog (Hh) pathway is a signaling cascade with a nonredundant function in the control of many developmental processes, also implicated in formation and progression of various cancers including RMS. Patched1 (Ptch1) together with smoothened (Smo) forms a receptor complex for the Hh pathway. Distinct mutations in key components of the Hh signaling cascade, for example mutations in Ptch1, have been identified in human cancers that result in pathological activation of the Hh pathway. In RMS, aberrant activation of the Hh cascade has been detected in primary specimens and has been attributed to genetic inactivation of Ptch1 or of suppressor of fused (SUFU) (like Ptch1, a negative regulator of Hh signaling), or to Gli1 amplification [51-53]. RMS resembling the embryonal subtype has been shown to develop in Ptch1 heterozygous mice and overexpression of Ptch1 and Gli1 mRNA has been reported in RMS [35]. Small molecule inhibitors of the Hh pathway have recently been shown to exhibit antitumor activity, suggesting the importance to identify subgroups of patients that harbor constitutively elevated Hh signaling and therefore susceptible to pathway inhibition.

The naevoid basal cell carcinoma syndrome (NBCCS) is characterized by developmental defects and a predisposition to the development of certain tumours, such as basal cell carcinoma, medulloblastoma and meningioma, and potentially fetal rhabdomyomas and embryonal rhabdomyosarcomas. It is caused by mutations in the hedgehog receptor *ptch* gene. Tostar and collaborators analysed *ptch* status in an

NBCCS patient with fetal rhabdomyoma and investigated whether deregulation of hedgehog signaling, as shown by altered expression of hedgehog pathway components and/or genetic imbalances, is a general finding in sporadic rhabdomyomas and rhabdomyosarcomas [35]. The NBCCS patient had a novel *ptch* germ-line mutation, 1370insT, and developed a fetal rhabdomyoma that harboured a 30 bp in-frame deletion in the second allele, resulting in homozygous inactivation of *ptch*. Sporadic rhabdomyomas and rhabdomyosarcomas showed overexpression of *ptch* (43/43) and *Gli1* (41/43) mRNA, as determined by *in situ* hybridization, indicating ongoing active hedgehog signalling. Immunohistochemical staining revealed a subgroup of fetal rhabdomyomas and embryonal rhabdomyosarcomas (12/34) lacking ptch immunoreactivity. Four of nine informative fetal rhabdomyomas and embryonal rhabdomyosarcomas showed loss of heterozygosity (LOH) in the *ptch* region with two of these (one fetal rhabdomyoma and one embryonal rhabdomyosarcoma) also showing LOH in the *SUFU* region. These findings suggest that haploinsufficiency for the two tumour suppressor genes *ptch* and *SUFU*, which are both active in the same signalling pathway, may be important for tumour development. Based on these results, the authors propose that the pathogenesis of rhabdomyoblastic tumours, particularly fetal rhabdomyomas and embryonal rhabdomyosarcomas, involves deregulation of the hedgehog signalling pathway [35].

Zibat and coworkers reported that marker genes of active Hh signaling, such as Patched1 (Ptch1), Gli1, Gli3 and Myf5, are expressed in E-RMS and fusion gene-negative A-RMS at significantly higher levels compared with fusion gene-positive A-RMS in two distinct cohorts of RMS patients [8]. Consistently, Gli1 expression correlates with Ptch1 expression in E-RMS and fusion gene-negative A-RMS, but not in fusion gene-positive A-RMS. In addition, expression levels of MyoD1 are significantly lower in E-RMS and fusion gene-negative A-RMS, pointing to an inverse association of Hh activation and early muscle differentiation. This study showed that high expression of Ptch1 or low MyoD1 expression significantly correlate with reduced cumulative survival in fusion gene-negative RMS and that activation of the Hh pathway in the group of fusion gene-negative tumors is associated with more aggressive tumors [8]. These findings have clinical relevance and important implications for molecular targeted therapies, such as small molecule Hh inhibitors.

2.1.9. Gli1, Gli2, Gli3

Gli1, Gli2 and Gli3 are the three members of the Gli family and are transcriptional factors. They have DNA zinc–finger domains and a characteristic 18 aminoacid region. Gli (glyoma associated oncogene) contain five zinc-finger domains, and are localized on chromosome 12q13.3 and 12q14.1. They are expressed in embryonal carcinoma cells. Gli3 is expressed as an 8.5kb mRNA in tissues such as testis, myometrium, placenta and lung. It maps on chromosome 7p13. Gli1 is associated to glyoma, Gli3 is not related to glyoma or other forms of neoplasia.

Gli3 is essential for Gli1 expression in the somites during muscle formation [54], however no correlation between RNA expression levels of these genes in the RMS samples was found. This finding suggests that Gli1 expression depends on cellular Gli3 levels predominantly in a developmental context with a higher level of non-redundancy under these conditions.

An inverse association between Gli1 and MyoD1 expression has been reported. This is consistent with previous studies, showing that the Hh pathway negatively regulates myogenesis and early muscle differentiation in muscle progenitor cells [54], and that Gli1 directly suppresses MyoD-mediated transcriptional activation and thus inhibits myoblast differentiation [55].

Data reported by Zibat [8] showed that in general E-RMS and fusion gene-negative A-RMS highly express Gli1, Gli3, Ptch1 and Myf5, whereas fusion gene-positive A-RMS do not. Thus, the authors identified subgroups of RMS cases that exhibited high Hh pathway activity, that is, E-RMS and fusion gene-negative A-RMS. Coregulation of individual genes was detected; Gli1 correlated with Ptch1 expression, when the overall RMS sample set was analyzed. When RMS subgroups were assessed, this correlation was detected in E-RMS and in fusion gene-negative A-RMS. Ptch1, Gli3, Myf5 and MyoD1 were identified as class predictors with acceptable or excellent discrimination for fusion gene-negative versus gene-positive cases [8].

2.1.10. Myf5

Myf5 (myogenic factor 5) is a protein-coding gene, occupying, together with MYOG and MyoD1, a muscle-specific gene promoter core region during myogenesis. It acts as a transcriptional activator, promoting transcription of muscle-specific target genes, playing a role in muscle differentiation. It induces fibroblasts to differentiate into myoblasts. Myf5 gene has been mapped to human chromosome 12 by Braun and collaborators [56]. Cupelli and coworkers mapped the Myf5/Myf6 gene cluster to 12q21 between D12S350 and D12S106, the 2 genes lying within 6.5 kb of each other [57].

An analysis by McDermott and colleagues identifies Myf5 as an excellent class predictor for RMS based on the fusion gene status or on histology. Myf5 has been shown to be a downstream target of Gli1 during muscle development, at least in the somite epaxial muscle progenitors in mice. In addition, its expression is dependent on Gli3 activity during embryonic muscle formation [54].

Myf5 overexpression was also recently reported in zebrafish model of E-RMS (58) underscoring the relevance of Myf5 in these tumors. Importantly, all but one A-RMS and E-RMS cases were correctly classified by means of Myf5 expression. This set of data confirms in an independent cohort of RMS patient that Myf5 serves as an excellent class predictor for RMS [8].

2.1.11. The RAS Family of Proteins

The acronym RAS indicates a group of three, highly related proteins, namely HRAS, KRAS and NRAS. They all are widely expressed in tissues and organs, and belong to the small GTPase class of proteins; their role is the transmission of signals inside the cell, mediated by conformational changes caused by GTP hydrolysis to form GDP. Their name derives from “rat sarcoma”, that indicates how and where the

first two members of the family (HRAS and KRAS) were first discovered [28] The identification of the third member occurred later and it was named NRAS upon its isolation inside human neuroblastoma cells [29]. In general terms, when appropriate signals arrive to the cell, the role of RAS proteins is to activate themselves through the GTP-GDP transition, and consequently activate a cascade of intracellular events (such as activation of MAP kinases) (59) that ultimately promote cell proliferation. Mutations permanently activating RAS proteins had been long associated to neoplastic transformation [30, 31]; indeed, RAS proteins control, either directly or indirectly, crucial cell metabolic pathways, including actin function, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. At the molecular level, RAS proteins (and in general, all small GTPases) exist in two alternative forms. When they are bound to GTP, they are in the active state; when they host GDP, they are not active. Thus, the switch between active and inactive state is ultimately related to the phosphorylation state of the bound guanine. The activation of RAS members is mediated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs); the balance between GEF and GAP activity determines the guanine nucleotide status of RAS, and consequently its activation state.

HRAS maps to position 11p15.5. It is involved in a wide number of normal cellular processes, and its upregulation may be sometimes physiological, as during rat liver regeneration [60]. Instead, its abnormal deregulation is usually associated with cancer formation or development. Mutations at position 61 were found in urinary tract tumors [33]. G13R mutation showed constitutive activation of the MAPK and PI3K signaling pathways. Taken together, these data indicate that codons 12/13 and 61 are 'hotspots' for mutations that activate their malignant transforming properties in all RAS members. In particular, for HRAS gene, gly12val is frequently associated with bladder carcinoma, [61]. An analysis of previous literature allowed to conclude that HRAS mutations in codons 12/13 are present in 2 to 3% of all types of tumors, while mutations in codon 61 are present in 1.4% of tumors, being almost all of them malignant. Mutations in HRAS are also responsible of Costello syndrome [62], a multiple congenital anomaly and mental retardation syndrome.

KRAS maps to position 12p12.1. Two isoforms are described: KRASA and KRASB, derived by an alternative splicing of the KRAS gene at exon 5. Exon 6 contains the C-terminal region in KRASB, whereas it encodes the 3'-UTR of KRASA. The differing C-terminal regions of these isoforms are subjected to different post-translational modifications causing important functional effects and leading to alternative trafficking pathways and protein localization [63]. Similarly to HRAS, also deregulated KRAS is frequently involved in neoplastic transformation; in fact, 17 to 25% of all human tumors harbor an activating KRAS mutation [64]. In addition to the above mentioned positions 12/13 and 61, additional critical regions for KRAS oncogenic activation include codons 59 and 63 [65]. These activating mutations cause the accumulation of the RAS-GTP (active) form inside the cell by impairing its intrinsic GTPase activity and conferring resistance to GTPase activating proteins [66]. Expression of KRAS variant G12D in the colonic epithelium of a mouse model stimulates cellular hyperproliferation in a Mek-dependent manner [67]. KRAS function also in controlling intracellular glucose metabolism; a mutated KRAS is able to upregulate GLUT1, allowing the cells to increase glucose uptake and glycolysis and to survive in low-glucose conditions, suggesting that glucose deprivation might drive the acquisition of KRAS pathway mutations in human tumors [68]. In man, KRAS mutation were identified in carcinomas and sarcomas [69], as in many different tumors and Costello syndrome [70].

NRAS maps to position 1p13.2. As for the other family members, also NRAS plays central roles in cell proliferation and its uncontrolled activation may lead to neoplastic transformation. Mutations in position 17 (in all RAS members), but particularly ser17asn (S17N) produces dominant-inhibitory proteins with higher affinities for exchange factors than normal RAS; in this situation, RAS proteins cannot interact with downstream effectors and therefore form unproductive complexes, preventing activation of endogenous RAS [71]. NRAS role in human cancer had been identified for many tumors, including sarcoma cell lines [72].

Dysregulation of the RAS cascade is involved in the pathogenesis and/or progression of embryonal RMS and this pathway (including *FGFR4*, *RAS*, *NF1*, and *PIK3CA genes*) is mutationally activated in at least 45% of P-F Negative tumors [27]. Constitutive RAS-mediated signal flow downregulates myogenic factors such as MyoD1 and myogenin, thus inhibiting myogenic differentiation and differentiation-associated apoptosis [73]. Moreover, experimental studies suggested that that RAS activation is sufficient to initiate tumorigenesis *in vivo* in embryonal RMS [74].

Martinelli and collaborators documented that somatic missense mutations in RAS genes represent a recurrent event in pediatric embryonal RMS, accounting for approximately one-fourth of cases [16]. These authors investigated the prevalence of HRAS, KRAS and NRAS mutations in 31 pediatric primary embryonal RMSs. They identified HRAS and NRAS lesions in 3.2% and 19.4% of tumors, respectively; no mutation was observed to affect the KRAS gene. All mutations were missense changes, affected the glutamine residue at codon 61, and introduced a positively charged aminoacid residue. No defects of RAS gene at codons 12 and 13, as reported by others, were found. Genotyping of available DNAs from circulating leukocytes of 3 patients with a mutation-positive RMS, demonstrated the absence of the mutated allele in each tested case, providing evidence that the mutations were somatic events acquired in cancer cells. Although genitourinary bladder/prostate localization is reported in this study, no further relationship is available between findings and the anatomical site of occurrence, due to the limited number of cases.

RAS gene involvement often is specific for different tumor types. HRAS mutations are most frequent in bladder and kidney carcinoma, while KRAS mutations predominate in colorectal, pancreatic, endometrial, lung, and cervical cancers, and NRAS lesions are more common in melanoma, liver carcinoma, lymphoid and myeloid malignancies. The aforementioned study [16] documented that, among the RAS family, NRAS is most commonly affected in embryonal RMS.

In this study patients carrying a RAS or a PTPN11 mutation had a higher overall survival probability (88% vs. 57%). The present and available data support the idea that dysregulation of RAS signaling plays a key role in RMS development. The analysis of a more numerous series is necessary to establish definitely whether these gene lesions actually have a significant impact on embryonal RMS pathogenesis and/or progression. In these patients, novel and innovative therapies must be attempted and a more detailed understanding of the molecular mechanisms and underlying gene defects is required to achieve future progress in novel target-specific therapeutic approaches [16].

2.1.12. NF1

The NF1 gene encodes neurofibromin, a cytoplasmic protein that is predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. It is a multidomain molecule, which regulates several intracellular processes (RAS-cAMP pathway, ERK/MAP kinase cascade, cytoskeletal assembly) [75], encoded by a gene having a complex organization [75, 76]. The role of NF1 protein as a tumor suppressor gene has been repeatedly demonstrated, along with its role as a negative regulator of RAS [77, 78]. The frequency on NF1 mutations in human malignancies is extremely high, with approximately 50% of all NF1 patients presenting with novel mutations [79]; indeed, less than 100 constitutional mutations had been reported so far [80].

Neurofibromatosis type 1 (NF1) is a common autosomal dominant syndrome affecting 1/3,500 individuals worldwide [1]. Clinical features of the disease are pigmentary anomalies, skinfold freckling and Lisch nodules [2], learning disabilities, orthopedic problems, and benign and malignant tumors [3, 4]. NF1 patients develop neural tumors more frequently than normal population. Rhabdomyosarcoma (RMS), Wilms tumor and acute and chronic leukemia have also been reported in association with NF1. RMS is the most frequent nonneurogenic sarcoma occurring in NF1 after malignant peripheral nerve sheath tumor. A population based study estimated that 1.5% of NF1 patients develop rhabdomyosarcoma [81]. The urogenital tract is the most commonly affected site of RMS in NF1 patients [82].

An inherited germline mutation at one allele, followed by a somatic inactivation of the second allele, seems to be related to the NF1 inactivation. The constitutional *NF1* mutations of NF1 patients with tumors are unclear [83]. An origin of NF1-associated RMS from neural crest cells rather than from mesoderm has been suggested by studies on mouse models, implying that they are part of the NF1 neurocristopathy [84, 85]. Hartley *et al.* reviewed case records of 157 children with soft tissue sarcoma in the Manchester Children's Tumour Registry between 1954 and 1983. During 124 interviews with families, four of these children were identified as having NF1. All four were boys, very young at diagnosis, and had rhabdomyosarcomas of the bladder or prostate. This finding suggested that NF1 may be associated with soft tissue sarcoma in children more commonly than previously thought and that affected children may be at increased risk of developing further malignancies [86]. More recently, two other studies mentioned NF1 patients with RMS of the bladder and prostate, respectively [82, 85].

Oguzkan and coworkers presented two NF1 cases with RMS and discussed their analysis for loss of heterozygosity (LOH) in the *NF1* gene locus [82]. They found all microsatellite markers showing hemizygosity in both blood and tissue samples of one patient and identified a large deletion in the *NF1* gene associated with the NF-NS patient with RMS. Neither LOH nor deletion was found in the other patient. In cases of concomitant RMS and NF1, the tumor predominantly affects urogenital organs, most often during early childhood. Due to the rarity of both RMS and NF1, it has been difficult to describe the prevalence, clinical characteristics and outcomes of such an association. The Intergroup Rhabdomyosarcoma Study IV (IRGS-IV) reported an incidence of NF1 in children diagnosed with RMS as 0.5%. This incidence is higher than that in the general population and an apparent predominance of bladder or prostate as the primary site of RMS in NF1 patients has also been noted. The *NF1* gene is believed to somehow promote the development of a specific type of RMS in the urogenital system [10, 13, 87, 25, 26]. NF1 cases reported in this study presented with RMS of the bladder. One patient featured two of the NF1 diagnostic criteria: *café au lait* spots and inguinal freckling. In addition, the patient had Noonan syndrome (NS) traits. Patients with characteristics of both NF and NS have been described in the literature [15, 41-47]. Investigation of some families has shown that NF1 and NS segregate as independent autosomal dominant traits. NS is not linked to the *NF1* locus in families that do not manifest NF1 features. A large deletion was found in the NF1 gene associated with a NF-NS patient with RMS. Although *NF1* gene mutations have been reported in NF-NS patients, no relationship has been demonstrated with mutations and the NF-NS phenotype. Large deletions in the NF1 gene are associated with tumor development at early ages and are associated with the abnormal facial appearance observed in some patients. The NF1 gene locus is distinct from the genetic changes described in alveolar and embryonal rhabdomyosarcoma; however, a large deletion in the *NF1* gene has been reported in two NF1 patients with RMS. The molecular pathogenesis of neural and nonneural tumors associated with NF1 is largely unknown and further studies will be needed to determine critical pathways in NF1-linked tumorigenesis, which may involve tumor suppressor genes and oncogenes or modifier genes that can affect the NF1 phenotype. Identification of genetic events in NF1 tumors may provide clues about etiology, therapeutic targets and risk of malignant transformation [82, 85].

Several studies discuss the involvement of p53 tumor suppressor gene in the pathogenesis of RMS in NF1 patients and various findings point to mutations hyperactivating the oncogene RAS (e.g., loss of *NF1* gene) cooperating directly with p53 mutations in the malignant transformation [85, 88, 89]. Inactivation of *NF1* and/or *TP53* has been shown in NF1 associated malignancies. Mutations which activate the proto-oncogene *RAS*, such as loss of *NF1*, may cooperate with inactivating mutations of the *p53* tumour suppressor gene during malignant transformation, as suggested by several models of tumorigenesis. Deletions of the entire *NF1* gene have been found to be over represented in the germline of NF1 patients with malignant peripheral nerve sheath tumours [88].

It is currently unclear whether constitutional deletion of other tumor suppressor genes is involved in these cases or whether the deletion in itself predisposes to a second hit in a second copy of the *NF1* or another gene, such as TP53 [89]. Lampe and collaborators detected a heterozygous microdeletion of their entire *NF1* gene in three familial cases of a mother and two children, one of which developed a prostate RMS. This is the first reported case of a rhabdomyosarcoma in a child with a germline deletion of the entire *NF1* locus. Since such microdeletions have been found in up to 10% of all NF1 cases, FISH studies in children with this condition, who are facially dysmorphic and have significant learning difficulties, should be considered, although there is no evidence that formal screening for malignancies associated with NF1 improves outcome [85].

2.1.13. PTPN11

Tyrosine-protein phosphatase non-receptor type 11 (PTPN11) is also known as protein-tyrosine phosphatase 1D (PTP-1D), protein-tyrosine phosphatase 2C (PTP-2C) or tyrosine phosphatase SHP2 (SHP2). It is encoded by the *PTPN11* gene, mapping to chromosome location 12q24.13. The protein-phosphatases are a heterogeneous group of enzymes whose function is to regulate the phosphorylation state of target proteins; in this perspective, the activity of phosphatases is sometimes considered antagonistic to that of kinases. Mammalian protein-tyrosine phosphatases usually are either transmembrane receptor containing linked cytoplasmic catalytic domains, or intracellular phosphatases; PTPN11 is an intracellular enzyme [90], that is widely expressed in human tissues and is particularly abundant in heart, brain, and skeletal muscle. It is required for RAS-ERK MAP kinase (MAPK) cascade activation (see also the section on RAS genes) and for this reason the conditions linked to its mutation are part of the RASopathies, a group of developmental syndromes caused by germline mutations in genes that alter the RAS subfamily and Mitogen-activated protein kinases (MAPK) that control signal transduction. This protein is the main cause of some human syndromes and diseases.

Activating mutations of the phosphatase activity of PTPN11 had been identified also in neuroblastoma, melanoma, acute myeloid leukemia, breast cancer, lung cancer, colorectal cancer [91] and in the *H. pilori*-mediated gastric cancer [92]. Taken together, these data support the hypothesis that PTPN11 is a proto-oncogene whose malfunction might increase cell proliferation; however, a recent report suggests that the same gene may also be considered a tumor suppressor, at least in some situations, such as in hepatocellular carcinoma [93].

Although a study on a small RMS cohort PTPN11 mutational screening did not reveal any cancer-associated mutation [94], a PTPN11 lesion was documented in a 2-year-old girl with stage IV embryonal RMS [95]. and in a 2-year-old boy with a Group II embryonal RMS, who did not show any clinical features suggestive of Noonan syndrome (NS), and in which the somatic origin of the defect was documented by mutational analysis of genomic DNA from normal tissue, demonstrating absence of the mutated allele [16].

Martinelli and colleagues investigated the impact of RAS and PTPN11 mutations on overall survival (OS) of patients with embryonal RMS. Their findings showed that patients carrying a *RAS/PTPN11* gene mutation tended to have a better outcome, with a higher OS probability compared to patients without mutations (88% vs. 57%), although statistical significance was not documented due to the relatively small size of the study cohort. Although this study gives further evidence that PTPN11 defects do not play a key-role in embryonal RMS, the possible involvement of other transducers in the signaling pathway cannot be ruled out [16].

2.1.14. CTNNB1

The CTNNB1 gene encodes β-catenin, a protein regulating the coordination of cell–cell adhesion and gene transcription in humans.. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer [96, 97]. Mutations in this signaling molecule have been described in rhabdomyosarcoma and recently were found in three P-F negative tumors (3%), one of which occurred in a fusion-negative alveolar RMS tumor [27].

2.1.15. FBXW7

This gene encodes a member of the F-box protein family, which is characterized by an approximately 40 amino acid long motif, the F-box. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination [98]. This gene has been recently implicated in RMS, showing a 7.4% frequency of somatic mutations in P-F negative RMS [27].

2.1.16. PDGFRA

This gene encodes a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family. These growth factors are mitogens for cells of mesenchymal origin. The identity of the growth factor bound to a receptor monomer determines whether the functional receptor is a homodimer or a heterodimer, composed of both platelet-derived growth factor receptor alpha and beta polypeptides.

As other tyrosine kinase genes, *PDGFRA* is recurrently mutated in P-F negative RMS (1.4%) [27].

2.1.17. ERBB2/4

ERBB2, a known proto-oncogene, is located at the long arm of human chromosome 17 (17q12). The ErbB family is composed of four plasma membrane-bound receptor tyrosine kinases, the other members being epidermal growth factor receptor, erbB-3 (neuregulin-binding; lacks kinase domain), and erbB-4. All four contain an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain that can interact with a multitude of signaling molecules and exhibit both ligand-dependent and ligand-independent activity. ErbB2/HER-2 can form heterodimers with any of the other three receptors and is considered to be the preferred dimerisation partner of the other ErbB receptors. Dimerisation results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and initiates a variety of signaling pathways [99]. Recurrent

mutations of this genes have been reported in 1.4% of P-F negative RMS [27].

2.1.18. BCOR

BCOR is a transcriptional repressor that interacts with both class I and II histone deacetylases. Somatic mutations in *BCOR* have been described in different pediatric tumors, including acute myeloid leukemia, retinoblastoma and medulloblastoma. A novel finding is the discovery of recurrent mutations in *BCOR* affecting 7.4% of PFN tumors. The discovery of its recurrent alteration in rhabdomyosarcoma reinforces its role as a potential therapeutic target [27].

3. UROTHELIAL NEOPLASMS

In the first 2 decades of life, urothelial carcinoma of the bladder is rare.. Since 1950, less than 100 cases have been reported in patients less than 30 years, and even less in children and adolescents, including patients with a neurogenic bladder who has undergone bladder augmentation, who have an increased risk for bladder cancer [100].. Most of the series are small and describe these tumors as being characteristically superficial and low grade (I-II). The etiology, invasive potential, treatment and surveillance of high-risk superficial transitional cell carcinoma of the bladder in children are not well established due to its rarity.

Papillary urothelial neoplasm of low malignant potential (PUNLMP) has been recently defined in the WHO 2004 classification systems for bladder tumours. According to the definition, PUNLMP is a noninvasive papillary urothelial neoplasm composed of multilayered urothelium with minimal or absent cytologic atypia, and is thought to present low risk of progression [101]. PUNLMP usually affects adults older than 20 years and is extremely rare in children younger than 10 years. A case of a 9-year-old child with bladder PUNLMP has been reported by Gao and coworkers, while Stanton and collaborators found one PULNMP and two LGPUC, low-grade papillary urothelial carcinoma [102, 103] From 65 children with polyps or masses in the urinary bladder only 7 urothelial tumors were identified, including 1 low-grade papillary urothelial carcinoma, by Huppmann and collaborators [2]. Nonetheless, in the Surveillance, Epidemiology and End Results (SEER) database (1973 to 2003), of 140 bladder tumours affecting patients younger than 18 years old, 50.7% were diagnosed as PUNLMP. Thus, PUNLMP is regarded as the most common bladder tumour within this age group [103, 104]. Early symptom is almost invariably gross hematuria, while in some cases the child may present with signs of urinary infection [105].

The mainstay of diagnosis is ultrasound followed by cystoscopy. Most pediatric bladder carcinomas are successfully treated by transurethral resection, and endoscopic resection proved effective in most reports. Because of low grade and superficial spreading, prognosis is good and tumor recurrence and deaths are infrequent in the first 2 decades of life, although some cases of higher grade have been reported [6, 100, 101, 106, 107].

Squamous cell carcinoma and more aggressive carcinomas, however, have been observed and may require a more aggressive surgical approach and chemotherapy [102, 106, 108, 109]. Follow up is based on periodic clinical and ultrasound evaluation. Urine cytological examination is ineffective. Periodic cystoscopy is indicated only after clinical or ultrasonographic suspicion of recurrence [6].

3.1. Genes in Urothelial Neoplasms

Urothelial neoplasms in children and young adult patients are rare. Although data regarding these tumors in children are limited, they seem to show a different behavior, with a lower rate of recurrence and progression than those of adults. Molecular data on these tumors in the pediatric age are very scarce. A study on molecular characteristics of urothelial neoplasms from patients under age 30 years was performed by Williamson and colleagues; the Authors used UroVysion fluorescence in situ hybridization (to analyze chromosomes 3, 7, 17, and 9p21 status) and DNA mutational analysis for the FGFR3 and TP53 genes [158]. In patients aged 6-26 years, seventeen tumors were identified, including low-grade papillary urothelial carcinoma (n=10), high-grade papillary urothelial carcinoma (n=5), urothelial papilloma (n=1), and papillary urothelial neoplasm of low malignant potential (n=1). In this study no tumor showed mutations of either FGFR3 or TP53. Under the age of 19 years, no tumor showed molecular abnormalities, supporting the idea that mutations of the FGFR3 and TP53 genes are rare or absent in urothelial neoplasms of young patients. These findings supported the hypothesis that an age of 19-20 years separates distinct molecular pathways of urothelial carcinogenesis [110] (Table **1**).

Similarly, a study on 14 patients aged 4 to 19 years, found no mutations in FGFR3, deletions of chromosome arms 9p, 9q or 17p, MSI or MRP loss (mismatch repair proteins (MRPs) hMSH2, hMLH1, and hMSH6), or HPV positivity in any of the patients Three cases showed chromosome alterations in CGH analyses, urothelial dedifferentiation with CK20 overexpression, or aneuploidy, and one TP53 mutation with TP53 overexpression was found. Urothelial neoplasms in patients younger than 20 years are mostly low grade and are associated with a favourable clinical outcome. Genetic alterations frequently seen in older adults are extremely rare in young patients. Urothelial neoplasms in children and young adults appear to be biologically distinct and lack genetic instability in most cases [111] (Table **1**).

3.1.1. p53

A significant association was found between urothelial tumors and a mutation in the p53 gene, of which a molecular and functional characterization has been illustrated above. In adults, this mutation is observed in bladder cancers with a significant genetic difference (p53 overexpression) between PUNLMP and papillary low grade TCC. Only 10% of PUNLMPs have been reported to be P53 positive, suggesting that p53 mutation does not play a role in the development of transitional tumors. Conversely, 75% of the papillary low grade TCC tumors revealed p53 overexpression, showing a crucial role for p53 mutation in further tumor progression from PUNLMP to low grade TCC [112].

A study by Linn and colleagues, from 73 tumors in patients younger than 30 years, found 81% of them to be low grade pTa tumors. Alterations in the p53 gene can be found in the majority of tumors, suggesting that the modification of

Table 1. Urothelial neoplasms. Expression comparison for most common genes mutated in adult BC.

Gene or Alteration	Expression in Children	Expression in Adults
FGFR3	Normal (111)	Overexpression
TP53	Overexpression or normal	Overexpression
CK20	Overexpression/aneuploidy/normal (111)	Overexpression

this gene may not correlate with poor prognosis tumors [113].

In a reported case by Gao and collaborators, the immunohistochemical staining showed that tumour cells were p53 weak positive, CK20 negative and a Ki-67 proliferative index below 1%. The UroVysion assay reported that chromosome 3, 7, 17 and 9 were normal [103].

3.1.2. p16/Ink4

p16/Ink4, also known as cyclin-dependent kinase inhibitor 2A, multiple tumor suppressor 1 (CDKN2A) is an oncosuppressor protein encoded by the *CDKN2A* gene, mapping to position 9p21.3 of the human genome. Through alternative splicing, CDKN2A may generate several mRNA transcripts; of them, at least three encode polypeptides [114]. It is a cell cycle regulator able to slow down the cell cycle progression from the G1 to the S phase. p16 acts mainly as an inhibitor of cyclin-dependent kinases such as CDK4 and CDK6 [115], which in turn phosphorylate retinoblastoma (RB) protein, one of the key proteins for the exit from G1 [116]; moreover, the inhibition of these two kinases blocks their interaction with cyclin D, that indirectly controls pivotal transcription factors, such as E2F1. The gene also contains another open reading frame coding for the ARF protein [116]. Consequently, another oncosuppression route derives by the ability of p19/ARF to bind MDM2, which remains locked inside the nucleolus instead of moving to the cytoplasm; thus, MDM2 cannot induce p53 degradation, and this enhances p53 function as a pro-apoptotic protein [117]. p16 may also activate a G2 cell cycle arrest thanks to its ability to inhibiting the activation of cyclin B1/CDC2 complexes [118]. While the overexpression of p16 is associated to cell senescence, the deletion or mutation of CDKN2A is frequently associated to neoplastic transformation, thus this gene is classified as an oncosuppressor. Indeed, p16 function impairing is evident in several cancer lines, including bladder cancer [119]. On the contrary, it has been reported that ARF degradation is inhibited in cancer cells [120], suggesting that p16 and p19/ARF may act in complementary, inversely related cell cycle control pathways.

p16/Ink4 was identified in four pediatric patients so far, aged 10-18, all males; they were collectively described in the work by Wild and collaborators [111]. Two of the patients had pTa low grade bladder cancer, one had a pTa high grade and the last a PUNLMP. Alterations in protein presence were assessed cytologically, using multicolour fluorescence *in situ* hybridization (FISH) using the UroVysion™ probe set; the presence of a single signal in at least 7/50 cells (>14%) was considered positive for the deletion of 9p21 region containing p16.

3.1.3. CK20 (Cytokeratin 20)

CK20 is a type of intermediate filament protein encoded by the *KRT20* gene and maps on chromosome 17q21.2. CK20 is expressed in gastric and intestinal mucosa and in the superficial - occasionally intermediate - cells of the normal urothelium of the bladder. Aberrant CK20 expression has been documented in urothelial carcinoma and has proved useful as an ancillary diagnostic aid for urinary bladder tumor in adults. There is a concordant expression of CK20 in the primary cancer and its matched lymph node metastasis and CK20 expression in metastatic urothelial carcinoma duplicates the expression of the same markers in the primary tumors. Therefore, immunohistochemical staining of metastatic tumors CK20 may be helpful for differential diagnosis in metastatic tumor of ambiguous origin [121]. Wild *et al.* analysed urothelial tumours from 14 patients aged 4 to 19 years, including immunohistochemistry for TP53, Ki-67, CK20 and the mismatch repair proteins (MRPs) hMSH2, hMLH1, and hMSH6, FGFR3 and TP53 mutation screening, comparative genomic hybridization (CGH), UroVysion FISH analysis, polymerase chain reaction for human papillomavirus (HPV), microsatellite analysis using the NIH consensus panel for detection of microsatellite instability (MSI) and six markers for loss of heterozygosity on chromosome arms 9p, 9q, and 17p. Three cases showed chromosome alterations in CGH analyses, or aneuploidy, and one showed TP53 mutation with TP53 overexpression. CK20 overexpression was found in the full thickness of urothelium only in two males of 18 and 19 years with pTa, low grade tumors. Genetic alterations frequently seen in older adults are extremely rare in young patients. Urothelial neoplasms in children and young adults appear to be biologically distinct and lack genetic instability in most cases [111].

4. MALIGNANT RHABDOID TUMOR (MRT)

Malignant rhabdoid tumour is a highly aggressive malignancy of early childhood. Approximately 80% of cases occur under 2 years of age. The median age at diagnosis is 11 months, ranging between the ages of 6 and 12 months [122].

The tumor can be classified based on its location as renal and extrarenal. Spinal cord and central nervous system (CNS) are the most common extrarenal lesions, usually referred to as atypical teratoid/ rhabdoid tumors (AT/RTs), Such tumours may arise in almost any site, including the extremities, brain, and heart. Extrarenal rhabdoid tumours generally have a histological appearance similar to renal rhabdoid tumours. Debate has been focused on whether AT/RTs are the same as rhabdoid tumours of the kidney The recent recognition that both CNS AT/RTs and MRTs have

deletions of the INI1 gene in chromosome 22 indicates that rhabdoid tumours of the kidney and brain are identical or closely related entities. Rhabdoid tumours at both locations possess similar histologic, clinical, and demographic features. Most patients with renal MRT present with haematuria and may develop hypercalcaemia secondary to elevated parathyroid hormone levels [123]. Pathological features of MRT of the bladder are similar to its renal counterpart, which is prone to necrosis and haemorrhage. MRT shows immunoreactivity to vimentin (which may show paranuclear staining), cytokeratin, epithelial membrane antigen, desmin, and neurofilament. 80% of patients develop metastases to the lungs and, less frequently, to the liver, abdomen, brain, lymph nodes, or skeleton [124]. Prognosis is poor, with mortality occurring within 12 months of diagnosis and scarce data on 5-year survival. To our knowledge, 9 cases of malignant rhabdoid tumour of the bladder have only been reported, mainly focusing on its pathological aspects. Most patients were within 6 years of age, while in 2 patients the tumor occurred at 9 and 17 years [125-127]. Malignant rhabdoid tumour has the worst prognosis among malignant tumours of the urinary tract in infancy and childhood. The treatment approach is different for rhabdoid tumour than for other bladder tumours, usually involving partial cystectomy and chemotherapy. The prognosis of children with malignant rhabdoid tumour is poor, therefore, making an early and accurate diagnosis is essential [128]. Genes important for MRT in childhood are illustrated in (Table **2**).

4.1. Genes in MRT

4.1.1. SMARCB1/INI1

The unifying features of both extracranial MRT and atypical teratoid/rhabdoid tumors are the exon deletions/mutations of the SMARCB1 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1) gene located in 22q11.23 and resulting in loss of SMARCB1/INI1 (integrase interactor 1) protein expression by immunohistochemistry [3].

The INI1 gene is a tumour suppressor gene that alters the conformation of the DNA histone complex so that transcription factors have access to the target genes. INI1 is a nuclear antigen that is normally expressed in nucleated cells. The diagnostic feature of a rhabdoid tumour is the loss of normal nuclear INI1 expression. The common genetic basis for rhabdoid tumours is a deletion and/or mutation of the INI1 gene, leading to inactivation of the tumour suppressor gene SMARCB1, although some cases it may lack this mutation [129].

Patients with rhabdoid tumours harbor homozygous deletions or mutations of the INI1 gene in chromosome band 22q11.2. Thus, molecular genetic analysis of the INI1 locus has clinical utility in a diagnostic setting. The diagnostic feature of a rhabdoid tumour is the loss of normal nuclear INI1 expression. Thus, a positive INI1 stain result will show loss of nuclear INI1 staining, as suggested in a case report and review. The cystectomy specimen of this patient exhibited loss of INI1 staining [128]. 98% of rhabdoid tumors demonstrate SMARCB1 loss and concomitant deletions or mutations of the SMARCB1 gene [130].

A recent report of a SMARCA4/BRG1 mutation in a family with rhabdoid tumor suggests that there is at least one other rhabdoid tumor locus, which may account for a small number of SMARCB1 positive neoplasms [131]. The INI1 gene mutation has also been reported to be associated with germline mutation and predisposition to familiar cancers, therefore evaluation for a germ line SMARCB1 alteration may greatly aid risk stratification and family planning [3]. Furthermore, the critical role of hSNF5/INI1 in RTs has been advocated by Bourdeaut and colleagues, and knowledge on its functions might lead to possibly more molecularly targeted treatment approach [127].

Although usually the tumor has a sporadic origin, as documented in a reported case in which patient's peripheral blood was negative for the deletions observed in the specimen [3], an association with a germline deletion or mutation of SMARCB1 has been estimated in as many as 35% of rhabdoid tumors. In the presence of a SMARCB1 abnormality, further genetic testing should be warranted to rule out a germline mutation. A history of schwannomatosis is also an indication for genetic testing, due to its possible association with a germline SMARCB1 mutation, and the increased risk of developing a rhabdoid tumor [131]. The median age of children with rhabdoid tumor and a germline mutation or deletion is lower than that of children with apparently sporadic disease. In the future, treatment approaches may differ based on the presence of a germline SMARCB1 alteration, as these patients are younger, and already predisposed to cancer.

Alterations in genes involved in chromatin remodeling, and particularly in genes encoding SWI/SNF subunits, as

Table 2. Genes in pediatric malignant rhabdoid tumor.

Gene or Alteration	Expression
SMARCB1/INI1	In malignant rhabdoid tumor and epithelioid sarcoma, frequent alteration of the SMARCB1/INI1 tumor-suppressor gene and the loss of its protein have been demonstrated, indicating that this molecule could be an effective target of these sarcomas [165]. Malignant rhabdoid tumors (MRTs) are rare pediatric malignancies characterized by clinically aggressive lesions that typically show loss of SMARCB1 expression [166].
	The common genetic basis for rhabdoid tumors is a deletion and/or mutation of the INI1 gene on chromosome 22 (22q11), inactivating the tumor suppressor gene SMARCB1 [129].
SMARCA4	Rare rhabdoid tumors have mutation in SMARCA4 [167].

SMARCB1, are increasingly being identified in a wide variety of cancers, raising the possibility that epigenetic dysregulation may be a central mechanism of oncogenesis. Near-uniform biallelic inactivating mutations in *SMARCB1* are a hallmark of RTs.

From a study on whole exome sequencing in 35 cases of MRT, several important considerations were raised. Based on the finding that *SMARCB1* is the sole gene recurrently mutated at high frequency in extremely aggressive and lethal RTs, and in some cases may be the only mutated gene, the authors cannot exclude the existence of mutations in noncoding portions of the genome or regulatory elements or in mutations in low coverage areas. Further, balanced translocations or inversions should be taken in consideration. Nonetheless, occult events could cooperate with *SMARCB1* loss. Another consideration raised by the authors is that the developmental stage/epigenetic state may serve a contributory role. During development, there is relative enrichment of minimally differentiated cell populations that have a high proliferative capacity. Consequently, it is possible that developmentally restricted or lineage-specific populations of cells characterized by a certain epigenetic state are particularly susceptible, such that mutation of a single chromatin remodeler can drive transformation. This is consistent with a mouse model a specificity in lineage effects has been found, which could explain why mutation of *SMARCB1* is largely restricted to RTs and a few other cancers [132].

Furthermore, contributions from germ line events are not to be excluded, although it seems unlikely that germ line alterations are essential for cancer formation driven by *SMARCB1* loss. Families with findings consistent with gonadal mosaicism predisposing to rhabdoid tumor have been reported by different authors. Sevenet and coworkers reported gonadal mosaicism and rhabdoid tumor in two families with multiple affected siblings and normal parents [133]. A case of gonadal mosaicism and SMARCB1 mutation has also been reported in a family with schwannomatosis [134]. Increased risk for rhabdoid tumor and schwannomatosis in the presence of germline SMARCB1 mutations should be taken into consideration when planning to have more children, warranting genetic counseling [131].

These findings from RTs demonstrate the role of mutations in the SWI/SNF chromatin remodeling complex as a potent drivers of cancer. Understanding their contributions to oncogenesis has the potential to achieve the development of targeted therapies for the wide variety of SWI/SNF mutant cancers.

5. INFLAMMATORY MYOFIBROBLASTIC TUMOR (IMT)

Inflammatory myofibroblastic tumor is a rare myofibroblastic spindle cell tumor, which occurs in different anatomic sites, including the urinary bladder. IMTs in the bladder localize at numerous sites [135], but most often at the dome [136]. Clinical presentation of IMT of the bladder may vary; symptoms may include dysuria, painless hematuria, pelvic pain or symptoms of urinary tract obstruction [137].

Tumors affecting this site usually appear as polypoid intraluminal or submucosal masses with or without extension into the perivesical fat [138], although a tough, diffuse lesion featuring uneven thickening of the bladder has been described [139]. Histologically, inflammatory myofibroblastic tumor is characterized by a proliferation of spindled cells arranged in cellular fascicles admixed with inflammatory cells including plasma cells, and lymphocytes. Similarly to other sites, in the urinary bladder these tumors show infiltrative growth, increased cellular density, cytologic atypia, mitotic activity, mucosal ulceration and necrosis, mimicking malignant spindle cell tumors such as leiomyosarcoma, sarcomatoid carcinoma and rhabdomyosarcoma. The vast majority of these tumors show a good prognosis, although occasional tendency to recurrence following surgical excision. IMTs have been successfully treated with steroids, nonsteroidal inflammatory drugs, radiotherapy and chemotherapy [140]. Nevertheless, the treatment of choice for IMT remains complete surgical excision.

5.1. Genes in IMT

Inflammatory myofibroblastic tumor shares immunophenotypic features with other malignant tumors, including the expression of cytokeratins, smooth muscle antigens and desmin. Positive immunohistochemistry staining for vimentin, alpha-smooth muscle actin and epithelial membrane antigen usually characterizes the tumor cells. On the other hand, staining for keratins (MNF116 and CK19), desmin, myoglobin, alpha-sarcomeric actin, glial fibrillary acidic protein, synaptophysin, S-100, chromogranin, CD68, MAC-387 and alpha-1 antitripsin are usually negative [141].

Because of its cytologic features and infiltrative nature, IMT of the bladder may be difficult to be distinguished histologically from other sarcomatous proliferations such as embryonal rhabdomyosarcoma. The morphologic and immunophenotypic similarities between this tumor and other malignant urinary bladder tumors can lead to a misdiagnosis, resulting in unnecessary radical surgery. Therefore, there is need for a more specific marker of inflammatory myofibroblastic tumor. Recently, anaplastic lymphoma kinase (ALK) gene translocations or ALK protein expression in IMT has been reported, especially in patients of relatively young ages. IMT in children and young adults often contain clonal cytogenetic rearrangements that activate the ALK receptor tyrosine kinase gene in chromosome band 2p23 [142]. There are only a few reports mentioning IMT of the bladder and series of patients in the pediatric age are even more limited [143]. However, ALK expression in IMT of the bladder has also been reported in children [144]. Genes important in IMT are reported in (Table **4**).

5.1.1. ALK

ALK is the gene coding for the anaplastic lymphoma kinase, also known as ALK tyrosine kinase receptor or CD246 (cluster of differentiation 246). Its location in the human genome is 2p23.2-23.1; molecularly, it shows greatest sequence similarity to the insulin receptor subfamily of kinases. This enzyme is expressed in the small intestine, testis, and brain but not in normal lymphoid cells, and it is commonly accepted that misexpression of this protein, even in truncated forms, may lead to malignant transformation. Indeed, in several reports, the ALK gene is fused with other genes as a consequence of chromosome translocations. In

these cases, the catalytic domain of the enzyme works under the control of the promoter of the fused gene, causing ALK function deregulation. Examples involve ALK/EML4 in lung tumor [145] and breast cancer [146]; ALK/NPM1, ALK/ALO17 and ALK/CLTC in T-cell lymphomas [147]; ALK/TPM3, ALK/TPM4 and ALK/CARS in inflammatory myofibroblastic tumor [147]; ALK/VCL in pediatric renal cell carcinoma [148]. Of course, also point mutations in the coding sequence of the gene had been linked to tumorigenesis. In neuroblastoma, mutations causing constitutive phosphorylation activity were described in several cases [149], missense mutations were identified at position F1174 and, less frequently, at position R1275 [150]. Interestingly, also the increase in copy number of the ALK gene (otherwise, of normal sequence) is a recognized cause of neoplastic transformation, as indeed found for breast cancer [151] and neuroblastoma [152]. Being a protein kinase, the targets of ALK are numerous. A specific search of such targets had been performed using functional RNA interference as a screening tool in anaplastic large-cell lymphoma (ALCL) samples [153]; among the others, a critical role was assigned to CEBPB and BCL2A1 that were necessary to induce cell transformation and/or to sustain growth and survival in these cell lines. Other identified partners are IL22R1 and IL22 that play a role in the inflammation in ALK-positive ALCL [154]. Protein expression and ALK rearrangements were investigated by Sukov and colleagues [155], comparing bladder MRT to other malignant bladder tumors. Immunohistochemistry showed cytoplasmic staining for ALK-1 in 13 of 21 (62%) tumors. ALK-1 staining was not identified in any other tumor types. ALK-1 staining in inflammatory myofibroblastic tumors varied from diffuse to very focal, ranging from 5 to 75% of the tumor, median 30%. Often, only a few spindle cells demonstrated ALK-1 positivity, whereas the majority of adjacent cells were negative for ALK-1 staining. The ALK-1-positive cells typically demonstrated a slightly more atypical morphology with more abundant cytoplasm, irregular cytoplasmic contour, and large nuclei with occasional nucleoli compared to ALK-1 negative cells. ALK rearrangements were detected by fluorescence in situ hybridization (FISH), demonstrating that all cases of inflammatory myofibroblastic tumor with ALK-1 expression carried ALK rearrangements. Leiomyosarcoma, sarcomatoid carcinoma, embryonal rhabdomyosarcoma and reactive myofibroblastic proliferations were negative for ALK-1 protein and ALK rearrangements. Immunohistochemistry showed no significant differences in markers of muscle, epithelial, neural, and follicular dendritic cell differentiation between inflammatory myofibroblastic tumor with and without ALK gene rearrangements, and between inflammatory myofibroblastic tumor and spindle cell malignancies. However, coexpression of cytokeratin and muscle-specific antigens was unique to inflammatory myofibroblastic tumor, observed in approximately half the tumors. These findings indicate that detection of ALK protein and *ALK* gene rearrangements are useful in distinguishing IMT from spindle cell malignancies in the urinary bladder. Furthermore, ALK rearrangement appear to be the primary mechanism for ALK activation and IMT likely represents a heterogeneous group of spindle cell proliferations with the majority associated with ALK translocations, and the remaining associated with other etiologies [155].

Previous studies showed that *ALK* activation is a recurrent oncogenic event in IMTs, in some cases accomplished by chromosomal fusion with the *TPM4* or *TPM3* loci. The authors suggest that either TPM3 or TPM4 can contribute oligomerization domains to ALK fusion oncoproteins in IMTs. In other cases, *ALK* is likely activated by fusion with other genes.

Based on this study, it seems that a substantial subset of IMTs will contain TPM3-ALK and TPM4-ALK fusions, whereas other cases will likely contain *ALK* fusions with other genes. Still other IMTs probably lack *ALK* fusion genes. This pathogenetic heterogeneity, similarly to that seen in anaplastic large-cell lymphomas, seems to discriminate between ALK-negative IMTs, diagnosed in 40- to 50-year-old patients, and ALK-positive IMTs, observed in patients younger than 30 years old. These data suggest that IMTs in children and young adults might depend generally on ALK activation, whereas IMTs in older adults might represent a different disease and/or have different transforming mechanisms. However, additional studies are required to determine whether ALK status correlates with histopathological features and clinical behavior in IMT [156]. Other authors described a novel fusion gene in an IMT tumor specimen in a 12-year-old male with a bladder IMT. They identified the fibronectin 1 gene (FN1) as a fusion partner of ALK. Further studies on this novel fusion, FN1-ALK, resulting in ALK overexpression, could clarify the causes of IMT and facilitate development of novel therapeutics [157].

In a recent report a patient with *ALK*-translocated IMT, carrying the*RANBP2* fusion partner, showed a sustained partial response to the ALK inhibitor crizotinib (PF-02341066, Pfizer) as compared with no observed activity in another patient without the *ALK* translocation. These results interestingly suggest a therapeutic strategy for genomically identified patients [158].

Although there are only few reports on IMT of the bladder in children, similar findings are described and ALK alterations apply to the same diagnostic and potential therapeutic significance [143, 159].

5.1.2. Vimentin, SMA

Three are the main components of eukaryotic cytoskeleton: microfilaments (principal component: actin), microtubules (tubulin) and intermediate filaments. Vimentin (VIM) is a type III intermediate filament mapping to chromosome location 10p13 [160]. The molecular organization of the gene includes nine exons and only one encoded polypeptide [160]. Typically, it is found in mesenchymal tissues, and for this reason it is broadly used as a specific marker for mesenchimally-derived tissues and, consequently, for sarcomas. Interestingly, it has been recently demonstrated that VIM might be involved in tumorigenesis: its depletion might impair the formation of the autophagy-inhibitory beclin-1/14-3-3/vimentin intermediate filament complex, which in turn would be no more a target of the antiapoptotic Akt kinase, an oncoprotein [161]. To date, a specific role in human pathogenesis for VIM has been found only in one patient affected by cataracts [162].

In various reports expression of vimentin, SMA and desmin has been demonstrated in IMT of the bladder in pe-

diatric patients. Six cases of ten examples of a pseudosarcomatous myofibroblastic proliferation occurring in the urinary bladder of children aged 2 to 16 years have been reported by Saavedra and collaborators [163]. Studies by immunocytochemical methods showed expression of vimentin and muscle-specific actin. In addition, two of these cases expressed desmin and two others cytokeratin [163]. In a series of 11 pediatric cases of bladder myofibroblastic tumors, the immunohistologic profile of the proliferating cells was characterized by positive reactions to vimentin, muscle-specific actin, alpha-smooth-muscle actin, polyclonal desmin, and keratin [164]. Despite these findings in pediatric bladder IMTs, the involvement of these genes in the pathogenesis of IMT remains unknown.

CONCLUSION

RMS is the most frequent tumor of the bladder in children, although literature specifically referring to this localization is scarce. Nonetheless characterization of this disease relies on the distinction between P-F and P-F negative RMS and on the knowledge that bladder RMS is essentially embryonal or P-F negative in nature. P-F and P-F negative genotypes seem to have a distinct relationship between mutational frequency and age, suggesting that P-F negative tumors require the accumulation of mutations before presentation. A number of genetic alterations already known or recently identified, including *FGFR4*, *IGF1R*, *PDGFRA*, *ERBB2*/*4*, *MET*, NF1, *MDM2*, *CDK4*, *PIK3CA*, are targeted by approved or developing therapeutics that could immediately inform clinical trials in rhabdomyosarcoma. Further studies on the discovered mutations in genes such as CTNNB1*, BCOR, FBXW7* and others may provide targets for novel treatments in patients with rhabdomyosarcoma.

Characteristic of RTs are biallelic inactivating mutations in *SMARCB1*, a gene encoding the SWI/SNF chromatin-remodeling complex, in which the possibility of epigenetic dysregulation as a central mechanism of oncogenesis has been implicated. *ALK* gene rearrangements are the most relevant features of IMT, useful in distinguishing it from spindle cell malignancies in the urinary bladder and for further improvements in novel therapies.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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