

REVIEW

REVIEW OF PROGNOSTIC AND PREDICTIVE ASPECTS OF MUTATED TP53 IN WILMS' TUMOR BIOLOGY WITH MORPHOLOGICAL REPORT AND MOLECULAR ANALYSIS OF 37-YEAR-OLD MAN'S NEPHROBLASTOMA

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Here we review prognostic and predictive aspects of mutated TP53 in Wilms' tumor biology on the basis of the morphological report and molecular analysis of adult nephroblastoma (diffuse blastemal pattern) of a 37-year-old man. Among quite different proteins, TP53 affects expression of several genes such as hypoxia inducible proteins GLUT1 and EPO as well as multidrug resistance (MDR) mediated by P-glycoprotein (Pgp/MDR1) and multidrug-resistant related protein (MRP1), with certain clinical implications. TP53 mutation was found both in our primary tumor (c.746G>T p.R249M frequency 92%) and in nodal metastasis (c.746G>T p.R249M frequency 90%), and the common polymorphism p.P72R in the same gene was revealed with frequency of about 97% in both primary tumor and metastatic disease with appliance of NGS technology (IonTorrent – LifeTechnology) using Ion AmpliSeq Cancer Hotspot Panel v2.

Key words: nephroblastoma, histopathological typing, staging, TP53 mutation.

Introduction

Wilms' tumor is a common malignant neoplasm of childhood, while it is incidental in adulthood with a frequency of approximately 3% of all reported cases [1]. Onset of both childhood and adult Wilms' tumor is preceded by mutation of the WT1 gene [2]. However, WT1 mutations are detected in only 10% up to

15% of pediatric Wilms' tumors. In addition, adult Wilms' tumor was also reported to harbor a novel germline mutation in intron 1 of WT1: IVS1-6 C-->A [2]. In contrast to pediatric tumors, the pre-operative detection of adult Wilms' tumor (AWT) is particularly worrisome as radiological imaging is not specific enough to distinguish nephroblastoma from the more frequent renal cancers of adulthood [1].

The features of adult nephroblastoma include heterogeneous appearance, calcifications, fields of necrosis and hemorrhagic foci in magnetic resonance (MR) that enables correct staging of the tumor [3]. However, no kind of imaging – whether it is combined MR, ultrasonography (US) and/or computed tomography (CT) – provides sufficiently specific results to favor a clinical diagnosis of AWT over incomparably more common renal carcinomas [3]. AWT is an extremely rare and aggressive renal malignancy with a few well-established prognostic factors of nephroblastoma [4]. Among them there are certainly staging and histopathological typing of adult nephroblastoma with a potent impact on patients' survival [5]. Namely, if the Wilms' tumor is bilateral, patients' survival is generally two-fold shorter [5]. Furthermore, extracapsular extension and blood vessel invasion of blastemal nephroblastoma was associated with sudden death a 77-year-old male patient during the first course of postoperative chemotherapy [6]. It is difficult to estimate the average survival of patients with AWT, but it is worth mentioning that four of the five patients died in 12 months after nephrectomy and the tumor progressed in all of them despite treatment with nephrectomy, radiotherapy and chemotherapy [7]. Limitation to the renal parenchyma and epithelial type of nephroblastoma of a 47-year-old woman were related to favorable prognosis: no tumor progression for 5 years before surgery and 2-year-long disease-free survival afterwards without any follow-up therapy [6]. That is why it is important to include such details as histopathological typing with presence of eventual anaplasia and staging e.g. bilaterality, extracapsular extension and blood vessel invasion of blastemal nephroblastoma. Lungs are the most common site for metastasis of pediatric nephroblastoma, and it seems to be the same for adult Wilms' tumor, with 38 cases of lung metastasizing AWT being recorded in the English literature [8]. The other frequent sites of metastasis are liver and lymph nodes, and rare locations are bones or skeletal muscles as a vertebral metastasis of adult nephroblastoma with favorable histology in a 74-year-old woman [9]. In contrast to childhood nephroblastoma, AWT has no specific guidelines for postoperative treatment and multimodal therapy is usually initiated immediately after surgery, particularly if not only a renal mass but also abdominal lymphadenopathy and bilateral pulmonary deposits are present [10]. CDVC (cyclophosphamide, doxorubicin, Vepesid, carboplatin) is another scheme of chemotherapy, given to a 25-year-old man with nephroblastoma stage IV [11]. Relapsed nephroblastoma could be managed with surgical eradication of the relapsed tumor mass followed by high-dose chemotherapy or radiation therapy and subsequent allogenic bone marrow transplantation (BMT) with successful prolongation

of survival by at least a few years [12]. It should also be mentioned that in relapsed nephroblastoma with lung and pleural metastases of a 37-year-old woman a partial remission was noted with appliance of recombinant interferon-alpha [13]. That is why we aimed to review features of prognostic and predictive significance in an example of adult nephroblastoma (diffuse blastemal pattern) with extensive molecular analysis of multiple genes with a special focus on P53 to help the most effective post-surgical therapy of a 37-year-old man in the described case.

Case description

Macroscopic and microscopic findings

The tumor was at least stage III according to the 5th protocol National Wilms' Tumor Study Group NWTSG-5). The tumor presented a classical triphasic pattern (Fig. 1A–D; epithelial component – Fig. 1A, C; stromal component – Fig. 1B) with predominance of diffuse blastemal type (Fig. 1B–D). The blastema component included fields of small cells with hyperchromatic nuclei, numerous mitotic figures, and hardly any cytoplasm that were closely packed in diffuse pattern. The epithelium of the nephroblastoma was arranged into small tubules and slightly collapsed small cysts lined by relatively tall cells with elongated nuclei with some wedging and molding. However, in sampled sections areas of anaplasia were not present. The tumor was characterized by a high mitotic index: on average 8 mitoses per 1 HPF in blastema of the tumor (which equaled 80 mitoses/10 HPF). Necrosis comprised up to 15% of tumor area, but precise determination of the extent of necrosis was not possible due to partial autolytic change of the tumor that affected up to approximately 10% of the neoplasm. In one of the sampled sections the ureter was surrounded by texture of nephroblastoma with neoplastic invasion of adventitia and muscularis of the ureter. There was perinephric and renal sinus fat neoplastic infiltration. There was neoplastic involvement of adventitia of the renal vein with vascular and lymphatic invasion. The tumor invaded through the renal fibrous capsule and infiltrated nerve bundles of the renal hilum. The smallest peripheral margin was 0.05 cm (the distance from the inked surface of surgically removed adipose tissue).

Nodal metastases were noted in 2 of 6 examined lymph nodes. In addition there were two metastatic neoplastic tumors with residual lymphoid texture at the periphery of the smaller tumor. They were located in the close vicinity of adrenal and nerve ganglia (neoplastic infiltrates closely adhere to adrenal and nerve ganglia without evident infiltration of their texture). Vimentin was strongly positive in the stromal component of the tumor, and it was expressed partially in epithelial texture (Fig. 2A, B). Desmin was partially

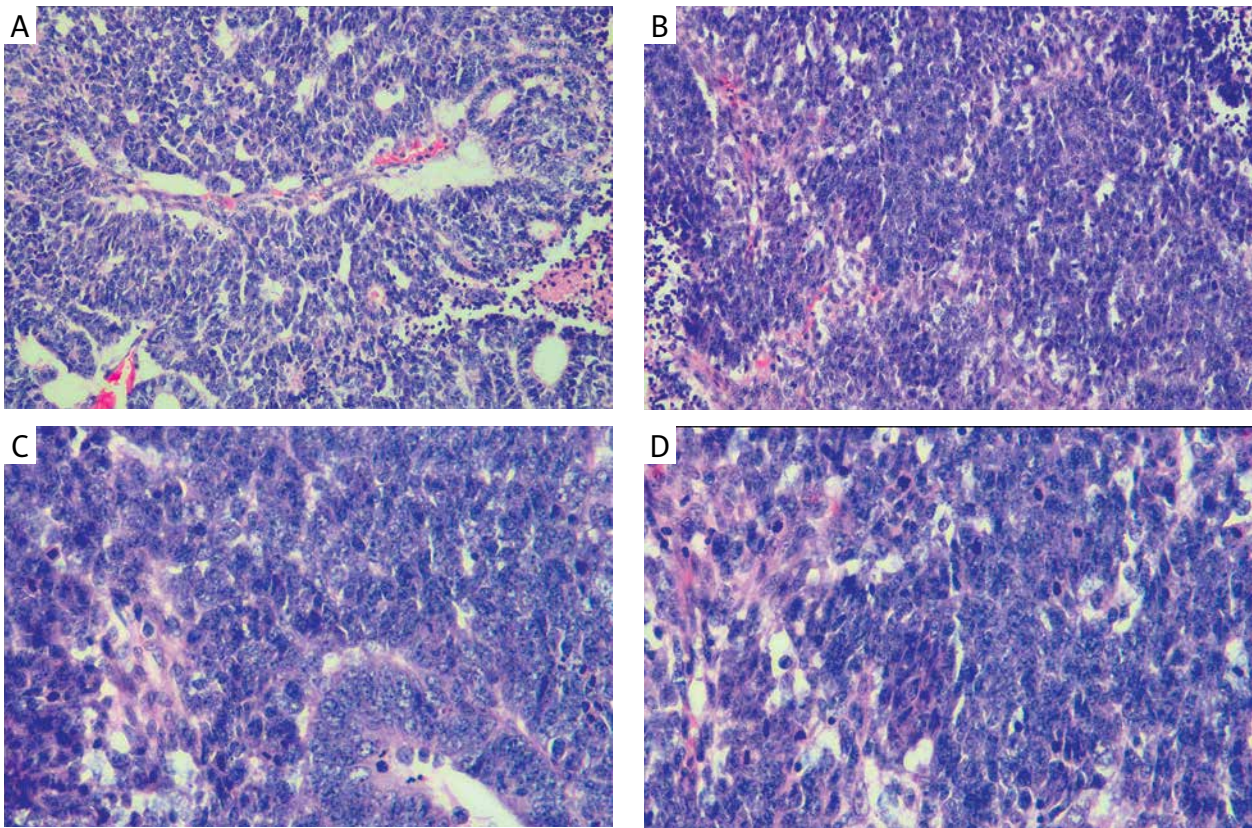


Fig. 1. Architectural diversity and cytological atypia of nephroblastoma (HE stain): A) typical epithelial pattern of nephroblastoma (magnification 200 \times), B) typical triphasic pattern (epithelial, blastemal and stromal) (magnification 200 \times), C) epithelial and blastemal pattern (magnification 400 \times), D) blastemal pattern with high mitotic activity without signs of anaplasia (magnification 400 \times)

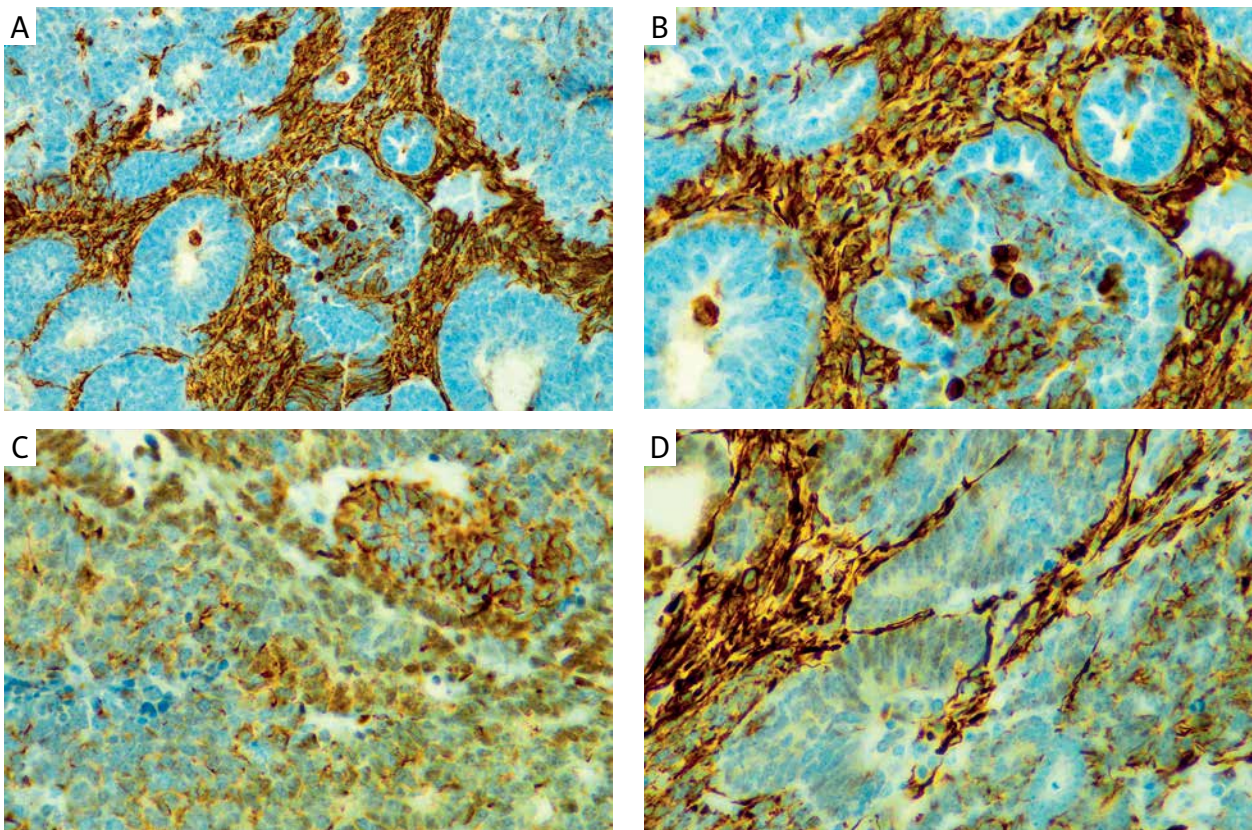


Fig. 2. Immunoprofile of nephroblastoma. A, B) Vimentin reactivity of stromal and partially in epithelial component of nephroblastoma (magnifications 200 \times , 400 \times), C) WT1 immunostain of epithelial, blastemal and stromal components (magnification 400 \times), D) WT1 immunostain of epithelial and stromal components (magnification 400 \times)

positive in stroma texture of the tumor [not shown]. With application of anti-N WT1 antibody, the WT1 marker was positive in epithelial texture and tumor blastema as well as in tumor stroma (Fig. 1C, D) and there was a mixed cytoplasmic and nuclear distribution of the protein with predominance of cytoplasmic location.

Molecular analysis

Independently from immunohistochemical evaluation gene profiling was performed. Status of *ABL1*, *EZH2*, *JAK3*, *PTEN*, *AKT1*, *FBXW7*, *IDH2*, *PTPN11*, *ALK*, *FGFR1*, *KDR*, *RB1*, *APC*, *FGFR2*, *KIT*, *RET*, *ATM*, *FGFR3*, *KRAS*, *SMAD4*, *BRAF*, *FLT3*, *MET*, *SMARCB1*, *CDH1*, *GNA11*, *MLH1*, *SMO*, *CDKN2A*, *GNAS*, *MPL*, *SRC*, *CSF1R*, *GNAQ*, *NOTCH1*, *STK11*, *CTNBN1*, *HNF1A*, *NPM1*, *TP53*, *EGFR*, *HRAS*, *NRAS*, *VHL*, *ERBB2*, *IDH1*, *PDGFRA*, *ERBB4*, *JAK2*, *PIK3CA* was studied by means of NGS technology (IonTorrent- LifeTechnology) using Ion AmpliSeq Cancer Hotspot Panel v2. This test covered hot spots localized in the 50 most often mutated oncogenes and tumor suppressors. Screening structures of all these genes, we detected only TP53 mutation both in primary tumor (c.746G>T p.R249M frequency 92% of mutated allele) and in nodal metastasis (c.746G>T p.R249M frequency 90% of mutated allele) (Fig. 3), and the common polymorphism p.P72R in the same gene with frequency of about 97% in both primary tumor and metastatic disease.

Discussion

The spectrum of histopathological morphology is the same for nephroblastomas of adulthood and childhood as both of them constitute the same entity, but schemes of postoperative therapy harbor greater toxicity in adults than in children [1]. The case presented by us showed a blastemal dominant histology in spite of the fact that epithelial type is the most common in adult nephroblastoma [14]. In addition, the high number of mitoses (8 mitoses per 1 HPF) definitely exceeds the number of 20 mitoses per 10 HPF, and the threshold of over 20 mitoses was a feature found exclusively in intermediate or high risk nephroblastomas and was additionally correlated with CD44 expression in a study of 39 cases [15]. Epithelial type of nephroblastoma is distinguished by positivity for WT-1 from papillary renal cell carcinoma, but it can resemble the malignant counterpart of metanephric adenoma, that is, “metanephric adenocarcinoma” [14].

Differential diagnosis also includes desmoplastic small round cell tumor (DSRCT) (EWS/WT1), which is determined and identified by EWS-WT1 translocation that leads to fusion of the Ewing’s sarcoma (EWS) gene with the Wilms’ tumor (WT1) and rarely occurs in adolescence and early adulthood [16]. Thus, differential diagnosis with small round blue cell tumor (SRBCT) might be a challenge due to its WT1 positivity, but SRBCT still has typical clinical and morphological features that are more or

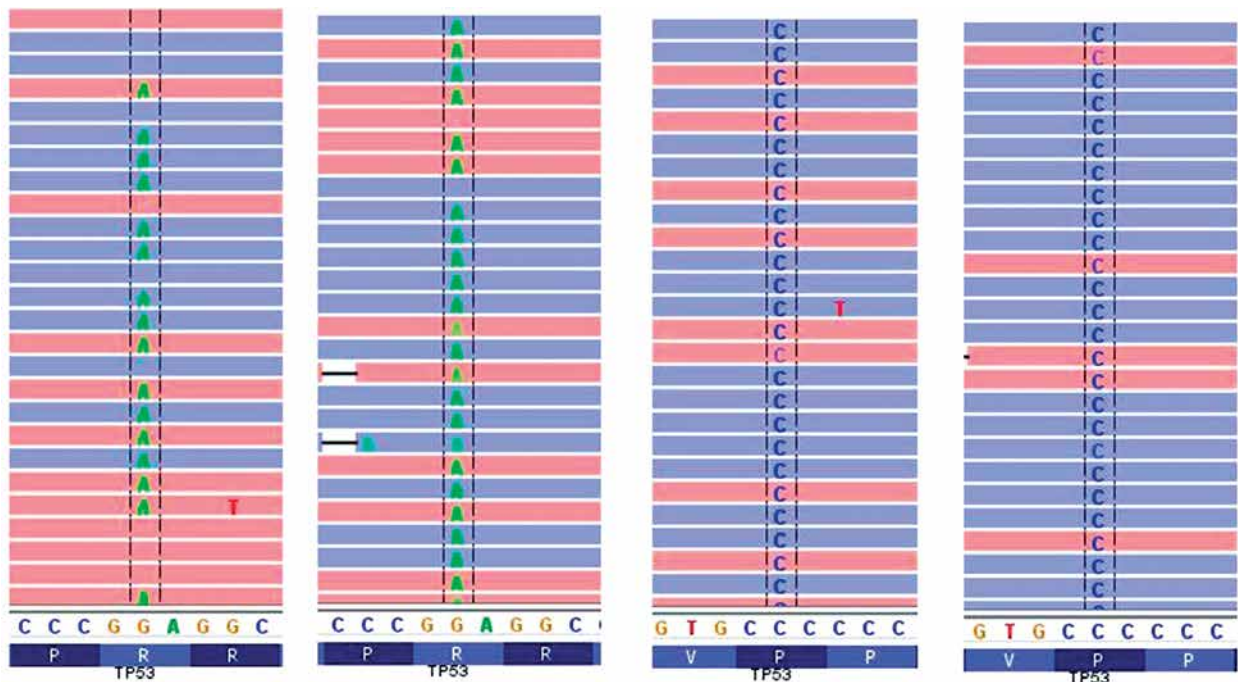


Fig. 3. Mutations detected in TP53 using the IT-PGM platform, as visualized in Integrative Genomic Viewer: p.R249M (c.746G>T) mutation detected in tumor (A) and in nodal metastasis (B) respectively and p.P72R (c.215C>G) polymorphism detected in tumor (C) and in nodal metastasis (D) respectively

less distinct in each case [17]. Of course, there can be encountered serious diagnostic pitfalls whenever overlapping morphology is presented by a tumor and mandatory immunohistochemical profiling is not sufficient to remove diagnostic doubts [17]. Particularly, morphology of DSRCT overlaps with diffuse blastemal type of nephroblastoma [18], which was diagnosed in our present case. Therefore a panel of CD99, WT1 protein, desmin, myogenin, NB84, and IN11 is applied in differential diagnosis of Wilms' tumor from other small round blue cell tumors [17]. While there is varied coexpression of desmin including dot-like pattern and cytokeratin both in DSRCT and blastema of Wilms' tumor, such an immunoprofile was historically attributed to DSRCT [16]. Furthermore, selective WT1 carboxy-terminus immunoreactivity is diagnostic for DSRCT, while dual immunoreactivity for the WT1 amino-terminus and carboxy-terminus supported the diagnosis of nephroblastoma [18].

In our case, anti-N terminus antibodies were applied to produce both predominantly cytoplasmic and slightly nuclear WT1 protein immunostaining of nephroblastoma cells. The WT1 protein's exclusive nuclear distribution is a feature of anti-C WT1 staining, while it can be mixed nuclear/cytoplasmic or only cytoplasmic if anti-N WT1 antibody is applied [17, 19]. Although Wilms' tumor 1 (WT1) protein was primarily recognized as a specific marker of nephroblastoma, some other tumors may share WT1 protein expression [19]. However, histopathological HE appearance of tumor comprised, besides predominant blastemal morphology, two other classical nephroblastoma components: stromal and epithelial ones. Thus, the H&E picture was characteristic enough for a straightforward diagnosis of nephroblastoma, so appliance of WT1 staining was of only confirmative value in our case. That is why WT1 analysis was not further developed, particularly at the gene level. *TP53* mutations are supposed to be associated with anaplasia in Wilms' tumor (anaplastic subtype or diffuse anaplasia) [5, 20]. However, in our case there was no anaplasia but there was a high frequency of *TP53* mutation in tumor tissue. It is possible that the wild type allele was lost during carcinogenesis in tumor tissue. However, this speculation requires further molecular studies. On the other hand, we could make the conclusion that p.R249M *TP53* mutation detected in the tumor of our case appears to be one of the earliest mutations in development of this tumor, because it was detected both in the primary and metastatic lesion in such a high percentage of tumor cells. Unfortunately, it was not possible to test DNA from a blood sample to distinguish germline or somatic status of this mutation. It was suggested that *TP53* mutations and gene polymorphisms (PIN2, PIN3, and PEX4) affected the risk of development, pa-

tient's age at onset of the tumor, and survival in WT [5]. That is why Andrade *et al.* performed sequencing of *TP53* exons 2-11 in 46 blood DNA samples and 31 fresh tumor DNA samples from 52 patients with WT to detect *TP53* pathogenic missense mutations (p.V197M, p.R213Q, p.R248W, and p.R337C) in up to 12.9% of samples [5]. The mutation (p.R249M) detected in our case is situated next to the p.R248W mutation mentioned above. Moreover, a novel intronic mutation, IVS2 + 37C > T, was found in only one patient (2.2%) in blood samples [5]. Characteristically, the PIN3 duplicated allele was a feature of 20-month later occurring nephroblastomas. *TP53* somatic mutation was related to poor survival probability of 37.5% in reference to 85.0% for patients without somatic mutations with no statistical significance [5]. Occurrence of *TP53* PEX4 C was associated with higher risk for WT onset [5]. We detected polymorphisms in *TP53* p.Pro72Arg, although with an unknown impact on the development of AWT. In conclusion, our genetic analysis revealed mutation in the *TP53* gene, which suggests that carcinogenesis in the case of children with WT and AWT could follow a similar molecular background.

Diffuse anaplasia is an ominous prognostic factor in comparison with non-anaplastic tumors, or with focal anaplasia [5]. However, in our case no anaplasia was detected in the histopathological report. *TP53* mutations coexist with such an unfavorable morphology as diffuse anaplasia with poor patient outcome in nephroblastoma [18]. Thus, the *TP53* mutation status was determined in our case. Lurie *et al.* reported *TP53* deletion at 17p, focal gain of MYCN, recurrent genomic loss and under-expression mostly at 4q and 14q in anaplastic Wilms' tumors, while gain of 1q and loss of 16q were observed both in favorable and unfavorable morphology of Wilms' tumor [21]. In addition, *TP53* behavior seemed to be modified *in vitro* by a single nucleotide polymorphism of *TP53* encoding either arginine or proline at codon 72. Augmentations of the Arg allele and Arg/Arg genotype Arg72 were statistically significant with no significant correlation with age, stage, or disease recurrence of nephroblastomas with favorable histology [22]. Furthermore, wild-type *TP53* represses transcription of GLUT1 – a glucose transporter whose transcription is induced by hypoxia inducible factor 1 – so transcriptional activity of GLUT1 gets unblocked by a gene promoter in *TP53* mutated cancers [20]. Increased glycolysis and uptake of glucose via glucose transporter GLUT1 characterize metabolism of neoplastic cells of various cancers [20]. Expression of GLUT1 was higher in Wilms' tumors with diffuse anaplasia in comparison to non-anaplastic Wilms' tumors [20]. In addition, a proline residue at position 72 of *TP53* was associated with increased expression of GLUT1 in Wilms' tumors without anaplasia [20]. Thus,

GLUT1 emerges as a therapeutic target in case of resistance to conventional therapy because anaplastic Wilms' tumors were found to be 2-deoxy-2-((18)F) fluoro-d-glucose avid [20].

TP53 gene mutations are a hallmark of poor prognosis of Wilms' tumor as they coexist with diffuse anaplasia, pointing at close linkage of anaplasia and *TP53* mutations in case of bilateral nephroblastomas with anaplasia [23]. Moreover, it seems that reporting on *TP53* inactivating mutation underlies multidrug resistance (MDR) mediated by P-glycoprotein (Pgp/MDR1) and multidrug-resistant related protein (MRP1) [23]. Namely, 24% of tumor samples were Pgp/MDR1-positive, 48% were immunoreactive for MRP1, and only 8% were positively stained for wild-type *TP53*, with the finding of very low coexpression of MDR and wild-type *TP53* in a study of 25 pediatric nephroblastomas [24] (Chart 1). Thus, evaluation of genetic abnormalities is so pivotal in prognostic and predictive perspective in such a single tumor as described in our case.

The authors declare no conflict of interest.

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