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Original Article

Tissue expression of AT-rich interacting domain 1 alpha in Wilms tumor

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Abstract: Objective: AT-rich interacting domain 1 Alpha (ARID-1A) is a subunit of chromatin remodeler Switch/Sucrose Non-Fermentable (SWI/SNF) complex. It is involved in several important cellular processes such as cellular differentiation, regulation of cell cycle and restoration of DNA damage. Although higher mutation rate of ARID-1A is strongly determined in several human cancers, its significance has not been fully established in Wilms tumor (WT). The aim of this study was to determine the prognostic value of ARID-1A expression in WT. Materials and methods: Nuclear ARID-1A expression in 50 tissue samples harvested from children with Wilms tumor and its relationship with prognostic parameters were evaluated. Results: Tissue samples of 50 cases (male, n=23; 46%, and female, n=27:54%) with Wilms tumor with a mean age of 3.26±2 years were analyzed. Mean tumor size, and weight of kidneys were 9.16±2.9 cm in diameter and 478±312 gr, respectively. Thirteen (26%) cases were in stage I, 18 (36%) in stage II, 7 (14%) in stage III, 6 (12%) in stage IV, and 6 (12%) in stage V. Thirty-nine cases were alive (78%), while 11 cases (22%) were deceased. Mean overall survival time was 68.2±39.5 (2-148) months. ARID-1A expression was normal in most tumors, while it was decreased or negative in 14 tumors (28%). Statistically, ARID-1A expression was found to be correlated with the weight of the tumor (P=0.002) and survival (P=0.021). Conclusion: This study indicates that ARID-1A expression may be associated with development of Wilms tumor. However further studies are needed to clarify the importance of this relevance.

Keywords: Wilms tumor, nephroblastoma, ARID-1A

Introduction

Wilms tumor (WT) is the most common primary malignant renal tumor of childhood. The worldwide prevalence of WT is 1:10,000 among the population [1, 2]. WTs morphologically resemble embryonic kidneys with a disrupted architecture and they have been associated with undifferentiated metanephric precursors [2]. Tumorigenesis of WT can be considered as a disrupted nephrogenesis on which different transcription factors, proto-oncogens, and various types of growth factors are effective [3]. Currently, high cure rates can be achieved and multimodality treatment has resulted in a significant improvement in outcomes. Although survival rates in WT are nearly 90%, certain population of patients continues to experience poor survival and increased rates of relapse [4, 5].

AT-rich interacting domain 1 Alpha (ARID-1A), also known as BAF250a, SMARCF1, or p270, is a subunit of chromatin remodeler Switch/Sucrose Non-Fermentable (SWI/SNF) complex. This complex regulates gene transcription through its ATP- dependent chromatin- remodeling activity [6]. It is involved in several important cellular processes such as cellular differentiation, regulation of cell cycle and restoration of DNA damage [6, 7]. In recent decades, SWI/SNF complex also has attracted remarkable attention because subunits of the complex are collectively mutated in >20% of human cancers. Among the SWI/SNF subunits, ARID-1A has the highest mutation rate in human cancers [8]. It is reported that ARID-1A is mutated nearly in 50% of some subtypes of carcinomas. These mutations are typically nonsense or frame-shift, which decrease ARID1A protein expression [9, 10].

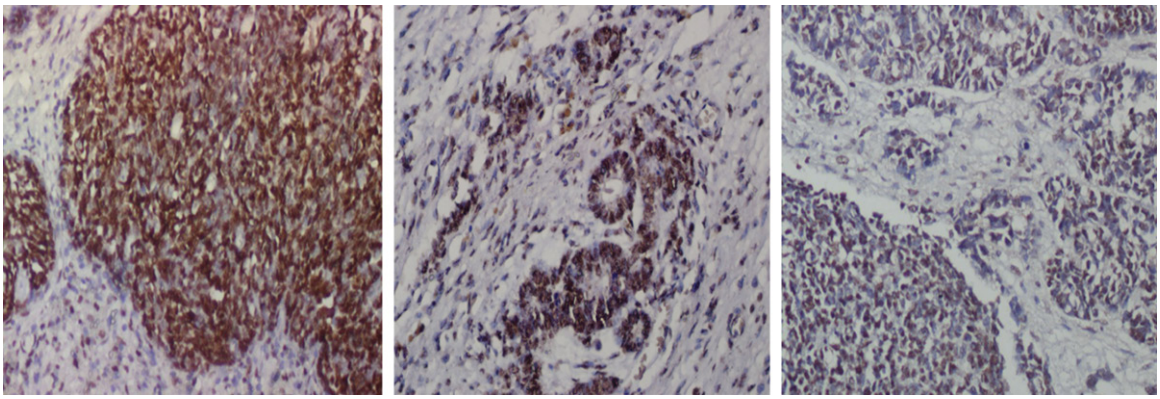

Figure 1. Normal nuclear ARID-1A expression in the blastemal component (at left), and in the epithelial component of a Wilms tumor specimen (in the middle). At right, a sample of negative ARID-1A expression (DABx200).

Hitherto, many parameters have been suggested as relevant markers for assessing the prognosis of the WT [1, 4, 5, 11-14]. But the presence of ARID-1A expression in WT has not been investigated widely. The aim of this study was to determine the importance of ARID-1A in Wilms tumor and also investigate the correlations between expression of ARID-1A and some clinical prognostic factors such as weight, stage and histological features of WTs.

Material and methods

WT resection specimens of 50 cases with WT diagnosed and treated in Dr. Behçet Uz Children's Education and Research Hospital between 1999 and 2014 were included in this study. The study was approved by the Local Ethics Committee of Tepecik Education and Research Hospital. The staging system developed by the National Wilms tumor Study Group (NWTS) was used to describe the extent of spread of these tumors.

For immunhistochemistry (IHC), hematoxylin and eosin (HE) staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. IHC was performed by the streptavidine biotin peroxidase method (Invitrogen, Camarillo, 85-9043, CA, USA). Serial 5-μm sections were obtained and these slides were incubated overnight at 60°C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat- induced epitope retrieval in the microwave (in 10 mM/L citrate buffer, pH 6.0, for 20 minutes, followed by cooling at room temperature for 20 minutes) and blocked for endogenous peroxidase and biotin. An affinity purified monoclonal mouse antibody against ARID-1A (Sigma, St. Louis, HPA005456) were used at a dilution of 1:200. Histochemical evaluation was made by an observer blinded to any of the clinical features. ARID-1A expressions were manifested as strong nuclear staining and we counted the rate of nuclear positivity. Strong nuclear staining of most tumor cells similar to the normal renal tissue was considered as a positive histochemical reaction. Strong focal staining, occupying less than 5% of the cells or weak staining, occupying less than 30% of tumor cells were considered as negative reaction (**Figure 1**).

For statistical analyses, Spearman Correlation analysis, Mann Whitney U test, Chi square test and Kaplan Meier survival analyses were performed using SPSS 20.0. *P* values less than 0.05 was considered to be statistically significant.

Results

Surgery, chemotherapy and radiotherapy were the treatment modalities which were applied alone or in combination to the total of 50 patients according to their individual features. We used the NWTS protocol with surgical approach first for the patients with unilateral tumors, but for the patients with bilateral tumors, pre-operative chemotherapy was added to the treatment protocol and the combination of drugs was changed. In addition, patients with unfavorable histology, and even some cases with localized diseases required radiation therapy [15]. Therefore all tumors

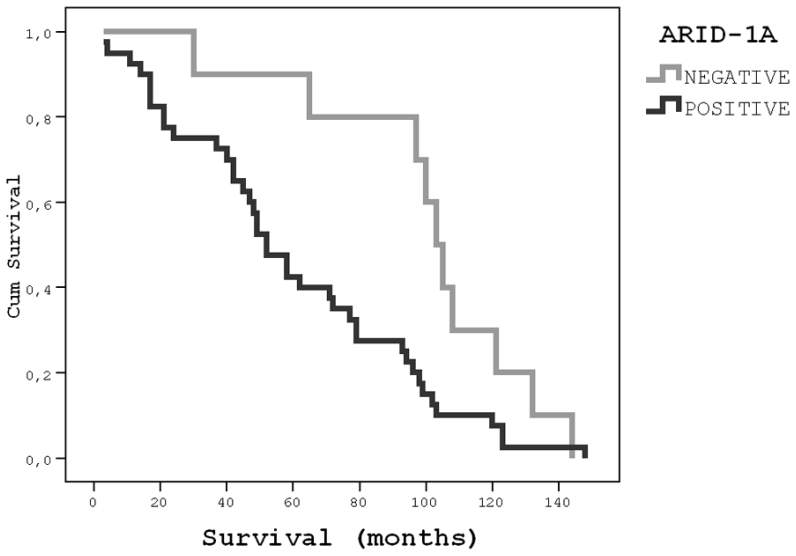

Figure 2. Graph demonstrating statistically significant correlation between tumoral ARID-1A expression and survival (Log Rank, P=0.021).

were classified as those with favorable or unfavorable histology. Thirty-nine (78%) cases had triphasic, and 11 (22%) cases biphasic tumors with a predominancy of blastemal component, and unfavorable histology. In the whole series, 11 patients died, and 3 of them exited because of complications of bilateral tumor. Four patients were lost secondary to conditions apparently unrelated to WT such as pneumonia, sepsis, hepatic insufficiency and veno-occlusive disease [11].

The study population consisted of 23 male (46%), and 27 (54%) female patients, with an overall mean age of 3.26±2 years (5 months-8 years). Tumors were right- (n=25:50%), and left-sided (n=19:38%) or bilateral (stage V) (n=6:12%) in respective number of cases. The mean tumor size, weight of affected kidneys 9.16±2.9 cm in diameter and 478±312 gr, respectively [15]. The distribution of cases according to disease stages was as follows: Stages I, n=13; 26%; II, n=18:36 %; III, n=7:14%, and IV, n=6. 12% Thirty-nine cases were alive (78%), while 11 cases (22%) were deceased. Mean overall survival time was 68.2±39.5 (3-148) months.

Generally, the rate of ARID-1A expression was similar in different components in the same tumor. In 14 cases (28%), expression of ARID-1A was decreased or negative at least in one of the tumoral components. The most serious defects of ARID-1A were determined in mesenchymal component.

Most prognostic parameters such as tumor diameter (P=0.308), patient age (P=0.369), therapy response (P=0.708) and survival rate (0.377) were not found to be associated with the ARID-1A expression by the Mann Whitney U and *chi*-square Analyses. Contrarily, there was statistical significance between expression of ARID-1A and kidney weight (P=0.002), as well as duration of the follow-up period (P=0.002). The mean weight of tumors with normal ARID-1A expression was 534±315 gr while it was 253.8±172.9 gram in those with abnormal ARID-1A expression. Similarly the overall survival was 60.1±37.1 months in patients with ARID-1A positive, and 100±32.7 months in cases with ARID-1A negative tumors. In addition a correlation existed between the epithelial ARID-1A expression and the survival (Log Rank, P=0.021) by Kaplan Meier Survival Analysis (**Figure 2**).

Discussion

The developmental pathways of kidney are regulated by many transcription factors, proto-oncogenes and growth factors [11, 15-17]. It has been suggested that WT is the direct result of maldevelopment of the embryonic kidney. It arises from pluripotent renal precursors which undergo excessive proliferation resulting in undifferentiated stromal components, blastemal cells and primitive epithelial structures. The presence of associated nephrogenic rests consisting of foci of persistent embryonic remnant in most WTs has led to the emergence of many fundamental insights such as the link between normal development and tumorigenesis. Hitherto, many factors involved in the survival and proliferation of WTs have been reported [1, 5, 11-13]. Since ARID1A has been described as a potential tumor suppressor gene, it may be suggested that defect of

ARID1A gene is associated with prognosis of WTs. Interestingly, to our knowledge this is the first study investigating the relationship between loss of ARID1A protein and prognosis or outcome in children with WT. In the present study, molecular genetic analyses for ARID-1A gene were not performed and this is a limitation of the study. Fortunately it is reported that mutations of the ARID-1A gene are typically nonsense or frame-shift, which cause loss of ARID1A protein expression [6-10].

It was previously implied that the most subunits of SWI/SNF chromatin remodeling complex play an essential role in the regulation of gene expression during development and differentiation of almost all tissues. Therefore deficiency of any subunits of the complex is linked with tumor susceptibility. It was proved that deficiency of ARID1A would increase the tumorigenic potential of cells. Similarly ARID-1A may play a critical role in the proliferation of differentiating nephroblasts which may promote the development of WT. It may be also concluded that deficiency of ARID1A is functionally similar to loss of WT1, and that ARID1A itself is a susceptibility gene for WT. Contrary, in a few studies, loss of ARID-1A expression was found to be an independent predictor for better prognosis [18-21]. In the present study, we have also speculated that ARID-1A loss is associated with better prognosis and ARID-1A could be involved in the progression of WT. Indeed, we found that the loss of ARID-1A expression is associated with longer survival and decreasing tumor size.

It has been shown that the mutations of the ARID1A gene frequently coexist with PI3K/AKT-pathway activating mutations of PIK3CA which leads to a downstream activation of the PI3K/AKT pathway [7, 18]. In summary, increased sensitivities of ARID1A-deficient cancer cells develop for PI3K- and AKT- inhibition [18]. Therefore dysfunctional ARID1A is associated with dysregulation of the PI3K/Akt signaling pathway, which may have a synergistic effect on tumor development. Moreover, an inverse correlation has been documented between ARID-1A and TP53 in uterine endometrioid, gastric, esophageal carcinomas, between ARID-1A and Phosphatase and Tensin homologue (PTEN) in colorectal and serous ovarian carcinomas, as well as between ARID-1A and microsatellite instability (MSI) in gastrointestinal cancers [18]. From the perspective of targeted therapy, ARID1A-mutated cells are more sensitive to PI3K/AKT inhibitors compared with ARID1A wild-type cells. Similarly, SWI/SNF mutated cancers also may be sensitive to DNA damage-inducing chemotherapeutics. Counterintuitively, ARID1A-mutated ovarian clear cell cancers typically lack genomic instability, and these tumors are less responsive to DNA damage caused by platinum-based chemotherapy. This suggests that the role of the SWI/SNF complex in DNA damage might be subunit- and/or tissue- dependent [22-25]. In the present study, we determined the highest survival rates in 14 patients (28%) with WT which presented with the expression defects of ARID1A protein. This finding suggested that the decreasing expression of ARID1A may play a protective role in tumorigenesis in WTs.

Hitherto, many of the genes implicated in Wilms' tumorigenesis have been implicated in the control of nephron progenitors or other processing pathways. Here, we discuss the relationship between ARID-1A and WT in the context of renal development. To our knowledge, it is the first investigation about the relationship between WT and ARID-1A expression which has been extensively studied in several tumors. However, further research is required to define how ARID-1A status can be used as a clinical advantage in WT.

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Disclosure of conflict of interest

None.

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