Immunohistochemical expression of P53 protein in histologically favorable Wilms tumor and its relationship to tumor stage at presentation

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Abstract

Objective
Wilms tumor, as the most common renal tumor of children, has been associated with chromosomal abnormalities. Although a correlation between anaplasia and mutations of P53 tumor suppresser gene has been found in Wilms tumor, significance of these mutations in different clinical stages of favorable- Wilms tumor, remains largely unresolved. The goal of this study was to determine the frequency of P53 expression in histologically favorable Wilms tumors and its correlation to tumor-stage at presentation.

Materials and Methods
In this retrospective study, 48 cases of confirmed Wilms tumor with favorable-histology were retrieved from the files of departments of pathology in three hospitals in Mashhad University of Medical Sciences between 1990 and 2004. Histological characteristics and clinicopathological staging were in accordance with National Wilms Tumor Study guidelines. P53 expression was determined by the immunohistochemical method. For each section, the proportion of neoplastic cells exhibiting nuclear positivity was broadly quantified and their intensity of staining was charted, based on visual impression by two pathologists.

Results
A total of 48 cases of histologically favorable Wilms tumor were assessed. Eleven cases (23%) showed positivity for P53 which were 3 (27.3%) with stage II, 3 (27.3%) with stage IV, 2 (18.2%) with stage I, 2 (18.2%) with stage III and 1 case (9.1%) with stage V. The P53 immunopositivity was seen in 1-25% of tumor cells in 9 cases (18.8%), in 26 to 50% of tumor cells in 1 case (2.1%) and in >75% of tumor cells in the other one case (2.1%). The intensity of staining was moderate in 6 cases (12.5%), weak in 4 (6.3%) and strong only in one case (4.2%). The most common component with P53 immunoreactivity was blastemal in 11 cases (100%). In 4 cases (36.4%) there was also positivity in epithelial and in 2 cases (18.2%) in mesenchymal components.

Conclusion
We found no correlation of P53 immunoreactivity and its intensity to tumor stage at presentation in individuals with histologically favorable Wilms tumors (p=0.66, p=0.52 respectively).

Keywords: Wilms tumor, P53 Protein, Favorable-histology, Stage, Renal neoplasm.
Introduction

The p53 gene is located on chromosome 17p13.1, and it is the most common target for genetic alteration in human tumors. A little over 50% of human tumors contain mutations in this gene. It encodes for a 53 kD nuclear phosphoprotein which its major functional activities are cell-cycle arrest and initiation of apoptosis in response to DNA damage (1).

Alterations of the p53 gene have been extensively investigated in a wide variety of human malignancies. Overexpression of P53 protein as determined by immunohistochemical analysis has been documented to be associated with a biologically aggressive disease in patients with carcinoma of the breast, colon, and lung. However, data on childhood malignant solid neoplasms such as Wilms tumor is still limited (2).

Although a correlation between anaplasia and mutations of the p53 tumor suppressor gene has been found in Wilms tumor, significance of these mutations in different clinical stages of favorable-histology Wilms tumor remains largely unresolved (3). There are patients with histologically favorable Wilms tumor who show advanced stage at presentation or aggressive clinical course. In this study we decided to determine the frequency of P53 protein expression in patients with histologically favorable Wilms tumors and its correlation to tumor-stage at presentation.

Materials and Methods

In this retrospective study, 48 cases of confirmed Wilms tumor with favorable-histology (before administration of chemotherapy) were retrieved from the files of departments of pathology in three hospitals in Mashhad University of Medical Sciences between 1990 and 2004. Histological character and clinicopathological staging were in accordance with National Wilms Tumor Study guidelines.

One paraffin block of each tumor, previously fixed in 10% buffered formalin, was selected for review. Immunohistochemical determination of P53 protein was performed on 4μ thick sections of each block. Immunoperoxidase streptoavidin-biotin procedure was performed. After deparraffinization and hydration slides incubated with 3% hydrogen peroxide for 20 minutes. Antigenic retrieval was done by incubation with molar citrate buffer 1% (pH=6) in microwave for 20 minutes, then slides incubated with the DO-7 antihuman p53 monoclonal antibody (DAKO, Denmark) for 60 minutes at room temperature. In the next stage biotinylated link antimouse and antirabbit immunoglobulin and streptoavidin-peroxidas (DAKOLSAB 2 system, peroxidase kit, Denmark) was used.

Peroxidase was shown with diamino benzidin hydrochloride (DAB). Counter statin was done with hematoxylin Mayer and mounted in canada balsam. For negative control P53 antibody omitted in this process and for positive control adenocarcinoma of colon was used. Microscopic slides were evaluated and interpreted by two pathologists. For each tumor, the proportion of neoplastic cells exhibiting nuclear positivity was broadly quantified as 0%, 1% to 25%, 26% to 50%, 51-75% and >75%. The intensity of positive staining was charted as weak, moderate or strong based on visual impression. Statistical evaluation used the Chi-square test with p<0.05 considered significant.

Results

A total of 48 cases of Wilms tumor with favorable-histology were available for possible review. There were 25 females (52.1%) and 23 males (47.9%) with a F:M ratio of 1.08. The ages of patients ranged from 3 months to 9 years (median 36 months). Twelve cases (25%) were with stage I, 13 (27.1%) with stage II, 13 (27.1%) with stage III, 7 (14.6%) with stage IV and 3 (6.3%) with stage V at presentation.
Expression of P53 protein in histologically favorable Wilms tumor

Eleven cases (23%) showed positivity for P53 which were 3 (27.3%) with stage II, 3 (27.3%) with stage IV, 2 (18.2%) with stage I, 2 (18.2%) with stage III and 1 (9.1%) case with stage V. The P53 immunopositivity was seen in 1-25% of tumor cells in 9 cases (18.8%), in 26 to 50% of tumor cells in 1 case (2.1%) and in >75% of tumor cells in the other case (2.1%) (Fig. 1). The intensity of staining was moderate in 6 cases (12.5%), weak in 4 (6.3%) and strong only in one case (4.2%) (Fig. 2).

The most common component with P53 immunoreactivity was blastemal in 11 cases (100%). In 4 cases (36.4%) there was also positivity in epithelial and in 2 cases (18.2%) in mesenchymal components.

There was no relationship of P53 immunopositivity and its intensity to the likelihood of tumor stage at presentation ($X^2 = 2.44, p = 0.66$ and $X^2 = 11.04, p = 0.52$ respectively) (Fig. 3).

![Fig. 1: P53 immunopositivity in 26-50% (A), and >75% (B) of tumor cell nuclei (stereptavidin biotin immunoperoxidase).](image1)

![Fig. 2: Strong immunopositivity of tumor cell nuclei in blastemal and epithelial components (stereptavidin biotin immunoperoxidase).](image2)

Fig. 3: The P53 immunopositivity in relation to pathological stage in 48 cases of favorable-histology Wilms tumor.

**Discussion**

Wilms tumor is the most common primary renal malignancy of childhood. The association of Wilms tumor to genetic abnormalities is well documented. The two most recognized oncogen abnormalities in Wilms tumor are the Wilms tumor suppressor gene WT1 mapped to chromosome 11p13 and WT2 gene mapped to chromosome 11p15 that are associated with congenital anomalies. In addition to these two genes, loci at 1p, 7p, 19q and 16q, are also believed to harbor genes involved in the biology of Wilms tumor (4).

Mutations of the P53 tumor suppressor gene are also implicated in the pathogenesis of Wilms tumor. The p53 and WT1 gene encode transcription factors that have been shown to interact physically and to modulate each other’s function in some experimental situations (5, 6). Mutations of the p53 gene...
and/or up-regulation of p53 expression have been reported to occur in 60% of histologically unfavorable Wilms tumor and it identifies those patients with a shorter survival period (7, 8). There is also association of p53 expression to tumor stage in unfavorable Wilms tumors (9). On the other hand there are cases of histologically favorable Wilms tumor which show aggressive behavior like advanced stage at presentation.

The biologic mechanisms underlying the aggressive phenotype of some favorable-histology Wilms tumors are not known. Huang et al. have suggested that dysfunction of p53 tumor suppressor gene may contribute to vigorous growth in some of these neoplasms. Such dysfunction is linked to accelerated neovascularization via depletion of angiogenesis inhibitor thrombospondin-1 (TSP-1) and upregulated expression of vascular endothelial growth factor (VEGF) (10).

In previously published studies there were controversies in correlation of p53 expression to tumor stage at presentation in histologically favorable Wilms tumors. Cheah et al. in a study on 44 cases of Wilms tumor (both favorable and unfavorable histologies) (11) and Srendi et al. in a study on 97 cases mentioned that immunohistochemical detection of mutated p53 gene product in Wilms tumor seems to be associated with advanced disease and relapse and is a useful feature in the prognostic assessment (9).

On the other hand D'Angelo et al. in a study on 63 cases of unilateral histologically favorable Wilms tumor, did not find significant relation of p53 expression to stage at presentation (7).

Because of these controversies, we decided to investigate the hypothesis that patients with histologically favorable Wilms tumor who have advanced stage at presentation will over express P53 as determined by immunohistochemical analysis.

Prior studies using immunohistochemical analysis to determine P53 protein expression in patients with histologically favorable Wilms tumors reveal 4 to 20% of these tumors have significant P53 protein up-regulation. In our study 23% of cases (11 of 48) of histologically favorable Wilms tumors showed over expression of P53 protein.

We found no correlation of P53 immunoreactivity and its intensity to tumor stage at presentation in individual with histologically favorable Wilms tumors (p=0.66, p=0.52 respectively).

It is noteworthy to remind two points regarding the limitations of our study: although the immunohistochemical detection of P53 generally indicates genetic alteration in the p53 tumor suppressor gene, it must be confirmed by molecular investigations. Also in this study only one parameter of biological aggressiveness, tumor-stage at presentation, was evaluated; and other parameters such as responsiveness to treatment or relapse have not been mentioned. It seems that other complementary prospective studies are needed to determine the exact relationship between the overexpression of p53 gene and tumor aggressiveness in favorable-histology Wilms tumor.

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References