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Expression of TGF-β1 in Wilms' tumor was associated with invasiveness and disease progression

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Abstract *Objective:* To detect the expression of TGF-β1 in Wilms' tumor and association with disease progression.
Methods: Immunohistochemistry was used to examine TGF-β1 expression in 51 primary tumors and 17 invasions/metastases. Transient transfection was performed to establish Wilms' tumor cells with high TGF-β1 expression (TGF-β1-WT), and the expression level of TGF-β1 was detected by Western blot analysis. Invasive capacity of the transfected cells was evaluated by transwell analysis.
Results: The positive expression rate of TGF-β1 was 50.98% (26/51) and 82.35% (14/17) in primary WT tissues and associated invasive/metastatic tissues, respectively. The higher level of TGF-β1 expression in primary WT tumors was relative to invasion/metastasis ($p = 0.048$). The expression of TGF-β1 between primary WT and matched invasive/metastatic tissues was concordant ($p = 0.219$). TGF-β1-WT cells showed more invasive capacity than GFP-WT and WT cells. TGF-β1 expression status was associated with disease-free survival (DFS) (50.2 months vs. 75.4 months, $p = 0.022$) but not overall survival (OS) (62.3 months vs. 75.8 months, $p = 0.141$).
Conclusions: Positive expression of TGF-β1 in WT was correlated with tumor invasion and disease progression, which might be useful in identifying patients at high risk of unfavorable outcomes.

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Introduction

Wilms' tumor or nephroblastoma, is one of the most common pediatric malignant diseases, affecting approximately 1 in 10 000 children [1]. It is well known for its unpredictable course and tendency of tumor recurrence or metastasis, even years after primary treatment [2]. It is generally accepted that tumor stage and histological subtype are the major criteria predicting the clinical outcome of the tumor [1,3]. However, the biologic behavior of Wilms' tumor is difficult to prognosticate on the basis of histopathologic findings only [4,5]. As the mechanism of cancer recurrence or metastasis has not been fully clarified, further research should be conducted in this field.

Transforming growth factor beta 1 (TGF-β1) is a pleiotropic cytokine which plays a dual role in the process of carcinogenesis. Previous reports [6,7] have suggested that TGF-β1 has biphasic actions, which suppresses tumorigenesis at early stages, but promotes tumor progression at the later stages. TGF-β1 has been considered to be positively associated with tumor cell invasiveness and metastasis, as well as disease progression in various cancers [8–12]. As the expression status of TGF-β1 in Wilms' tumor has not been well clarified, we conduct this study to evaluate the role of TGF-β1 in Wilms' tumor progression.

Materials and methods

Patients

Details of 51 patients with Wilms' tumor who underwent radical nephrectomy during the period December 2005 to December 2012, as well as their paraffin-embedded tumor samples, were retrieved from our hospital. Patients who had received chemotherapy or radiotherapy before surgery were excluded from the research. Fourteen patients were diagnosed with local invasive or metastatic disease during nephrectomy by means of pathological detection, including three perirenal adipose capsule, one renal pelvis, one ureter, five lymph node, two renal vascular, two renal vascular embolus. Three patients had hematogenous metastasis, including two pulmonary metastasis and one clavicular metastasis post-nephrectomy. Retroperitoneal recurrence was detected in situ in eleven patients. Tumor rupture occurred in six patients in the process of surgery, of whom four patients suffered from tumor recurrence in the process of postoperative follow-up. All the patients received postsurgical treatment according to the guidelines of NWTS-5 (National Wilms' Tumor Study), as well as diagnosis criteria for histopathological type. Histopathological type and tumor stage were determined after pathological review. Clinicopathological factors including age, gender, stage and histopathological type were collected. The endpoint for disease-free survival (DFS) was the first documented day of recurrence, distant metastasis or death from the disease. Overall survival (OS) was calculated starting from the first surgery to the date of death caused by the disease or April 2013 for surviving patients.

Immunohistochemical staining

Immunohistochemistry was performed as previously described [12]. Primary antibody against TGF-β1 (1:100, rabbit polyclonal antibody (sc-146), Santa Cruz, USA) was used according to the manufacturer's instructions. The secondary antibody used was biotinylated goat anti-rabbit IgG (SP-9001, Zhong Shan, China). The negative control was carried out following the same steps but primary antibody was replaced by normal serum derived from the same species.

Staining assessment and scoring

Cells were considered to be positive stained in the cytoplasm and (or) nucleus (Fig. 1). Blind assessment was conducted by two investigators. Semi-quantitative immunoreactive score (IRS) was used as a scoring system [13]. Briefly, the IRS was calculated by multiplying the staining intensity (SI) (graded as: 0 = absent, 1 = weak, 2 = moderate and 3 = intense staining) and the percentage of positively stained cells (PP) (0 = 0–10% of stained cells, 1 = 11–50% of stained cells, 2 = 51–80% of stained cells and 3 = more than 80% of stained cells). Tumors were considered positive when IRS scoring was at least 2.

Wilms' tumor cell culture

All cell culture reagents were obtained from Gibco (Grand Island, NY, USA). After the primary Wilms' tumor tissue (case 14 in Table 1) was removed surgically, the friable tumor tissue was immersed and rinsed briefly in calcium-free Hanks solution with 1% penicillin and streptomycin. The tumor tissue was finely minced into fragments approximately 1 mm in diameter, and then digested using 0.25% trypsin/0.02% ethylene diamine tetraacetic acid (EDTA) solution for 5 min at 37 °C to obtain a single cell suspension. Further trypsin action was halted by the addition of an equal volume of DMEM/F12 medium with 10%FBS. The suspension was centrifuged at 800 g for 5 min and then the digested single cells were added to a series of 25-cm^2 culture flasks containing 4 ml DMEM/F12-based culture with 10%FBS. Cultures were maintained at 37 °C in a humidified atmosphere of 5%CO^2. The medium was freshened after the cells were cultured for 24 h to remove the suspended substance including debris and contaminating blood cells, and then fed fresh growth medium every 3 days. When the cells were confluent, the monolayer cells were detached using 1 ml 0.25% trypsin/0.02% EDTA and distributed to new flasks at a 1:3 subculture ratio. Cells used for the transfection were passages 2 to 3.

Construction of PcDNA3.1(+)/TGF-β1 and PcDNA3.1(+)/GFP and transient transfection

PcDNA(+) was purchased from Invitrogen (Cat No.:V790–20). TGF-β1 and GFP (GenScript, Nanjing, China) were then cloned into the plasmid using BmtI/HindIII and BmtI/BamHI, respectively. We verified the authenticity of the expression vector by sequencing the plasmid. The plasmid was transiently transfected into low TGF-β1