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Characterization of a WiT49 Cell Line Derived Orthotopic Model of Wilms Tumor

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Abstract

Wilms tumor is the most common malignant renal tumor in children. However, to date no Wilms tumor mouse model is available due to the lack of Wilms tumor cell lines. Herein for the first time we report an orthotopic xenograft mouse model utilizing the recently described Wilms tumor cell line WiT49. It has a high tumor occurrence rate (85%) without metastasis. Hematoxylin and eosin staining showed it is subcapsular in location and mainly biphasic with stromal and epithelial components while blastemal component is unappreciable. This model provides the prerequisite for the screening and development of new anti-tumor agents for Wilms tumor.

Keywords

Wilms tumor; orthotopic model; WiT49

Introduction

Wilms tumor is the most common renal malignancy in children. While improvement in survival has occurred in the last few decades, as a result of multimodal therapy (surgery, radiation and chemotherapy), novel therapeutic approaches have not been rapidly forthcoming. In large part progress has been hampered by a lack of appropriate cell lines and suitable preclinical models. Cell lines/models available to date have not been representative of classic Wilms tumor. Herein we report on the development of an orthotopic preclinical mouse model utilizing the recently described cell line WiT49, a Wilms tumor cell line derived from lung metastatic site [1].

Materials and Methods

Cell line

The cell line WiT49 was kindly provided by Dr. Herman Yeger (Hospital for Sick Children, Toronto, Canada) and cultured as described previously (1). Briefly, the cells were maintained in DMEM (Sigma, Saint Louis, MO) supplemented with 15% FBS (Hyclone,

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Conflict of Interest Statement

No conflict of interest exists in the submission of this manuscript.

Logan, UT), 6 µl/l 2-mercaptoethanol (Sigma), 1.2 µg/ml insulin (Sigma), 100 U/ml penicillin and 100 ug/ml streptomycin (Gibco, Grand Island, NY), and incubated in a humidified chamber at 37°C and 5% CO₂. Cells were plated in 100 mm culture dishes and passaged through trypsinization.

Animals

20 Six-week-old male athymic NCr-nu/nu nude mice (National Cancer Institute, Frederick, MD) were utilized to determine efficacy of establishing an orthotopic Wilms model. They were housed in a barrier facility and acclimated to 12-hour light/dark cycles for at least 24 hours before use.

Orthotopic Wilms tumor xenograft model

In vivo experiments were conducted according to our animal protocol approved by the Institutional Animal Care and Use Committee of University of Connecticut Health Center. Briefly, the mice were anesthetized with intraperitoneal injection of 1.25% avertin (0.5 ml/20 g mouse). A 1.0 – 1.5 cm incision was made parallel to the spine on the left flank and the thin muscle layer was split, gentle pressure on the abdomen results in exposing the left kidney through the incision. Three million of WiT49 cells in 30µl of PBS were injected into the renal subcapsule using a Hamilton Syringe (700 Series). The skin incision was then closed with 4–0 Polysorb suture (US surgical, Norwalk, CT), smeared with glue and covered with Tegaderm transparent dressing. Eight weeks later, the mice were sacrificed by carbon dioxide inhalation. Both the tumor-bearing kidney and uninvolved contralateral kidney from each mouse were harvested, taken pictures and fixed for hematoxylin and eosin staining. Representative sections from the liver and lung were evaluated for metastasis as was the peritoneal cavity.

Results

The Rate of Tumor Occurrence and Tumor Metastasis

Seventeen out of 20 mice (85%) had developed tumors locally in 8 weeks (Fig. 1). The contra-lateral kidney, liver and lungs showed no evidence of metastatic disease under hematoxylin and eosin staining evaluation. Similarly, the abdominal cavity was free of disease.

General Features of Wilms Tumor in an Orthotopic Xenograft Model

All Tumors were subcapsular in location. Similar to that in human, the tumor growth expands the subcapsular space and respects the native capsule without infiltration (Fig. 2A). The advancing border of the tumor shows minimal infiltration of the native renal parenchyma with no significant inflammatory host response (Fig. 2B).

Microscopic Features of Wilms Tumor in an Orthotopic Xenograft Model

The tumor is mainly biphasic with stromal and epithelial components while blastemal component is not appreciable.

The epithelial component includes tubules, glomeruloid structures and epithelial cystic structures (Fig. 2C–E). The larger cystic structures show intraluminal tumor growth as finger-like projections with stroma and rounded polypoid structures with stromal and epithelial elements. The larger polypoid structures tend to show cauliflower-like configuration with abundant chondromyxoid extracellular matrix material. Some cystic structures contain luminal proteinaceous fluid. Strands of fibrinoid material are noted in some of the tubulo-cystic luminal spaces. The tubular and cystic lining epithelium mostly

consists of one layer of cells with mild nuclear variability in size and shape. Focal areas of crowding/hyperplasia and fine papillations are present.

The stromal component of the tumor is solid and variably cellular. The stromal cells are plump and spindle-shaped and in areas chondromyxoid, showing moderate nuclear pleomorphism. Mitotic count is approximately 3 to 5 mitoses per 10 high power field (HPF). The extracellular matrix is variably loose/myxoid, hyaline to cartilaginous. Isolated tumor cells show nuclear pyknosis and karyorrhexis. Wide expanses of tumor necrosis are absent.

Discussion

Although effective treatment is available for the majority of Wilms tumor patients, the failure of some patients to respond to current treatments along with emerging data regarding long term risks of those treatments such as radiation-related scoliosis, gonadal failure, adverse effects on pregnancy, cardiomyopathy, and increased long-term risk of second malignancies [2–6], call for the development of new therapeutic approaches. A prerequisite for the successful screening and development of new anti-tumor agents is the availability of an appropriate animal model.

Orthotopic xenografts of human tumor cells in nude mice reproduce the histology and metastatic pattern of most human tumors at advanced stage. They more accurately reproduce the original disease state than the more commonly used subcutaneous xenografts in preclinical drug development [7]. However, to date no real orthotopic Wilms tumor xenograft model is available due to limited Wilms tumor cell lines.

Originally derived in 1971 as an anaplastic Wilms tumor, SK-NEP-1 was regarded as a Wilms tumor cell line [8]. Due to the fact that it could produce subcutaneous tumors, it had been commonly used to test many new anti-tumor and anti-angiogenesis agents [9–13]. However, recently Smith et al. used different molecular tools such as gene expression profiling, RT-PCR and DNA sequencing to reveal that SK-NEP-1 expresses EWS-FLI1 gene fusion transcripts, a characteristic of Ewing sarcoma [14]. Therefore, the observed biological features and treatment response patterns in prior publications utilizing SK-NEP-1 should be ascribed to Ewing family tumors, not Wilms tumor.

Another cell line extensively used as Wilms tumor was G401. It was obtained from a tumor of the kidney from a three-month-old child in 1975. It can form tumor rapidly in nude mice. However, in 1993 Garvin et al. found that the properties of G401 heterotransplant were consistent with a rhabdoid phenotype rather than those of a Wilms tumor by histology, ultrastructure, immunohistochemistry, and messenger RNA comparison analysis [15]. These findings call into question the research that has been performed using G401 cell line as a classic model of Wilms tumor [16–18]. As a consequence, investigators have had no bona fide Wilms tumor cell line available.

Interestingly, in 2003 Alami et al. characterized a Wilms tumor cell line derived from a first-generation xenograft of a human Wilms tumor lung metastasis, named WiT49 [1]. Its histologic appearance in monolayer culture showed multiple phenotypes resembling that of parent tumor. In their report they note that it expresses normal WT1, overexpresses IGFII, and carries a mutant p53, which are the three Wilms tumor markers. Also, it overexpresses hepatocyte growth factor (HGF) and its receptor met that was also observed in a series of metastatic Wilms tumor and negative in SK-NEP-1 cells [1]. Moreover, analysis performed by the COG biopathology center suggested that this cell line had loss of heterozygosity (LOH) at 1p and 16q (data not shown). However, this later finding is limited by the lack of normal DNA from the original patient. All of the above features make WiT49 a potentially acceptable tumor cell line model of Wilms tumor.

Here we established an orthotopic tumor model using WiT49 cells. By western blot analysis, these orthotopic tumors express p53, HGF and met proteins (Supplemental Figure 1), which recapitulates the molecular phenotype of WiT49 cells. More importantly, the histology features in those tumors were consistent with the expected properties of classic Wilms tumor. This finding fills the current void in the Wilms tumor murine models and provides the substrate for the screening and development of new agents for Wilms tumor therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. The appearance of the tumor-bearing kidney and uninvolved contralateral kidney in WiT49 orthotopic xenograft model. A representative figure taken by camera was shown. Uninvolved contralateral kidney (left); Tumor-bearing kidney (right) The finest scale bar represents 1mm.

Figure 2.

The microscopic structures of wilms tumor in WiT49 orthotopic xenograft model. The representative figures by hematoxylin and eosin staining were shown. A: the overall microscopic feature (x40); B: advancing front (x100); C: tubular differentiation (x200); D: glomeruloid structures (x400); E: bulky cauliflower-like growth within cystic spaces (x100).