



*Case Report*

## Tetraploidization in Wilms tumor in an infant

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**ABSTRACT.** Genetic instability is frequent in human cancer. Unscheduled tetraploidization can trigger cell transformation and tumorigenesis. We made a cytogenetic analysis by Giemsa-trypsin banding of a stage I, biphasic Wilms tumor diagnosed in a 10-month-old male. An evident karyotypic heterogeneity was found. Four different subclones of tumor cells were observed, with DNA content varying from diploid to near-tetraploid complements. The genetic events involved in the acquisition of aneuploidy in Wilms tumor remain unclear. We hypothesize that initial tetraploidization caused aberrant cell division, leading to abnormal chromosomal segregation, cell transformation and tumorigenesis.

**Key words:** Wilms tumor; Cancer biology; Cytogenetics;  
Tumor biology

## INTRODUCTION

Wilms tumor (WT), or nephroblastoma, is the most common malignant renal cancer to affect the pediatric population. Cytogenetic investigations on WT usually show nonrandom chromosomal changes characteristic of this tumor type. Numerical changes, mostly trisomies of chromosomes 7, 8, and 12, are particularly recurrent in this setting (Höglund et al., 2004).

Besides these structural cytogenetic observations, little is known about the genetic events involved in the acquisition of aneuploidy in WT. Changes in ploidy can result from several pathophysiological events, including aberrant polyploidization. Tetraploid cells represent an important intermediate on the route to aneuploidy and cancer. Unscheduled tetraploidy can arise through cell fusion, mitotic slippage or failure to undergo cytokinesis (Storchova and Kuffer, 2008).

In the present article, we describe the cytogenetic observation of tetraploidization and emergence of multiple subclones in WT occurring in an infant. Furthermore, the genetic implication of tetraploidization in human cancer, particularly in childhood WT, is also briefly reviewed.

## CASE REPORT

An abdominal mass was palpable in a routine pediatric consultation of a 10-month-old male. This child was the first son of an apparently healthy and non-consanguineous couple. Family history disclosed no other cases of childhood cancer.

On physical examination, the child was in good general condition. He had a slightly distended abdomen and a mass was felt throughout his left flank and pelvis. Abdominal ultrasound (US) showed a bulky lesion, heterogeneous and arising from the left kidney. A computed tomography (CT) scan depicted the predominantly solid aspect of the lesion with intense, irregular enhancement following contrast injection. The mass measured 8 cm in length, while the contralateral right kidney was normal. Chest CT and bone scintigraphy were normal. The child was submitted to a US-guided True-Cut® biopsy. Histological examination of the specimen revealed a biphasic tumor composed of a predominant blastematos component, admixed with epithelial tubular structures, resembling immature renal tubules. The diagnosis of a stage I biphasic WT was established. The therapeutic approach followed the non-metastatic SIOP-2001 protocol, which combines a four-week, two-drug, neo-adjuvant regimen (vincristine and dactinomycin), followed by surgical approach and adjuvant chemotherapy.

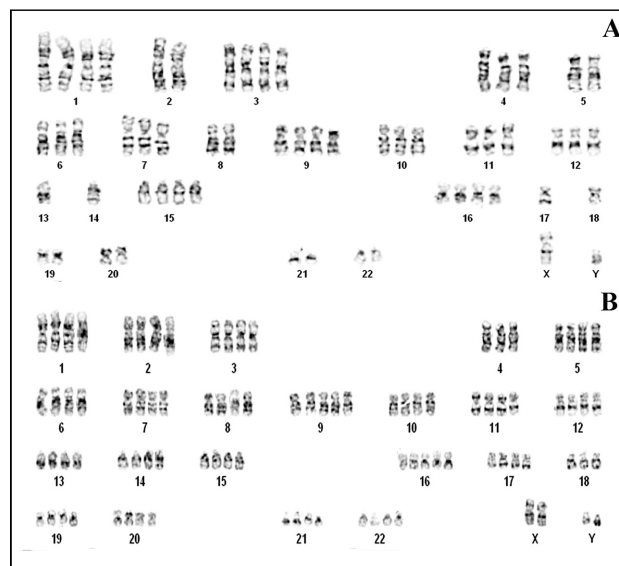
## CYTOGENETICS STUDIES

A fresh pre-therapy WT sample (adjacent to areas of tumor verified by frozen section) was aseptically collected and minced with scissors on a Petri dish. The minced pieces were divided into T30 flasks and enzymatically disaggregated for 2 h on 0.5% collagenase type IV (Sigma Chemical Co., St. Louis, MO, USA) in DMEM-F10 medium (Gibco BRL, Life Technologies, Carlsbad, CA, USA) supplemented with 15% fetal bovine serum (FBS). Following disaggregation, the cells were centrifuged and the collagenase solution removed and replaced with medium. Cultures were harvested after 1 week with an overnight colcemid (Gibco) treatment at a final concentration of 0.25 µg/mL. Cells were collected by trypsinization and centri-

fused at 1000 rpm. Trypsin activity was inhibited by resuspension in medium containing 10% FBS. The cells were pelleted by centrifugation, washed with phosphate-buffered saline, and resuspended in hypotonic medium (0.075 M KC1) for 20 min at 37°C. Following hypotonic treatment, preparations were fixed three times with methanol:acetic acid (3:1). Further, cytogenetic analysis was performed by Giemsa-trypsin banding (GTG-banding).

## RESULTS

Cytogenetic analysis of the WT sample showed karyotypic heterogeneity. Forty-one countable metaphase spreads were analyzed. Seventeen of these showed a normal complement of 46,XY while the rest showed near-diploid, near-triploid, and near-tetraploid chromosome numbers. Twelve metaphases (29%) displayed  $2n = 44-49$ , five metaphases (12%) showed  $2n = 58-80$  and the other 17% were near-tetraploid with  $2n = 81-93$  (Figure 1). No structural rearrangements were observed.



**Figure 1.** Partial results from conventional analysis of Giemsa-trypsin-banded Wilms tumor chromosome metaphase spreads. **A.** 58 <math>\langle 3n \rangle</math>, XY, -?X, -?Y, +1, -2, +3, -5, -8, +9, -13, -13, -14, -14, +15, +16, -17, -17, -18, -18, -19, -20, -21, -22 karyotypes. **B.** 92, XXYY, -4, +9, +16, -18 karyotypes.

## DISCUSSION

Most malignant tumors usually display genetic instability, which can occur at the level of mutations in individual genes, by multiple structural and numerical aberrations or as large-scale rearrangements with changes in cell ploidy. These genetic events may be responsible either for initiating or facilitating tumor progression (Storchova and Kuffer, 2008).

Wilms tumor is a malignant renal neoplasm derived from nephrogenic blastema. Besides the structural abnormalities that lead to homozygosity or hemizygosity for a small deletion on chromosome 11 (11p13) typically seen in WT, classical cytogenetic investigations

have shown that this tumor usually carries numerical chromosomal changes. At least two cytogenetic pathways exist, one dominated by gains, with chromosomes 7, 8 and 12 trisomies being the most common abnormalities observed, and another characterized by losses of 1p, 16q, 11p, 22q, and X (Höglund et al., 2004). However, despite the abundance of cytogenetic information, little is known about the genetic events involved in the acquisition of aneuploidy in WT.

In the present report, we describe the occurrence of WT in an infant. Tumor karyotype analysis revealed multiple neoplastic subclones, probably resulting from initial tetraploidization and abnormal chromosomal segregation.

Polyploid WT cases are relatively rare and have been estimated at only 8% of cases (Höglund et al., 2004). Even though a plethora of WT cases have been studied cytogenetically, no correlations between specific chromosome alterations and histologic subtypes have been found. Evidence for an association between a tetraploid DNA pattern and more aggressive malignancies with poor outcomes has been shown by flow cytometric analyses (Li et al., 1995; Betts et al., 1997; Iyer et al., 1999). Also, it has been shown that intratumor DNA ploidy heterogeneity is significantly associated with unfavorable outcomes and death (Yildiz et al., 1994), reflecting the high genomic instability that leads to a rapid progression of the disease.

Tetraploidy is highly prevalent in different forms of cancer, suggesting a role for this cell cycle state in promoting cellular transformation. Aberrant polyploidization can arise through several mechanisms including cell fusion, endoreplication, DNA decatenation, mitotic slippage, or failure to undergo cytokinesis (Storchova and Pellman, 2004). Tetraploid cells represent an important intermediate on the route to aneuploid status and cancer. The major source of instability in tetraploid cells is the presence of supernumerary centrosomes that lead to the formation of multipolar spindles and consequently, aberrant chromosome segregation (Storchova and Pellman, 2004; Gisselsson et al., 2008). The tetraploid-intermediate model is also supported by the fact that the number of chromosomes in cancer cells is often very high, which is difficult to explain by a repeated accumulation of chromosomes at each division (Ganem et al., 2007). Furthermore, the presence of four copies of certain chromosomes, as seen for the aberrant near-diploid and near-triploid clones in the present case, points to the rational conclusion that chromosome heterogeneity may have been a consequence of random chromosome losses following tetraploidization. This genetic event seems to have facilitated the emergence of a WT in our little patient and may also be potentially implicated in tumorigenesis in other cases of infantile WT.

### Conflict of interest statement

All authors have no conflict of interest to disclose.

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