the nurse cells and oocyte nucleus throughout oogenesis. To determine the fate of germinal vesicles after ablation, PZ107 egg chambers were recovered from host females at various times after ablation, fixed, and stained for β-galactosidase activity. In some egg chambers (n = 7), the oocyte nucleus was absent based on β-galactosidase staining, whereas in other egg chambers (n = 4) a stained nucleus remained. In four cases, β-galactosidase and *vasa* localization were examined in the same egg chamber after germinal vesicle ablation. In two of these cases, both were absent; in one case, the oocyte nucleus failed to stain for β-galactosidase and yet *vasa* protein was still localized apparently normally. In the fourth case, a β-galactosidase–positive oocyte nucleus was found even though *vasa* protein was not localized. This may indicate that there are multiple aspects of oocyte nucleus function which can be eliminated separately or in concert.

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Suppression of Tumorigenicity in Wilms Tumor by the p15.5-p14 Region of Chromosome 11

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Wilms tumor has been associated with genomic alterations at both the 11p13 and 11p15 regions. To differentiate between the involvement of these two loci, a chromosome 11 was constructed that had one or the other region deleted, and this chromosome was introduced into the tumorigenic Wilms tumor cell line G401. When assayed for tumor-forming activity in nude mice, the 11p13-deleted, but not the 11p15.5-p14.1–deleted chromosome, retained its ability to suppress tumor formation. These results provide in vivo functional evidence for the existence of a second genetic locus (WT2) involved in suppressing the tumorigenic phenotype of Wilms tumor.

WILMS TUMOR, A PEDIATRIC NEPHROBLASTOMA, originates from embryonal kidney stem cells and presents itself in either a classical form or a differentiated form (*1*). The classical form comprises embryonic blastema cells, whereas the differentiated form may contain a variety of cell types, including striated muscle, squamous epithelial, and cartilaginous cells. Wilms tumor occurs in either a familial or sporadic form; >95% of all Wilms tumors are of the sporadic form (*2*). Cytogenetic analysis of Wilms tumors from patients with the familial WAGR syndrome (Wilms tumor, aniridia, genital urinary abnormalities, and mental retardation) shows interstitial deletions of chromosome 11 that include 11p13 (*3*). Restriction fragment length polymorphism (RFLP) analysis revealed a loss of heterozygosity (LOH) of surrounding DNA markers in the 11p13 region from some sporadic Wilms tumors (*4*). Thus, the region may encode a tumor suppressor gene, the inactivation of which is required for progression to the malignant state. A candidate 11p13 Wilms tumor suppressor gene cDNA, named WT1, was isolated and is specifically expressed during normal kidney development (*5, 6*). However, RFLP analyses of sporadic Wilms tumors have also indicated a LOH of DNA markers in the 11p15 region, named WT2 (*7*), in the absence of any detectable alteration in the 11p13 region, suggesting the involvement of a second genetic locus in the etiology of Wilms tumor (*8*). In addition, two reports have excluded the involvement of both the 11p13 region and the 11p15 region in some familial Wilms tumors (*9*), suggesting the involvement of a third genetic locus.

We have investigated the regions of chromosome 11 that may contribute to suppression of the tumorigenic phenotype in the G401 Wilms tumor cell line. Copies of chromosome 11, with appropriate deletions, were transferred into G401 cells by the microcell-mediated chromosome transfer (MMCT) technique (*10*). The G401 cell line, isolated directly from an explant of a Wilms tumor from a 3-month-old male (*11*), is undifferentiated in culture, which is consistent with the establishment of other Wilms tumor cell lines (*12*). It is pseudodiploid with a single marker 12 chromosome and contains no cytogenetically detectable deletion or alteration of either chromosome 11 homolog. G401 is immortal in vitro and forms tumors when injected subcutaneously into nude mice. When a balanced X;11 translocated human chromosome [der(11)t(X;11)(q26;q23)] that contains >90% of chromosome 11 is introduced into G401.6TG.6 (HPRT-clone of G401) cells by MMCT, the tumorigenic potential of G401 cells is suppressed, whereas the introduction of "irrelevant" X and X;13 [der(13)t(X;13)(p22;q12)] chromosomes does not affect tumorigenesis (*13*). To further define the region that contains the tumor suppressor information for the G401 Wilms tumor cell line, we transferred a naturally occurring balanced X;11p translocated chromosome [der(11)t(X;11p)-(q21;q13)] from the human fibroblast cell line GM3322 into mouse A9 cells by MMCT, which produced monochromosome hybrid MCH 701.8. The X;11p chromosome was then transferred from MCH 701.8 into G401.6TG.6 cells. The human X;11p chromosome has been characterized cytogenetically and with 17 DNA markers that span the 11p chromosomal arm (*14*) (Fig. 1). The introduction of the X;11p chromosome into G401.6TG.6 resulted in the isolation of two microcell hybrids, MCH 706.1 and MCH 706.2, that contained 47 chromosomes and that were nontumorigenic when inoculated subcutaneously into athymic nude mice (Table 1). The spontaneous segregants of the X;11p chromosome from the nontumorigenic microcell hybrids were selected with the addition of 6-thioguanine to the medium. A segregant thus isolated, MCH 706.2.6TG, contained 46 chromosomes and reexpressed the malignant phenotype (Table 1). This constitutes further evidence of the specific correlation of suppression of tumorigenicity with the presence of normal genetic information on chromosome 11. These results define the region responsible for suppressing the tumorigenic phenotype of the G401 Wilms tumor to 11pter-q13 with an in vivo functional assay.

The introduction of the X;11p chromosome into G401.6TG.6 further localized the tumor suppressor gene but did not differentiate between the 11p13 and 11p15 regions or investigate cooperativity between the two loci. We therefore devised a procedure to enrich for deletion or rearrangement of a specific chromosomal arm (*14*). Two useful radiation-reduced microcell hybrids (XMCH 708.24 and XMCH 708.20) were generated by this method from MCH 701.8 (Fig. 1). The deleted X;11p chromosome present in XMCH 708.24 contained a simple interstitial deletion encompassing the p15.5::p14.1 region and retained an intact 11p13 region. The deleted X;11p chromosome present in XMCH 708.20 contained a

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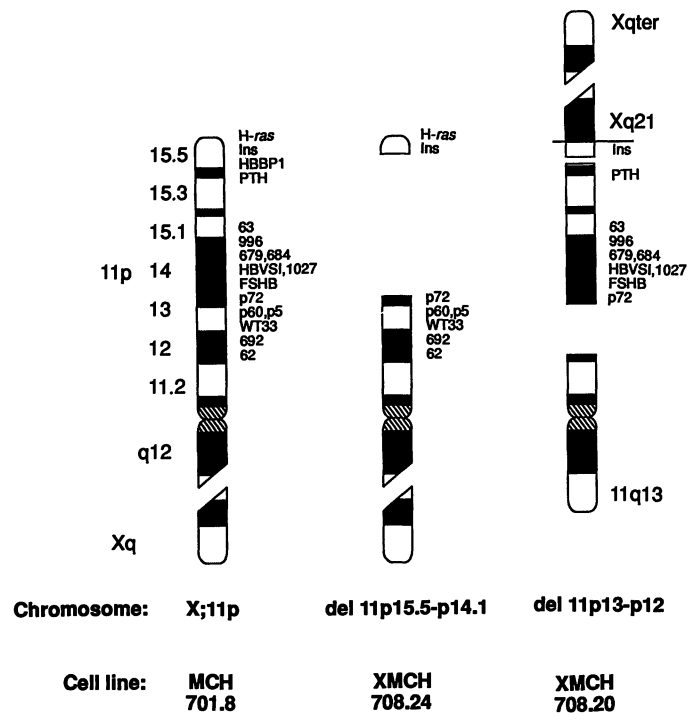

Fig. 1. Summary of Southern (DNA) blot and cytogenetic results of the X;11p human chromosome present in MCH 701.8 and the resulting deleted 11p13-p12 chromosome in XMCH 708.20 and the deleted 11p15.5-p14.1 chromosome in XMCH 708.24 after XMMCT treatment (*14*). The blank regions of the ideograms denote deletions detected by cytogenetic analysis, Southern blot analysis, or both. The DNA markers are positioned in their mapped position. The absence of a DNA marker denotes its loss on the chromosome, on the basis of Southern blot analysis. The blank diagonal truncation denotes a continuance of the Xq arm for diagrammatic simplification. The deleted X;11p chromosome in XMCH 708.24 contains a simple interstitial deletion encompassing the 11p15.5-p14.1 region. The deleted X;11p chromosome in XMCH 708.20 contains a complex rearrangement involving the inversion of the 11pter-q13 region with religation of the 11p15.5 subband to the Xq21 band. The horizontal line denotes the breakpoint and religation. In addition, the chromosome has undergone a deletion of the 11p13-p12 region and a submicroscopic deletion surrounding the HBBP1 locus, as detected by Southern blot analysis. Cytogenetic analysis was performed as described (*27*) on a minimum 25 metaphase chromosome spreads. The DNA markers used are as described (*14*). Insulin, Ins; hepatitis B viral insertion site I, HBVSI; follicle-stimulating hormone B chain, FSHB; β globin pseudogene 1, HBBP1; parathyroid hormone, PTH; and 62, 63, 679, 684, 692, 996, and 1027 (*29*), and p5, p72, p60 (*30*), random genomic DNA fragments mapped to chromosome 11 as indicated.

Table 1. Tumorigenicity of G401.6TG.6 microcell hybrids containing a radiation-reduced X;11p chromosome. The cell lines were maintained on Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hazleton) plus 2 mM L-glutamine, penicillin (100 IU/ml), streptomycin (0.1 mg/ml), and fungizone (amphotericin B; 0.05 μg/ml). MMCTs were performed as described (*9*). Biochemical selection of X;11p chromosomes was based on the presence of the HPRT gene on the Xq region of the chromosome and by the addition of hypoxanthine, aminopterin, thymidine (HAT) medium (*26*). Cytogenetic analysis was done as described (*27*) on a minimum of 25 metaphase chromosome spreads. The tumorigenic potential of each microcell hybrid was determined by subcutaneous inoculation of 1×10^7 cells into the ventral surface of multiple athymic nu/nu (nude) mice and examined on a regular basis for >60 days or until the nude mice were killed. A single small nodule was observed at greater than 85 days postinoculation in MCH 370.6. In addition, MCH 370.6 has demonstrated genomic instability during late passage numbers. Nude mice were maintained in accordance with NIH guidelines. All of the cell lines were routinely assayed for the presence of *Mycoplasma* by cultural methods, by the 4,6-diamidino-2-phenylindole (DAPI) method (*28*), or both, and were routinely negative.

Cell line	Chromosome 11 introduced	Modal chromosome number	Growth in selective HAT medium	Tumorigenicity*
G401.6TG.6		46	−	12/12
MCH 706.1	11p	47	+	0/8
MCH 706.2	11p	47	+	0/4
MCH 706.2.6TG.1		46	−	5/5
MCH 370.6	del p13-p12	47	+	1/7
MCH 370.m1	del p13-p12	47	+	0/4
MCH 370.m2	del p13-p12	47	+	0/4
MCH 370.m3	del p13-p12	47	+	3/4
MCH 369.6	del p15.5-p14.1	47	+	7/7
MCH 369.13	del p15.5-p14.1	47	+	7/7
MCH 369.18	del p15.5-p14.1	47	+	8/8

*Number of mice with tumors over total number tested.

deletion encompassing the p13::p12 region including the WT33 candidate Wilms tumor suppressor gene locus and a submicroscopic deletion surrounding the globin cluster at p15.5. In addition, the chromosome has an inverted 11p arm, which resulted in an acrocentric chromosome that has religated to the X chromosome arm between the insulin and H-*ras* loci (and also resulted in loss of the H-*ras* locus).

The deleted 11p15.5-p14.1 chromosome from XMCH 708.24 was transferred into G401.6TG.6 cells by means of MMCT and resulted in the isolation of three clones, MCH 369.6, MCH 369.13, and MCH 369.18. Cytogenetic analysis identified the presence of the deleted 11p15.5-p14.1 chromosome derived from XMCH 708.24 in all three microcell hybrids (Fig. 2). Subcutaneous inoculation of each of the cell lines into nude mice generated tumors (Table 1). Thus, a genetic element present in the 11p15.5-p14.1 region contributes to suppressing the tumorigenic potential of G401 cells. The deleted 11p13-p12 chromosome from XMCH 708.20 was transferred into G401.6TG.6 cells by means of MMCT and resulted in the isolation of one clone, MCH 370.6, and three mass populations, MCH 370.m1, 370.m2, 370.m3. All four cell lines were analyzed cytogenetically and contained the deleted 11p13-p12 chromosome from XMCH 708.20 (Fig. 2C). After subcutaneous inoculation of each of the cell lines into nude mice, MCH 370.6, MCH 370.m1, and MCH 370.m2 did not form tumors (Table 1). However, MCH 370.m3 was tumorigenic (Table 1). Cytogenetic analysis of a tumor reconstitute of MCH 370.m3 indicated that the introduced deleted 11p13-p12 chromosome was present and apparently intact. Transfer of this chromosome from MCH 370.m3 back into the A9 mouse cell line would allow the investigation of additional loss of DNA markers in the 11p15-p14 region incurred as a result of microcell transfer. The suppression of tumorigenicity of the G401 cells by the introduction of the deleted 11p13-p12 chromosome from XMCH 708.20 shows that the 11p13 region is not responsible for tumor suppression of the G401 Wilms tumor cell line.

Our results provide evidence from a functional in vivo assay for the presence of a second genetic locus (WT2), residing in the p15.5-p14.1 region of chromosome 11, that is involved in suppressing the tumorigenic phenotype of the G401 Wilms tumor cell line. Our data place this gene either between HBBP1 and insulin or between HBBP1 and 11p14.1. On the basis of RFLP analyses of sporadic Wilms tumors (*8*), this locus is probably located in the 11p15.5 region.

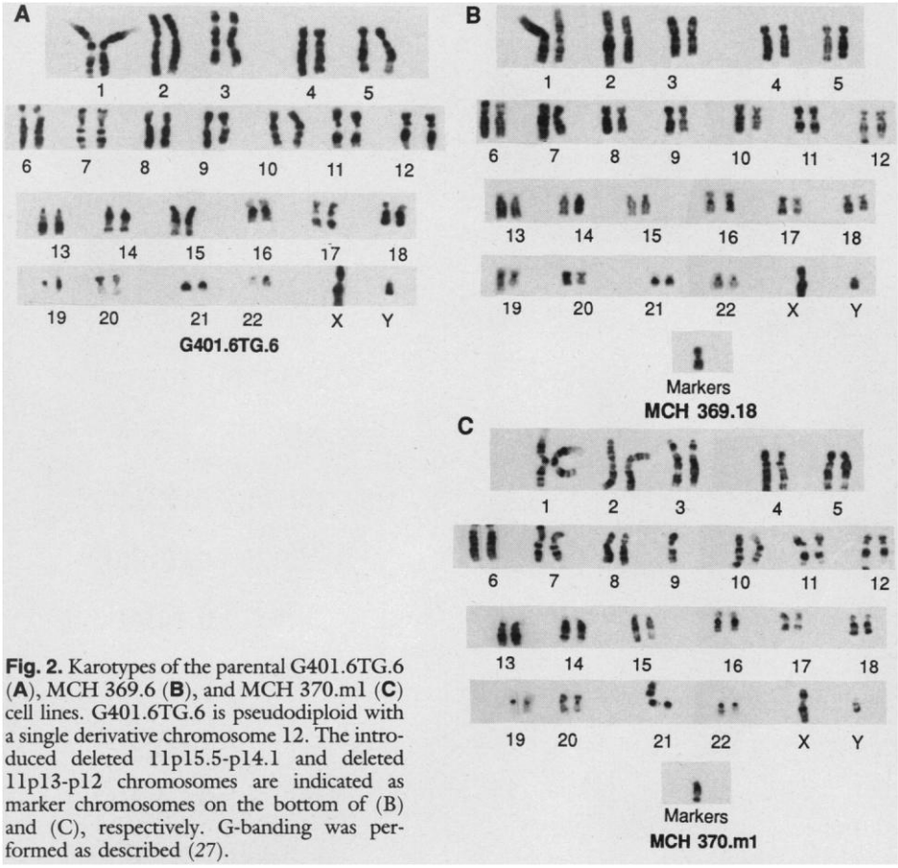

Fig. 2. Karotypes of the parental G401.6TG.6 (**A**), MCH 369.6 (**B**), and MCH 370.m1 (**C**) cell lines. G401.6TG.6 is pseudodiploid with a single derivative chromosome 12. The introduced deleted 11p15.5-p14.1 and deleted 11p13-p12 chromosomes are indicated as marker chromosomes on the bottom of (B) and (C), respectively. G-banding was performed as described (*27*).

Henry *et al.* (*15*) have suggested that genes located at both the 11p15 region and the 11p13 region may be involved in the etiology of Wilms tumor by cooperating with one another. However, the data from our in vivo system suggest that these two loci are separate and discrete key elements of different negative growth regulatory pathways. There is a possibility that the WT2 gene may positively regulate the expression of the endogenous G401 WT1 gene in vivo but not in vitro, because of additional signals. However, this explanation seems remote because the candidate WT1 gene is not transcribed in the tumorigenic G401.6TG.6 or nontumorigenic microcell hybrids (*13*) and expression of WT1 has been observed in some Wilms tumors (*16*). Moreover, another Wilms tumor cell line (GOS-4) has been established in culture that has a similar morphology to G401, contains two intact copies of WT1 but fails to express either, and is immortal in vitro and tumorigenic in vivo (*17*). GOS-4 provides additional evidence for the apparent lack of involvement of WT1 in some Wilms tumors.

On the basis of the functional data presented here, we propose a model in which at least two genetic subtypes of Wilms tumor exist, one involving the inactivation of a key element of a negative growth regulatory pathway located at 11p13 (WT1) and the other involving the inactivation of a separate negative growth regulator located at 11p15.5-p14.1 (WT2), which gives rise to a nephroblastoma. Furthermore, the WT2 gene may be a key growth regulatory gene involved in several other malignancies, including the following: adrenocortical carcinoma (*15*); Beckwith-Weidemann syndrome (*18*); bladder carcinoma (*19*); breast carcinoma (*20*); squamous-cell carcinoma, large-cell carcinoma, and adenocarcinoma of the lung (*21*); hepatoblastoma (*22, 23*); rhabdomyosarcoma (*24*); and testicular cancer (*25*).

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