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Research Articles

Introduction of a Normal Human Chromosome 11 into a Wilms' Tumor Cell Line Controls Its Tumorigenic Expression

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The development of Wilms' tumor, a pediatric nephroblastoma, has been associated with a deletion in the p13 region of chromosome 11. The structure and function or functions of this deleted genetic material are unknown. The role of this deletion in the process of malignant transformation was investigated by introducing a normal human chromosome 11 into a Wilms' tumor cell line by means of the microcell transfer technique. These variant cells, derived by microcell hybridization, expressed similar transformed traits in culture as the parental cell line. Furthermore, expression of several proto-oncogenes by the parental cells was unaffected by the introduction of this chromosome. However, the ability of these cells to form tumors in nude mice was completely suppressed. Transfer of other chromosomes, namely X and 13, had no effect on the tumorigenicity of the Wilms' tumor cells. These studies provide support for the existence of genetic information on chromosome 11 which can control the malignant expression of Wilms' tumor cells.

MALIGNANT TRANSFORMATION IN HUMAN CELLS APPEARS to be a multistep process (*1*). One set of genes implicated in this progression is the dominantly acting transforming genes that were initially isolated from both avian and mammalian retroviruses (*2*). Cellular homologs of these retroviral transforming genes, termed proto-oncogenes, have been identified (*3*). Activated forms of these cellular genes, called oncogenes, have been shown to induce neoplastic transformation of rodent cells in culture in an apparently dominant fashion (*3, 4*). Transforming ability of these oncogenes may be associated with changes as subtle as a point mutation as well as partial deletions, translocations, or overexpression of the relevant proto-oncogene product. Up to now, more than 25 different cellular oncogenes have been isolated from various

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sources, including many human tumors and tumor cell lines (*2, 3*). Many of these oncogenes have been shown to have the ability to induce transformation of cultured rodent cells when introduced by DNA transfection methods. In several instances it appears that these genes code for proteins involved in the control of cellular proliferation and development. Although the key functional role that any of these oncogenes play in the neoplastic process has not been determined, it has been shown that several families of oncogenes exist with respect to biochemical properties. These include cellular localization—for example, c-*myc* is a DNA-binding protein localized in the nucleus (*5*), *erb*-B is a truncated growth factor receptor found in the plasma membrane (*6*); and physiological properties such as protein kinase activity (*src*) and GTP (guanosine triphosphate)–binding ability (*ras*) (*7*).

In contrast, a second set of genes implicated in the etiology of human cancer has been postulated in which tumor-predisposing mutations are recessive to wild-type alleles (*8*). Although hypotheses for this class of genes were formulated on the basis of familial studies and observance of chromosomal deletions in familial cancers, other evidence for the genetic regulation of tumorigenic expression came from somatic cell hybrid experiments. Despite early studies which suggested that tumorigenicity behaved as a dominant genetic trait in hybrids derived from the fusion of mouse tumor cells with normal mouse fibroblasts (*9*), it was later shown that tumorigenic potential was initially suppressed in such hybrid cells. Reexpression of tumorigenicity was associated with a loss of chromosomes from the hybrid populations (*10*). Thus, the initial studies suggesting dominance of tumorigenic expression were compromised either because of rapid chromosome loss from the hybrid cell lines or because of the lack of refined cytogenetic techniques. Support for the notion of suppression of tumorigenic behavior of cancer cells was greatly strengthened by the use of intraspecific human cell hybrids. When malignant human cells were fused with normal human diploid fibroblasts, the resulting hybrid cells showed complete and stable suppression of the tumorigenic phenotype (*11*). Most important, the hybrid cells continued to behave as transformed cells in culture, indicating that the transformed and tumorigenic phenotypes are under separate genetic control (*12*).

There are familial forms in many types of pediatric cancers, including retinoblastoma, neuroblastoma, and Wilms' tumor (*13*). Cytogenetic analyses of tumor material from these patients led to the identification of chromosomal deletions that are associated with these diseases. In particular, deletions in chromosome 11p13 have been associated with Wilms' tumor, and deletions in chromosome 13q14 have been associated with retinoblastoma (*14*). These data suggest that two copies of a "recessive cancer" gene must be lost or inactivated in order for any of these cancers to develop (*8*). In certain cases patients carried a deletion in one chromosome in the constitutional tissue which could account for increased risk of tumor development. The loss of genetic information being associated with the neoplastic behavior of cancer cells is consistent with the phenomenon of genetic elements that control neoplastic expression, as illustrated in the somatic cell hybrid studies described above.

While somatic cell hybridization has proved to be a useful method for initial studies of the genetic analysis of human malignancy, specific questions about individual chromosomes and genes that play a role in the control of malignant expression are difficult to answer by this procedure. The more refined technique of microcell hybridization has been developed which allows the introduction of a single chromosome into a recipient cell (*15*). Thus, one can study the effects of the introduction of a particular single chromosome into a recipient cell. We report here the introduction of a chromosome 11 derived from cultured normal human diploid fibroblasts into a Wilms' tumor cell line via the procedure of microcell hybridization. While these microcell hybrids appear to retain their transformed phenotype in culture, they are totally suppressed in their ability to form tumors. The transfer of other single human chromosomes derived from normal fibroblasts had no effect on tumorigenic expression of the Wilms' tumor cells.

Table 1. Characterization of parental and microcell cell lines. G401, a cell line derived from a 3-month-old patient with Wilms' tumor, was obtained from the American Type Culture Collection (Rockville, Maryland). For microcell hybridization studies, a hypoxanthine phosphoribosyltransferase (HPRT)–deficient variant, G401-6TG C6, was isolated following treatment with MNNG and selection in medium containing 6TG at 10 μg/ml. Three different mouse cell lines containing individual human chromosomes were used as donor cells for microcell hybridization studies. These include 110.1, containing a single human t(X;11) chromosome (*17*); MCH 200-10, containing a single X chromosome; and CF 25-8, which contains a single human t(X;13) chromosome. The t(X;11) chromosome contains the entire p arm and the majority of the q arm of chromosome 11 (*16*). The microcell hybridization technique of Fournier and Ruddle as modified by Saxon *et al.* (*15, 28*) was used to obtain the various microcell hybrids. All cell lines were propagated in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 10 percent fetal calf serum (J. R. Scientific). The 110.1/G401 microcell hybrids were maintained in medium containing the HAT selective ingredients of Szybalski *et al.* (*29*). All cell lines were monitored for mycoplasma infection by both cultural and biochemical methods (*30*).

Cell line	Modal chromosome number*	Growth in selective medium		Chromosome introduced	Tumorigenicity§
		HAT†	6TG‡		
G401-6TG C6	46	−	+		12/12
110.1/G401.1	47	+	−	t(X;11)	0/12
110.1/G401.2	47	+	−	t(X;11)	0/12
110.1/G401.5	47	+	−	t(X;11)	0/12
G401.2/6TG.1	46	−	+		8/10
G401.5/6TG.3	46	−	+		6/6
MCH/G401.1	47	+	−	X	6/6
MCH/G401.2	47	+	−	X	6/6
MCH/G401.3	47	+	−	X	6/6
25-8/G401.1	47	+	−	t(X;13)	6/6
25-8/G401.2	47	+	−	t(X;13)	6/6

*The karyotypic analysis was performed as in Fig. 1. Modal chromosome counts were based on an examination of at least 20 metaphase spreads. †HAT medium consisted of regular growth medium plus $3.2 \times 10^{-5}M$ thymidine, $1 \times 10^{-4}M$ hypoxanthine, $2 \times 10^{-6}M$ aminopterin, and $1 \times 10^{-4}M$ glycine. ‡6TG medium consisted of regular growth medium containing 6-thioguanine at 10 μg/ml. §Tumorigenicity was measured by the ability of a cell line to form a progressive tumor in *nu/nu* (nude) mice. Each inoculum consisted of 1×10^7 cells injected subcutaneously into either newborn or weanling animals. Animals were examined weekly for tumor formation for up to 6 months, at which time the animals were killed.

Chromosome analysis of parental and microcell hybrids. A hypoxanthine phosphoribosyltransferase–deficient ($HPRT^-$) variant of the Wilms' tumor cell line G401 was shown to have a pseudodiploid karyotype (Fig. 1A) with two cytogenetically normal copies of chromosome 11 and a single marker chromosome $12q^+$ evident. Microcell hybrids were generated by transferring a single copy of a human t(X;11) chromosome (11pter > 11q23::Xq26 > Xqter) (*16*) from a mouse A9 cell containing only this single human chromosome (*17*) via the microcell transfer technique. This t(X;11) chromosome is a chromosome 11 whose q arm terminus has been replaced by the Xq26-Xqter portion of the X chromosome, which includes the hypoxanthine phosphoribosyltransferase (HPRT) locus. The microcell hybrids were confirmed by cytogenetic analysis to have a modal chromosome number of 47 and contained the t(X;11) chromosome in addition to the two copies of chromosome 11 originally present in the Wilms' tumor cell (Fig. 1, B to D). In the case of the clone 110.1/G401.2, a deletion has occurred in the q arm of the chromosome, presumably during the microcell hybridization process. Other microcell hybrids were generated where either a single copy of the human X chromosome was introduced or a single

copy of a t(X;13) chromosome (Xqter > Xp22::13q12 > 13qter). The HPRT gene localized on the fragment of X, which had translocated to both the t(X;11) and t(X;13) chromosomes, provided the selectable marker necessary to select clones of Wilms' tumor cells into which these chromosomes had been transferred.

The microcell hybrid series 110.1/G401 were grown in medium containing 6-thioguanine (6TG) in order to select for $HPRT^-$ clones which had lost the t(X;11) chromosome, presumably via chromosome nondisjunction. Such variants arose in the 6TG medium with a frequency ranging from 1×10^{-5} to 1×10^{-6}. Loss of the t(X;11) chromosome was confirmed by karyotypic analysis.

Tumorigenicity is suppressed in the t(X;11) microcell hybrids. The tumor-forming capability of the various cell lines was assayed by inoculation of the cells into *nu/nu* (nude) mice. The parental G401-6TG C6 cell line formed large progressive tumors in 100 percent of the animals within 2 to 4 weeks after subcutaneous inoculation of 1×10^7 cells. However, all three t(X;11) microcell hybrids consistently failed to form tumors in either newborn or weanling nude mice over an observation period of 6 months (Table 1). Thus, the introduction of the human fibroblast-derived chromosome t(X;11) into this Wilms' tumor cell line resulted in complete suppression of the tumorigenic potential of these cells.

Two important, alternative explanations for these results must be considered. The first is the trivial caveat that the introduction of any chromosome would be sufficient to abrogate the tumorigenic potential of the Wilms' tumor cells. The second possibility is that the small portion of the q arm of the human X chromosome is responsible for these observations. We have investigated these alternatives by generating two additional sets of microcell hybrids. The first set consists of Wilms' tumor cells into which the normal human X chromosome was introduced via microcell transfer. A second set of microcell hybrids was also produced in which a human fibroblast-derived chromosome t(X;13) was introduced into the G401 parental cells. The rationale for testing the effect of transfer of a complete X chromosome is that the t(X;11) chromosome contains the q terminus of chromosome X, which may contain the tumor-suppress-

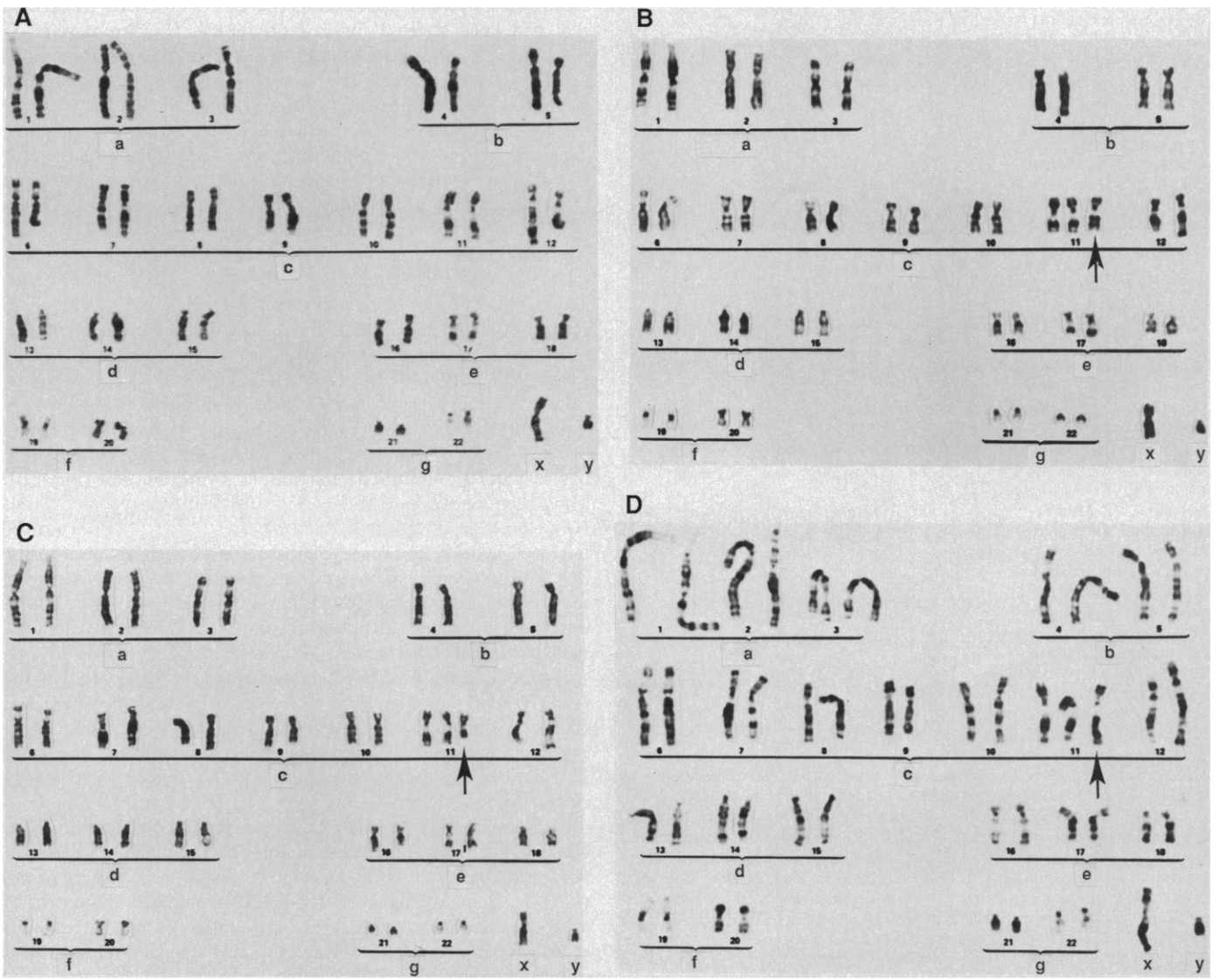

Fig. 1. Karyotypic analysis of the Wilms' tumor cell line and the microcell hybrids. Representative karyotypes of the Wilms' tumor cell line, G401-6TG C6 (**A**), and three individual microcell hybrids, 110.1/G401.1 (**B**), 110.1/G401.2 (**C**), and 110.1/G401.5 (**D**) are presented. Cells were treated with colcemid (0.5 μg/ml) for 1 hour at 37°C. After trypsinization, the cells were suspended in a hypotonic solution of 0.075*M* KCl for 45 minutes. The cells were then fixed with Carnoy's fixative and placed on clean glass slides, which were incubated at 37°C for 24 hours, treated with 0.1% trypsin for 15 to 60 seconds, stained with 5% Giemsa, and dried in air. Metaphase spreads were visualized and photographed [Nikon microscope (×1000)]. Arrow indicates the translocated human t(X;11) chromosome.

ing genetic information. The t(X;13) chromosome was selected because it carries the rb-1 gene, which has been mapped to 13q14 (*18*) and is implicated in the development of retinoblastoma (*19*). We therefore considered whether a chromosome containing another putative "tumor-suppressor" genetic element would influence the tumorigenic potential of Wilms' tumor cells, and found (Table 1) that these microcell hybrids were as tumorigenic as the parental G401 cells when injected into nude mice. Thus, it appears that the introduction of these other human chromosomes does not control the tumorigenic potential of the Wilms' tumor cells.

If the normal human chromosome t(X;11) carries information that modulated the tumorigenic expression in Wilms' tumor, then the loss of this chromosome should result in the reexpression of tumorigenicity in those cells. This hypothesis was examined by selecting variant cells from the microcell hybrids which had regained the ability to grow in growth medium containing 6TG at 10 μg/ml.

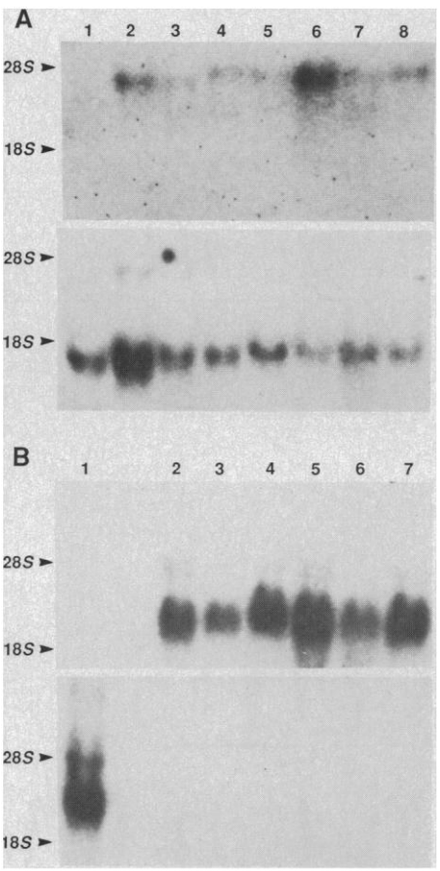

Fig. 2. Proto-oncogene expression in parental and microcell hybrid lines. To examine the expression of specific proto-oncogenes in the parental and microcell hybrid cells, Northern blot analysis was carried out. RNA from each cell line was isolated by the guanidium isothiocyanate method (*32*). Total RNA samples were separated by electrophoresis through 1 percent agarose gels containing 2.2*M* formaldehyde. After transfer to nitrocellulose, RNA blots were prehybridized at 42°C for 2 to 4 hours and hybridized with the appropriate radioactive probe for 18 hours at 42°C. After being washed, filters were exposed to XAR-5 film at −70°C for either 1 day (N-*myc* and thymidine kinase transcripts) or 4 days (c-*sis* and c-*myc* transcripts). Radioactive DNA probes were labeled (*33*) with the use of the "random primer" technique with the Klenow fragment of DNA polymerase and hexadeoxynucleotide primers (*33*). (**A**) Hybridization to a 1.2-kb v-*sis* probe (*34*) is shown in the top panel, and hybridization to a 1.4-kb thymidine kinase cDNA probe (*35*) after removal of the v-*sis* probe is shown in the bottom panel. Each RNA sample (10 μg) was run on a "minigel" and treated as above. (Lane 1) HeLa was used as a negative control for *sis* expression; (lane 2) A172, a human glioblastoma line (*36*) was used as a positive control; (lane 3) G401; (lanes 4 to 6), microcell hybrid clones 110.1/G401.1, -.2, and -.5, respectively; (lane 7) 110.1/G401.5 6TG.3; (lane 8) 25-8/G401.1 [t(X;13)]. The position of the 28*S* and 18*S* ribosomal RNA bands after staining with acridine orange is indicated. (**B**) Hybridization to 1.5-kb human c-*myc* exon 2 probe (Amersham) is shown in the top panel while a 1.0-kb human N-*myc* cDNA probe (ATCC) was used for hybridization (after removal of c-*myc*) in the bottom panel. (Lane 1) Y79, a human retinoblastoma cell line that contains amplified copies of the N-*myc* oncogene and overexpresses N-*myc* mRNA was used as a positive control (*37*). (Lane 2) G401; (lanes 3 to 5) microcell hybrid clones 110.1/G401.1, -.2, and -.5, respectively; (lane 6) 110.1/G401.5 6TG.3; (lane 7) 25-8/G401.1 [t(X;13)].

When these cells were tested for tumor-forming ability in nude mice, they were highly tumorigenic (Table 1). These data indicate that the continued presence of a single copy of the normal t(X;11) chromosome is required to suppress the tumorigenic potential of this Wilms' tumor cell line.

Characterization of the in vitro properties of the microcell hybrids. Most studies with somatic cell hybrids have indicated that malignant × normal hybrid cells that have lost their tumor-forming ability retain the expression of their transformed phenotypic traits in culture (*10, 12*). Examination of our microcell hybrids generated also showed that many traits of transformation continue to be expressed although certain quantitative differences were observed (Table 2). No differences were observed in morphology, levels of production of plasminogen activator, or distribution of fibronectin on the cell surface. Although some variation was seen in the saturation densities of the various clones, no correlation could be made with tumorigenic potential. We did observe a measurable and reproducible decrease in the ability of the 110.1/G401 microcell hybrids to grow in soft agar compared to the parental G401 Wilms' tumor cells. Partial restoration to the G401 level occurred in cells that had been selected for loss of the t(X;11) chromosome in medium containing 6TG; under these conditions normal human diploid fibroblasts fail to grow in soft agar. Thus, it would seem that the introduction of the t(X;11) chromosome into the Wilms' tumor cells has little effect on their growth behavior in culture despite the definite effect upon the ability to form tumors in nude mice.

Oncogene expression. Overexpression or altered expression of oncogenes has been reported to occur in many human tumor tissues (*3*). In the case of Wilms' tumor overexpression of the N-*myc* gene has been reported (*20*). We examined expression of this oncogene in the G401 Wilms' tumor cell line and the t(X;11) microcell hybrids and did not detect expression of N-*myc* in any of the cell lines examined (Fig. 2B). Nisen and colleagues reported an inverse relation between N-*myc* and c-*myc* expression and showed that tissue from one Wilms' tumor had an undetectable level of N-*myc* expression and a high level of c-*myc* expression (*20*). In agreement with this latter observation we found that there was equivalent expression of c-*myc* in the Wilms' line G401 and all of the t(X;11) microcell hybrids; and an indication of significant overexpression (Fig. 2B). Thus, there does appear to be an inverse relation between levels of N-*myc* and c-*myc* expression in both the Wilms' cell line and the microcell hybrids. However, there is clearly no significance to the lack of expression of N-*myc* or overexpression of c-*myc* with respect to neoplastic behavior of the cells since both the tumorigenic Wilms' tumor cell line and the nontumorigenic microcell hybrids exhibit the same pattern of expression.

During a general screen of proto-oncogene expression in the G401 Wilms' tumor cell line, c-*sis* messenger RNA (mRNA) was expressed, an unexpected finding in that c-*sis* is not a commonly expressed oncogene in tumor tissue (*21*), although Tatosyan *et al.* (*22*) have reported expression in one poorly differentiated kidney carcinoma.

McCormick and co-workers have reported that transfection of the v-*sis* oncogene into human diploid fibroblasts resulted in the acquisition of an anchorage-independent phenotype (*23*). Because of the variability in the soft agar cloning of G401 compared to the t(X;11) microcell hybrids the c-*sis* gene expression was examined. The c-*sis* is expressed in all the cell lines (Fig. 2A) with no apparent difference in expression between the Wilms' tumor cell line G401 and most of the t(X;11) microcell hybrid derivatives. The one microcell hybrid that shows increased expression of c-*sis* (110.1/G401.5) has low clonability in soft agar. Thus, the observed differences in soft agar clonability do not seem to be associated with corresponding changes in the levels of c-*sis* expression in the cells.

Table 2. In vitro growth characteristics of the G401 cells and t(X;11) microcell hybrids. The morphology of all cells was epithelial-like. To establish saturation densities for the G401 cells and t(X;11) microcell hybrids, cells were plated in 60-mm petri dishes at a density of 1×10^5 cells per petri dish in growth medium plus fetal calf serum (10 percent) (FCS). The following day the cells were washed with serum-free medium and then each dish received growth medium and either 0.5 or 5.0 percent FCS. After 7 days, when the dishes had reached confluence, the cells were removed and counted. Data from the average number of cells in triplicate dishes grown in 0.5 percent FCS and 5.0 percent FCS are shown. The expression of fibronectin on the cell surface was visualized by immunofluorescence with a mouse antiserum to human fibronectin (*12*). The production of plasminogen activator (PA) was measured by the release of iodinated fibrin from fibrin-coated dishes after incubation with serum-free supernatants from each cell line in the presence of human plasminogen. The assay was normalized for units of PA activity by measuring the total radioactivity released after incubation with known amounts of urokinase (*31*). Anchorage-independent growth was assayed by the cloning efficiency of each cell line in 0.3 percent soft agar as previously described (*12*). Data are expressed as average values (percentage of 5×10^4 cells seeded) of duplicate plates.

Cell types	Saturation density (cells/dish)		Fibronectin expression	PA production (total units/culture)	Growth in soft agar* (%)
	0.5% FCS	5.0% FCS			
G401-6TG C6	3.1×10^5	2.5×10^6	−(+)†	340	9.8
110.1/G401.1	1.8×10^5	1.5×10^6	−(+)	331	2.1
110.1/G401.2	1.7×10^5	1.0×10^6	−(+)	ND‡	1.4
110.1/G401.5	ND	ND	−(+)	464	0.5
110.1/G401.2 6TG.1	4.0×10^5	2.5×10^6	−(+)	646	3.9
110.1/G401.5 6TG.3	2.1×10^5	1.1×10^6	−(+)	703	ND

*One representative experiment of three conducted. †While most of the cells were negative for fibronectin expression, occasional small islets of cells displayed fibronectin filaments. ‡ND, not done.

The expression of a number of other proto-oncogenes that have been associated with human tumors was also examined. These include Ha-*ras*, Ki-*ras*, N-*ras*, *src*, and *erb*-B. No detectable expression of Ki-*ras*, *src*, or *erb*-B was found in any of the cell lines. Both Ha-*ras* and N-*ras* were expressed at the same level in each of the cell lines and were not overexpressed. Thus, there was no obvious correlation between expression of any of the proto-oncogenes examined and tumorigenic potential.

Tumor-suppressor genes. In the early seventies, Knudson postulated that two "hits" in a recessive gene were required for the development of human retinoblastoma (*8*). Comings also proposed a somatic mutation theory of cancer where both the loss of regulatory genes and the concomitant activation of transforming genes had a role in the development of tumors (*24*). In both theories there is the association of loss-of-function mutations with mechanisms including point mutation, frameshift mutation, and deletion. During the last decade there have been many reports of chromosome deletions associated with human malignancies including retinoblastoma and associated malignancies, Wilms' tumor, neuroblastoma, small cell lung carcinoma, and acoustic neuromas (*14, 25*). The best characterized are those of the Rb locus and the Wilms' tumor locus where restriction fragment length polymorphism (RFLP) probes have shown homozygosity of alleles in the region of these loci in the tumor tissue compared to heterozygosity in the constitutional cells (*26*). However, only circumstantial evidence exists for genes at these loci that function to regulate neoplastic behavior. Friend *et al.* have described the isolation of a complementary DNA (cDNA) fragment that detects a chromosomal segment having properties of the Rb gene (*27*). As yet no functional studies of this gene have been reported.

Our studies provide direct evidence that genetic information present on human chromosome 11 completely suppresses the malignant phenotype of Wilms' tumor cells. The fact that the introduction of a normal chromosome X or t(X;13) did not affect the tumor-forming ability of the Wilms' tumor cell line indicates that the addition of a random chromosome to these cells does not result in the suppression of tumorigenicity. The addition of a fibroblast-derived human translocation chromosome t(X;13) containing the Rb locus did not affect the tumorigenic potential of these cells. Although we show that a gene or genes on chromosome 11 can modulate the tumorigenic potential of Wilms' tumor cells, we are at this time unable to ascertain, with the exception of X and 13, whether other chromosomes also have genetic elements capable of suppressing the tumor-forming ability of these cells.

Furthermore, our data do not conclusively establish the 11p13 region as the area of interest. Further studies involving the introduction of smaller segments of the human chromosome 11 are needed to show whether the genetic material in the 11p13 region is responsible for control of tumorigenic potential. However, the region of the q arm of 11 that was missing from the t(X;11) translocated chromosome can already be removed from consideration. Also, further genetic information is missing from the q arm of chromosome t(X;11) in the nontumorigenic 110.1/G401.2 microcell hybrid (Fig. 1C). Our initial cytogenetic analysis indicates that as much as 50 percent of the genetic material has been lost from the q arm.

The transfer of chromosome t(X;11) into Wilms' tumor cells results in the suppression of tumorigenicity with little or no effect on the parameters of a number of transformed traits expressed in culture, with the exception of growth in soft agar. The same separation of genetic control of the transformed and tumorigenic phenotypes is well known for tumorigenic × normal whole cell hybrids (*12*). Thus, the Wilms' tumor–suppressor gene would seem to regulate a late step in the progression to the malignant state rather than one of the initial preneoplastic stages.

Although many of the cellular functions of oncogenes have been identified, their role in the development of human cancer has not been determined. The expression of a variety of oncogenes which have been associated with human cancers was examined in the G401 parental cells and the microcell hybrids. Several pertinent conclusions could be reached from the proto-oncogene expression assays. No detectable expression of N-*myc* was found in the Wilms' tumor cell line or any of the microcell hybrid derivatives. Although an inverse correlation could be found between N-*myc* and c-*myc* mRNA levels, no alteration in the ratios was found in tumorigenic versus nontumorigenic cells and therefore it seems unlikely to be a parameter of significance in the malignant expression of Wilms' tumor cells. The variability in ability to grow in soft agar seen with the microcell hybrid clones did not correlate with differences in levels of c-*sis* expression as may have been expected (*23*). Although no differences in expression of any of the proto-oncogenes tested correlated with tumorigenic potential our study has clearly not exhausted the number of possible proto-oncogenes that can be tested. Whether other known or undiscovered oncogenes are involved in the control of these cellular parameters warrants further investigation.

The difficulty in isolating tumor-suppressor genes lies in the lack of information about the function or the product of these genes. The technique of microcell hybridization thus provides a powerful method for identifying changes caused by the presence of these recessive genes. Further characterization of these sets of cell lines should add to our understanding of the process of malignant transformation. In addition, similar approaches can be used to determine whether tumor-suppressor genes that control the malignant potential of other cancers are present on other chromosomes, such as chromosome 13 in retinoblastoma or chromosome 1 in neuroblastoma.

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