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Genetics

The progesterone receptor gene maps to human chromosome band 11q13, the site of the mammary oncogene *int-2*

(mouse mammary tumor virus/human breast cancer/restriction fragment length polymorphism)

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ABSTRACT **Progesterone is involved in the development and progression of breast cancers, and progesterone receptors (PR) are important markers of hormone dependence and disease prognosis. We have used a human PR cDNA probe, genomic DNA blotting of a series of Chinese hamster–human cell hybrids, and *in situ* hybridization to map the human PR gene to chromosome 11, band q13. This band also contains the human homolog of the mouse mammary tumor virus integration site, *int-2*, which surrounds a protooncogene thought to be involved in the development of murine mammary cancers. That these two genes share the same chromosomal location raises important questions about their possible linkage and about the relationship between the mammary-specific oncogene and the steroid hormone in the development, growth, and hormone dependence of human breast cancers.**

Progesterone and breast cancer have been linked since the concept of hormone-dependent cancers was first elaborated (1–3). The steroid hormone has been implicated in both the genesis and the extinction of experimentally induced mammary tumors (2–5). In women, progesterone is often considered to have a protective effect against cancer development (6) and the presence of progesterone receptors (PR) in human breast cancer is associated with hormone dependence and prolonged survival (7, 8).

The role of viral agents in the induction of human breast cancers is still unclear. In mice, integration of the murine mammary tumor virus (MMTV) proviral DNA within a limited domain of host genomic DNA appears to be required for induction of mammary tumors. Two such domains have been described, designated *int-1* and *int-2* (9, 10). The distribution and integration of the provirus in different mouse mammary tumors fall into two oppositely oriented clusters in the *int-2* locus spanning at least 25 kilobases (kb) (11, 12). This dispersal of the viral integration sites has led to the hypothesis that the MMTV provirus acts as an enhancer for transcription of nearby cellular genes, one of which, a candidate *int-2* protooncogene, is centered within the *int-2* locus (12).

Although all the functional studies relating MMTV integration and *int-2* expression have been performed in mice, a homolog of *int-2* exists in human DNA and has been mapped to chromosome 11 band q13 (13). We now report that the human progesterone receptor gene shares the same chromosome location. This proximity raises important questions about the relationship between the mammary-specific oncogene and the steroid hormone in regulating the genesis, growth, and hormone dependence of human breast cancers.

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EXPERIMENTAL PROCEDURES

Somatic Cell Hybrids and DNA Preparation. A series of human–rodent cell hybrids used in the present study was derived from several fusions involving various Chinese hamster ovary CHO-K1 auxotrophic mutants and human fibroblasts or lymphocytes. The human chromosome content in these hybrids and the methods of analyses have been described (14–20). All of the hybrids were derived from human–CHO fusions except 32-1A, which was a human–mouse (A9) cell hybrid. Human cell line HT1080 (21) and the CHO auxotrophic mutant Gly^-A (22) were used as genomic DNA controls for human and Chinese hamster, respectively. The procedures for preparing DNA from cultured cells, cell hybrids, and human lymphocytes have been described (19, 23–25).

PR Gene Probes. The human PR clone (hPR-50) was isolated from a T47D-pCD library (26) containing 2×10^6 recombinants. The library was screened with a chicken PR probe (CPR 19), which is 3.3 kb long and contains the highly conserved C1 and C2 regions (27). hPR-50 consists of 1075 base pairs (bp), of which 960 bp represent 3′-coding sequence and the remainder is 3′ untranslated region. The authenticity of the clone was verified by DNA sequence analysis (B.W.O., unpublished data) and by comparison to the chicken progesterone receptor (27). The translated part of hPR-50 cDNA contains the C2 region (185 bp), which has 98% homology to the corresponding region in the chicken PR cDNA. An additional sequence located at the extreme 3′-coding region shows a 90% level of homology between hPR-50 and the cDNA to the authentic chicken progesterone receptor. hPR-50 was subcloned into pGEM4 (Promega Biotec, Madison, WI), the DNA was labeled with ^{32}P by nick-translation, and 10^6 cpm of probe per ml was added to the hybridization solution.

Southern Blot Analysis of Genomic DNAs from Human, CHO-K1, and Cell Hybrids. The procedures for Southern blot analysis were similar to those described (19, 24, 25). Briefly, 10–15 μg of genomic DNA was digested with restriction enzymes, electrophoresed on a 0.8% agarose gel, transferred to either nitrocellulose filter or GeneScreen*Plus* membrane (DuPont), hybridized to ^{32}P-labeled progesterone receptor probe at 42°C for 16 hr in the presence of dextran sulfate, washed at either 55°C (40 min) or 70°C (2 hr), dried, and then exposed to Kodak XAR film at −70°C for 16 hr to 4 days with an intensifying screen.

***In Situ* Chromosome Hybridization.** The procedures used for *in situ* hybridization to metaphase chromosomes were

Abbreviations: MMTV, murine mammary tumor virus; CHO, Chinese hamster ovary; PR, progesterone receptor(s); RFLP, restriction fragment length polymorphism.

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similar to those described by Harper and Saunders (28). Briefly, human chromosomes were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes, synchronized with methotrexate and thymidine, and incubated with Colcemid (0.1 μg/ml) for 1 hr before harvest. Slides were treated with RNase (100 μg/ml) for 1 hr at 37°C, rinsed in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), and dehydrated in ethanol. The chromosomes were denatured in 7% formamide/2× SSC at 70°C for 2 min and then dehydrated in ethanol. The PR probe was labeled with [^{3}H]dCTP, [^{3}H]TTP, and [^{3}H]dATP by the random-primer labeling technique of Feinberg and Vogelstein (29, 30). Hybridization was carried out at 37°C for 18 hr in 50% formamide/10% dextran sulfate/2× SSCP (1× SSCP is 0.12 M NaCl/0.015 M sodium citrate/0.02 M sodium phosphate, pH 5.6)/denatured salmon sperm DNA (1 mg/ml)/1× Denhardt's solution (50× Denhardt's solution is 1 g of bovine serum albumin, 1 g of polyvinylpyrrolidone, and 1 g of Ficoll in 100 ml of H_2O). After hybridization, the slides were washed extensively in 50% (vol/vol) formamide/2× SSC at 41°C, then in 2× SSC at 41°C and at room temperature, followed by dehydration in ethanol and air drying. Slides were coated with Kodak NTB2 nuclear track emulsion; stored at 4°C for 7, 10, or 14 days; and developed in Kodak Dektol. The slides were stained with Wright's stain or with Fisher's Giemsa in phosphate buffer at pH 6.8.

RESULTS

Southern Blot Analysis of Human, CHO-K1, and Cell Hybrids Using a Human PR cDNA Probe. Upon *Hin*dIII digestion, human genomic DNA exhibited three bands of 4.7, 3.7, and 2.8 kb that hybridized to the human PR probe (Fig. 1*A*, lane 1). *Hin*dIII-digested CHO-K1 DNA produced three hybridizing bands at different positions—6.8, 5.8, and 2.3 kb (Fig. 1*A*, lane 9)—indicating homology between the hamster PR gene sequences and the human PR probe. A typical hybridization profile for cell hybrids is shown in lanes 2–8. Positive hybrids CP4-1 (lane 2), CP12-1 (lane 3), CP15-1 (lane 4), J1 (lane 5), and J1-7 (lane 7) contained both human and CHO hybridizing bands, and negative hybrids J1-1 (lane 6) and J1-11 (lane 8) displayed only CHO bands.

Fig. 1*B* shows the same nitrocellulose filter used in Fig. 1*A* but subjected to high-stringency wash. After exposure to x-ray film overnight and development as shown in Fig. 1*A*, the same filter was washed at 70°C for 2 hr, dried, and exposed to x-ray film for 4 days. As a result of this high-stringency washing condition, the less homologous CHO bands disappeared, and only the human sequences in HT-1080 and the positive hybrids remained.

Table 1 presents hybridization results from 14 cell hybrids using the human PR probe. Synteny analysis based on the human chromosome content in the hybrids localized the PR gene to human chromosome 11. Definitive evidence came from the positive hybridization of hybrid J1, which contains human chromosome 11 as its only human genomic material (18).

Regional Assignment of the PR Gene to the Long Arm of Human Chromosome 11 Using Cell Hybrids Containing Parts of Chromosome 11. Hybrid subclones derived from J1 containing partial human chromosome 11 were used for a regional assignment of the PR gene on chromosome 11. The results are summarized in Table 2. Since the PR gene was present in J1-7, which has lost the entire short arm of chromosome 11, and is absent in J1-11, which has lost a large portion of the long arm, it may be concluded that the PR gene is located on the long arm of chromosome 11 region q13–qter.

Further Refined Regional Assignment of the PR Gene to Chromosome 11 q13 by *in Situ* Hybridization to Metaphase Chromosomes. To refine the regional localization of the PR gene on the long arm of chromosome 11, we performed *in situ* hybridization to metaphase chromosomes prepared from human lymphocytes. In the 27 spreads with grains on chromosome 11, 53% (17/32) of the grains were found predominantly in the long arm of chromosome 11, with a peak in q13 (Fig. 2).

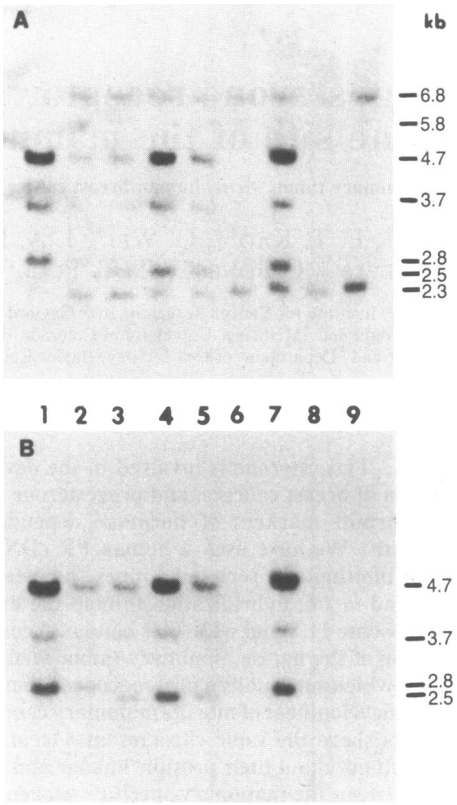

FIG. 1. Southern blot analysis using the human PR probe hybridized to *Hin*dIII-digested genomic DNAs from various cell hybrids and the parental cells. Lanes: 1, human; 2, CP4-1; 3, CP12-1; 4, CP15-1; 5, J1; 6, J1-1; 7, J1-7; 8, J1-11; 9, CHO-K1/Gly-A. (*A*) Blot showing hybridization followed by standard washing conditions: three times at room temperature for 5 min each in 2× SSC/0.1% $NaDodSO_4$, followed by another three washes at 65°C for 15 min each in 0.1× SSC/0.1% $NaDodSO_4$. (*B*) Same blot as in *A* but subjected to high-stringency washing: 70°C for 2 hr in 0.1× SSC/0.1% $NaDodSO_4$.

Restriction Fragment Length Polymorphism (RFLP). In Fig. 1, a RFLP was observed for one of the human hybridizing bands after *Hin*dIII digestion of 2.8 kb for HT1080 and CP4-1, and 2.5 kb for CP12-1, CP15-1, J1, and J1-7. To determine the frequency of this RFLP in the human population, we examined DNA samples from lymphocytes of 26 random adult individuals using Southern hybridization analysis with *Hin*dIII as the restriction enzyme and the PR cDNA as the radioactive probe. Fig. 3 shows the band patterns of four individuals, two homozygous for the 2.8-kb allele (lanes 1 and 2), one homozygous for the 2.5-kb allele (lane 4), and one heterozygous for both the 2.8- and 2.5-kb alleles (lane 3). Of the 52 chromosomes (26 individuals × 2 = no. of chromosome 11) examined, 41 generated the 2.8-kb fragment, while 11 generated the 2.5-kb fragment. Hence, the *Hin*dIII fragments were at least dimorphic and had a frequency of 21% (11/52) of the minor allele in the population studied. This level of allelic frequency makes the RFLP of the PR gene reasonably informative as a genetic marker.

DISCUSSION

The data presented here provide a genetic relationship augmenting the previously established physiological relation-

Table 1. Assignment of the human PR gene to chromosome 11 by molecular hybridization and synteny analysis

Cell hybrid	Human chromosome 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Human PR gene*
CP4-1	−	+	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	+
CP5-1	+	−	−	−	+	−	−	+	+	−	−	+	−	+	+	−	+	−	+	−	+	+	−	−
CP6-1	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	−
CP12-1	−	+	−	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+
CP14-1	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	−
CP15-1	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	−	−	−	+
CP17-1	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
CP18-1	+	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	+	+	+	−	−	−	−	+
CP26-1	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+
CP27-1	−	+	+	+	+	+	+	−	−	−	−	+	+	+	−	+	−	+	−	−	+	−	+	−
CP28-1	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−
CP29-1	−	+	+	+	+	−	−	−	−	−	+	+	−	+	+	+	−	+	+	−	+	+	+	+
32-1A	−	+	−	+	+	−	+	−	+	+	−	−	−	−	+	+	−	−	−	−	+	−	−	−
J1	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+
Concordant hybrids, no.	6	8	7	6	5	7	6	8	6	8	14	7	8	7	8	7	6	6	6	5	3	8	8	
Concordant frequency, %	43	57	50	43	36	50	43	57	43	57	100	50	57	50	57	50	43	43	43	363	21	57	57	

*Presence (+) or absence (−) of the hybridizing human bands using the human PR probe.

ship between progesterone and mammary cancer. The various human–hamster cell hybrids used here illustrate some of the powers of genetic analyses contributed by these approaches that can be applied to many systems.

We have used a cloned human PR gene probe in Southern blot hybridization analysis of a series of somatic cell hybrids for the assignment of the gene to human chromosome 11. In addition, by using a series of cell hybrids containing partial human chromosome 11, we have regionally mapped the PR gene to the long arm of chromosome 11, region q13–qter. This same series of deletion hybrids has been used previously for regional assignment of several other genes to the region 11q13–qter on the long arm of chromosome 11, including uroporphyrinogen I synthase (31), apolipoprotein A-I (15), and the skeletal muscle glycogen phosphorylase (or myophosphorylase) (32). Finally, we have used an *in situ* hybridization technique to assign the PR gene to a more refined region on the long arm, band q13.

We have shown that the PR probe exhibits RFLP for a *Hin*dIII site (Fig. 3) with two alleles present at frequencies of 79% and 21% in the population studied. This makes the probe useful in linkage analysis of the long arm of chromosome 11. Further tests with other restriction enzymes may reveal additional polymorphic sites. The opportunity also exists for a comprehensive survey of the association between the RFLP and familial predisposition to breast cancer.

Striking sequence homologies have been described in the cysteine-rich DNA-binding domains of the steroid receptor genes (27, 33, 34), and of c-*erbA* and a hepatitis B virus DNA integration sequence (35). Despite this sequence conservation, the genes are scattered among six different chromosomes of the human genome: The glucocorticoid receptor gene on chromosome 5 (33), the estrogen receptor gene on chromosome 6 (34), c-*erbA* on chromosome 17 (35), *ORF* on chromosome 3 (36), the vitamin D_3 receptor gene on chromosome 12 (B.W.O., unpublished data), and the PR gene on chromosome 11. We suggest that the DNA-binding domain, domain 2 of steroid receptors (37), represents a primordial gene. This genetic unit was duplicated and dispersed throughout the genome, where subsequent exon recruitment provided the additional functional domains for this family of genes, analogous to the recruitment involved in assembly of the low-density lipoprotein receptor gene (38).

Table 2. Regional assignment of the human PR gene on chromosome 11 using cell hybrids containing parts of chromosome 11

Cell hybrid	Portion of chromosome 11 retained	Human PR gene
J1	Intact	+
J1-1	None	−
J1-7	p11–qter	+
J1-11	pter–q13	−

Although linkage conservation has been found for genes on portions of the long arm of human chromosome 11 and mouse chromosome 9 (39), the *int-2* gene has been mapped to mouse chromosome 7 (40). The location of the PR gene in the mouse genome would be of considerable interest, since it would indicate whether the link between the *int-2* gene and the PR gene is obligatory for the development of tumors and, perhaps of greater significance, for the acquisition of hormone dependence.

Chromosome 11 band q13 contains the human homolog of *int-2*, a limited region of chromosomal DNA defining the

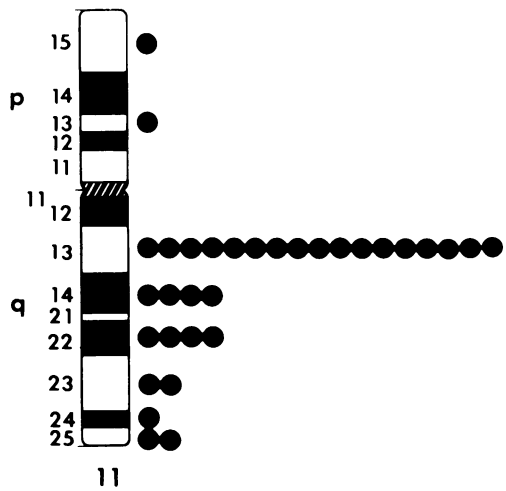

FIG. 2. Localization of the human PR gene to the long arm of chromosome 11 band q13 by *in situ* hybridization.

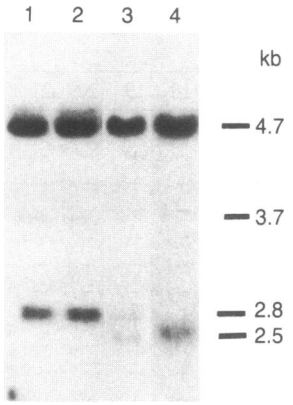

FIG. 3. Representative hybridization profile showing RFLP at a *Hin*dIII site in human genomic DNA hybridized to the human PR probe. Individuals 1 and 2 are homozygous for the 2.8-kb fragment, individual 3 is heterozygous for the 2.8- and 2.5-kb fragments, and individual 4 is homozygous for the 2.5-kb fragment.

integration site of MMTV. Provirus insertion in the mouse *int-2* activates expression of the cellular *int-2* gene located within this locus, whose protein product may be involved in the genesis of murine mammary carcinomas (11). In addition to mouse and human, genes homologous to *int-2* have been found in a variety of mammalian species, but not, interestingly enough, in other classes or phyla (13). If the insertion mutagenesis model holds for MMTV-induced mouse mammary tumors, the assignment of both the PR gene and *int-2* to the same band on human chromosome 11 makes a multistep model involving oncogene activation coupled to hormonal regulation an attractive one for the genesis and progression of human breast cancers (41). In particular, this relationship might explain the fact that these tumors are of the hormone-dependent type.

Several mechanisms can be proposed for the link between *int-2* and PR. Their relationship may be analogous to that for the v-*erbB* gene, which encodes a truncated epidermal growth factor receptor (42)—namely, that the *int-2* gene and PR are structurally related. In that case, the PR gene, or a related DNA sequence, might itself be a site for viral integration. Such a mechanism resembles that proposed for the etiology of human hepatocellular carcinoma, where the hepatitis B virus DNA integration site has been located in a sequence homologous to the DNA-binding domain of steroid receptor genes (36). Perhaps a more likely mechanism for the interaction of the PR gene and *int-2* relates to the suggestion of Peters *et al.* (41) that either activation of RNA and protein synthesis from an oncogene within *int-2*, or the target of the oncogene product, is under hormonal control. This suggestion is based on the finding that certain MMTV-induced tumors are dependent on pregnancy hormones—of which progesterone is a major contributor. Furthermore, progesterone, acting through its receptor, is a known regulator of MMTV expression (43). Can progesterone modulate *int-2* expression through this pathway? Can the *int-2* product or the MMTV enhancer regulate PR expression?

Further understanding of the relationship between PR, *int-2*, and mammary cancers requires exact sequence determinations of all of the genetic loci in the 11q13 band. Standard sequencing procedures applied over so large an interval represent a formidable task. However, the procedure using cell hybrids described previously (25, 44) promises to make such delineation feasible. By use of the differential specificity of human and Chinese hamster repetitive sequences, it has been demonstrated that regions of different sizes can be isolated from different positions on human chromosome 11 (44). The use of repetitive sequences in conjunction with pulsed-field gel electrophoresis (45) appears to offer increased resolving power to the point where standard sequencing operations will be readily applicable. This method can also be used to show possible relationships among PR, *int-2*, and *bcl-1* (46), an oncogene located at a breakpoint in 11q13 associated with B-cell malignancies.

Although the PR and *int-2* genes are assigned to the same cytogenetic band, the molecular distance between the two genes remains to be determined. However, the involvement of *int-2* in the genesis of mouse mammary carcinomas, and the involvement of progesterone and PR in human breast cancers, suggests the possibility that the geometric closeness of these genes in the human genome may not be coincidental and that a cis-acting regulatory model involving a hormone and an oncogene could account for the development and the hormone dependence of human breast cancers.

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