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Human Pregnancy-specific β_1-Glycoproteins Are Coded within Chromosome 19

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Summary

We have isolated and characterized cDNAs that code for apoproteins having amino acid sequences highly similar to pregnancy-specific β_1-glycoproteins (PSβG). cDNAs coding for PSβGs, as well as the cDNA clone reported here, are members of the carcinoembryonic antigen (CEA) gene family. The previous localization of CEA-related genes to human chromosome 19, and the high level of DNA sequence conservation in the CEA family, suggested that the PSβG genes are also located on this chromosome. We demonstrate here that chromosome 19 is indeed the site of PSβG sequences. Our finding is in contrast to the recently reported indication that pregnancy-specific glycoproteins are encoded in chromosomes X and 6.

Introduction

Carcinoembryonic antigens (CEAs) constitute a family of membrane-bound and secreted glycoproteins that are abundant in human tumors and fetal tissue (Gold and Freedman 1965. By immunological criteria, there are a dozen or more members of the CEA family that react with monospecific anti-CEA antibodies (Shively and Beatty 1985). This observation can now be explained by the extensive amino acid sequence similarities displayed among the extracellular domains of 180-kD CEA, nonspecific cross-reacting antigen (NCA), and the transmembrane (TM) CEAs (Barnett et al. 1988, 1989). From Southern hybridization with a conserved disulfide loop-domain DNA probe, we have estimated that there are 8–10 different CEA-family-member genes that appear to be clustered in human chromosome 19 (Kamarck et al. 1987). While cloning and characterizing CEA-related genes, we isolated cDNAs encoding a protein, termed $PS_{K\alpha}$, that differs considerably from 180-kD CEA, NCA, and the TM-CEAs in amino acid sequence but that is nonetheless clearly related to CEA in structure. Restriction-site mapping and sequence analysis of $PS_{K\alpha}$ cDNA, and examination of its derived amino acid sequence, indicate that $PS_{K\alpha}$ belongs to the same class of pregnancy-specific β_1-glycoproteins as do the antigens coded for by the PSG16 and PSG93 cDNAs that have been identified as CEA family members by Watanabe and Chou (1988*a*).

Chan and Qui (1988) recently reported that PSβG gene sequences are located on human chromosomes X and 6, a finding that is not expected on the basis of the location of other CEA genes. However, using both loop domain- and 3′ untranslated region (UTR)–specific probes from $PS_{K\alpha}$ cDNA, we demonstrate here that the PSβG-related genes are indeed localized on human chromosome 19. This finding of synteny of all members of the greater CEA family suggests that the emergence of the CEA family is fairly recent in evolution.

Material and Methods

A λgt11 cDNA library prepared from poly A^+ RNA of KG-1 erythroleukemia cells was obtained from Clontech Laboratories (Palo Alto) and was screened for CEA homologues by hybridization with the generic CEA loop-domain probe, LV7 (Kamarck et al. 1987). *Eco*RI inserts from LV7-positive phage were subcloned in the plasmid Bluescript® (Stratagene, San Diego). For hybridization probes, plasmid inserts were purified in 1%

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low-melting-temperature agarose gels and were radiolabeled according to the method of Feinberg and Vogelstein (1984).

DNA samples were prepared from a panel of mouse-human somatic cell hybrid lines. The human-chromosome content of each line in the panel was established by isozyme analysis (Ryan et al. 1983), and use of the panel in CEA gene mapping has been described elsewhere (Kamarck et al. 1987). Conditions for hybridization of probes to hybrid cell and normal human DNAs have been given by Kamarck et al. (1987).

Results

Figure 1 aligns restriction maps of the PSβG-like cDNA, $PS_{K\alpha}$, isolated by us from the erythroleukemia cell line KG-1, and the placental pregnancy-specific β_1-glycoprotein cDNA, PSG93, characterized by Watanabe and Chou (1988*b*). This comparison demonstrates that $PS_{K\alpha}$ is a homologue of PSG93, on the basis of the positions of restriction-enzyme cleavage sites. Similarities include conserved restriction sites in the 5′ UTR near the beginning of translation and within the coding region, an *Eco*RI site at the point of translation termination, and conserved restriction sites in the 3′ UTR. Restriction-site differences between $PS_{K\alpha}$ and PSG93 do exist, indicating that the cDNAs do not represent the same gene product. Overall, the $PS_{K\alpha}$ gene is clearly a member of the same PSβ-glycoprotein gene family as is PSG93. Since $PS_{K\alpha}$ was isolated by hybridization with a CEA gene probe, it is therefore also a member of the extended family of CEA genes.

The *Eco*RI restriction site at the point of translation termination allows a convenient separation of the $PS_{K\alpha}$ cDNA into (*a*) the coding region plus the 5′ UTR and (*b*) the 3′ UTR. We hybridized these as separate radiolabeled probes to duplicate arrays of somatic cell hybrid DNAs digested with *Bam*HI. The hybrid cell panel is essentially the same as the one that was used to locate CEA gene family members to chromosome 19 (Kamarck et al. 1987). Figure 2 shows that both cDNA segments of $PS_{K\alpha}$ cDNA hybridize exclusively to human DNA and to DNA from the hybrid cell lines identified as containing human chromosome 19 (table 1). Thus, most, if not all, members of the CEA gene superfamily are syntenic.

The Southern blot of figure 2*A* shows that the coding-region probe of $PS_{K\alpha}$ cDNA hybridizes strongly to three major DNA segments, suggesting that there may be a small number of PSG genes. At the same time, there is weaker hybridization of the probe to *Bam*HI segments between ~4 and 2 kb that are similar in size to those detected by the generic CEA probe LV7 (Kamarck et al. 1987). These bands of low hybridization intensity almost certainly represent segments of the CEA, NCA, and TM-CEA genes, since their cDNAs have ~60% DNA sequence similarity to PSG93 cDNA (Watanabe and Chou 1988*b*).

More than one restriction segment of chromosome 19 DNA hybridizes with the 3′ UTR probe (fig. 2*B*),

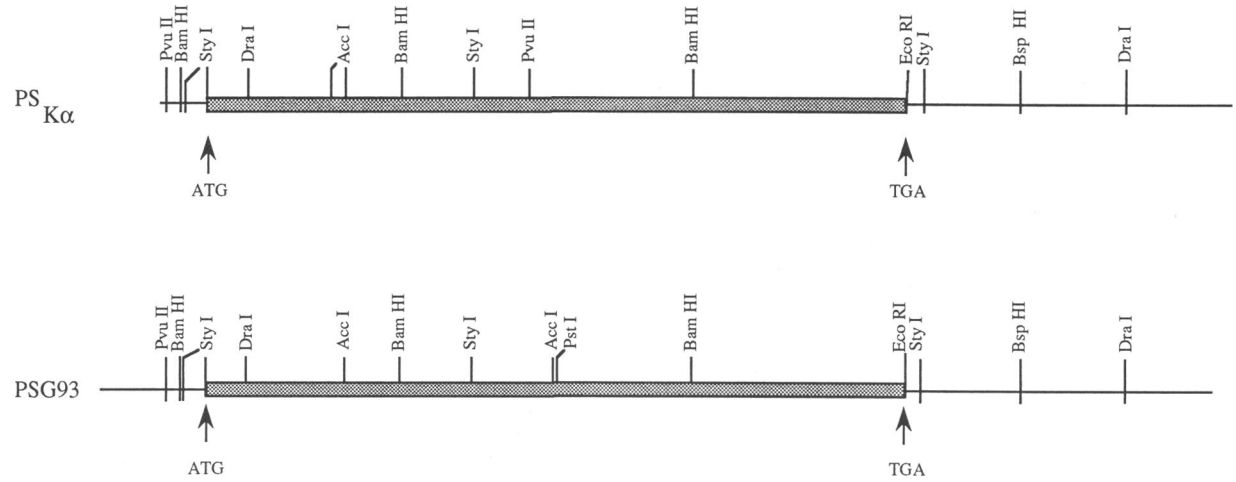

Figure 1 Comparison of $PS_{K\alpha}$ and PSG93 cDNAs. The two cDNA diagrams are aligned with respect to the translation initiation (ATG) and termination (TGA) signals, determined from DNA sequence analysis of $PS_{K\alpha}$ (authors' unpublished data) and PSG93 (Watanabe and Chou 1988*b*). Selected restriction sites are shown to demonstrate the overall similarity of the two cDNA clones. The *Eco*RI site at the point of termination separates each cDNA into (*A*) a 5′ UTR plus coding region segment and (*B*) a 3′ UTR segment.

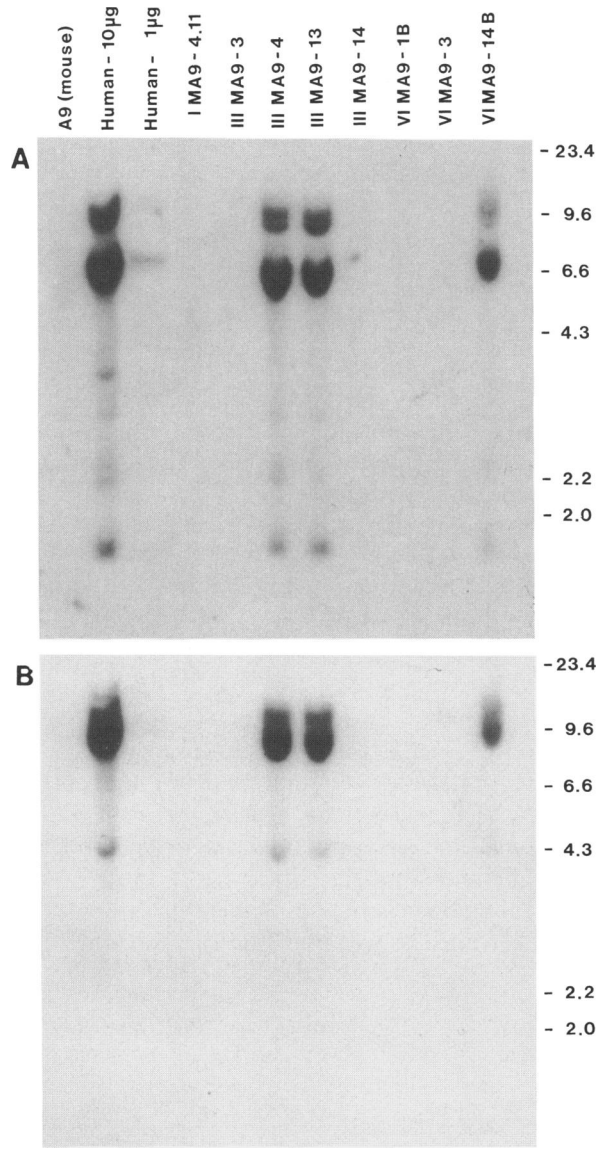

Figure 2 Southern blot analysis of $PS_{K\alpha}$ cDNA probes hybridized to a panel of somatic cell DNAs. Mouse-human hybrid cell DNAs were digested with *Bam*HI, electrophoresed in duplicate 1% agarose gels, and blotted onto nylon filters. DNAs on each filter were challenged with radiolabeled segments of $PS_{K\alpha}$ representing (*A*) the 5′ UTR plus coding region or (*B*) the 3′ UTR (see fig. 1). Somatic cell hybrid line designations, as well as mouse and human control DNAs, are indicated above each lane. The sizes of bacteriophage λ *Hin*dIII segments are on the right. The human chromosome content of each hybrid cell line is given in table 1.

so that there may be either an intron within the corresponding portion of genomic DNA or a small number of PSG genes that have closely related 3′ UTR sequences. The latter possibility is consistent with the small number of sequence differences seen between the 3′ UTRs of PSG93 and $PS_{K\alpha}$ cDNAs (data not shown). The presence of a number of restriction-site differences between $PS_{K\alpha}$ and PSG93 coding regions further suggests that these are coded for by separate transcription units and are not likely to be allelic variants.

Discussion

The concept of a cluster of CEA-related genes on chromosome 19 is consistent with models suggesting a recent evolution and expansion of this gene family (Paxton et al. 1987). In addition to the CEA genes, other members of the immunoglobulin supergene family that are localized to chromosome 19 include the genes for myelin-associated glycoprotein (MAG; Barton et al. 1987) and intercellular adhesion molecule–1 (ICAM-1; Katz et al. 1984).

It is interesting that in situ hybridization of a 3′ UTR probe from NCA cDNA to human chromosomes (Zimmermann et al. 1988) has suggested that there are two loci for NCA genes, one in the short arm of chromosome 19 and one in the long arm. The apparent presence of two separate NCA-related loci can also be explained, however, as being due in part to cross-hybridization between the NCA probe and PSG sequences. We have noted in comparing the cDNA sequences of NCA (Neumaier et al. 1988) and PSG93 (Watanabe and Chou 1988*b*) that there is 83% nucleotide sequence homology between the two in an ~500-bp length of 3′ UTR. On the basis of both this and indications that PSβG is more diverged from CEA, NCA, and TM-CEA than any of the latter is from each other, we propose that pregnancy-specific β_1-glycoprotein genes occupy a locus in the short arm of chromosome 19, while the other members of the CEA family are clustered in the long arm.

We cannot easily explain Chan and Qui's (1988) localization of pregnancy-specific β_1-glycoprotein genes to the X chromosome and to chromosome 6, as our results are completely incompatible with theirs, for the following reasons: (1) The human X chromosome is selected for in all of the hybrid cell panel we used, but only the lines that have human chromosome 19 in common give a hybridization signal. (2) chromosomes 6 and 19 are present together only in lines III MA9-13 and VI MA9-14B, and no line that has human chromosome 6 but not 19 gives a signal. While we used Southern blots of a somatic cell hybrid panel to map PSβG genes, the chromosomal localization studies of Chan and Qui were performed by hybridization to DNA of

Table 1

Assignment of PSβG Genes to Human Chromosome 19

	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	PSβG
I MA9-4.11	+	+	−	+	+	+	−	−	−	−	−	−	−	−	+	−	+	+	−	+	+	−	+	−
III MA9-3	−	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−
III MA9-4	−	+	−	−	−	−	−	+	−	−	−	+	+	+	+	−	+	+	+	−	+	−	+	+
III MA9-13	−	+	−	+	−	+	−	−	+	−	+	+	+	+	+	+	−	+	+	−	+	−	+	+
III MA9-14	+	−	−	+	−	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+	+	−	+	−
VI MA9-1B	−	+	+	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−
VI MA9-3	+	−	+	+	−	−	−	+	−	−	−	+	+	+	−	+	+	+	−	−	−	−	+	−
VI MA9-14B ...	+	−	+	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	+	+	+
Concordant	3	5	3	2	5	4	4	5	6	5	6	5	5	6	5	4	4	5	8	3	6	6	3	
Discordant	5	3	5	6	3	4	4	3	2	3	2	3	3	2	3	4	4	3	0	5	2	2	5	

Note.—Shown is the distribution of human chromosomes among the hybrid cell lines. Also given are the results of the Southern blot hybridizations shown in fig. 2. $PS_{K\alpha}$ cDNA hybridization is concordant with chromosome 19.

chromosome-specific recombinant libraries dotted onto nylon membranes. It is possible that another DNA present in some libraries that is homologous to a component of the cDNA probe used by Chan and Qui could account for their finding.

References

Barnett, T., S. Goebel, M. A. Nothdurft, and J. Elting. 1988. Carcinoembryonic antigen family: characterization of cDNAs coding for NCA and CEA and suggestion of nonrandom sequence variation in their conserved loop-domains. Genomics **3**:59–66.

Barnett, T., A. Kretschmer, D. Austen, S. Goebel, J. Hart, J. Elting, and M. Kamarck. 1989. Alternative splicing accounts for the multiple mRNAs that code for novel members of the CEA family. J. Cell Biol. **108**:267–276.

Barton, D., M. Arquint, J. Roder, R. Dunn, and U. Francke. 1987. The myelin-associated glycoprotein gene: mapping to human chromosome 19 and mouse chromosome 7 and expression in *quivering* mice. Genomics **1**:107–112.

Chan, W.-Y., and W.-R. Qui. 1988. Human pregnancy-specific β1 glycoprotein is encoded by multiple genes localized on two chromosomes. Am. J. Hum. Genet. **43**:152–159.

Feinberg, A., and B. Vogelstein. 1984. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.

Gold, P., and S. Freedman. 1965. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J. Exp. Med. **122**:439–466.

Kamarck, M., J. Elting, J. Hart, S. Goebel, P. M. M. Rae, M. A. Nothdurft, J. Nedwin, and T. Barnett. 1987. Carcinoembryonic antigen family: expression in a mouse L-cell transfectant and characterization of a partial cDNA in bacteriophage λgt11. Proc. Natl. Acad. Sci. USA **84**:5350–5354.

Katz, F., M. Parkar, K. Stanley, L. Murray, E. Clark, and M. Greaves. 1984. Chromosome mapping of cell membrane antigens expressed on activated B cells. Eur. J. Immunol. **15**:103–106.

Neumaier, M., W. Zimmermann, L. Shively, Y. Hinoda, A. Riggs, and J. Shively. 1988. Characterization of a cDNA clone for the nonspecific cross-reacting antigen (NCA) and a comparison of NCA and carcinoembryonic antigen. J. Biol. Chem. **263**:3202–3207.

Paxton, R., G. Moser, H. Pande, T. Lee, and J. Shively. 1987. Sequence analysis of carcinoembryonic antigen: identification of glycosylation sites and homology with immunoglobulin supergene family. Proc. Natl. Acad. Sci. USA **84**:920–924.

Ryan, J., P. E. Barker, K. Shimuizu, M. Wigler, and F. H. Ruddle. 1983. Chromosomal assignment of a family of human oncogenes. Proc. Natl. Acad. Sci. USA. **80**:4460–4463.

Shively, J., and J. Beatty. 1985. CEA-related antigens: molecular biology and clinical significance. CRC Crit. Rev. Oncol. Hematol. **2**:355–399.

Watanabe, S., and J. Chou. 1988*a*. Human pregnancy-specific β_1-glycoprotein: a new member of the carcinoembryonic antigen gene family. Biochem. Biophys. Res. Commun. **152**:762–768.

_____. 1988*b*. Isolation and characterization of complementary DNAs encoding human pregnancy-specific β_1-glycoprotein. J. Biol. Chem. **263**:2049–2054.

Zimmermann, W., B. Weber, B. Ortlieb, F. Rudert, W. Schempp, H.-H. Fiebig, J. Shively, S. von Kleist, and J. Thompson. 1988. Chromosomal localization of the carcinoembryonic antigen gene family and differential expression in various tumors. Cancer Res. **48**:2250–2554.