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CHROMOSOMAL ASSIGNMENT OF THE HUMAN HOMOLOGUE ENCODING SGP-2

Kevin Slawin, Ihor S. Sawczuk, Carl A. Olsson and Ralph Buttyan*

Department of Urology, Columbia University
College of Physicians and Surgeons
New York, NY 10032

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Although originally characterized as a constituitively expressed gene product in mammalian Sertoli cells, sulfated glycoprotein-2 (**SGP-2**) has gained widespread attention due to its remarkably rapid and sizable induction in numerous types of mammalian cells undergoing apoptosis, or programmed death. In order to identify the chromosomal assignment for the human homologue of SGP-2, we performed Southern blot analysis of Bgl II restricted genomic DNA extracted from a panel of cloned hamster-human hybrid cell lines and screened for the presence of restriction fragments homologous to SGP-2. The results of this analysis indicate that the human homologue of SGP-2 resides on chromosome 8.

The sulfated glycoprotein-2 (**SGP-2**) gene product is synthesized and secreted in large amounts by normal mature mammalian Sertoli and epididymal epithelial cells (1,2). Quite remarkably, a gene product sharing extensive sequence homology with SGP-2, initially referred to as testosterone-repressed prostate message-2 (TRPM-2), was shown to be intensely expressed in a wide variety of mammalian cells undergoing apoptosis, a process of active cell death (3). Because of this unique activity, and the resultant markedly enhanced levels of the encoded protein in regressing tissues, and in the serum and urine of experimentally injured rats (4,5), it has been suggested that the SGP-2 gene product may serve as a useful clinical marker for degenerative disease conditions in humans.

*To whom correspondence should be addressed.

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Subsequent screening of a lambda GT10-rat genomic DNA library utilizing TRPM-2 cDNA as a probe allowed the isolation of eight different primary genomic clones (6). These 8 primary isolates were variants of a single genetic unit. Since the size of the restriction endonuclease fragments containing the coding sequences for SGP-2, as determined from the genomic clones, matched the size of the restriction fragments hybridizing to SGP-2 cDNA on a Southern blot containing digested rat DNA, we concluded that SGP-2 and TRPM-2 are encoded by a single gene. As part of our further characterization of this gene and its products, we attempted to identify the chromosomal assignment for the human homologue of SGP-2 by analysis of a panel of human-hamster hybrid cell lines, each carrying a defined subset of human chromosomes.

Materials and Methods

Animals and Animal Care. Mature, 3-day castrated Sprague-Dawley rats (350-375 gm) were utilized as the source of regressing ventral prostate tissue. Laboratory rats were maintained in facilities according to the N.I.H. Guidelines for Care and Use of Laboratory Animals. Food and water were available **ad libitum**. Castration was performed under sodium pentobarbitol anesthesia through a scrotal incision. Ventral prostate tissue was recovered from sodium pentobarbitol-euthanized animals and was stored at -85° C.

RNA extraction and cDNA synthesis. RNA was extracted from pulverized frozen ventral prostate tissue using the method of Cathala, et al. (7) and, as previously described (3), Poly $(A)^+$ mRNA was isolated from total RNA following oligo dT-cellulose chromatography and was copied to cDNA with the use of AMV reverse transcriptase according to the method of Gubler and Hoffman (8).

Generation, Labeling and Hybridization of the SGP-2 probe. Double stranded linear cDNA encoding SGP-2 was generated by PCR elongation of first-strand regressing rat ventral prostate cDNA (9). Primers were selected to allow amplification of a 1367 bp fragment that included a majority of the protein-coding region of SGP-2 mRNA. 100 ng of amplified DNA was subsequently labeled with ^{32}[P] by a nick-translation procedure (10). Labeled probe was denatured by boiling and then hybridized overnight at 68oC to Southern blots in a buffer consisting of 6X SSC, 5X Denhardt's solution, 0.5% SDS and 5 mM EDTA. The blots were subsequently washed at 60°C in a series of solutions containing progressively diluted amounts of SSC to 0.5X. These blots were exposed to Kodak XAR-5 film for development.

Southern Blots of Human-Hamster Hybrid Cell Panel. A Southern blot containing a variety panel of restriction endonuclease-digested

DNAs from human cells (normal B-lymphocytes) or from hamster cells (Chinese hamster ovary, CHO-K1) was obtained from BIOS Corporation (New Haven, CT). In addition, Southern blots containing Bgl II-digested DNA from a panel of 25 different clonal hamster-human hybrid cell lines (11,12) was obtained from BIOS Corp. Each human-hamster cell line had been karyotyped (11) to determine the human chromosomal complement carried by that line.

Results and Discussion

Polymerase chain reaction generated SGP-2 cDNA probe was hybridized to a panel of human and hamster genomic DNAs, each digested with a variety of different restriction endonucleases. The cDNA probe hybridized with two Bgl II restriction fragments in human DNA, including one prominent band at approximately 4.5 kb and one minor band estimated at 7.3 kb. The cDNA probe also hybridized

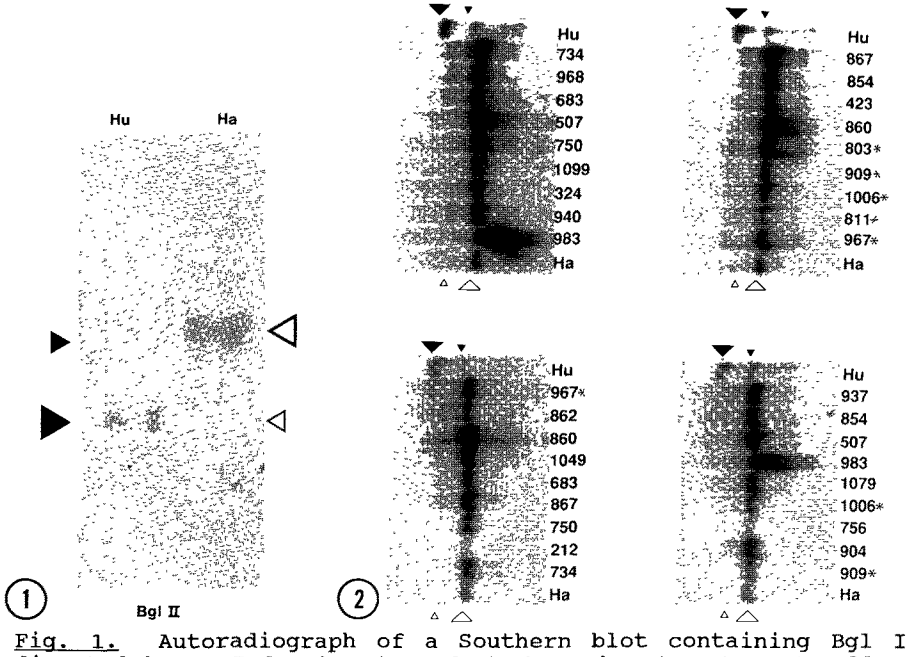

Fig. 1. Autoradiograph of a Southern blot containing Bgl II-digested human B lymphocyte DNA (Hu) or hamster CHO-KI cell DNA (Ha) hybridized to a radiolabeled SGP-2 cDNA probe. Arrowheads indicate position of major and minor bands.

Fig. 2. Autoradiograph of Southern blot panels containing Bgl II-digested DNAs from 25 different clonal isolates of hamster-human hybrid cells (indicated by their numeric designation at the top of the lane) and hybridized to a radiolabeled SGP-2 cDNA probe. DNAs showing the presence of the characteristic human 4.5 kb band are indicated by an asterisk. Hybrid cell DNA lanes are flanked by Bgl II-digested human cell DNA (Hu) or hamster DNA (Ha).

to two Bgl II restriction fragments in hamster DNA, including one band with intense hybridization estimated at 7.5 kb and one minor band estimated at 5 kb. Therefore, the human homologue of SGP-2 could be distinguished from the hamster variant by the presence of a 4.5 kb Bgl II restriction fragment (figure 1).

Genomic DNAs extracted from a panel of 25 different clonal hamster-human hybrid cell lines (11,12) were each digested with Bgl II, transferred to nitrocellulose filters, and hybridized with the radiolabeled SGP-2 cDNA probe (figure 2). There was complete concordance among all the hybrid clones examined for the

Table 1

CORRELATION OF HUMAN SGP2 HOMOLOGOUS SEQUENCES WITH HUMAN CHROMOSOMES IN HAMSTER X HUMAN SOMATIC CELL HYBRIDS

	GENE/CHROMOSOME				
HUMAN CHROMOSOME	**+/+**	**+/-**	**-/+**	**-/-**	**% DISCORDANCY**
1	0	5	3	17	32
2	0	5	1	19	24
3	0	5	4	16	36
4	2	3	0	20	12
5	3	1	13	2	74
6	1	4	3	17	28
7	1	4	1	19	20
8	**5**	**0**	**0**	**20**	**0**
9	0	5	2	18	28
10	0	5	3	17	32
11	1	4	3	17	28
12	0	5	3	17	32
13	1	4	5	15	36
14	1	4	6	14	40
15	1	4	2	17	25
16	1	4	1	18	21
17	1	4	1	19	20
18	1	4	3	17	28
19	1	4	6	14	40
20	0	5	3	17	32
21	1	4	6	14	40
22	1	4	3	17	28
X	2	3	1	19	16
Y	0	5	4	16	36

Note. Discordancy table demonstrating the cosegregation of the human SGP-2 homologue with human chromosome 8. Hybrids in which a particular chromosome was present only in part, or in fewer than 10% of cells were excluded. Discordancy represents the presence of the gene in the absence of the chromosome (+/-) or absence of the gene despite the presence of the chromosome (-/+), and the sum of these numbers divided by the total number of hybrids examined (x100) represents percent discordancy.

cosegregation of the human specific 4.5 kb fragment and human chromosome eight. All other autosomes, as well as the sex chromosomes, were excluded by at least three or more discordant hybrids, or a minimum 12% discordancy (table 1). Therefore, our analysis indicates that the human homologue of SGP-2 resides on human chromosome 8.

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