boundary. A model that supposes an episode of intense volcanism in latest Cretaceous time would generate a rich spectrum of climatic and biological effects that would fit well into what is already known about the geologic history of that period.

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Oncogene from Human EJ Bladder Carcinoma Is Located on the Short Arm of Chromosome 11

Abstract. *The human cellular homolog of the transforming DNA sequence isolated from the bladder carcinoma cell line EJ was localized on the short arm of human chromosome 11 by Southern blot analysis of human-rodent hybrid cell DNA. This locus contains human sequences homologous to the Harvey murine sarcoma virus v-Ha-*ras *oncogene.*

Cellular transforming genes isolated from neoplasms are DNA sequences defined by their ability to induce transformation of tissue culture cells (*1*). The resulting transformed cells can be tumorigenic in nude mice (*2*). Spontaneous, chemically or virally induced neoplasms of different mammalian species contain such transforming sequences (*1–3*), some of which have been isolated from several human tumor cell lines (*4*). The oncogene of the human bladder carcinoma cell lines EJ and T24 were isolated by DNA-mediated gene transfer into NIH 3T3 mouse fibroblasts (*5–7*). These biologically active cloned transforming genes contain sequences present in normal cellular DNA. We investigated the chromosomal location of the normal sequences homologous to the transforming gene of the human EJ bladder carcinoma cell line.

We used Southern blot analysis to examine 24 different human-rodent hybrid cell lines that were derived from fusions between established Chinese hamster or mouse cell lines and normal diploid human fibroblasts or leukocytes (*8*). The hybrid cell lines retained different but overlapping complements of human chromosomes, so that any given pattern of hybridization could be unambiguously correlated with the presence of a specific human chromosome. Chromosome analysis and isozyme characterization of the hybrids was carried out at the time when DNA was extracted (*8*). Nitrocellulose filters were prepared with restriction endonuclease–cleaved DNA's from the hybrids and the parental cell lines from which they were derived. The filters were incubated with labeled probes made from clones of the biologically active 6.6-kb Bam HI fragment of the transforming gene (pEJ6.6) (*5*).

Under stringent conditions of hybridization, the pattern produced by normal human DNA is consistent with the existence of single-copy reactive sequences. A single, intense major restriction fragment was visualized in the five human donor DNA's cleaved with either Hind III or Eco RI (lanes 1 and 2 in Fig. 1A and lanes 1, 7, and 8 in Fig. 1B). Two human donor DNA's cleaved with Bam HI or Bgl II revealed the presence of two restriction fragments of the same intensity; these can be accounted for by restriction fragment length polymorphisms (lane 5 in Fig. 1C and lane 6 in Fig. 1D) (*6*). In addition to these strong signals, minor bands could also be detected in most autoradiograms. Cross-reacting sequences of varying number and intensity could be detected in Chinese hamster DNA, but they did not overlap with the human fragments detected in these blots (lane 3 in Fig. 1A and lanes 2 and 9 in Fig. 1B).

Hybrids were scored for the presence of the major fragment or the polymorphic alleles. The results (Table 1) indicate that sequences homologous to the bladder carcinoma transforming gene segregate only with human chromosome 11. The hybridization pattern was discordant with the presence of any other human chromosome in at least 40 percent of the hybrid lines. Moreover, the presence of a second closely related locus on another human chromosome could also be excluded; no positive signal in the position of the major human band was obtained in the absence of human chromosome 11 when various other human chromosomes were present. (However, parts of human chromosomes 7, 9, and 10 that were involved in complex rearrangements could not be formally ruled out.) The presence of cross-hybridizing extrachromosomal sequences is unlikely; the same hybridization pattern was observed with either nuclear DNA or total cellular DNA derived from the same hybrid line (lanes 2 and 3 in Fig. 1C).

A subset of 14 somatic cell hybrids was then used for regional localization on chromosome 11. Eight of these hybrid cell lines contained only parts of chromosome 11 (Fig. 2). The results indicated that the homologous sequences are located on the short arm of chromosome 11. Two mouse-human hybrids (series VII) had retained only one human chromosome, the result of a translocation between almost the entire short arm of chromosome 11 (region A in Fig. 2) and part of the long arm of chromosome 17 carrying the cytoplasmic thymidine kinase gene (*9*). The 6.6-kb Bam HI restriction fragment homologous to the probe is carried by this derivative chromosome, which can be selected in hypoxanthine-aminopterin-thymidine (HAT)-containing culture medium (lanes 2 and 3 in Fig. 1C) or selected against in the

presence of bromodeoxyuridine (BrdU). One Chinese hamster–human hybrid of series XV had retained the human No. 11 short arm (region B in Fig. 2) translocated to a hamster chromosome and had lost the long arm, as indicated by lack of expression of a long arm marker (*10*). DNA from this hybrid gave a positive result when tested with the probe (lane 6 in Fig. 1A and lane 5 in Fig. 1B). Expression of the lactate dehydrogenase (LDH) A isozyme, known to be coded for by a gene on 11p12 (Fig. 2) (*11*), was used as a marker for the presence of the short arm in all hybrids studied.

Further regional mapping information was obtained from series XXI hybrids in which the human donor carried a balanced reciprocal translocation t(11;15)(p11;p12); the two human chromosomes 11 are morphologically distinguishable, one being normal and the other being distributed between the two products of the reciprocal translocation. The derivative chromosome 11 contains region C, and the derivative chromosome 15 contains region D (Fig. 2). The human donor DNA cleaved by Bam HI or Bgl II revealed the presence of two fragments of different molecular weight but the same relative intensity (as in lane 5 in Fig. 1C and lane 6 in Fig. 1D). The two fragments segregated in hybrids that

Table 1. Correlation between presence of each human chromosome and result of hybridization in 21 Chinese hamster–human somatic cell hybrids. Chinese hamster–human hybrids derived from fusion experiments XII, XIII, XV, and XXI were constructed by fusion of four different human donors (three males and one female) and two different established Chinese hamster cell lines. Methods for propagation in culture, chromosome analysis, and isozyme characterization has been reported (*8*). Detailed karyotype analysis was carried out on the cell population used for DNA extraction and for enzyme analysis.

Hybridization	Presence of chromosome	Human chromosomes																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
+	+	4	6	9	6	8	3	4	11	4	5	9	9	4	9	6	6	8	6	4	6	8	10	7	6
−	−	7	8	4	8	5	4	7	3	7	8	5	6	4	3	5	6	7	5	5	5	4	3	5	6
+	−	6	6	2	5	4	5	7	1	8	7	0	3	8	3	5	6	4	6	8	5	4	2	5	1
−	+	2	1	5	1	4	4	0	5	0	0	0	3	5	5	4	3	2	4	4	4	4	6	3	1
Number of discordant clones		8	7	7	6	8	9	7	6	8	7	0	6	13	8	9	9	6	10	12	9	8	8	8	2
Number of informative clones*		19	21	20	20	21	16	18	20	19	20	14	21	21	20	20	21	21	21	21	20	20	21	20	14

*Data for chromosomes involved in rearrangements or present at a frequency less than 0.1 were excluded.

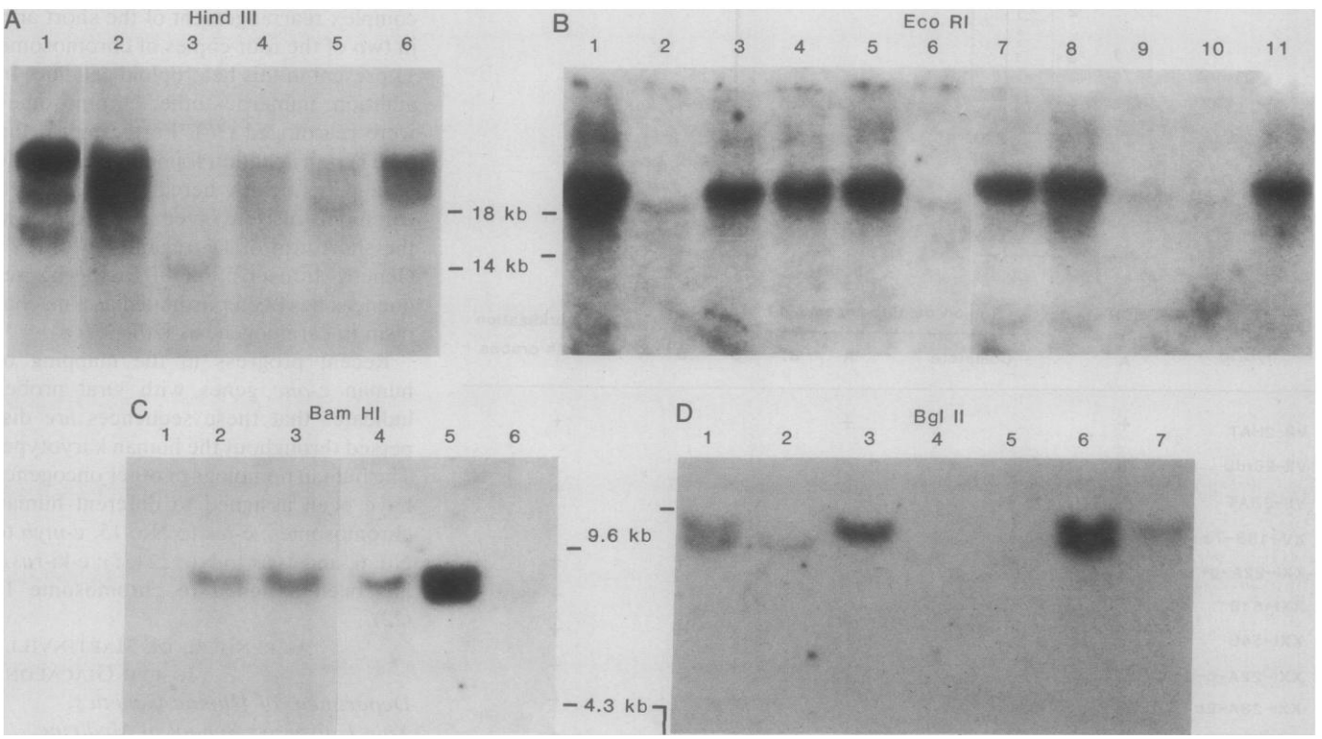

Fig. 1. Restriction enzyme analysis of somatic cell hybrid DNA. High molecular weight nuclear DNA (10 μg) was digested to completion with Hind III, Eco RI, Bam HI or Bgl II, as recommended by the manufacturer (Bethesda Research Laboratories and New England Biolabs), subjected to electrophoresis in 0.7 to 1 percent agarose, and transferred to nitrocellulose filters (Schleicher & Schuell BA 85). All DNA's were analyzed with probes pEJ6.6 (A) and the biologically inactive equivalent pEC (B–D) (*23*). Hybridization was carried out with probes labeled with ^{32}P by nick translation to a specific activity of 2×10^8 to 3×10^8 cpm/μg. Filters were washed at high stringency with a final wash in 0.1 strength standard saline citrate and 0.1 strength sodium dodecyl sulfate at 68°C. Illustrations are derived from different autoradiograms. Lanes A4 to A6 and lanes B3 to B5 were obtained with different digests of the same hybrids from series XV. They were derived from a fusion between human donor cells TH5 (lanes A2 and B1) and Chinese hamster cells V79/380-6 (lanes A3 and B2). The negative hybrid in lane B6 is of same derivation. Hybrids in lanes B10, B11, and C4 were the products of fusion between human donor cells MN4 (lanes B7 and C5) and Chinese hamster cells (lanes B9 and C6). Lane C1 contains the mouse donor DNA of hybrids VII-3HAT whose nuclear DNA (lane C3) or total cellular DNA (lane C2) are shown. Hybrids in lanes D1 to D4 and lane D7 are XXI-51B, XXI-42A, XXI-22A-g-1a, XXI-23A-2c, XXI-54B; they were derived from a fusion between human donor cells KG7 (lanes D6 and A1) and Chinese hamster cells Don/a-23 (lane D5).

contained only one or the other chromosome 11 short arm (lanes 2 and 7 in Fig. 1D). The lower molecular weight fragment was carried by the morphologically normal chromosome 11, and the higher one segregated with the derivative chromosome 15. In four hybrids, the derivative chromosome 11 containing the long arm region (region C in Fig. 2) was retained in the absence of both the normal chromosome 11 and the other translocation chromosome. These hybrids did not contain the LDHA gene and did not hybridize with the bladder carcinoma oncogene probe (as in lane 4 of Fig. 1D). All of our results taken together indicate that the cellular homologous sequences are on the short arm of chromosome 11 (region p11→p15) (Fig. 2). The oncogene of this bladder carcinoma is homologous to the v-Ha-*ras* oncogene of Harvey murine sarcoma virus (*12*). Consequently, we have mapped the human c-Ha-*ras 1* gene (*13*). We did not detect any gene amplification (*14*) [the intensity of hybridization being roughly proportional to the frequency of the respective chromosome in each hybrid line (data not shown)], and we found the same degree of restriction fragment length polymorphism previously reported (*6*).

Our results are relevant to other tumor-related genes and to specific chromosome rearrangements associated with malignancy. The region (11p11→p15) to which we have assigned the c-Ha-*ras 1* oncogene contains the LDHA gene, the non-α-globin gene complex, a locus for catalase, and several loci for cell surface antigens (*10, 15*). In addition, this region contains several loci related to cell growth or malignancy, including the structural gene for insulin (*15, 16*) and genes that predispose to Wilms' tumor, an embryonic kidney tumor, and that also cause aniridia. Both Wilms' tumor and aniridia occur when these genes are present in a hemizygous condition due, for example, to deletion of one allelic region (*17*). Whether the location of c-Ha-*ras 1* coincides with the region (distal band 11p13) pathogenetically important for the aniridia–Wilms' tumor association remains to be determined.

Multiple restriction fragment length alleles are detectable with both the insulin and β-globin-region probes (*15*). Therefore, our assignment of the bladder carcinoma oncogene to the same region will allow the establishment of linear order and recombinational distances between c-Ha-*ras 1* and these other two polymorphic loci by family linkage studies.

Since certain malignancies are associated with specific chromosomal rearrangements, we have carried out a karyotype analysis of the EJ bladder carcinoma cell line to determine whether breaks in chromosome 11 had led to detectable rearrangements. We found a complex rearrangement of the short arm in two of the four copies of chromosome 11 present in this heteroploid cell line. In addition, numerous other chromosomes were rearranged (*18*). Furthermore, the t(3;11) translocation found in tumor cells of a patient with hereditary renal cell carcinoma also involved a breakpoint in the short arm of 11 (region 11p15) (*19*). Genetic transposition of oncogene sequences has been postulated as a mechanism in carcinogenesis (*20*).

Recent progress in the mapping of human c-*onc* genes with viral probes indicates that these sequences are dispersed throughout the human karyotype. The human homologs of other oncogenes have been assigned to different human chromosomes: c-*fes* to No. 15, c-*myb* to No. 6, and c-*sis* to No. 22 (*21*); c-ki-*ras 2* has been assigned to chromosome 12 (*22*).

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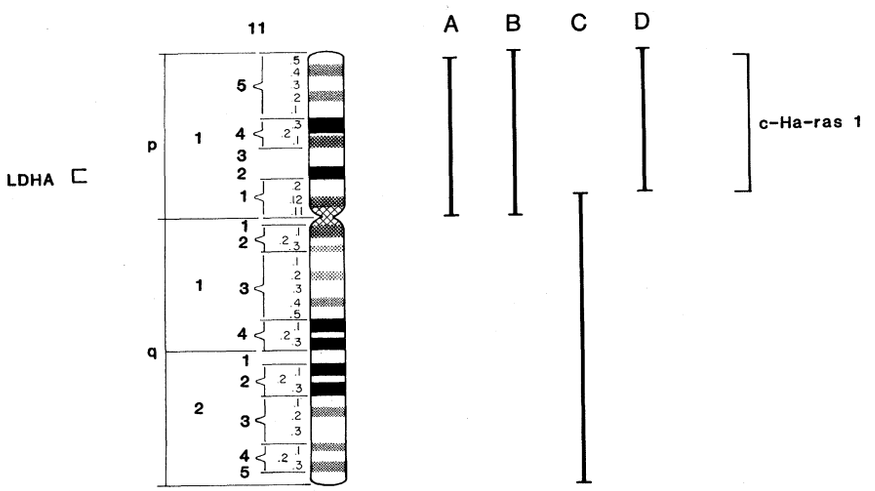

Somatic cell hybrid	LDH	Region of chromosome 11 present					Hybridization with probes
	A	Complete	A	B	C	D	
VII-2HAT	+		+				+
VII-2BrdU	−						−
VII-3HAT	+		+				+
XV-18B-7a	+			+			+
XXI-22A-g-1a	+				+	+	+
XXI-51B	+	+				+	+
XXI-54B	+					+	+
XXI-22A-c-2c	−				+		−
XXI-23A-2c	−				+		−
XXI-23A-2d	−				+		−
XXI-61A	−				+		−
XXI-33A-b	−						−
XXI-42A	+	+					+
XXI-22A-h	+	+			+	+	+

Fig. 2. Regional mapping of bladder carcinoma oncogene. Ideogram of human chromosome 11 shows high-resolution Giemsa banding patterns (*24*). The bracket on the right defines region 11p11→p15 that contains c-Ha-*ras 1* sequences based on the presence (+) or absence (−) of hybridization with bladder carcinoma oncogene probes. The bracket on the left denotes location of LDHA gene in band 11p12.

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Drug-Induced Alterations of Cytokeratin Organization in Cultured Epithelial Cells

Abstract. *The distribution of keratin intermediate filaments, previously considered static in organization and imperturbable by conventional drugs used to alter the structure and organization of the cytoskeleton, can be altered significantly by treatment with colchicine and cytochalasin D. The loss of microfilaments and microtubules converts the keratin cytoskeleton from a branching, even distribution to a series of starlike structures whose filaments are maintained by multiple membrane attachment sites. These findings provide a means for manipulating cytokeratin organization to investigate the role of keratins in cytoskeletal structure and function.*

Although most types of intermediate filaments (IF's) are known to be structural components of the vertebrate cytoskeleton, their role in the function of the cytoskeleton has yet to be established. Immunological and biochemical evidence has revealed at least five distinct classes of IF's: keratin filaments, vimentin filaments, desmin filaments, glial filaments, and neurofilaments (*1–3*). Defining the structural and functional relations among IF's and between IF's and the two other major cytoskeletal filament systems, microtubules and microfilaments, is an important area of research in cell biology. Keratins are of particular interest in studying these relations because they (i) are found as cytoskeletal elements (cytokeratins) in virtually all higher vertebrate epithelial cell types both in vitro and in vivo (*4–7*); (ii) represent a system of tonofilaments whose organization is characterized in numerous epithelia (*8–12*); (iii) are found in tumor cells of epithelial origin (*1, 13*); and (iv) are the major products of differentiation for the epidermal cells of the integument (*14*).

We report that the distribution of cytokeratin IF's can be significantly altered by treatment with a combination of colchicine and cytochalasin D. Cytokeratins in transformed and fetal mouse epidermal cells undergo a rapid transition from an even distribution (Fig. 1A) to a lattice of keratin fibers interconnecting membrane-associated focal centers (Fig. 1, B and C).

Keratin filaments were identified by using indirect immunofluorescence microscopy with antiserum to α-keratin isolated from avian stratum corneum (*15*). Rabbit antiserum to chicken α-keratin, like antiserums to mammalian α-keratins, cross-reacts with keratins from other higher vertebrates, including mice. Stably transformed cultures of the KLN 205 clone of a squamous cell carcinoma (*16*) derived from a DBA/2 mouse tumor were grown on glass cover slips and maintained in Leibovitz-15 medium supplemented with 10 percent fetal bovine serum. Primary cultures of DBA/2J mouse epidermal cells from 14-day fetuses were grown under the same culture conditions.

Both cell types were treated for 2 hours at 37°C with cytochalasin D (0.5 μg/ml) in 1 percent dimethyl sulfoxide (DMSO), colchicine (40 μg/ml), or a combination of the two. Controls included untreated cells, cells treated with 1 to 10 percent DMSO, and cells treated with colchicine and 1 percent DMSO. The cells were fixed in methanol and made permeable with acetone for 5 minutes each at −20°C and air-dried before being processed for immunofluorescence. Indirect immunofluorescence with preabsorbed rabbit antiserum to chicken α-keratin or with serum from unimmunized rabbits demonstrated no fluorescence. Control cells and cells treated with cytochalasin D or colchicine alone displayed a normal cytoplasmic distribution of keratin filaments (Fig. 1A).

Cytochalasins and colchicine derivatives have been used to study the roles of microfilaments and microtubules, respectively, in cell morphology, motility, and division (*17–20*). These antimitotic drugs have been tested for indirect effects on the various IF systems with limited success (*7, 21*). Only an effect by Colcemid on vimentin filament distribution has been reported (*21*). Neither Colcemid nor cytochalasin B alone elicits any change in the organization of keratin filaments (*7, 21*). However, we have induced rearrangement of cytokeratins by disrupting the integrity of microtubules and microfilaments simultaneously. The depolymerizing effects of colchicine and cytochalasin D have been confirmed in both KLN 205 carcinoma cells and fetal mouse epidermal cells by indirect immunofluorescence with antiserum to actin or tubulin. Cytochalasin D elicits extensive surface retraction of spread epithelial cells. As a result, the margins of the cells retain contacts with other cells only at focal adhesive sites similar to those seen between KLN 205 cells (Fig. 1D). However, the organization of keratins throughout the cytoplasm is generally undistrubed unless both cytochalasin D and colchicine are used (Fig. 1A).

The effect of the combination of colchicine and cytochalasin D on the organization of cytokeratins suggests some kind of direct interaction between keratin filaments and microtubules and microfilaments. Alternatively, changes in