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Assignment of the Urokinase-Type Plasminogen Activator Receptor Gene (PLAUR) to Chromosome 19q13.1-q13.2

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Summary

The urokinase-type plasminogen activator receptor (uPAR) is a key molecule in the regulation of cell-surface plasminogen activation and, as such, plays an important role in many normal as well as pathological processes. We applied a cDNA probe from the corresponding gene (PLAUR) in a location analysis using a panel of human/rodent cell hybrids and in a multipoint linkage analysis of 40 CEPH families. These two independent studies both found PLAUR to be located on chromosome 19. The cell hybrid study suggested that PLAUR is located at chromosome 19q13-qter, and the multipoint analysis indicated that PLAUR is located at chromosome 19q13.1-q13.2 and surrounded by DNA markers in the following way (with distances given in recombination fractions): D19S27–.11–CYP2A–.06–PLAUR–.03–D19S8–.04–APOC2–.24–PRKCG. Further, a ligand-binding study performed on cell hybrids verified the species specificity of the uPAR and confirmed the chromosome assignment.

Introduction

Cell-surface plasminogen activation is involved in normal and pathological processes such as gametogenesis, trophoblast implantation, tissue-destructing diseases, and malignant tumors in which cells assume an aggressive, invasive behavior which requires regulated extracellular proteolysis (Reich 1978; Danø et al. 1985; Blasi et al. 1987). Plasmin is the final product of a reaction that takes place at the cell surface and which is regulated in a very complex way, involving two types of plasminogen activators, proenzyme activation, plasminogen activator inhibitors, the substrate and specific receptors for plasminogen and plasminogen activator (Reich 1978; Danø et al. 1985; Blasi et al. 1987; Blasi 1988; Miles and Plow 1988). A specific receptor for the urokinase-type plasminogen activator (uPAR) (Bajpai and Baker 1985; Stoppelli et al. 1985; Vassalli et al. 1985), is at the core of this regulation. The uPAR is a 313-residue-long (Roldan et al. 1990) glycoprotein attached to the cell surface via a glycolipid anchor (Ploug et al. 1991). The gene coding for the uPAR is called "PLAUR," and this study reports on the chromosomal localization of PLAUR, as part of a project aiming at an understanding of its role in physiologic homeostasis as well as in the common tissue-destructive diseases mentioned above. A more precise, regional localization of PLAUR and its relation to surrounding DNA markers is also determined.

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Material and Methods

Somatic Cell Hybrids Used in Mapping Studies

Hybrid cells. — The cell hybrids, which were produced by polyethylene glycol–mediated fusion of rodent cell lines and human fibroblasts, have been described elsewhere (Kruse et al. 1988). Cell hybrid DNA was a gift from Drs. K.-H. Grzeschik and H. H. Ropers.

Southern transfer, and filter hybridization. — Restriction-enzyme digestion of hybrid cell DNA, Southern transfer, and filter hybridization were carried out according to methods described elsewhere (Southern 1975; Al-

dridge et al. 1984; Kruse et al. 1988). The probe used was puPAR-2, which is a 600-bp *Bam*HI fragment of the puPAR-1 cDNA clone (Roldan et al. 1990). The DNA probe was labeled with ^{32}P by nick-translation, to a specific activity of more than 10^8 cpm/μg.

Ligand-binding Study

Material.—Human urokinase-type plasminogen activator (uPA) was obtained from Lepetit SpA (Milan). The amino terminal fragment (ATF) was provided by Dr. Jack Henkin (Abbott, USA). Chromosome 19 probe D19S8 was purchased from ATCC (Rockville, MD). Cell lines HeLa and mouse Ltk$^-$ were obtained from Dr. Carlo Croce (Philadelphia). The hybrids PT30 cl.5 and PAFxBalb cl.3 have been described elsewhere (Tripputi et al. 1985).

Cell fusion.—Hybrids HeLa × L1 and HeLa × L3 were obtained by fusing HeLa with mouse Ltk$^-$ cells by using polyethylene glycol 4000. Hybrids were selected in HAT medium in the presence of 10 μM ouabain.

Ligand-binding studies.—Iodination of ATF and binding of iodinated ATF were carried out according to methods described elsewhere (Stoppelli et al. 1986) but without acid washing, as HeLa cells produce no uPA.

Linkage Analysis

The RFLPs used for the linkage analysis have been described by Børglum et al. (1991, and in press). DNA samples for linkage analysis were obtained from CEPH (Centre d'Etude du Polymorphisme Humain, Paris). Five-microgram portions of the DNA samples were digested with appropriate restriction enzymes according to the manufacturers' recommendations and were analyzed by Southern blotting as described above. Pairwise and multilocus linkage analyses were performed using the LINKAGE 4.7 package of computer programs (Lathrop and Lalouel 1984; Lathrop et al. 1984) using data from the CEPH data base Ver3. Gene order was examined by multilocus likelihood calculations using the ILINK and LINKMAP programs. Genetic distances were calculated using Haldane's mapping function, which converts recombination frequencies to genetic distances in Morgans.

Results and Discussion

Hybridization to Somatic Cell Hybrid DNA

DNA from 15 different human/rodent cell hybrids was analyzed by Southern blotting after digestion with the restriction enzyme *Hin*dIII (examples are shown in fig. 1). When a 600-bp cDNA fragment of puPAR-1, called "puPAR-2," was used as the probe for PLAUR, two hybridizing bands were revealed in human genomic DNA, whereas one cross-hybridizing band was seen in hamster (not shown in fig. 1), and no bands were visible in mouse DNA. In table 1 the segregation of the human-specific bands and the chromosomal contents of the hybrid cells are compared. For any given hybrid, only chromosomes totally absent or present in at least 30% of the metaphases examined were taken into account, while those detected in less than 30% of the metaphases examined were considered uninformative. The results in table 1 show concordant segregation of chromosome 19 and PLAUR, whereas other chromosomes show at least 21% discordant segregation. One of the hybrids, 790 × 175 K6, contains only the chromosome 19 fragment 19q13-qter. The PLAUR probe also hybridizes to this hybrid, leading to the conclusion that PLAUR is located on chromosome 19q13-qter.

Ligand-binding Study

To confirm the chromosome assignment, four human/mouse cell hybrids were examined—along with the two parentals HeLa and Ltk$^-$—for their expres-

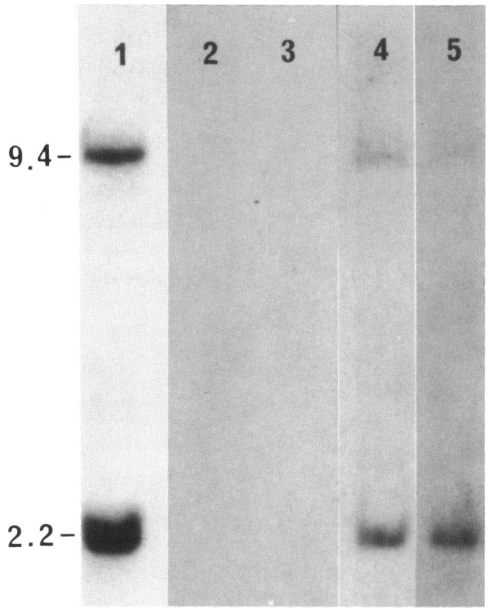

Figure 1 Detection of PLAUR sequences by Southern blot analysis with probe puPAR-2. Lane 1, Human DNA. Lane 2, RAG (mouse). Lane 3, Hybrid 749. Lane 4, Hybrid 790 × 175 K6. Lane 5, Hybrid 445 × 393 K1.

Table 1

Analysis of Human Chromosomes and PLAUR in 15 Rodent-Human Cell Hybrids

A. Human Content in Hybrids

Human-Rodent Hybrid	Results in Human Chromosome[a]																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	PLAUR[b]
445 × 393 K1	+	+	+	+	+	+	+	−	−	−	+	−	−	/	−	−	−	−	+	−	/	−	+
697 × 175 K36	−	/	−	+	−	+	−	−	−	−	−	−	−	+	/	/	−	−	−	−	/	/	−
617 × 347 K6	−	−	−	−	−	/	−	−	−	−	/	+	−	−	/	/	/	/	−	/	+	−	−
422 K2	−	−	+	−	+	+	−	+	+	−	+	−	+	/	−	/	−	−	−	+	+	+	−
749	+	−	+	+	+	+	+	−	−	−	/	+	−	+	−	/	−	+	−	−	+	/	−
494 × 393 K6	−	+	−	−	−	−	−	−	−	+	−	−	/	−	−	−	−	−	−	−	−	−	−
790 × 175 K6	−	−	−	−	−	−	−	−	−	−	−	−	+	−	/	−	−	−	+[c]	−	+	−	+
750	/	−	−	/	/	−	−	−	−	−	+	−	−	+	/	−	+	−	−	/	−	/	−
PI-RAG-72	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+[d]	−	−	+	−	−	−	+	−
GO-RAG-4	−	−	+	/	−	−	/	+	−	−	−	−	−	+	+	−	−	+	−	−	−	+	−
GM194-RAG-7	−	−	+[e]	+	+	−	−	−	−	/	−	−	−	−	−	−	−	/	+	−	+	−	+
GM194-RAG-5-5	−	−	+[f]	+	+	+	+	−	−	+	−	+	+	+	−	+	−	−	−	+	+	+	−
GM97-RAG-8-13	+[g]	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	+	+	−
GM89-A9-9c-7	−	−	+	+	−	−	+	−	−	−	+	−	−	+	+	/	−	+	/	+	+	+	−
MS58-A9-26	−	−	−	−	−	−	−	−	−	−	+	−	+	+[h]	+	−	−	−	−	−	−	−	−

B. Summary of Segregation

Concordance-Discordance	No. in Human Chromosome																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
+ / +	1	1	1	2	2	1	1	0	0	0	1	0	1	0	0	0	0	0	3	0	2	0
− / −	8	10	6	5	7	6	7	9	9	10	6	8	8	3	5	6	10	7	11	7	6	3
+ / −	2	1	5	5	4	5	4	3	3	2	4	4	3	7	3	1	1	4	0	3	5	6
− / +	2	2	1	1	1	2	2	3	3	2	2	3	2	2	2	3	3	2	0	3	0	3
Total:																						
Concordant	9	11	7	7	9	7	8	9	9	10	7	8	9	3	5	6	10	7	14	7	8	3
Discordant	4	3	6	6	5	7	6	6	6	4	6	7	5	9	5	4	4	6	0	6	5	9

[a] A plus sign (+) denotes that the chromosome was detected in at least 30% of hybrid cells; a slash (/) denotes that the chromosome was detected in less than 30% of hybrid cells; and a minus sign (−) denotes that the chromosome was not detected.
[b] Analysis was performed by Southern blot analysis with probe puPAR-2, as described in Material and Methods.
[c] Hybrid contains only region q13-qter of chromosome 19.
[d] Hybrid contains only region q13-qter of chromosome 15.
[e] Hybrid contains only region q21-qter of chromosome 3.
[f] Hybrid contains only region pter-q21 of chromosome 3.
[g] Hybrid contains only region pter-q12 of chromosome 1.
[h] Hybrid contains only region pter-q32 of chromosome 14.

sion of the human uPAR, the presence of chromosome 19, and the species specificity of uPA binding (Appella et al. 1987; Estreicher et al. 1989). The data are reported in table 2.

All the hybrids except PAF × Balb clearly bind human iodinated ATF. Mouse uPA did not inhibit the binding to the HeLa × L1 hybrid, which demonstrates the species specificity. The presence of chromosome 19 in HeLa × L1 but not in PAF × Balb confirms the chromosome assignment of PLAUR (table 1).

It was further verified that the uPAR molecule expressed in the HeLa × L1 hybrid cells was indeed the uPA receptor as expressed by HeLa cells. This was done by cross-linking experiments on cells that had bound iodinated ATF and by analysis of the cross-linking products by SDS-PAGE (data not shown).

Two-Point Linkage Analysis

The cDNA probe, puPAR-2, reveals RFLPs in digestions of human DNA with restriction enzymes *Pst*I

Table 2

Binding of ^{125}I-ATF by Human-Mouse Somatic Cell Hybrids

Cell Line	Bound ^{125}I-ATF When Concentration of Unlabeled Competitor[a] Is (specific cpm)				Status of Chromosome 19[b]
	0 nM	.1 nM	1.0 nM	10.0 nM	
HeLa	16,500	17,600	7300	750	+
HeLa × L1[c]	3,900	3,800	1,200	0	+
PT30 c1.5	5,100	ND	ND	0	ND
HeLa × L3	6,000	ND	ND	0	ND
PAF × Balb c1.3	150	ND	ND	0	–
Ltk$^-$	90	ND	ND	0	–

Note.—ND = not determined.

[a] Human uPA.

[b] Analysis was performed by Southern blot analysis with probe D19S8 as described in Material and Methods.

[c] No competition of ATF binding was observed with mouse uPA.

and *Eco*RI. *Pst*I detects two polymorphisms, each with two alleles. The alleles (with size and frequency shown in parentheses) are A1 (4.9 kb; .85), A2 (4.5 kb; .15) and B1 (1.9 kb; .92), B2 (2.1 kb; .08). *Eco*RI yields one polymorphism with two alleles: C1 (4.2 kb; .92) and C2 (4.4 kb; .08). The *Eco*RI polymorphism was found to be in absolute linkage disequilibrium with the B polymorphism of *Pst*I. Of the 40 CEPH families, 24 were informative with either the *Pst*I A or the *Eco*RI/*Pst*I B polymorphism. Only two of the families were informative with both systems. On this basis the two-point linkage analysis showed an overwhelming degree of linkage to chromosome 19: of the 26 chromosome 19 DNA markers in the CEPH data base, 15 showed linkage to PLAUR, with lod scores above 3 (and, not seldom, well above 10). Six of the DNA markers are presented in table 3, with physical localization and lod scores at different recombination fractions relative to PLAUR.

Multipoint Linkage Analysis

In order to localize PLAUR in detail, relative to already localized chromosome 19 markers, a reference five-point genetic map was constructed using five DNA markers from the CEPH data base. By multipoint linkage analysis the most likely order of these five loci, which all show a high degree of linkage to PLAUR (table 3), was found to be D19S27, CYP2A, D19S8, APOC2, and PRKCG. This order was favored by odds of 140:1 over any alternative order and is in agreement with both the consortium physical map and the consortium genetic map (Le Beau et al. 1989). The best estimates of the recombination fractions between the five markers were found to be D19S27–.10–CYP2A–

Table 3

Two-Point Linkage Analysis between PLAUR and Six Marker Loci

Physical Localization (locus)	Lod Score at Recombination Fraction of									Maximum Lod Score	Maximum Recombination Fraction	Confidence Limits
	.00	.001	.01	.05	.10	.15	.20	.30	.40			
19p13.2-q12 (D19S27)	$-\infty$	−6.87	−.03	4.11	5.23	5.43	5.21	4.06	2.24	5.43	.14	.06–.27
19q13.1-q13.2 (CYP2A)	$-\infty$	12.04	17.57	19.65	18.72	16.93	14.73	9.59	4.06	19.65	.05	.02–.10
19q13.1–q13.2 (ATP1A3)	$-\infty$	13.36	15.10	15.32	14.37	13.09	11.60	8.15	4.10	15.47	.03	.004–.10
19q13.2 (D19S8)	$-\infty$	17.74	20.37	20.80	19.52	17.76	15.71	10.98	5.52	20.98	.03	.007–.08
19q13.2 (APOC2)	$-\infty$	5.81	9.57	11.27	11.04	10.24	9.14	6.35	3.03	11.31	.06	.02–.15
19q13.4 (PRKCG)	$-\infty$	−39.66	−18.90	−5.33	−4.59	1.72	2.74	2.98	1.08	3.10	.36	.16–.38

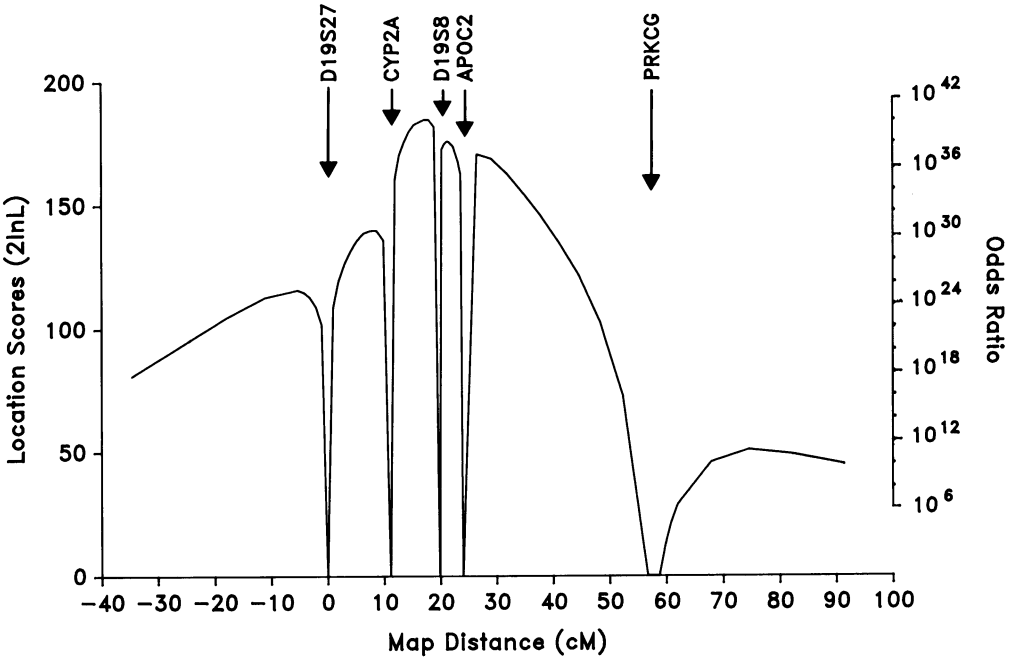

Figure 2 LINKMAP analysis of location of PLAUR, with respect to linkage map of five marker loci: D19S27, CYP2A, D19S8, APOC2, and PRKCG. The genetic distance, measured from locus D19S27, is expressed, in centiMorgans, on the horizontal axis. The right vertical axis is the odds ratio for the location of PLAUR at a given distance, compared with a location of PLAUR at an infinite distance from the five fixed markers. The left vertical axis is the location score, defined as twice the natural logarithm of the odds ratio.

.08–D19S8–.04–APOC2–.24–PRKCG, and these distances were used in the following LINKMAP analysis.

A six-point analysis was performed using the LINKMAP program, which calculates the likelihood for any position of PLAUR with respect to one fixed map of five markers. This analysis yielded the location-score curve shown in figure 2, which demonstrates a peak location score of 185 between CYP2A and D19S8, corresponding to a multipoint lod score of 40.2. The most likely location outside this interval is between D19S8 and APOC2, with a peak location score of 176, corresponding to odds of 90:1 for the location between CYP2A and D19S8.

An additional six-point analysis using the ILINK program was performed to calculate the most probable gene order and the most likely estimates of the recombination fractions. This analysis too showed that PLAUR is located between CYP2A and D19S8, with odds of 26:1 over the second most likely location (data not shown).

Thus, we appoint the following order, with the recombination fractions in between: D19S27–.11–CYP2A–.06–PLAUR–.03–D19S8–.04–APOC2–.24–PRKCG. From this genetic localization and the already known physical localization of the DNA markers, it follows that PLAUR must be located in 19q13.1-q13.2. In conclusion, both the two independent mapping studies presented here (i.e., the cell hybrid study and the multipoint linkage analysis) agree on the chromosomal localization of PLAUR, which is further confirmed by the ligand-binding study.

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