Rapid Publications

Human Insulin Receptor Gene

Data Supporting Assignment to Chromosome 19

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SUMMARY

Somatic cell hybrid clones constructed by crossing human skin fibroblasts with mouse L cells have been examined for expression of human insulin receptors, using a monoclonal antibody directed against the human insulin receptor. Data obtained in this study support the assignment of the human gene for the insulin receptor to chromosome 19. DIABETES 1985; 34:816–20.

The first step in insulin action involves binding to a specific high-affinity receptor on the cell surface. The human insulin receptor is an intergral membrane glycoprotein ($M_r \sim 350,000$–$400,000$) that is composed of two α-subunits ($M_r \sim 125,000$–$135,000$) and two β-subunits ($M_r \sim 90,000$), held together by disulfide bonding.[1–3] The insulin receptor possesses tyrosine-specific protein kinase activity that is activated by binding of insulin to the receptor.[4–7] Activation of the tyrosine-specific protein kinase activity very likely represents a key mechanism for transmembrane signaling by the hormone. Recently Ullrich et al.[8] have isolated and sequenced a complementary DNA (cDNA) clone for the human placental insulin receptor gene. Metabolic labeling and affinity labeling studies,[9–11] and the amino acid sequence deduced from the nucleotide sequence of the cDNA,[8] have indicated that the α- and β-chains are synthesized as a single high-molecular-weight precursor polypeptide with the α-chain at the amino end and the β-chain at the carboxyl end. Based on the DNA sequence, the predicted molecular weight of the unglycosylated α-chain is 82,400 and the β-chain is 69,700.[8] The β-chain contains a transmembrane domain and a cytoplasmic protein kinase domain that has sequence homology with other tyrosine-specific protein kinases, including the epidermal growth factor (EGF) receptor, and a number of retroviral oncogenes including the v-*src* oncogene of Rous sarcoma virus and the v-*ros* oncogene of avian sarcoma virus UR2.[8] Evidence suggests that the insulin binding site resides primarily on the external α-subunit domain.[1,8]

Insulin resistance at the tissue level is important in the etiology of type II (non-insulin-dependent) diabetes mellitus[12] as well as a number of rare syndromes including lipoatrophic diabetes, leprechaunism, and syndromes of insulin resistance and acanthosis nigricans (reviewed in refs. 13 and 14). A decrease in insulin receptor numbers or a qualitative change in insulin receptors has been demonstrated in a number of these disorders. In some of these disorders the deficiency of insulin receptors is clearly a secondary consequence of other metabolic problems. For example, in type B syndrome of insulin resistance and acanthosis nigricans, the decrease in insulin receptor numbers and insulin resistance are caused by circulating autoantibodies directed against the insulin receptor.[13] However, evidence suggesting an intrinsic defect in the insulin receptor has been obtained for two other rare syndromes, leprechaunism and type A syndrome of insulin resistance and acanthosis nigricans. Qualitative or quantitative defects in insulin receptors have been found in fibroblasts or lymphocytes cultured from three patients with leprechaunism accompanied by extreme insulin resistance (reviewed in ref. 14). Insulin resistance has also been observed to be correlated with a defect in insulin receptors on fibroblasts or lymphocytes cultured from several patients with type A syndrome of insulin resistance and acanthosis nigricans.[15–19] In one of these cases a family study strongly suggested a genetic basis for the deficiency in insulin receptors.[17] It would be of considerable interest to determine whether any of these disorders is caused by a mutation in the insulin receptor gene or its controlling elements.

Southern blot analysis suggests that there is a single copy of the insulin receptor gene per haploid chromosome set,[8] and very recently, Yang-Feng et al.[20] have used recombinant DNA methods to provisionally assign the receptor gene to

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human chromosome 19. We report here data obtained by a different method that supports assignment of the receptor gene to chromosome 19.

MATERIALS AND METHODS

Isolation of hybrid clones. Hybrid clones were isolated by crossing diploid human skin fibroblasts with two derivatives of the mouse L cell line: A9, which contains a mutation in the gene for hypoxanthine-guanine phosphoribosyl transferase (HPRT) and B82, which contains a mutation in the gene for thymidine kinase (TK).[21] Hybrid clones (Table 1) were selected in HAT medium[21] and karyotyped by the fluorescence banding procedure as described previously.[22] Strain GM969 normal diploid human skin fibroblasts were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey.

Cell culture and preparation of crude membrane fractions. Hybrid cells were grown to confluence in 10–15 10-cm dishes containing minimal essential medium (MEM) supplemented with 0.1 mM nonessential amino acids, 10% fetal bovine serum (Gibco, Grand Island, New York), penicillin (71 U/ml), streptomycin (100 μg/ml), and HAT.[21] The human fibroblasts, A9 cells, and B82 cells were grown in the same medium minus HAT. For preparation of crude membrane fractions, cells were placed on ice, washed twice with phosphate-buffered saline (PBS), and removed from the dishes by scraping. All subsequent operations were performed at 4°C. Cells were recovered by centrifugation, resuspended in 10 ml of hypotonic buffer [8.5 mM tris-HCl, 3.0 mM NaCl, 1.0 mM glucose, 0.2 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM benzamidine-HCl, 10 μg/ml phenylmethyl sulfonylfluoride (PMSF), pH 7.8], and allowed to swell for 10 min. The cells were then homogenized with a Dounce homogenizer, and the homogenate was centrifuged for 10 min at 581 × *g*. The pellet was discarded and the supernatant was centrifuged for 1 h at 50,000 × *g*. Pellets from the high-speed spin were stored frozen at −70°C before use.

Membrane solubilization. Membrane pellets were thawed, suspended in 250 μl of solubilization buffer (50 mM tris-HCl + 1 mM Triton X-100, pH 7.5), and incubated at 23°C for 15 min with frequent agitation. The solubilized membranes were then centrifuged in an airfuge at 30 psi (149,000 × *g*) for 20 min to remove insoluble particulate material. The clear supernatants were assayed for the presence of human insulin receptors as described below. Protein in the solubilized membrane fractions was measured by the method of Lowry et al.[23], as modified by Wang and Smith.[32]

Binding of ^{125}I-insulin to solubilized insulin receptors and immunoprecipitation of the insulin-receptor complex. Porcine insulin (Elanco, Indianapolis, Indiana) was iodinated by the stoichiometric method to a specific activity of approximately 150 μCi/μg, as previously described.[24] Solubilized membrane preparations (40–150 μg protein) were incubated with ^{125}I-insulin (50,000 or 500,000 cpm) in assay buffer (50 mM tris-HCl, 0.1% bovine serum albumin, 70 U/ml bacitracin, pH 7.7) in a 200-μl total volume, in the absence or presence of 2 μg of unlabeled insulin. Parallel controls were also performed in which membrane protein was omitted. A 20-μl aliquot of a 1/40 dilution of αIR-1[25,26] clarified ascites in 50-fold diluted normal mouse serum was then added, and incubation was continued at 4°C for 6 h. Parallel controls were performed using a class-matched γG1 (kappa) subclass ascites (MOPC 21). At the end of the 6-h incubation, 20 μl of a 1/3 dilution of goat anti-mouse antiserum was added, and incubation was continued for 16 h at 4°C. Assay buffer (1 ml, 4°C) was added to each tube, and precipitates were collected by centrifugation in a microfuge (8730 × *g*) for 10 min at 4°C. Supernatants were removed, and radioactivity in the pellet was determined with a Beckman Biogamma-2 gamma counter (efficiency 78%) (Beckman Instruments, Fullerton, California).

RESULTS AND DISCUSSION

In this study we used the αIR-1 anti-human insulin receptor monoclonal antibody[25,26] to detect insulin receptors in solubilized membrane preparations. This antibody offered two distinct advantages for this study. First, it exhibits very little, if any, cross-reactivity with rodent insulin receptors[25] and can thus be used to detect human insulin receptors on the surface of human × rodent hybrid cells. Second, the αIR-1 antibody exhibits a relatively high degree of specificity for the insulin receptor.[26] The insulin-like growth factor I (IGF-I) receptor has a subunit structure that is homologous to the insulin receptor, and IGF-I receptors bind insulin with a low affinity.[27,28] Furthermore, indirect evidence suggests that the insulin and IGF-I receptors may share some immunologic determinants.[29] However, because of the high affinity of binding of insulin to its own receptor and the specificity of the αIR-I antibody for the insulin receptor, immunoprecipitation assays performed with ^{125}I-insulin as ligand and the αIR-1 antibody detect primarily or exclusively insulin receptors.[26]

In initial experiments (Table 2), solubilized membrane preparations were incubated in the presence of 50,000 cpm (0.2 ng) of ^{125}I-insulin, and the ^{125}I-insulin–receptor complexes were immunoprecipitated. Controls included (1) parallel assays performed in the presence of a high concentration of competing unlabeled insulin, (2) parallel assays in which insulin–receptor complexes were mock precipitated with a class-matched control monoclonal antibody (MOPC 21) not directed against the insulin receptor, and (3) control assays in which the receptor was omitted. Cells were scored as positive for the human insulin receptor if the amount of ra-

TABLE 1
Human chromosome content of hybrid cells

Hybrid clone	Human chromosomes	Human insulin receptor
CF33-16	2, 3, 5, 6, 8, 11, 12, 14, 16, 17, 18, 19, 21, 22, t(1; X)(1pter → 1q12::Xq26 → Xqter)	+
CF67-7	7, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, X	+
CF31-1	3, 4, 5, 6, 10, 11, 12, 14, 15, 18, 21, 22, t(X; 20)(Xqter → Xcen::20cen → 20pter), 1p	−
CF67-2	1, 4, 7, 8, 10, 13, 14, 16, 17, 18	−
CF80-8	6, 7, 18, 21, Y, t(17; 20)(17pter → 17q25::20q131 → 20qter)	−

TABLE 2
Immunoprecipitation of ^{125}I-insulin-receptor complexes

Cells	Radioactivity precipitated (counts/5 min)	P	Human insulin receptor
Human fibroblasts			
Experiment 1			
αIR-1	2057 ± 117	< 0.01	+
+ Insulin control	1065 ± 42		
MOPC 21	1042 ± 19		
− Receptor blank	1004 ± 110		
Experiment 2			
αIR-1	1983 ± 61	≈0.01	+
+ Insulin control	1222 ± 59		
− Receptor blank	1202 ± 9		
A9			
Experiment 1			
αIR-1	1153 ± 105	0.2 > P > 0.1	−
+ Insulin control	997 ± 24		
MOPC 21	1079 ± 47		
− Receptor blank	988 ± 65		
Experiment 2			
αIR-1	908 ± 45	0.6 > P > 0.4	−
+ Insulin control	875 ± 50		
− Receptor blank	941 ± 59		
B82			
αIR-1	1191 ± 209	0.7 > P > 0.6	−
+ Insulin control	1280 ± 10		
MOPC 21	1605 ± 375		
− Receptor blank	1406 ± 290		
Hybrid clone CF67-7			
Experiment 1			
αIR-1	1003 ± 37	0.02 > P > 0.01	+
+ Insulin control	782 ± 66		
MOPC 21	695 ± 28		
− Receptor blank	791 ± 41		
Experiment 2			
αIR-1	857 ± 61	0.02 > P > 0.01	+
+ Insulin control	673 ± 7		
MOPC 21	719 ± 11		
− Receptor blank	701 ± 16		
Experiment 3			
αIR-1	1457 ± 78	0.05 > P > 0.02	+
+ Insulin control	1280 ± 33		
MOPC 21	1186 ± 22		
− Receptor blank	1187 ± 28		
Hybrid clone CF33-1			
Experiment 1			
αIR-1	726 ± 30	0.4 > P > 0.3	−
+ Insulin control	755 ± 21		
MOPC 21	749 ± 52		
− Receptor blank	771 ± 3		
Experiment 2			
αIR-1	686 ± 27	0.7 > P > 0.6	−
+ Insulin control	663 ± 84		
MOPC 21	632 ± 7		
− Receptor blank	649 ± 46		
Experiment 3			
αIR-1	594 ± 66	0.3 > P > 0.2	−
+ Insulin control	516 ± 2		
MOPC 21	652 ± 30		
− Receptor blank	556 ± 42		

^{125}I-insulin-receptor complexes were immunoprecipitated as described in MATERIALS AND METHODS. Radioactivity precipitated with αIR-1 was compared with + insulin control by *t*-test, and P-values are shown in column 3.

dioactivity precipitated by the αIR-1 antibody was significantly greater for receptors incubated with ^{125}I-insulin than receptors incubated with ^{125}I-insulin plus unlabeled insulin. Using this criterion, normal diploid human skin fibroblasts were clearly positive for human insulin receptors, while the A9 and B82 derivatives of the mouse L cell line were clearly negative (Table 2). Two human fibroblast × L cell hybrids containing different complements of human chromosomes were also assayed for human insulin receptors by this method (Table 2). Hybrid clone CF67-7 was scored as a clear positive

TABLE 3
Immunoprecipitation of ^{125}I-insulin-receptor complexes

Cells	Radioactivity precipitated (counts/5 min)	P	Human insulin receptor
Human fibroblasts			
Experiment 1			
αIR-1	6331 ± 23	$P < 0.01$	+
+ Insulin control	1637 ± 156		
MOPC 21	2553 ± 555		
− Receptor blank	3173 ± 371		
Experiment 2			
αIR-1	6292 ± 792	$P < 0.01$	+
+ Insulin control	2864 ± 40		
− Receptor blank	2822 ± 94		
A9			
αIR-1	4352 ± 255	$0.6 > P > 0.5$	−
+ Insulin control	4595 ± 449		
− Receptor blank	5140 ± 176		
Hybrid clone CF33-16			
αIR-1	3257 ± 340	$P < 0.01$	+
+ Insulin control	1531 ± 73		
MOPC 21	1838 ± 184		
− Receptor blank	1581 ± 118		
Hybrid clone CF67-7			
αIR-1	4553 ± 126	$P < 0.01$	+
+ Insulin control	2707 ± 94		
MOPC 21	2151 ± 19		
− Receptor blank	2463 ± 525		
Hybrid clone CF33-1			
αIR-1	1479 ± 91	$0.3 > P > 0.2$	−
+ Insulin control	1350 ± 83		
MOPC 21	1420 ± 31		
− Receptor blank	1661 ± 230		
Hybrid clone CF67-2			
Experiment 1			
αIR-1	2310 ± 504	$0.8 > P > 0.7$	−
+ Insulin control	2195 ± 194		
MOPC 21	2643 ± 58		
− Receptor blank	2348 ± 154		
Experiment 2			
αIR-1	1842 ± 95	$0.4 > P > 0.3$	−
+ Insulin control	2068 ± 349		
MOPC 21	2312 ± 171		
− Receptor blank	2413 ± 47		
Hybrid clone CF80-8			
Experiment 1			
αIR-1	3154 ± 331	$0.5 > P > 0.4$	−
+ Insulin control	2869 ± 349		
MOPC 21	2463 ± 24		
− Receptor blank	2680 ± 157		
Experiment 2			
αIR-1	4650 ± 550	$0.2 > P > 0.1$	−
+ Insulin control	4135 ± 247		
MOPC 21	3917 ± 183		
− Receptor blank	3576 ± 80		

^{125}I-insulin-receptor complexes were immunoprecipitated as described in MATERIALS AND METHODS. Radioactivity precipitated with αIR-1 was compared with + insulin control by *t*-test, and P-values are shown in column 3.

in this assay, while hybrid clone CF31-1 was scored as a clear negative.

We found subsequently that the sensitivity of the assay could be improved by using 500,000 cpm rather than 50,000 cpm of ^{125}I-insulin in the assay. Five human fibroblast × L cell hybrid clones were assayed for human insulin receptors by the improved method (Table 3). Two of the hybrids, CF67-7 and CF33-16, yielded a positive result, while three others, CF31-1, CF67-2, and CF80-8, yielded negative results. An examination of the karyotypes of the five hybrids (Table 1) indicates that human chromosome 19 is the only possible chromosomal assignment for the insulin receptor gene.

Yang-Feng et al.[20] have recently used a cDNA probe[8] to provisionally assign the human insulin receptor gene to the short arm of chromosome 19. The possibility that the probe used in their study was actually derived from the IGF-I receptor gene has not been conclusively ruled out. The present results, obtained by a different approach, support the assignment of the insulin receptor gene to chromosome 19.

Further genetic studies will be required to establish

whether any of the human syndromes characterized by insulin resistance are caused by mutations in the insulin receptor gene or its controlling elements. Interestingly, myotonia dystrophica has been mapped by linkage analysis to the short arm of chromosome 19.[30] Myotonia dystrophica is characterized by insulin resistance, and a study of 12 patients with myotonia dystrophica indicated a reduction of insulin binding to circulating mononuclear blood cells.[31] It would be of considerable interest to determine whether the gene for myotonia dystrophica and the gene for the insulin receptor are genetically linked.

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