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Brief Communication

Chromosomal Localization of the Gene for a Human Cytosolic Thyroid Hormone Binding Protein Homologous to the Subunit of Pyruvate Kinase, Subtype M_2

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Abstract—*A cDNA for the gene that encodes a human cytosolic thyroid hormone binding protein (p58) recently has been isolated and sequenced. Analysis of the p58 sequence indicates that it is identical to the subunit of pyruvate kinase, subtype M_2. By in situ hybridization, the gene for p58 was mapped to 15q24-25. This localization shows that the p58 gene is not linked to the L-type of pyruvate kinase, which is located on chromosome 1. The p58 gene was found to be activated in several forms of cancer. Current localization will permit us to assess the effect of alterations involving chromosome 15 on the structure and activity of the p58 gene in neoplasms or chromosome syndromes.*

INTRODUCTION

Cytosolic thyroid hormone binding protein is widely distributed in the tissues of many species (1). In rat brain, liver, and heart, it was found to be developmentally regulated (2–4). At the present time, its function(s) is not clearly understood. It was postulated to serve as a buffer, regulator, or transporter, providing readily available 3,3′,5-triiodo-L-thyronine (T_3) to nuclei for gene regulatory activities (1).

Recently, a human cytosolic thyroid hormone binding protein (p58) was purified to homogeneity from human epidermoid carcinoma cells (5). It has a molecular weight of 58,000 and consists of a single polypeptide chain. p58 is not posttranslationally modified by glycosylation, sulfation, or phosphorylation (6). Using two monoclonal antibodies, a cDNA for p58 was isolated and characterized recently. Sequence analysis indicated that p58 is homologous to a subunit of pyruvate kinase, subtype M_2 (PKM_2). p58 is a monomer that has ~5% of the enzymatic activity of tetrameric pyruvate kinase PKM_2. Tetrameric PKM_2 does not bind T_3. Conversion of the monomeric p58 to tetrameric PKM_2 is reversible and is under the control of fructose 1,6-bisphosphate. Fructose 1,6-biphosphate activates the pyruvate kinase activity by facilitating the association of monomeric p58 to form tetrameric PK. The conversion is inhibited by T_3 and its analogs in a dose-dependent manner (7).

PK (ATP:pyruvate 2-*O*-phosphotransferase, EC 2.7, 1.40) catalyzes the conversion of phosphoenol pyruvate to pyruvate with the generation of ATP. There are four mammalian isoenzymes of pyruvate kinase known, each of which consists of four identical or nearly identical subunits with the molecular

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weight of each subunit ranging from 57 to 60 kDa (8). The four isoenzymes are designated at L, R, M_1, and M_2. The L type is mostly present in liver; R is found exclusively in erythrocytes; M_1 is mostly in muscle; and M_2 is widely found in many tissues such as kidney, intestine, lung, fibroblasts, testis, adipose tissue, and stomach. In tumors or regenerating liver, M_2 is increased (8). In some neoplasias such as meningiomas, malignant gliomas, and rhadomyosarcomas, a switch from M_1 toward M_2 was found (9, 10). The L- and R-type PKs differ from M_1- and M_2-type PKs in their electrophoretic, kinetic, and immunological properties. The cDNAs and genomic clones for rat L, R, M_1, and M_2 have been isolated and sequenced (11–13), and the cDNA for the human L type also was obtained (14). The L- and R-type and M_1- and M_2-type PKs are under the control of different genes. The L and R type of rat pyruvate kinase are produced from a single gene by use of different promoters, whereas the M_1 and M_2 type are produced from the same gene by alternative RNA splicing.

Using a human–mouse hybrid clone, human PKM_2 has been assigned on 15q22-qter (15–17). However, these previous studies have not assigned its subregional location. Recently, PKM_2 was localized to 15q22 (13). The present study used the full-length cDNA for p58 and a 3′ end 0.838-kb fragment of p58 cDNA as probes to map its locus by in situ hybridization. We found that p58 is located on the long arm of chromosome at the region q24-25.

MATERIALS AND METHODS

A full-length cDNA encoding human p58 (TC6) and a PstI–PstI 0.838-kb fragment (see Fig. 1) were used as probes. TC6 was isolated from a cDNA library prepared by using polyadenylated RNA extracted from a human thyroid carcinoma as described by Kato et al. (7). The 0.838-kb fragment was obtained by restricting the TC6 with PstI and

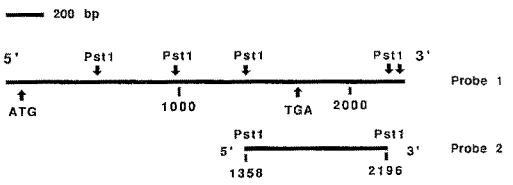

Fig. 1. Schematic diagram of the probes used in the mapping of p58. Probe 1 is the full-length cDNA (TC6). Probe 2 is the PstI–PstI-restricted fragment with a size of 0.838 kb located at the 3′ end of p58 cDNA. Between ATG and TGA is the coding region for p58.

purified by eluting from an agarose gel after electrophoresis. The probes were nick-translated with all four ^{3}H-nucleotides to a high specific activity of 1.5×10^7 cpm/μg DNA and 1.7×10^7 cpm/μg DNA for TC6 and the 0.838-kb fragment, respectively.

For in situ hybridization, chromosome preparations obtained from synchronized normal leukocyte cultures were pretreated with RNAase and denatured in 70% formamide at 70°C (18). The hybridization solution contained 50% formamide, 5% dextran sulfate, 5 mM EDTA, 2× Denhardt's, 300 mM NaCl, 30 mM sodium citrate, 50 μg/ml single-stranded salmon sperm DNA, and 20 mM phosphate buffer (pH 6.4), and a volume equivalent to 5×10^5 cpm of the labeled probe was layered on each slide. The slides were covered with cover slips and incubated in a moist chamber at 40°C for 20 h. After 50% formamide and 2× SSC washes at 42°C, the hybridized slides were coated with nuclear track emulsion NTB-2 (Kodak, Rochester, New York) diluted 1:1 with H_2O and stored desiccated at 4°C. After 14 days, slides were developed, stained, and spreads exhibiting silver grains on the chromosomes were photographed. To obtain the G-banding for individual chromosome identification, the slides were destained, treated with a solution of 0.03% trypsin–0.012% EDTA (Gibco, Grand Island, New York), and restained with 0.25% Wright stain in 0.06 M phosphate buffer (1:3, pH 6.8) (19, 20). Previously photographed chromosome spreads were relocated, and a second photomicrograph

displaying G-bands was used for grain localization.

RESULTS

In situ hybridization was carried out by first using a ^{3}H-labeled full-length cDNA (TC6) as a probe (Fig. 1). As shown in the photomicrographs (Fig. 2A and B), grains were identified before and localized after G-banding. Analysis of 102 labeled chromosome spreads and the distribution of 220 grains on G-banded chromosomes revealed three sites of hybridization (Figs. 2 and 3A): a major site at 15q24-26 (70 grains, 32% of the total) and two minor sites at 1p11-12 (29 grains, 13% of total) and at 6p15.3 (24 grains, 10% of total (Fig. 3A). Together, these sites accounted for 55% of all grains scored. The rest of the grains were distributed randomly over the remainder of the complement (Fig. 3A).

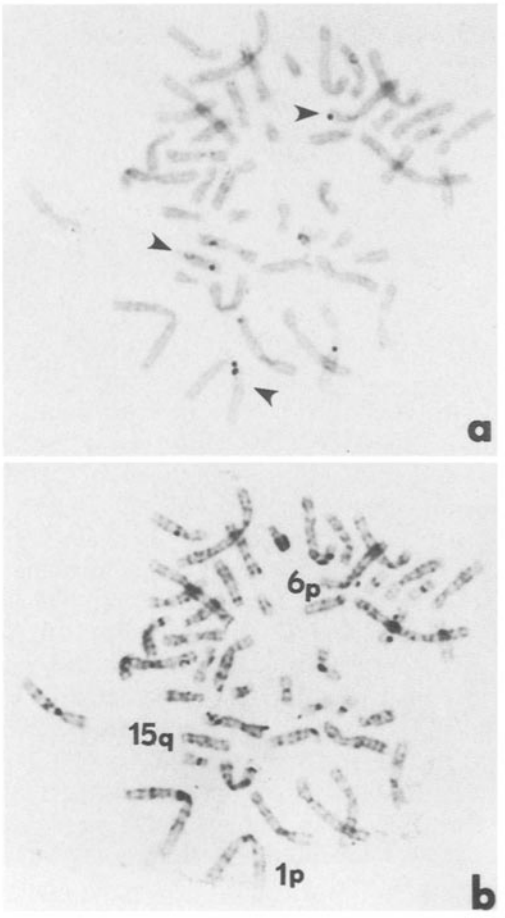

Fig. 2. (A) Human metaphase after in situ hybridization with a full-length cDNA probe (probe 1). The arrows indicate the labeling at three specific sites. (B) After G-banding the labeled chromosomes are identifiable. Grains are localized on the short arms of chromosomes 1 and 6 and on the long arm of chromosome 15.

Since p58 is homologous to a subunit of PKM_2, the three sites identified could be due to cross-hybridization with other members of the PK multigene family or other unidentified homologous genes. Comparison of p58 to the human L type of pyruvate kinase indicated there is a 41% sequence similarity (14). However, the three sites also could represent that p58 has multicopy genes. To differentiate these possibilities, a probe that has the least sequence similarly to L and R type pyruvate kinase also was used for in situ hybridization. Comparison of sequences showed that the pyruvate kinase genes have the highest divergence at or near the 3′ end. We, therefore, used the 0.838-kb (PstI–PstI) fragment located at the 3′ end of p58 cDNA as a probe (see Fig. 1). This 0.838-kb fragment only has a 26% sequence similarity to the corresponding region of human L type pyruvate kinase (14).

Using the 0.838-kb fragment as a probe, the in situ hybridization was carried out identically to that described for the full-length cDNA probe. After analysis of 98 metaphases, a total number of 217 grains were localized. Fifty-nine grains (27% of total) were found to concentrate on chromosome 15. The remainder of the grains were distributed randomly over the rest of the complement as shown in Fig. 3B. Figure 4 shows the grain distribution on chromosome 15; 76% of the grains found in chromosome 15 were clustered at bands 24-25 with a peak at 15q24. Based on the data from both probes we assigned p58 gene at 15q24-25.

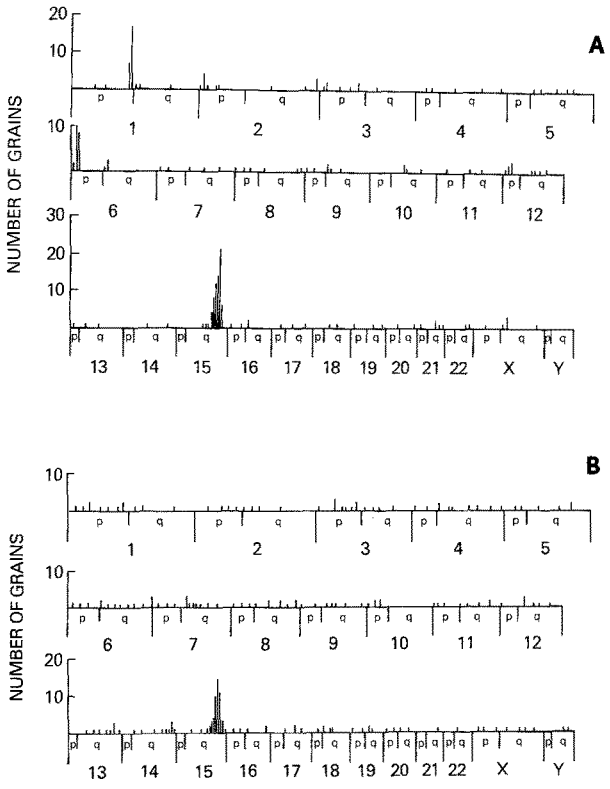

Fig. 3. Grain distribution on a 400-band ideogram after in situ hybridization. (A) The probe is a full-length cDNA (probe 1). One major and two minor sites of hybridization were found on chromosome 15 and chromosome 1 and 6, respectively. (B) The probe is the 0.838-kb fragment (probe 2). Only one hybridization site was found on chromosome 15.

DISCUSSION

In the present study, two probes were used to localize to the p58 gene. Using the full-length cDNA, the majority of grains were found to be concentrated on 15q24-26 with a small fraction of the probe localized on chromosomes 1 and 6. The localization of p58 was further defined by using a p58-specific probe (0.838-kb fragment) with which no other hybridization site was identified. Thus, the p58 locus was clearly mapped to 15q24-25. These results eliminated the possibility that the minor sites identified by the full-length cDNA were due to a multicopy distribution of the p58 gene. Rather, it most likely represents cross-hybridization with other genes that have homologous sequences. The identity of these genes remains to be established.

Since p58 is a subunit of PKM_2, our results confirmed the earlier findings in which PKM_2 was reported to be on 15q22-qter (15–17). More recently, Tani et al. reported the localization of the human PKM_2 gene to 15q22 by in situ hybridization (13). Tani et al. isolated PKM_2 DNA from human liver, whereas p58 was isolated from human thyroid carcinoma. Comparison of the cDNA-derived protein sequences showed that the p58 sequence differs from that of liver in amino acids 102 (Tyr → Ile), 131 (Leu → Val), and 378 (His → Asn) (7, 21). However, one would not expect that the three amino acid differences in sequence could lead to different localization on the long arm of chromosome 15. Our assignment was based on consistent results obtained with two different probes. The explanation for the discrepancy between the present results and those of Tani et al. is unknown.

The finding that p58 is located in

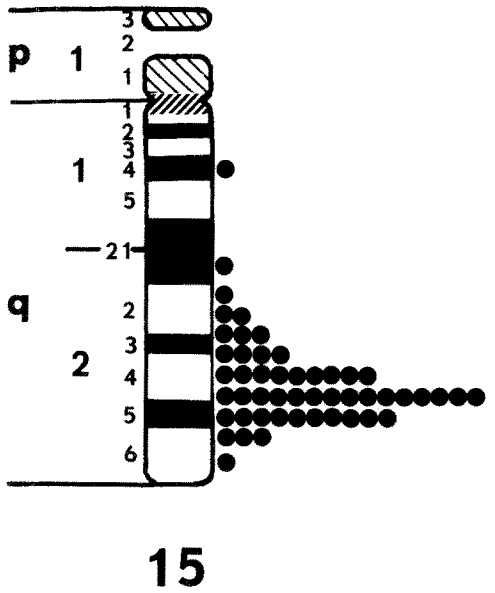

Fig. 4. Grain distribution on chromosome 15 with the 0.838-kb fragment as a probe. The grains were clustered in the region 15q24-25 with a peak at 15q24.

15q24-25 raises the possibility that this gene may serve as a useful marker for Tay-Sachs disease. Hexosaminidase A, the enzyme lacking in patients with Tay-Sachs disease, is also located in the same chromosomal region (15q23-24) as the p58 gene (22, 23).

Increased p58 expression has been detected in meningiomas, malignant gliomas, and rhadomyosarcomas (8–10). However, the mechanism by which the expression of p58 is increased is not currently understood. Moreover, whether the p58 gene is involved in carcinogenesis and tumor progression is unknown. *Fes* protooncogene (24, 25) and the gene encoding human myeloid membrane antigen gp150 (26), which is involved in myeloid malignancies, are located very close to the p58 gene. Both genes, as well as p58, are rather distant from the breakpoint in reciprocal translocation 15;17 specific for acute promyelocytic leukemia (27). This translocation is the only recurrent structural alteration of chromosome 15 in human cancer. The mapping of p58 to 15q24-25 should help future studies in elucidating the relationship between activation of the p58 gene and tumor growth.

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