Molecular Cloning of Transferrin Receptor 2

A NEW MEMBER OF THE TRANSFERRIN RECEPTOR-LIKE FAMILY*

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Hiroshi Kawabata‡, Rong Yang, Toshiyasu Hirama, Peter T. Vuong, Seiji Kawano, Adrian F. Gombart, and H. Phillip Koeffler§

From the Cedars-Sinai Medical Center, Department of Medicine, Division of Hematology/Oncology, Burns and Allen Research Institute, University of California Los Angeles School of Medicine, Los Angeles, California 90048

Transferrin receptor (TfR) plays a major role in cellular iron uptake through binding and internalizing a carrier protein transferrin (Tf). We have cloned, sequenced, and mapped a human gene homologous to TfR termed TfR2. Two transcripts were expressed from this gene: α (~2.9 kilobase pairs), and β (~2.5 kilobase pairs). The predicted amino acid sequence revealed that the TfR2-α protein was a type II membrane protein and shared a 45% identity and 66% similarity in its extracellular domain with TfR. The TfR2-β protein lacked the amino-terminal portion of the TfR2-α protein including the putative transmembrane domain. Northern blot analysis showed that the α transcript was predominantly expressed in the liver. In addition, high expression occurred in K562, an erythromegakaryocytic cell line. To analyze the function of TfR2, Chinese hamster ovary TfR-deficient cells (CHO-TRVb cells) were stably transfected with FLAG-tagged TfR2-α. These cells showed an increase in biotinylated Tf binding to the cell surface, which was competed by nonlabeled Tf, but not by lactoferrin. Also, these cells had a marked increase in Tf uptake, which was competed by nonlabeled Tf, but not by lactoferrin. Taken together, TfR2-α may be a second transferrin receptor that can mediate cellular iron transport.

Iron is essential in a wide variety of cellular processes including oxidative phosphorylation and DNA synthesis. Our knowledge concerning cellular iron transport has been markedly advanced by the recent discoveries of several genes such as HFE, associated with hereditary hemochromatosis (1), and divalent metal transporter (DMT1/Nramp2), a transmembrane iron transporter (2, 3). One of the well-studied key molecules involved in iron uptake is transferrin receptor (TfR)1 (reviewed in Ref. 4 and 5). On the cell membrane, the TfR homodimer binds to two differferic transferrin (Tf) molecules, resulting in internalization of the complex. In the endosome, iron is released from Tf in a pH-dependent manner and is transported into the cytosol by DMT1/Nramp2 (6). The iron is utilized as a cofactor by heme, aconitase, cytochromes (reviewed in Ref. 7), and ribonucleotide reductase (8), or it may be stored in ferritin molecules. The affinity of diferric Tf to TfR is modulated by HFE (9).

Although TfR-mediated endocytosis is the major pathway for cellular iron uptake, cells can also obtain iron through TfR-independent pathways from iron-bound Tf or from inorganic irons (10–13). These processes are thought to be through fluid-phase endocytosis, passive perfusion, or other membrane-based transport systems, and no other receptor for Hf has been reported to date. In this study, we describe the cloning of a new TfR-like family member, TfR2, which may mediate the cellular uptake of iron via a new pathway.

EXPERIMENTAL PROCEDURES

Cell Lines—ML-1, NB4, Kasumi 3 (myeloid leukemia), and both CHO-TRVb (TfR-deficient Chinese hamster ovary) and TRVb-1 (human TfR stably transfected TRVb) cells were kindly provided by Drs. J. Manomet (14), M. Lanette (15), H. Asou (16), and T. E. Magew (17), respectively. All of the other cell lines were obtained from American Type Culture Collection (Manassas, VA). Human mononuclear cells were isolated from the blood of a normal volunteer by centrifugation on a Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) gradient at 400 × g for 30 min. Informed consent was obtained from the individual.

Molecular Cloning of cDNA and Genomic DNA—Complementary DNA libraries were constructed from TF-1 (erythroid leukemia) and HL60 (myeloid leukemia) cells using a commercial kit (Marathon cDNA Amplification Kit; CLONTECH, Palo Alto, CA) and used for 5'- and 3'-rapid amplification of cDNA ends (RACE) reactions to obtain a full-length cDNA clone. Primers (Table I, primers A and B) were used for 5' RACE, and primers (Table I, primers C and D) were used for 3' RACE, respectively. The products of RACE reactions were subcloned into the pigEM-Teasy vector (Promega, Madison, WI). We isolated two transcripts of 2.9 kb (α transcript) and 2.5 kb (β transcript) from the TF-1 and HL60 cDNA libraries, respectively.

Genomic DNA was isolated from a human genomic library (Lambda FIX II Library; Stratagene, La Jolla, CA) using a 2.2-kb fragment of the 3'-end of the TfR2 cDNA as a probe (as shown in Fig. 1A). After restriction enzyme mapping, a 3.85-kb fragment that included exons 4–6 was subcloned into the pl vectors (α transcript) and 2.5 kb (β transcript) from the TF-1 and HL60 cDNA libraries, respectively.

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Northern Blot and RT-PCR Analyses—Northern blot and RT-PCR analyses were performed as described previously (18). Human tissue Northern blot membranes and cDNAs were purchased from OriGene (Rockville, MD). For Northern blot analysis, two TfR2 cDNA fragments (Probe-1 and Probe-2 shown in Fig. 1A), a human β-actin cDNA fragment (OriGene), and an approximately 300-base pair TfR2 cDNA fragment were used as probes. For RT-PCR, the α form-specific primers (primers A and D) and the β form-specific primers (primers C and E)
Genomic structure of TfR2 gene. A, map of the TfR2 gene. An approximate 16-kb genomic fragment was cloned from a human genomic library (Genomic Clone 1), and restriction enzyme sites were mapped. A 3.85-kb fragment of the Genomic Clone 1 (shown as a shaded bar) was subcloned into the pBluescript II(-) plasmid and sequenced. The exon-intron borders shown in this figure were based on data deposited in the GenBank (accession number AP053356), with some modifications based on our data. The α transcript contains 18 exons (closed boxes on the line). The β transcript lacks exons 1–3 and has an additional 142 bases at the 5'-end of exon 4 (an open box on the line). The boxes indicate the primer sequences used to synthesize the α and β transcripts, respectively, by RT-PCR. Putative translation initiation codon for the β transcript is shown as bold ATG. Guanines at -3 and +4, which are consistent with Kozak's sequence for this initiation codon, are underlined.

were used (Fig. 1B). Conditions for amplification were 35 cycles of 94 °C for 30 s, 56 °C for 40 s, and 72 °C for 1 min. As a control, glyceraldehyde-3-phosphate dehydrogenase was amplified in a separate reaction using primers, 5'-CCATGGAAGGCTGGGG-3' and 5'-CAAAATGTCATGGATGACC-3' for 27 cycles.

Transfection and Immunoblotting—CHO-TRVb cells were maintained in P12-nutrient mixture (Life Technologies, Inc.) supplemented with 5% fetal bovine serum. An amino-terminal FLAG-tagged TfR2α cDNA was subcloned into pcDNA3 (Invitrogen, Carlsbad, CA). This plasmid (10 μg) was transfected into CHO-TRVb cells using Lipofectin (Life Technologies, Inc.). For transient expression, cells were harvested 48 h after the transfection. We also isolated a stably expressed clone using G418 (200 μg/ml), and the protein expression was confirmed by immunoblotting using anti-FLAG (M5) antibody (Eastman Kodak, New Haven, CT). Immunoblot analysis was performed as described previously (19).

Flow Cytometric Analysis of Tf Binding to the Cell Surface—Approximately 3 × 10^6 cells were incubated with 5 μg/ml biotinylated human holo-Tf (Sigma) in 500 μl of minimum Eagle's medium α (Life Technologies, Inc.) in either the presence or absence of nonlabeled human holo-Tf (Sigma) or human lactoferrin (Lf) (Calbiochem, San Diego, CA) for 30 min on ice. After two washes with phosphate-buffered saline supplemented with 0.1% bovine serum albumin, the cells were incubated with streptavidin-phycocerythrin (DAKO). The cells were washed twice and subsequently analyzed by flow cytometry.

Analysis of Tf-mediated Iron Uptake—^59Fe-Tf was prepared by the method described previously (20), except that we used 0.4 mCi of ^59FeCl3 (NEN Life Science Products, Boston, MA) instead of 0.1 mCi of ^59FeCl3. A specific activity of 27,000 cpm/μg was obtained. Cells were incubated with ^59Fe-Tf in minimum Eagle's medium α in either the presence or absence of a 200-fold excess of nonlabeled holo-Tf at 37 °C with 5% CO2. After washing with phosphate-buffered saline, the cells were lysed with 0.1 N NaOH, and the radioactivity was counted using a liquid scintillation counter.

RESULTS

Molecular Cloning, Chromosomal Mapping, and the Genomic Structure of the TfR2 Gene—During 5'-RACE, while attempting to isolate genes encoding new transcriptional factors, we serendipitously cloned an 831-base pair human cDNA fragment that had significant amino acid homology to the middle portion of the TfR protein from the TF-1 cDNA library. We obtained an approximately 2.9-kb cDNA sequence for TfR2 from this library (α form; GenBank accession number AF067864). A cDNA clone encoding the putative full-length coding sequence was created by polymerase chain reaction using 5' and 3' gene-specific primers. When we used a HL60 cDNA library for cloning TfR2, the 5'-RACE products were shorter than those from the TF-1 library, and the sequences around the 5'-end were different (β form; Fig. 1B).

According to the radiation hybrid panel analysis, TfR2 mapped on chromosome 7q22, between the D7S651 and WI-5853 markers.

The restriction enzyme mapping and partial sequencing of a 16-kb genomic DNA clone and comparison with the genomic sequence in GenBank (accession number AP053356) deposited by Gleockner et al. (21) revealed that the α form consisted of 18 exons (Fig. 1). However, some differences between their predicted exon-intron borders and ours were noted. Our DNA sequence contained an additional 298 nucleotides in the 3'-untranslated region (nucleotides 2580–2877). The 5'-end and lacked 18 nucleotides in exon 18 (nucleotides 1053–1133 in the TfR2-α) and 18 nucleotides in exon 18 (between nucleotides 2163 and 2184) as compared with their predicted mRNA sequence. This resulted in a 27-amino acid addition and a 6-amino acid deletion for our predicted TfR2-α protein. Also, our mRNA sequence contained an additional 298 nucleotides in the 3'-untranslated region (nucleotides 2580–2877).

The β form, which may be an alternative product of splicing or promoter usage, lacked exons 1, 2, and 3, and its first exon (exon 4 of the α form) had an additional 142 nucleotide bases at the 5'-end (Fig. 1). No typical iron-responsive element was present in the untranslated regions of either of the TfR2 transcripts (22).

The Primary Structure of TfR2 Proteins—The predicted amino acid sequence of TfR2-α is shown in Fig. 2. The hydrophobic stretch of residues from 81 to 104 following a pair of arginines represents the predicted transmembrane domain. It
is located close to the amino terminus, similar to the transmembrane domains of human TfR and prostate-specific membrane antigen (PSMA) (shaded section in Fig. 2) (23, 24). By analogy to TfR and PSMA, TfR2-\(\alpha\) probably is a type II membrane protein. Therefore, residues 1–80 of TfR2-\(\alpha\) may be the cytoplasmic domain, and residues 105–801 may be the extracellular domain. In the extracellular domain, amino acid sequence homologies between TfR2-\(\alpha\) and either TfR or PSMA were quite high. The extracellular domain of TfR2-\(\alpha\) was 45% identical and 66% similar with that of TfR. With PSMA, the identity was 27%, and the similarity was 60%. The cysteines at positions 89 and 98 of TfR form disulfide bonds, resulting in

![Fig. 2. Deduced amino acid sequence of TfR2-\(\alpha\) aligned with those for the human TfR and PSMA proteins. Identical residues are boxed. Hydrophobic amino acid stretches located in the putative transmembrane portions are shaded. The internalization motif of TfR and the correspondingly similar motif of TfR2-\(\alpha\) are double underlined. Predicted initial methionine of TfR2-\(\beta\) is shown as a bold letter.](image)

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| TfR2-\(\alpha\) | TfR | PSMA |
|---|---|---|
| 1 | M | M | M |
| 51 | FCPNEGRPSGGNPGPSRNPLLWPL | GEDRRPRLTGG | MFLGFL |
| 35 | HVEMLRMAEADNTANAKVTKPSC | KESGGIGTYTATVFLCFTMG |
| 2 | W | W | W |
| 101 | YVAF-RSC-QAGGDSVLVSD | EYFPODLLHOG-YSLQMAFLQF |
| 85 | YLGCKSGVEPTEKLAGEPSYPRE | FGPPFPAPAARLWLYLRKKLE |
| 37 | E | E | E |
| 148 | LGSLFEDTFR | GAAATAAGGAASLMKTH | A |
| 135 | LDELKANTEKFLYNTFQP | AGTQGNSWEGOSLMLA |
| 62 | W | W | W |
| 193 | TVYGLCOF | EPDPVPGYFPLQSGSATNV |
| 185 | QHYNKSD | XDB-QNSKLIVNYDDKRL |
| 112 | H | H | H |
| 242 | T | T | T |
| 290 | T | T | T |
| 161 | FSAFPQGMPEDLRLRAGVDPY | EALRLVNLVSR |
| 265 | AEVKANAEKLNASGLVY | Y | Y |
| 211 | GNVKNAAGLSLAGPSEPDA | FGYKYSQGNY |
| 325 | L | L | L |
| 300 | GDPFTPGFSP | NQFQFFPVQVA | K |
| 311 | APASSWREG | LPV | |
| 413 | IFCIEGEBSPDHYVY | GQADRDAWGPAKSA | V |
| 398 | IFVGKCFEP | VHEPVDFPD | A |
| 308 | VTLLNAG | P | |
| 405 | N | N | N |
| 463 | LDGDHFHSKL | PXSSLSK | |
| 456 | LGSNVEK | KSLLY | |
| 408 | E | E | E |
| 500 | V | V | V |
| 537 | SGMPRSISKL | ESNEDVL | |
| 604 | YEMNHKLQGGL | PAVQA | |
| 573 | YKEL- | I | |
| 507 | ETY-I | EYFDPMK | YHLY |
| 632 | RHIGN | FEQ | |
| 650 | SFVDNLQ | H | |
| 606 | KYADKY | I | |
| 701 | R | R | R |
| 724 | LHRLR | SNNSSPTGAT | S |
| 715 | NNLRLRGN | | |
| 732 | F | F | F |

| TfR2-\(\beta\) | TfR | PSMA |
|---|---|---|
| 1 | I | DO |
| 8 | D | D | D |
| 56 | S | S | S |
| 147 | D | D | D |
| 230 | H | H | H |
| 314 | V | V | V |
| 375 | G | G | G |
| 557 | A | A | A |
| 640 | L | L | L |
| 728 | S | S | S |

In the extracellular domain, amino acid sequence homologies between TfR2-\(\alpha\) and either TfR or PSMA were quite high. The extracellular domain of TfR2-\(\alpha\) was 45% identical and 66% similar with that of TfR. With PSMA, the identity was 27%, and the similarity was 60%. The cysteines at positions 89 and 98 of TfR form disulfide bonds, resulting in
Homodimerization. Two cysteines at positions 108 and 111 in TfR2-α are located in an analogous region and may serve a similar function. In addition, TfR2-α contains the motif YQRV (amino acids 23–26) in the middle of the cytoplasmic domain, which may function as an internalization signal, similar to the YTRF motif in TfR (Fig. 2, double underlined) (25–27).

The β transcript lacks exons 1–3, which encode the entire transmembrane and cytoplasmic domains as well as a part of the extracellular domain including the two cysteines at 108 and 111. The additional 142-nucleotide 5′-sequence in exon 4 does not contain an initiation codon. Translation probably starts at the ATG located at nucleotide 542, which is in frame with the α transcript open reading frame. The predicted initial methionine is shown in Fig. 1B, Exon 4 and Fig. 2 as bold ATG and M, respectively. This ATG contains a G at positions −5 and +4, indicating that it is an ideal start site for translation (28). Hence, the predicted protein product of the β transcript would lack both a transmembrane domain and signal peptide, resulting in a possible intracellular protein.

Characterization of TfR2 mRNA Expression—Northern blot analysis of poly(A)⁺ RNA from human tissues showed that a 2.9-kb mRNA for TfR2 was expressed predominantly in the liver and to a lesser degree in the stomach (Fig. 2A). This corresponded with the length of TfR2-α cDNA isolated from TF-1 cells. In addition, faint bands at 4 kb (stomach) and 1.7 kb (liver, lung, small intestine, stomach, testis, and placenta) were observed. These bands may reflect the presence of additional alternative forms of TfR2 mRNA. Northern blot analysis of total RNA of various cell lines revealed high expression of TfR2-α in K562 cells (erythroleukemia) and HepG2 cells (hepatoblastoma) (Fig. 3B). The expression levels of TfR2-α were not always correlated with those of TfR (Fig. 3B). No transcripts corresponding to TfR2-β (2.5 kb) were observed by Northern blot analysis.

To compare the expression of the α and β transcripts, RT-PCR was performed using specific primers for each form. Using a human tissue cDNA panel as a template, the expression of the α form was limited to the liver, spleen, lung, muscle, prostate, and peripheral blood mononuclear cells (Fig. 4A). On the other hand, expression of the β form occurred in all of the tested human tissues. Human cancer cell lines from various tissues were studied for expression of the two transcripts. Most of the cell lines expressed both transcripts; however, two cell lines, SK-Hep1 (hepatoma) and ML-1 (myeloblast), lacked the β transcript (Fig. 4B).

Tf Binding to the TfR2-α-transfected Cells—To analyze the function of TfR2-α, we stably transfected CHO-TRVb cells, which lack functional TfR, with FLAG-tagged TfR2-α. The cell surface Tf binding was examined using biotinylated Tf and flow cytometry. Neomycin-resistant control cells were almost negative for the cell surface Tf binding (Fig. 5A, left panels). TRVb-1, human TfR stably transfected cells, was positive for cell surface Tf binding, which was competed by nonlabeled Tf, but not by Lf (Fig. 5A, center panels). In the CHO-TRVb cells stably expressing TfR2-α, the mean level of cell surface Tf binding was clearly higher than that of the control cells (Fig. 5A, right panels, solid lines). In competition experiments, a 10-fold excess of nonlabeled Tf clearly inhibited the binding of biotinylated Tf, but even a 100-fold excess of Lf did not inhibit the binding (Fig. 5A, right panels, broken lines). Tf binding to the TfR2-α cells was also examined in a transient expression system using CHO-TRVb cells, and the levels of Tf binding to the cell surface were consistently as follows: TfR cells > TfR2-α cells > pcDNA3 cells (data not shown).

Tf-mediated ⁵⁵Fe Uptake of the TfR2-α-transfected Cells—Human TfR and TfR2-α stably transfected CHO-TRVb cells

Fig. 3. Northern blot analysis. A, multiple tissue blots of human mRNA were hybridized with a TfR2 probe (Probe-1 in Fig. 1). Membranes were hybridized in the same bottle at the same time, and the autoradiograms were developed after a 12-h exposure. B, 30 μg of total RNA from cell lines were loaded in each lane and hybridized with a TfR2 probe (Probe-2) and a TfR probe. A β-actin probe was used as a control for all blots. Size markers or the positions of ribosomal RNAs are indicated on the left.
were incubated with \(^{55}\text{Fe}-\text{Tf}\) and \(^{55}\text{Fe}\) uptake was measured. Neomycin-resistant CHO-TRVb cells were used as controls. Tf-mediated \(^{55}\text{Fe}\) uptake by the TRV2-α cells was comparable to that by TfR cells, and both were clearly higher than that by control cells (Fig. 5B). Competition by a 200-fold excess of nonlabeled Tf almost completely blocked \(^{55}\text{Fe}\) incorporation in these three cell lines after a 5-h incubation (Fig. 5B). Despite the absence of functional TfR, Tf-mediated \(^{55}\text{Fe}\) uptake was also seen in the control TRVb cells to a slight extent, as reported previously by Chan et al. (12).

**Dimerization of the FLAG-tagged TfR2-α Proteins Expressed in Mammalian Cells**—Cell lysates from the cells transiently transfected with the FLAG-tagged TfR2-α plasmid were examined by immunoblotting using anti-FLAG antibody (Fig. 5C). Two closely migrated bands of \(-105\) kDa were observed under reducing conditions (lane 2). When 2-mercaptoethanol was omitted from the sample loading buffer, the doublet of \(-105\) kDa decreased, but a protein of \(-215\) kDa appeared (lane 3). Faint bands of \(-260\) kDa and \(-125\) kDa were also seen under nonreducing conditions (lane 3, arrows).

**DISCUSSION**

The primary structure of the TRV2-α protein deduced from its mRNA is similar to that of TfR (see “Results”). Also, our results showed that TRV2-α had a similar function to TfR with respect to Tf binding and Tf-mediated iron uptake. However, the mechanisms that regulate expression of TfR and TfR may be different. Levels of the TfR protein are regulated post-transcriptionally through iron-responsive elements in its 3’-untranslated region, to which iron regulatory protein (IRP)-1 and IRP-2 can bind. In cells lacking sufficient iron, IRPs bind to iron-responsive elements of TfR mRNA and stabilize these transcripts. In the presence of excess intracellular iron, IRPs
are released, leading to degradation of the TJR mRNA. In rapidly growing cells, the proto-oncogene c-myc represses H-ferritin and increases IRP-2. The latter may enhance TJR protein expression (29). Neither the 3'- nor the 5'-untranslated regions of the TJR2 mRNAs have a detectable iron-responsive element-like structure, suggesting that another mechanism(s) may regulate TJR2 expression.

The size of the FLAG-tagged TJR2-a expressed in mammalian cells is ~105 kDa in the presence of a reducing agent and is ~215 kDa in the absence of a reducing agent (Fig. 5C), indicating dimerization of TJR2-a through disulfide bonds. The size of FLAG-tagged TJR2-a monomer, ~105 kDa, is larger than that calculated from the amino acid sequence (~90 kDa). This may reflect post-translational modifications of the protein such as glycosylation. Four putative N-glycosylation sites (amino acids 240, 339, 540, and 754) occur in the TJR2-a protein. Hence, the double bands of ~105 kDa shown in Fig. 5C may be due to different states of glycosylation. In addition, faint bands of ~260 kDa and ~125 kDa just above the clear bands of ~215 kDa and ~105 kDa, respectively, were observed under nonreducing conditions (Fig. 5C, lane 3). These faint bands may reflect the interaction of TJR2-a with a small protein (~20 kDa) through disulfide bonds.

Northern blot analysis using normal human tissue poly(A)+ RNA showed that the liver was the only tissue tested that prominently expressed TJR2-a (Fig. 3A). Also, TJR2-a was expressed highly in the K562 cell line, which is capable of hemoglobin synthesis (Fig. 3B). This result suggests that erythroid hematopoietic cells may express high levels of TJR2-a. The major product of red blood cells is hemoglobin, which contains abundant iron, and if TJR2-a is involved in iron transport, it would be expected to be strongly expressed in these cells. In erythroid cells, Cotner et al. (30) predicted the presence of an alternative form of TJR using a set of monoclonal antibodies against TJR. Their findings may be ascribed to TJR2-a.

We cloned two different forms of transcripts from the TJR2 gene: α and β. Two different transcripts are also expressed from the human PSMA/NAAG-peptidase gene (24, 31), the only known homolog of TJR. Because the expression of PSMA is high in prostate cancer, the antibody against PSMA was approved for use as an imaging agent to detect prostate cancer metastasis (32). The shorter form of PSMA lacks the 5'-end encoding the transmembrane domain (33), similar to the β form of TJR2. Nearly a 100-fold difference in the ratio of expression of the longer and the shorter form of PSMA mRNA has been reported during progression of prostate cancer, with the shorter form predominant in normal cells, and the longer form predominant in the cancer cells (34). Using the extremely sensitive RT-PCR method, we could distinguish expression of the α and β forms of the TJR2 gene. The expression of the α (longer) form was detected in the liver, spleen, lung, muscle, prostate, peripheral blood mononuclear cells and most human cancer cell lines from various tissues (Fig. 4). The β form was distributed more widely. All of the human tissues tested and most of the human cell lines expressed this β-transcript (Fig. 4).

We mapped TJR2 to chromosome 7q22. Deletion or loss of heterozygosity of this chromosomal region has been reported in several malignant diseases including myelodysplastic syndromes, acute myeloid leukemia, as well as breast, ovarian, and pancreatic cancers (35–39). Additional studies are required to determine whether TJR2 mutations occur in these cancers.

To investigate the function of TJR2, both Tf and Lf were considered as candidate ligands of TJR2. Lf is another Tf family member. The CHO-TRVb cells transfected with FLAG-tagged TJR2-a showed higher levels of Tf binding to the cell surface than did the control cells (Fig. 5A). This indicates that FLAG-tagged TJR2-a was expressed on the cell surface and was bound by Tf. This binding was effectively competed by nonlabeled Tf but not by Lf (Fig. 5A). This indicates that Tf can bind to TJR2-a more specifically than can Lf. In addition, Tf-mediated iron uptake by TJR2-a-transfected cells was obviously higher than that of control cells (Fig. 5B). These results indicate that TJR2 may be involved in another iron transport pathway, which is not identical to that of TfR.

However, if the only ligand for TJR2-a is Tf, and the main function of TJR2-a is cellular iron uptake, why do the cells have two different receptors for Tf? TJR2-a may simply be another transferrin receptor with a different affinity. The fate of the TJR2-a complex on the cell surface may be different from that of the Tf/TfR complex. The putative internalization motif of TJR2-a is not identical to that of TfR, and even a minor difference of the internalization motif may result in different destinations of the endosomes (27). Still, the possibility exists that TJR2-a has another specific ligand other than Tf. We have identified the murine TJR2 transcript from MEL cells, and found that it is highly homologous to human TJR2 in both nucleotide and protein sequences but clearly distinct from murine TJR2.2 TJR knockout mice died in utero with defective erythropoiesis and neurological abnormalities (13). This indicates that murine TJR2 cannot fully compensate for the loss of the function of TJR. Does TJR2-a bind to HFE, which normally forms a complex with TJR on the cell membrane? Can TJR2-a form a heterodimer with TJR? Elucidation of the precise role of TJR2 may provide an important step for clarifying the mechanisms and the regulation of cellular iron uptake.

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