Transferrin receptor 2 protein is not expressed in normal erythroid cells

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Human TFR2 (transferrin receptor 2) is a membrane-bound protein homologous with TFR1. High levels of TFR2 mRNA were found mainly in the liver and, to a lesser extent, in erythroid precursors. However, although the presence of the TFR2 protein in hepatic cells has been confirmed in several studies, evidence is lacking about the presence of the TFR2 protein in normal erythroid cells. Using two anti-TFR2 monoclonal antibodies, G/14C2 and G/14E8, we have provided evidence that TFR2 protein is not expressed in normal erythroid cells at any stage of differentiation, from undifferentiated CD34⁺ cells to mature orthochromatic

erythroblasts. In contrast, erythroleukaemic cells (K562 cells) exhibited a high level of expression of TFR2 at both the mRNA and the protein level. We can therefore conclude that an elevated expression of TFR2 protein is observed in leukaemic cells, but not in normal erythroblasts. The implications of this observation for the understanding of the phenotypic features of haemochromatosis due to mutation of the TFR2 gene are discussed.

Key words: erythropoiesis, haemochromatosis, iron, liver, transferrin, transferrin receptor.

INTRODUCTION

TFR2 (transferrin receptor 2) is a new member of the TFR family fortuitously cloned in 1999 during an attempt to identify new transcription factors [1]. Two transcripts of these genes were expressed: α and β . The predicted amino acid sequence of the TFR2- α protein revealed that this is a type II membrane protein, sharing 45 % identity and 66 % similarity in its extracellular domain with TFR1 [1]. The TFR2- β protein lacked the N-terminal portion of the TFR2- α protein including the transmembrane domain [1]. Studies *in vitro* have demonstrated that the expressed TFR2 protein mediates transferrin-iron uptake into the cells, but has an affinity for transferrin that is 30-fold lower than TFR1 [2]. Unlike TFR1, TFR2 mRNA lacks iron regulatory elements, and TFR2 expression may be regulated by the cell cycle rather than by intracellular iron status [3].

Additional differences between TFR1 and TFR2 concern mRNA tissue distribution, as evaluated both by Northern blot analysis and by RT-PCR (reverse transcriptase–polymerase chain reaction). Using these techniques, it was shown that the TFR2 mRNA is detected predominantly in the liver and, among a large panel of cell lines, only in the K562 erythroleukaemia cell line and HepG2 hepatoblastoma cells [1]. Other studies have shown high levels of TFR2 expression in early erythroid cells and in primary leukaemic blasts, mostly derived from the FAB M6 erythroleukaemia subtype [4,5]. Finally, TFR2 expression was also observed in the small intestine, although only at the level of crypt cells [6].

The function of TFR2 appears to be different from that of TFR1. In fact, TFR1 knockout mice did not survive beyond embryonic day 12.5 because of severe anaemia and neurological abnormalities, which clearly indicates that murine TFR2 cannot fully compensate for the functions of TFR1 [7]. Moreover, mice with only one functional TFR1 allele exhibit a phenotype associated with mild tissue-iron depletion, whereas disabling mutations of the TFR2 gene result in haemochromatosis type-3, a genetic form

of iron overload exhibiting a clinical picture similar to HFE (haemochromatosis gene product)-associated hereditary haemochromatosis, including hepatic iron loading [8–10]. Furthermore, target mutagenesis of the murine TFR2 gene produces haemochromatosis, characterized by periportal hepatic iron loading [11]. These observations clearly indicate that TFR2 is involved in iron homeostasis under physiological conditions. This conclusion is also supported by a recent study showing a co-localization of TFR2 and HFE in crypt duodenal cells [6]. Accordingly, it was suggested that TFR2 may function as an iron-sensor mechanism.

Studies of the TFR2 protein have been limited by the lack of specific reagents. Recently, antibodies specifically reacting with the human TFR2 have been reported [12,13]. These reagents were clearly useful in terms of determining more precisely the pattern of tissue distribution of this receptor, which seems to be limited to hepatocytes, crypt duodenal cells and erythroleukaemia cells [12], and its subcellular localization, showing that the receptor is localized to the cell membrane and to some punctate perinuclear subcellular compartments, seemingly corresponding to endocytic vesicles [12,13].

In the present study we have characterized the pattern of expression of the TFR2 protein in normal erythroid cells. To perform these studies, we took advantage of the availability of a large panel of anti-TFR2 mAbs (monoclonal antibodies) developed by some of the present authors [12]. Our results have shown that, in normal erythroid cells, TFR2 is expressed at low levels at the mRNA level, but the TFR2 protein remains virtually undetectable during all stages of differentiation.

EXPERIMENTAL

Antibodies

Anti-TFR2 antibodies (clones G/14C2 and G/14E8) have been reported and characterized in detail in a previous study [12].

Abbreviations used: AML, acute myeloid leukaemia; BFU-E, burst-forming unit-erythroid; HFE, haemochromatosis gene product; mAb, monoclonal antibody; PNGase F, peptide N-glycanase F; RT-PCR, reverse transcriptase–PCR; TFR(1/2), transferrin receptor 1/2; TRITC, tetramethylrhodamine β -isothiocyanate.

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Cell lines

Erythroleukaemic K562 cells and hepatoblastoma HepG2 cells were grown in RPMI 1640 medium containing 10 % (v/v) fetal calf serum.

Erythroid cell cultures

Human CD34⁺ progenitor cells were purified from peripheral blood by positive selection using the midi-MACS immunomagnetic separation system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. CD34⁺ progenitors were cultured in serum-free medium in the presence of various recombinant human cytokine combinations. Serum-free medium was prepared as follows: Iscove's modified Dulbecco's medium was supplemented with BSA (10 mg/ml), pure human transferrin (0.7 mg/ml), insulin (10 µg/ml), human low-density lipoprotein (40 µg/ml), sodium pyruvate (10⁻⁴ M), L-glutamine (2 × 10⁻³ M), rare inorganic elements supplemented with iron sulphate (4 × 10⁻⁸ M) and nucleosides (10 µg/ml each). For erythroid lineage culture, serum-free medium was supplemented with 0.01 unit/ml interleukin-3, 0.001 ng/ml granulocyte/macrophage colony-stimulating factor (GM-CSF) and 3 units/ml erythropoietin to induce uncontaminated uni-lineage differentiation [14,15]. The purity of the erythroid progeny generated in uni-lineage erythroid cultures was assessed by staining of the cells with anti-(glycophorin A) (97.5 ± 2 % positive cells) and anti-CD15 mAbs (< 2 % positive cells).

The differentiation stage of erythroid cells was evaluated by May-Grunwald Giemsa staining and cytological analysis.

Subcellular fractionation

Subcellular fractionation was performed according to a procedure reported previously [16]. Briefly, cells were washed twice with PBS and lysed for 30 min at 4 °C with a hypotonic buffer containing 20 mM Tris/HCl, pH 7.4, 2 mM EDTA, 0.1 µg/ml PMSF, 1 mM sodium orthovanadate (all these reagents were purchased from Sigma, St Louis, MO, U.S.A.) and a cocktail of protease inhibitors (Roche Diagnostics, Mannheim, Germany; catalogue no. 1 836 170).

Lysates were centrifuged at 1000 g for 10 min at 4 °C to separate supernatant from nuclear pellet. The supernatant was then centrifuged at 100 000 g for 1 h at 4 °C to produce a nucleus-free membrane fraction. The resulting pellet (membrane fraction) was resuspended in lysis buffer and stored at -80 °C before use. The supernatant (cytosolic fraction) was concentrated by trichloroacetic acid/sodium deoxycholate precipitation, before SDS/PAGE analysis.

Immunostaining and Western blotting

Immunofluorescence detection of TFR2 was performed on cells smeared on glass slides by cytocentrifugation. Briefly, cytospin preparations of the cells were fixed in 2 % (v/v) paraformaldehyde at 4 °C for 10 min, permeabilized for 5 min at room temperature with 0.1 % (v/v) Triton X-100 and then extensively washed in PBS. The slides were then pre-incubated for 10 min at room temperature with purified human immunoglobulins (50 µg/ml) in PBS containing 2 mg/ml BSA (Sigma) to reduce non-specific binding of primary antibodies, washed with PBS and then incubated for 30 min at room temperature with primary antibodies (mAbs anti-TFR2, clone G/14C2 or G/14E8). After extensive washing in PBS, the slides were incubated with TRITC (tetramethylrhodamine β-isothiocyanate) or FITC-labelled F(ab')₂ fragments of affinity-purified goat anti-mouse IgG diluted 1:40

(Dakopatts, Copenhagen, Denmark). After extensive washing in PBS, the slides were mounted in anti-fade glycerol mounting medium (Molecular Probes, Eugene, OR, U.S.A.) and examined under an Olympus inverted microscope equipped for confocal microscopy.

Western blotting experiments have been performed on whole-cell extracts as well as on subcellular fractions obtained from K562, HepG2 and erythroid cells. Cellular fractions were prepared as described above, while total-cell extracts were prepared by standard techniques. Briefly, cells were washed twice in cold PBS and then lysed for 20 min at 4 °C with a buffer containing 20 mM Hepes, 50 mM NaCl, 10 mM EDTA, 2 mM EGTA and 0.5 % (v/v) Nonidet P40, supplemented with 0.5 mM dithiothreitol, 10 mM sodium molybdate, 10 mM sodium orthovanadate, 100 mM NaF, 0.5 mM PMSF and a cocktail of protease inhibitors (Roche). Lysates were centrifuged at 5000 g for 10 min and the supernatants were separated into aliquots and stored at -80 °C.

Aliquots of total-cell extracts (approx. 30 µg) and of cellular fractions (approx. 20 µg) were run on a 7 % NuPAGE Tris-acetate gel (Invitrogen, Carlsbad, CA, U.S.A.) under denaturing and reducing conditions, and trans-blotted on to PVDF membranes (Invitrogen). Non-specific binding of antibody to the membrane was blocked by a 2 h incubation with 5 % (w/v) non-fat dried milk (Bio-Rad, Richmond, CA, U.S.A.)/0.05 % (v/v) Tween 20 in PBS. The membrane was subsequently incubated overnight with the primary anti-TFR2 mAbs G/14C2 or G/14E8 diluted 1:4 (clone supernatants) in TBST buffer [10 mM Tris/HCl (pH 8.0)/150 mM NaCl/0.05 % (v/v) Tween 20] containing 1 % (w/v) dried milk and then for 1 h with anti-mouse horseradish-peroxidase-conjugated secondary antibody (Bio-Rad) diluted 1:3000. Immunoreactivity was detected by using an enhanced chemiluminescence (ECL[®]) detection kit (Pierce, Rockford, IL, U.S.A.).

Deglycosylation experiments

Deglycosylation experiments were performed according to the manufacturer's instructions. Briefly, cell lysates from either K562 or HepG2 cells were boiled for 10 min in the presence of 0.5 % (w/v) SDS and 1 % (v/v) 2-mercaptoethanol to denature the proteins. The denatured samples were then incubated in 1 × G7 buffer (50 mM sodium phosphate, pH 7.5; New England Biolabs, Beverly, MA, U.S.A.), 1 % Nonidet P-40 and a mixture of 1000 units of PNGase F (peptide N-glycanase F; New England Biolabs) and 250 m-units of neuraminidase (Sigma) overnight at 37 °C. After digestion, samples were precipitated with 10 mM sodium deoxycholate in 50 % trichloroacetic acid for 1 h at 4 °C, and then centrifuged at 16 000 g for 15 min at 4 °C. The resulting pellet was washed once with cold acetone and finally suspended in sample buffer for Western blot analysis.

RT-PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen, Nilden, Germany) according to the manufacturer's protocol. Approx. 0.8 µg of total RNA was reverse-transcribed (SuperScript[™] III RNase H reverse transcriptase; Invitrogen), with oligo(dT) as the primer. After glyceraldehyde-3-phosphate dehydrogenase normalization [17], an aliquot of reverse-transcribed RNA was amplified for each sample. For RT-PCR, TFR2-α-specific primers were used [1]. Conditions for amplification were as follows: 38 cycles of 94 °C for 30 s, 56 °C for 40 s and 72 °C for 1 min. PCR was performed in a total volume of 50 µl using a PTC-100 DNA thermal controller (MJ Research, Inc., Watertown, MA,

Figure 1 TFR2- α mRNA expression in normal erythroid cells

Representative results on freshly purified human CD34⁺ progenitor cells induced to uni-lineage erythroid differentiation (E) and analysed on different days of culture (from day 0 to day 13). Aliquots of reverse-transcribed RNA from 5×10^4 cells were collected at the indicated days and amplified by 38 PCR cycles based on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) normalization. K562 cells were used as a positive control.

U.S.A.); 10 μ l of each sample was separated in a 2% (w/v) agarose gel, transferred to a nylon filter and hybridized with a specific probe end-labelled with [³²P]ATP and polynucleotide kinase. A negative control lacking template RNA or reverse transcriptase was included in each experiment. The level of the transcripts was quantified with a laser densitometer (LKB).

RESULTS

Normal erythroid cells express low levels of TFR2- α mRNA

In our first series of experiments, we have evaluated the level of TFR2- α mRNA in CD34⁺ cells that were induced for uni-lineage erythroid differentiation, and analysed first the differentiation of burst-forming unit-erythroid (BFU-E) cells into pro-erythroblasts (from day 0 to day 7), and then the terminal maturation of erythroblasts from basophilic erythroblasts to late orthochromatic erythroblasts (from day 8 to day 13). These experiments, performed by semi-quantitative RT-PCR, provided evidence that TFR2- α was initially undetectable in CD34⁺ cells, was induced at day 6 of culture, and then remained expressed up to the terminal maturation stage (day 13; Figure 1). Interestingly, the level of TFR2- α mRNA observed in normal erythroid cells was markedly lower than that observed in the erythroleukaemic K562 cell line.

Reactivity of different anti-TFR2 antibodies with normal and malignant erythroid cells

In a first set of experiments we have evaluated the reactivity of a series of leukaemic cell lines, HepG2 hepatoblastoma cell line and normal erythroid cells with two anti-TFR2 mAbs, G/14C2 and G/14E8, previously reported and characterized in detail [12]. Among the various cell lines tested, only erythroleukaemic cell lines (K562 and HEL), but not myeloid cell lines (HL60, U937, NB4) and lymphoid cell lines (Jurkat, Daudi), displayed reactivity with these two antibodies (Figure 2). In particular, only K562 cells displayed a strong reactivity with the two antibodies (Figure 2), whereas HEL cells were only weakly labelled (results not shown). In line with previous studies, HepG2 cells exhibited a clear reactivity with both antibodies (Figure 2). Surprisingly, erythroid cells analysed at different differentiation stages, i.e. at the BFU-E stage (CD34⁺ cells), at the pro-erythroblast stage (day 7 of culture) and at the orthochromatic erythroblast stage (day 13), do not exhibit any reactivity with both these antibodies (Figure 2). These

Figure 2 Flow cytometry analysis of TFR2 expression on the membrane of differentiating erythroid cells

K562 cells, HepG2 cells, purified CD34⁺ cells, immature and mature erythroblasts were labelled with either G/14C2 or G/14E8 anti-TFR2 mAbs using an indirect immunofluorescence technique, and analysed for fluorescence emission using a flow cytometer. Immature and mature erythroblasts were derived from erythroid uni-lineage cultures at days 7 and 14 respectively. Filled-in histograms indicate the fluorescence observed in cells incubated with irrelevant mouse IgG (negative control); histograms without shading show the fluorescence observed in cells incubated with anti-TFR2 mAbs.

findings were confirmed in erythroid cultures derived from both adult and cord blood CD34⁺ cells.

In addition to the study on intact cells, we have explored the reactivity of the two anti-TFR2 mAbs by confocal microscopy on cells fixed and permeabilized. In line with a previous report [12], only the G/14C2 antibody was suitable for immunofluorescence analysis on fixed cells. This analysis provided evidence that in both K562 and HepG2 cells a marked cytoplasmic reactivity was observed, with a pattern of labelling suggesting a localization of the TFR2 protein at the level of a microvesicular cytoplasmic compartment (Figure 3A). To better define the subcellular localization of TFR2, double labelling experiments were performed. In the first set of experiments, K562 cells were incubated with FITC-labelled transferrin (shown in Figure 3 in green) for 15 min at 37 °C, fixed and then incubated with anti-TFR2 mAb (shown in red); the TFR2 cytoplasmic staining only marginally overlaps with the vesicular sites occupied by endocytosed transferrin (Figure 3B). In the second set of experiments, K562 cells were labelled with Sytox, a DNA-labelling reagent (shown by the green coloration) and with the anti-TFR2 mAb (shown in red); the reactivity of the anti-TFR2 mAb was confined to cytoplasmic compartments and does not label the nucleus, a phenomenon

Figure 3 Immunofluorescence analysis of TFR2 in cells fixed and permeabilized

K562, HepG2 and normal erythroid cells were stained with the G/14C2 mAb, followed by anti-mouse FITC or TRITC. From the top to the bottom, the panels represent the following: (A) top row, left to right, single staining of K562, HepG2 and normal erythroblasts (E) respectively with anti-TFR2 mAb; (B) middle row, double staining of K562 cells with anti-TFR2 (shown in red) and with transferrin (FITC); an intercalating agent able to label DNA (shown in green); (C) bottom row, double staining of K562 cells with anti-TFR2 (red) and with an intercalating agent (Sytox) able to label DNA (shown in green).

observed for several proteins involved in iron transport or storage (Figure 3C). Normal erythroid cells grown *in vitro* from adult peripheral blood CD34⁺ cells displayed a faint cytoplasmic reactivity, mainly localized at the level of the perinuclear area (Figure 3A, right panel).

TFR2 protein is undetectable in normal erythroid cells

In a previous study [12], several of the present authors characterized a series of anti-TFR2mAbs. Two antibody clones (G/14C2 and G/14E8) seemed particularly interesting, given their reactivity against NIH-3T3 cells transduced with the human TFR2 gene [12]. Preliminary Western blotting experiments showed that, using cell extracts from the erythroleukaemic K562 and HepG2 hepatoma cell lines, two bands of approx. 95 kDa and 66 kDa were observed when blots were reacted with the G/14C2 antibody, whereas only the 95 kDa band was detected with the G/14E8 antibody (Figure 4A). Interestingly, when blots of normal erythroid cells were reacted with the G/14C2 antibody only the 66 kDa band was observed, whereas no TFR2 band (95 kDa) was detected using the G/14E8 antibody in cell extracts derived from these cells (Figure 4A).

Since TFR2 has four putative N-linked glycosylation sites and the 95 kDa species is known to be glycosylated [3], we performed deglycosylation experiments to verify the glycosylation of the 95 kDa and 66 kDa bands. We treated cell extracts derived from either HepG2 or K562 cells with a mixture of PNGase F and neuraminidase, and then we evaluated the size of TFR2 by Western blotting: after N-glycosidase digestion, the 95 kDa band was shifted to a position at approx. 80 kDa (Figure 4B), whereas the 66 kDa band remained unmodified after deglycosylation (Figure 4B). Treatment of HepG2 cells with 1-deoxynojirimycin, an inhibitor of N-glycosylation, inhibited the formation of the 95 kDa

band, but not of the 66 kDa band (results not shown). Altogether, these observations indicate that the 95 kDa band, but not the 66 kDa band, is glycosylated.

The 95 kDa band conforms to the expected molecular mass of wild-type TFR2- α , although it is unclear whether the 66 kDa may represent a molecular species related to the TFR2. To define more precisely a possible role for the 66 kDa band, we performed subcellular fractionations of K562, HepG2 and normal erythroblasts into two fractions: a cytosolic and a membranous fraction. In K562 and HepG2 cells, the 95 kDa band was detected only in the membrane-enriched fraction, and was virtually absent in the cytosolic fraction (Figure 4C). On the other hand, the 66 kDa band was detected in the cytosol, but not in the membranous compartment (Figure 4C).

DISCUSSION

Most haemochromatosis patients are homozygous for a unique mis-sense mutation occurring at the level of HFE, a gene encoding an atypical major histocompatibility class I molecule [18]. A second gene was found to be mutated in a small number of patients with a non-HFE-linked form of haemochromatosis, autosomal-recessive iron-loading disorder. These patients carry mutations in the TFR2 gene, which encodes a protein that is closely related to transferrin receptor-1. Despite this similarity, the function of TFR2 has not been established and it remains unclear how mutations in TFR2 lead to haemochromatosis, since the expected function of this receptor should consist of mediating transferrin-iron uptake. Individuals homozygous for a truncation mutation in TFR2 have a clinical profile similar to that of HFE-associated hereditary haemochromatosis, including hepatic iron loading limited to parenchymal cells [8–10]. Furthermore, targeted mutagenesis of the murine TFR2 gene produces a haemochromatosis

Figure 4 Western blotting analysis of TFR2 protein in cell extracts of normal erythroblasts, K562 and HepG2 cells

Cellular lysates were electrophoresed, transferred to a PVDF membrane and probed with the anti-TFR2 antibodies G/14C2 and G/14E8. (A) Total cellular lysates derived from K562, HepG2 and normal erythroblasts were probed with G/14C2, G/14E8 and β -actin antibodies. To the left of the gel, the positions of molecular-mass markers are indicated. (B) Total cellular extracts derived from K562 or HepG2 cells were incubated (+) or not (–) with PNGase F and neuraminidase, electrophoresed, transferred and then probed with either G/14C2 or G/14E8 antibodies. (C) Total cellular lysates derived from K562 cells were analysed before or after separation into two fractions: cytoplasmic (cyt) and membranous (mem). (D) Subcellular fractions and total cellular lysates derived from HepG2 cells were probed with G/14E8 and β -actin antibodies. (E) Subcellular fractions and total cellular lysates derived from normal erythroblasts were probed with G/14C2 and β -actin antibodies.

phenotype, associated with hepatic iron loading [11]. To explain these observations, it was suggested that TFR2 may act as a modulator of hepcidin (a circulating antimicrobial peptide produced in the hepatocytes in response to inflammatory stimuli and to iron, which acts as a negative regulator of iron release from intestinal and microphagic cells) synthesis: inappropriate hepcidin synthesis would lead to increased iron influx in parenchymal hepatic cells, following excessive iron release from intestine and macrophages [19]. However, these mice do not exhibit any abnormality at the level of the erythropoietic system (haematocrits, haemoglobin levels, erythrocyte indices and reticulocyte counts), and this finding was explained by the activity of the TFR1, which is highly expressed in erythroid cells [11].

The findings of the present study help to shed light upon this observation. In fact, we have observed that in normal erythroid cells the TFR2 protein is not expressed at any stage of differentiation, and therefore it is not surprising that an inactivating mutation of the TFR2 gene does not affect erythroid cell homeostasis.

Previous studies on normal erythroid cells have been mainly based on the analysis of TFR2- α mRNA, showing that this mRNA species is present at low levels in these cells [4]. However, in this study the only demonstration of expression of the TFR2 protein in normal erythroblasts was obtained by an immunohistochemical staining using a rabbit anti-TFR2 antibody [4]. Using the G/14C2 mAb, we also observed a faint reactivity of normal erythroblasts using immunofluorescence labelling. This reactivity, however, could not be ascribed to TFR2, since Western blotting experiments have provided clear evidence that this mAb in erythroid cells cross-reacts with a non-membrane-bound 66 kDa protein and not with the TFR2, which is absent or present at extremely low, undetectable levels in these cells. Therefore, on the basis of these observations it is clear that the supply of iron to developing erythroid cells is regulated only by TFR1, which is hyper-expressed in these cells [17], whereas the expression and function of TFR2 are limited to hepatic cells.

In contrast with normal erythroid cells, leukaemic erythroid cells express elevated levels of TFR2. This finding was based on the analysis of both erythroleukaemic cell lines [3,4] and primary leukaemic blasts [4,5]. The studies carried out on blasts derived from AMLs (acute myeloid leukaemias) have clearly indicated that, although the majority of these patients exhibit high levels of TFR2 mRNA [20], the highest TFR2 expression was observed in M6 AMLs. However, these findings were based only on the analysis of TFR2 at the mRNA level, and studies at the protein level are required to evaluate whether the expression of this receptor is deregulated in leukaemic cells.

On the basis of these observations, we conclude that in normal tissues the expression of TFR2 protein is restricted to very few tissues, among which are hepatocytes [3] and enterocytes [6], whereas erythroid cells, in spite of a low expression of the TFR2 mRNA, do not possess detectable TFR2 protein. Therefore the function of the TFR2 protein must be related to the mechanism of iron absorption (enterocytes) and storage (hepatocytes). Concerning iron absorption, it was observed that TFR2 interacts with HFE at the level of an early endosomal compartment, distinct from that which involves TFR1, and may constitute a selective transport pathway for the delivery of transferrin iron to the intestine and for the regulation of the rate of iron absorption by mature enterocytes [6]. However, the main physiological function of TFR2 is mediated at the level of the liver, where this receptor acts in the context of an iron-sensing pathway. At the level of hepatocytes, TFR2 forms heterodimers with TFR1: these heterodimers have a role in a sensing mechanism for transferrin saturation and, through an unidentified signalling pathway, control the synthesis of general regulators of iron metabolism, selectively expressed in the liver, such as hepcidin [21] and haemojuvelin/HFE2 [22]. Experiments are underway to define the signalling properties of TFR2 in hepatic cells.

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