Why do humans need two types of transferrin receptor? Lessons from a rare genetic disorder

The notion that humans have two types of transferrin receptor is rather recent. The classical transferrin receptor (TFR1) is a key molecule, essential for iron uptake through its endosomal cycle.1 The second transferrin receptor (TFR2) was only recently cloned, independently by two groups. Glockner et al. cloned the whole region of chromosome 7q22 which contains TFR2 as well as other important genes, such as erythropoietin in 1998.2 One year later Kawabata et al. serendipitously cloned the gene during an effort to identify new transcriptional factors involved in cancer.3

At a protein level the two receptors share moderate homology. Like TFR1, the predicted TFR2 protein has short cytoplasmic and transmembrane domains and a large extracellular domain. The amino acid identity between the two receptors reaches 45% in the extracellular region.4 However, compared with TFR1, TFR2 has distinct structural features (Table 1). The most significant are the tissue specific expression, which is restricted to the liver, and the lack of iron-regulation. At variance with TFR1, TFR2 has no iron responsive elements (IRE) in its RNA untranslated regions. As IRE are able to interact with iron regulatory proteins (IRP1 and IRP2), it follows that TFR2 is not iron-regulated at a post-transcriptional level.5 Another distinctive feature of TFR2 is its inability to bind HFE, while HFE-binding is a constitutive feature of TFR1.6 Since, if hyperexpressed in cell cultures, TFR2 is able to bind transferrin and to internalize iron,7 it was initially interpreted as a subsidiary tool for cellular iron uptake. Experiments in iron-loaded and in Hfe−− mice led to the hypothesis that iron uptake through TFR2 might be one mechanism leading to liver iron accumulation.8 This interpretation was hardly convincing in the light of the observation that TFR2 did not compensate for the lack of TFR1 in TFR1-deficient mice, which died before embryonic day 12.5 of severe iron-deficient anemia and central nervous system abnormalities.9 These observations underlined the essential role of TFR1 in iron uptake and suggested a different function for TFR2.

The role of TFR2 in iron metabolism remained unclear until we identified mutations of TFR2 in patients with HFE-unrelated hemochromatosis (now called type 3 hemochromatosis, OMIM # 604250). Using a positional candidate cloning approach we showed that hemochromatosis in 2 Sicilian families was linked to chromosome 7q22 and that all the affected subjects had TFR2 inactivating mutations in the homozygous state.10 The phenotype of iron loading following its inactivation was inconsistent with the idea of TFR2 as an iron uptaker, but rather pointed to it having a regulatory function on iron homeostasis.

A mouse model homozygous for Y245X, a mutation orthologous to the first mutation reported in humans (Y250X), developed the same features of iron loading observed in patients, confirming the regulatory role of TFR2 in rodents.11 Studies on the tissue distribution of TFR2 protein using specific monoclonal antibodies and immunohistochemical techniques revealed major staining in the human liver and duodenal cells.12 It was subsequently shown that TFR2 co-localizes with HFE in duodenal crypts,13 although a direct binding between the two proteins has been excluded in vitro.14 A puzzling features of TFR2 remains its expression in K562 and in primary leukemic blasts, especially M6 type,15 whereas in normal erythroid cells the TFR2 protein is not expressed at any stage of differentiation.16 Exposure of K562 cells to transferrin-bound iron induced a significant up-regulation and relocalization of membrane TFR2,17 whereas after addition of apo-transferrin the expression of TFR2 was unmodified. Whether this has some physiological relevance in early erythropoiesis is presently unknown.

The discovery of TFR2 and of type 3 hemochromatosis has spread some excitement in the scientific community, since at that time it was recognized that a proportion of hemochromatosis patients had no HFE mutations. However, the expectation that TFR2 might account for all these cases was not met by the finding that type 3 hemochromatosis is rare. Worldwide screening of non-HFE patients for Y250X (the first identified mutation of TFR2) did not reveal a single positive case.1819 However, the finding of other rare TFR2 mutations in non-HFE hemochromatosis patients both in Italy19 and elsewhere20 confirmed the association of TFR2 with hemochromatosis. All the mutations so far reported are private and, as shown in Figure 1, spread along the entire gene sequence.

Preliminary data suggested that TFR2-hemochromatosis might be restricted to Italy and Europe. Surprisingly, an identical (AVAQ 594-597) deletion, originally described in Italians,19 was found in a Japanese family.21 In this issue of the Journal (page 302) Koyama et al. enrich the number of TFR2 mutations. They report the molecular study of nine Japanese patients with a clinical diagnosis of hemochromatosis. None had HFE mutations, but two had novel TFR2 mutations. The first, a 41-year old patient with cirrhosis and diabetes, was homozygous for a 1469T→G nucleotide change, which causes the substitution of arginine for leucine at position 490 of the protein (L490R). This mutation was found in association with a previously described polymorphism 714C→G (I123V). The second, a 58-year old patient with cirrhosis, diabetes and skin pigmentation, was homozygous for 1665delC leading to a premature stop at valine 561 (V561X). The latter mutation produces a truncated protein, as occurs in the cases of E60X, C130X, Y250X (Figure 1). L490R is a missense change with a substitution of a neutral with a charged amino acid in a conserved residue. As in the cases of AVAQ deletion in the AVAQ motif and of Q690P, the novel L490R missense mutation targets a conserved residue of the TFR2 extracellular domain, which must have an important role in the protein.

Hemochromatosis is rare among Orientals and in particular Japanese, among whom another genetic form of iron overload is due to aceruloplasminemia.22 Since only one case of C282Y, a typical Caucasian mutation, has been reported,23 the present evidence is that the most common form of hemochromatosis in Japan is TFR2-related. How has our understanding of TFR2 function progressed since its discovery at the end of the last century? The major recent advance in elucidating iron regulation has been the discovery of the liver hepcidin peptide as the central effector of iron homeostasis.24 Hepcidin is also central to the pathogenesis of hemochromatosis, both of the severe juvenile type2526 and of the HFE-classic type.2728 Thus the question becomes: is TFR2 related to hepcidin? Data from mice29 and humans30 suggest that TFR2 contributes to modulating hepcidin production. Indeed hepcidin liver RNA is downregulated in TFR2-
and urinary hepcidin is low/absent in patients. Conversely, in TFR2-deficient subjects increased transferrin saturation does not induce hepcidin production through HFE. Interestingly, digenic inheritance of mutations of TFR2 and HFE may cause juvenile hemochromatosis. The future challenge is to understand the mechanisms of hepcidin regulation by HFE and TFR2. As far as we are beginning to understand why we have two distinct transferrin receptors.

**Table 1. Main differences between TFR1 and TFR2.**

<table>
<thead>
<tr>
<th>Features</th>
<th>TFR1</th>
<th>TFR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein family</td>
<td>TFRC</td>
<td>TFRC</td>
</tr>
<tr>
<td>Expression</td>
<td>ubiquitous</td>
<td>liver, duodenum</td>
</tr>
<tr>
<td>High expression</td>
<td>erythroblasts</td>
<td>hepatocytes</td>
</tr>
<tr>
<td>IRE elements</td>
<td>3'UTR (several)</td>
<td>none</td>
</tr>
<tr>
<td>Affinity for transferrin</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>HFE binding</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Knockout mouse</td>
<td>iron-deficient</td>
<td>iron overload</td>
</tr>
<tr>
<td></td>
<td>anemia lethal</td>
<td>iron overload</td>
</tr>
<tr>
<td>Mutations in humans</td>
<td>not reported</td>
<td>iron overload</td>
</tr>
</tbody>
</table>

TRFC: transferrin receptor; IRE: iron responsive elements; 3'UTR: 3' untranslated region.

deficient mouse and urinary hepcidin is low/absent in patients with TFR2 mutations. Recent findings in a HEPG2 cell line indicate that TFR2 might be a sensor of transferrin saturation, a function compatible with its low affinity for transferrin, as the TFR2 protein is stabilized in vitro the presence of diferric transferrin. Thus, if TFR2 modulates hepcidin, this effect would likely occur in response to transferrin saturation. TFR2 forms heterodimers with TFR1; these heterodimers might have a role in the sensing mechanism. However, if both HFE and TFR2 modulate hepcidin, their activities must be distinct, since a normal TFR2 does not compensate for HFE inactivation in C282Y patients. Conversely, in TFR2-deficient subjects increased transferrin saturation does not induce hepcidin production through HFE. Interestingly, digenic inheritance of mutations of TFR2 and HFE may cause juvenile hemochromatosis. The future challenge is to understand the mechanisms of hepcidin regulation by HFE and TFR2. As far as we are beginning to understand why we have two distinct transferrin receptors.

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**References**

The future of anticoagulation clinics: a journey to thrombosis centers?

Cumarins were discovered in the late 1930s as a result of decades of research spent identifying the cause of a hemorrhagic disease in cattle. At first they were used as rat poison, but from the mid 1950s they began to have some clinical impact. Since their efficacy was proved in several clinical studies, the use of coumarins, in particular warfarin, has increased progressively in many countries. Concomitantly with their clinical use, there was a need for more precise laboratory control, since bleeding can at times be fatal. Over the years the prothrombin time, as a monitoring test to tailor the dosage of oral anti-coagulants in the single patient, underwent a process of standardization, which was started in 1962 by Leon Poller. In 1983 Kirkwood proposed the international normalized ratio (INR) system, approved by the World Health Organisation. Despite a few limitations, the INR, recently reviewed by Poller, is currently the standard way to express the result of a prothrombin time test, and has served to validate the efficacy of oral anti-coagulants in a number of clinical studies.

The organization of anticoagulation clinics in Italy

The need for periodic monitoring and the complexity of the therapy have given rise to a whole culture on this topic and led to the creation of Centers for the surveillance of anticoagulation drugs in Europe and the US: the so-called anticoagulation clinics. In Italy the Federation for the Surveillance of Anticoagulated Patients (FCSA) was founded in 1989 with the aim of improving standardization in oral anticoagulation therapy in the country. From the initial 8 founding institutions, the Federation has grown into a network of more than 300 anticoagulation clinics spread over the country. From a survey performed by the FCSA in 2003 it emerged that these clinics were located in general laboratories (39%), transfusion services (15%), and departments of internal medicine (10%), hematology (9%), cardiology (8%), and angiology (4%). A minority (15%) have declared that they are thrombosis services. A more detailed survey will be necessary to know exactly how Anticoagulation Clinics actually work in terms of activities other than the surveillance of oral anticoagulation. Each year national congresses, courses, and workshops are held for physicians, technicians, and nurses. Many studies have been conducted and published by FCSA Centers in the past few years. These have dealt with several aspects of oral anticoagulant therapy, such as hemorrhagic and thrombotic complications; atrial fibrillation; different degrees of anticoagulation in prosthetic heart valves; malignancy; the elderly; computerized therapy management; and the patient’s own point of view.

A guide to oral anticoagulant therapy has recently been published. Moreover, studies on portable coagulometers and on the management of therapy using these devices have led to the publication of a consensus document by the FCSA on this topic. Nevertheless, for some time now a new concept has been taking shape: new anti-thrombotic drugs will replace Anticoagulation Clinics and perhaps cause their downfall, since it has been shown in clinical trials that these new drugs will probably render laboratory monitoring unnecessary in clinical practice. If we consider that...