

Transferrin Receptors and Hematopoiesis: Review

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The effectiveness of hematopoiesis depends on hematopoietic cell activity as well as on the presence of iron. Iron uptake is regulated by some of the members of the transferrin family of iron-containing proteins – transferrin and its receptors which assigns them a key regulatory role in hematopoiesis. Transferrins are expressed mainly in the liver, but small amounts arise also in the bone marrow, pancreas, testes, brain, spleen and kidneys. Experimental data show three types of transferrin receptors – transferrin receptor 1 (TfR1), transferrin receptor 2 (TfR2) and soluble transferrin receptor (sTfR). The expression of TfR1 and TfR2 is tissue- and cell cycle specific and is regulated by different control mechanisms, suggesting that they have different roles in iron metabolism. Cell surface TfR expression and concentration reflect iron requirements of the cells and they may be a useful marker for quantitative evaluation of the erythroid lineage, erythropoiesis and iron deficiency.

Key words: transferrin, transferrin receptor 1 (TfR1), transferrin receptor 2 (TfR2), soluble transferrin receptor (sTfR).

Introduction

Transferrin receptor is a cell membrane glycoprotein regulating cellular iron uptake by transferrin (Tf), a plasma protein which binds and transports iron [13]. Cell surface TfR concentration reflects iron requirements of the cell. Normally, about 60-70% of total body iron is incorporated in hemoglobin. Myoglobin, cytochromes and other iron-containing enzymes comprise 10% and the remaining 20-30% is stored in ferritin. Although iron bound to transferrin is less than 0.1% of the total body iron, it is the most important iron pool with the highest rate of turnover [13]. The transferrin binding site is situated on the extracellular domain of the receptor and each receptor subunit binds one transferrin molecule. Iron chelation by transferrin serves three main purposes: a) maintains Fe^{3+} in a soluble form under physiologic conditions, b) facilitates regulated iron transport and cellular uptake, and c) maintains Fe^{3+} in a redox-inert state, preventing the generation of toxic free radicals. Tf has an indirect defensive role against systemic infections by depriving the potential pathogens of extracellular iron, which is essential for their growth [4]. Under normal conditions, approximately 30% of the Tf iron-binding sites are saturated. In humans, values of Tf saturation <15% indicate iron deficiency, whereas >45% are consistent with iron overload [4].

According to the scientific data there are three types of transferrin receptors – transferrin receptor 1 (TfR1), transferrin receptor 2 (TfR2), and soluble transferrin receptor (sTfR).

1. Transferrin receptor 1 (TfR1)

Transferrin receptor (TfR) also known as CD71 is ubiquitously expressed at low levels on normal cells and at greater levels on cells with a high proliferation rate such as cells of the basal epidermis, intestinal epithelium, activated peripheral blood mononuclear cells, on cells that require large amounts of iron such as placental trophoblasts and maturing erythroid cells that require iron for heme synthesis. Mature erythroid cells do not express TfR. TfR expression has also been observed on nonproliferating cells such as the vascular endothelium of the brain capillaries, endocrine pancreas, seminiferous tubules of the testes, cells of the pituitary gland, luminal membranes of the breast, hepatocytes, Kupffer cells in the liver and tubules of the kidney [2, 5, 7]. Elevated levels of expression of the TfR are reported on cancer cells when compared to the normal cells [2]. Liu et al. demonstrate that during cell development from myeloid dysplasia to apparent leukemic cells, both CD71 and CD34 gradually increase and suggest that these molecules may be useful tools for understanding clonal development in leukemia [9]. According to Marsee et al. CD71 is a highly effective marker for the detection of cells of the erythroid lineage in bone marrow biopsy specimens as its immunoreactivity is restricted to erythroid precursors and exhibits a membranous and cytoplasmic staining pattern [10].

In humans, the TfR1 gene is located on human chromosome 3q29. TfR1 is the primary target of transferrin in the iron transport system and is expressed as a type II transmembrane glycoprotein composed of a disulfide-bonded homodimer on the surface of nearly every cell type. Each monomer (760 amino acids, molecular weight 90-95 kDa) contains a large extracellular C-terminal domain (671 amino acids) known as the ectodomain that contains the Tf-binding site, a single-pass transmembrane domain (28 amino acids), and a short intracellular N-terminal domain (61 amino acids). The ectodomain contains 3 N-linked glycosylation sites and one O-linked glycosylation site. Glycosylation at these sites is required for adequate function of the receptor [2]. TfR expression is primarily regulated at the post-transcriptional level in response to intracellular iron levels. The 3' untranslated region of the TfR transcript is large and plays an important role in the regulation of mRNA stability. This region contains 5 iron response elements (IRE) that consist of approximately 30 nucleotides each that form loop structures and are involved in the post-transcriptional regulation of TfR expression. These IRE are recognized by two RNA-binding iron regulatory proteins (IRP) [2]. Once Tf binds to the extracellular domain of the TfR on the plasma membrane, it changes its conformation and dimerizes. The Tf-TfR complex enters the endocytic pathway via endocytosis mediated by clathrin-coated pits and is transported to early endosomes. TfR1-mediated endocytosis is the only route for iron delivery to erythroid precursors [4]. Ablation of the TfR1 gene impairs erythropoiesis and neurologic development in mice and leads to embryonic lethality [8]. The Tf/TfR1 route is also essential for iron transport to the central nervous system (CNS) which is separated from the circulation by the blood brain barrier [4]. Astrocytes play a crucial role in providing iron to neurons, exporting the metal via ferroportin. Exported iron can bind to brain Tf, which is locally produced by the choroid plexus. Brain Tf delivers iron to TfR1-expressing cells, such as developing oligodendrocytes and neurons [4].

2. Transferrin receptor 2 (TfR2)

Transferrin receptor 2 (TfR2) is a 105 kDa type II transmembrane glycoprotein, member of the TfR family and homologous to TfR1. The TfR2's extracellular domain is

approximately 45% identical and 66% similar to the TfR1 ectodomain [2]. TfR2 binds to Tf with a 25-fold lower affinity and cannot substitute for TfR1, even though it is expressed in the erythroid compartment [12]. The gene for human TfR2 is located on human chromosome 7q22 [7]. The expression of TfR2 is restricted to hepatocytes, enterocytes of the small intestine and erythroid precursors and is not regulated by iron regulatory proteins (IRP) [2, 16]. Surface expression of TfR2 was also found on a wide variety of human cell lines derived from solid tumors and selected B and myeloid cell lines [2]. Expression of TfR2 increased in the liver during embryo development, whereas expression of TfR1 decreased. In the spleen, expression of TfR1 increased while the levels of TfR2 were constant during development [6]. Low expression of TfR transcripts was also found in lungs, testis, heart, duodenum, brain and kidneys [6]. In human bone marrow TfR2 protein staining was detected on erythroblasts but not on more differentiated erythroid cells. There was also staining on megakaryocytes, but not myeloid cells [6].

Forejtnikova et al. show that TfR2 and the receptor for erythropoietin (EpoR) are synchronously coexpressed during the differentiation of erythroid progenitors. TfR2 associates with EpoR in the endoplasmic reticulum and is required for the efficient transport of this receptor to the cell surface. Erythroid progenitors from TfR2^{-/-} knockout mice show a decreased sensitivity to Epo and increased circulating levels of erythropoietin (Epo) [3]. According to Nai et al. by modulating the Epo sensitivity of the erythroid precursors, TfR2 may act as a control system of red blood cells count to maintain a correct balance between their production and the available iron [11]. Erythroid TfR2 restricts Epo sensitivity to limit excessive erythropoiesis [12]. Wallace et al. suggest a possible regulatory role of TfR2 in Epo production by the kidney [18].

TfR2 and TfR1 differ in their expression during erythroid differentiation. TfR2 was reduced while TfR1 levels increased with differentiation in MEL cells and in human erythroid cells having the highest TfR2 expression in erythroblasts [6]. TfR2 mRNA expression is cell cycle dependent. In MG63 osteosarcoma cells, there was no expression in the G₀/G₁ phase and expression reached its highest level in the late G₁ phase, with a lower level of expression continuing through the remaining stages of the cell cycle. In contrast, TfR1 expression peaked at both late G₁ and G₂/M stages [17]. Inactivation of TfR2 leads to the development of hemochromatosis due to inappropriate hepcidin levels relative to body iron [14]. The authors show in a model of anemic mice that the absence of hematopoietic TfR2 delays erythroid differentiation and immature polychromatic erythroblasts accumulate in the spleen and bone marrow of anemic mice. The results demonstrate that erythroid TfR2 is essential for an appropriate erythropoietic response in iron-deficient anemia [14]. Along with hemojuvelin and HFE, TfR2 is regarded as an upstream regulator of iron signaling to hepcidin [12]. TfR2 is stabilized in response to high Tf saturation. Hepatic TfR2 promotes iron signaling to hepcidin to inhibit further iron fluxes to the bloodstream. Experimental data reveal that mice lacking Tmprss6 and TfR2 have splenomegaly and extramedullary hematopoiesis in the spleen [18]. The authors observe altered red-white pulp architecture, infiltration of red pulp with nucleated red cells and variable megakaryocyte infiltration. Flow cytometric analysis revealed increased proportions of cells in the immature erythroblast subpopulations I, II and III compared to wild type (control) mice and reduction in subpopulation IV, corresponding to mature erythrocytes, suggesting that TfR2 is required for terminal erythroblast differentiation [18]. Rishi et al. demonstrate that mice with similar knockout alleles fed an iron deficient diet significantly accumulated nucleated erythroid cells in their liver. The authors also show a significantly increased extramedullary hematopoiesis in the liver and spleen as compared to the control mice on the same diet [14]. The results suggest a key regulatory role of TfR2 in erythropoiesis.

3. Soluble transferrin receptor (sTfR)

TfRs are also present in the circulation and the circulating serum TfR (sTfR) level reflects total body TfR concentration [15]. Under normal conditions erythroid precursors – the erythroblasts are the main source of sTfR. Serum sTfR levels average 5.0 ± 1.0 mg/l in normal subjects. The most important determinant of sTfR levels is the bone marrow erythropoietic activity which can cause variations up to 8 times below and up to 20 times above average normal values [1]. Disorders of the bone marrow with reduced erythroid precursors are associated with low sTfR levels. The sTfR concentration begins to rise early in iron deficiency with the onset of iron-deficient erythropoiesis. It is also increased in patients with expanded erythropoiesis, including hemolytic anemias, myelodysplastic syndromes and use of erythropoietic stimulating agents [15]. sTfR level is decreased in cases of decreased erythropoiesis, bone marrow ablation with stem cell transplantation, aplastic anemia, pure red cell aplasia, etc. [15]. Measurements of sTfR are very helpful to investigate the pathophysiology of anemia, quantitatively evaluating the absolute rate of erythropoiesis and the adequacy of bone marrow proliferative capacity for any degree of anemia and to monitor the erythropoietic response to various forms of therapy, in particular allowing to predict response early when changes in hemoglobin are not yet apparent. With the exception of chronic lymphocytic leukemia (CLL) and high-grade non-Hodgkin's lymphoma and possibly hepatocellular carcinoma, sTfR levels are not increased in patients with malignancies [1].

Hematopoiesis depends on bone marrow hematopoietic cells' proliferative activity as well as on the effectiveness of iron uptake. Iron uptake is regulated by transferrin which binds to a transferrin receptor. Alterations in this receptor impair erythropoiesis and development in mice. In light of the current experimental data one can say that despite of the high homology between TfR1 and TfR2, both receptors exhibit tissue and cell cycle specific expression and regulate hematopoiesis, each in a different way. Ablation in one of the receptors cannot be compensated by the other which suggests that each one regulates the process in a unique way. TfR expression and cell surface concentration may be a useful marker for quantitative evaluation of the erythroid lineage, inefficient erythropoiesis and iron deficiency. Measurement of serum TfR will allow adequate evaluation of erythropoiesis, body iron demands as well the response to therapy in cases of hematological disorders.

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