

Renal Iron Metabolism: Transferrin Iron Delivery and the Role of Iron Regulatory Proteins

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In mammalian cells, iron is required for the function of many prosthetic groups, including heme and iron-sulfur clusters. Mammals absorb dietary iron and heme across the apical mucosa of duodenal epithelial cells using a Fe^{2+} transporter known as divalent metal transporter 1 (DMT-1; also known as solute carrier family 11 member 2, divalent cation transporter 1 (DCT1), and natural resistance associated macrophage protein 2) (1) or a specific heme transporter, heme carrier protein 1 (2). On the basolateral membrane, ferroportin (also known as mental transport protein 1 and iron regulated transporter 1) exports iron to the plasma (3), aided by hephaestin (4), which oxidizes ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. Serum transferrin (Tf), a 78-kD glycoprotein that is secreted mainly by the liver, binds one or two Fe^{3+} atoms. Each Fe^{3+} binds to four amino acid ligands from Tf and additionally binds a carbonate anion that stabilizes iron binding by providing two oxygen ligands. Carbonate binding completes occupancy of the six coordination positions of Fe^{3+} and thereby stabilizes binding of Fe^{3+} to Tf. Tf-bound iron circulates freely in the serum and extravascular spaces, and it serves as a source of iron for cells and tissues that are perfused by the systemic circulation, including liver, heart, muscle, kidney, and bone marrow (5). Excess intracellular iron is stored in ferritin, a heteropolymeric molecule that has a spherical shell structure and is composed of 24 H and L subunits, which can store up to 4500 iron atoms as a mineral core inside the shell (6). Iron can be released from ferritin when cells need more iron either when ferritin is degraded in the lysosome (7) or through a pore in the ferritin shell (8).

Most cells modulate iron uptake by regulating the amount of Tf receptor 1 (TfR1) (9) that they express on the plasma membrane. The TfR functions as a dimer, and each 90-kD monomer has a single transmembrane-spanning domain. Upon binding iron-bearing Tf, the TfR-Tf complex is internalized into an early endosome, where acidification facilitates release of Fe^{3+} from Tf and a reductase reduces Fe^{3+} to Fe^{2+} (10), which then can be exported into cytosol by DMT-1 in the late endosomal/lysoso-

mal compartment. A second TfR that is expressed mainly by hepatocytes, TfR2, is not regulated by intracellular iron levels and is unlikely to be important in renal iron metabolism (11).

Renal Filtration of Tf and Proximal Tubule Resorption

In normal urine, the quantity of Tf is approximately 0.32 to 0.47 mg/24 h (12), but in Fanconi syndrome, a disease that is characterized by generalized dysfunction of renal proximal tubules, urinary concentrations of Tf increase markedly (13). These findings undermine the commonly held belief that the high molecular weight of Tf prevents it from being filtered by the glomerulus (14). In the proximal tubule, the cubilin receptor, which is highly expressed on the apical membrane of kidney proximal tubules, is thought to mediate uptake of Tf (15). Although DMT-1 has been reported to localize to the apical membrane of proximal tubule cells (16), more recent studies suggest that DMT-1 localizes on the late endosomal and lysosomal membranes of proximal tubule cells, where it would facilitate the uptake of Tf-bound iron (17–19). In addition, when Tf is added to either the apical or the basolateral membrane of a polarized cell line that is derived from proximal tubule cells, WKPT-0293 Cl.2 cells, Tf is internalized from the apical membrane but not from the basolateral membrane (19). These findings also suggest that some Tf normally enters glomerular filtrate, but it is retrieved by specific receptor-mediated uptake in the kidney tubular system. It is interesting that TfR is expressed on the apical membrane of proximal tubule and collecting duct cells in mice (Figure 1), in distal convoluted tubules in the medulla of rats (20), and in all tubules in humans (21), offering a straightforward mechanism by which Tf can be retrieved from filtrate (Figure 2). It has been found that Tf is an essential growth factor in the development of kidney and differentiation of tubule (22), and retrieval of Tf from filtrate may be the major mechanism by which proximal tubule cells acquire the iron that they need (19).

In the kidney-derived cell line MDCK, TfR localizes to the basolateral membrane (23). It is not known which of the many cell types in kidney gave rise to MDCK cells, and although the localization of TfR in MDCK cells has been studied extensively, its physiologic role in renal iron metabolism is not clear.

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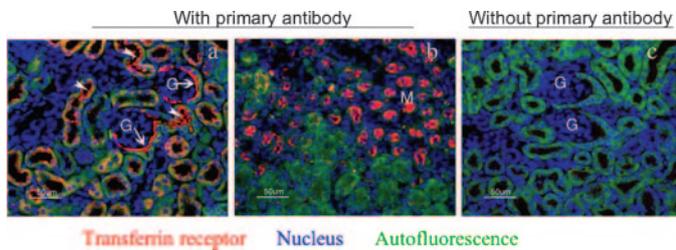


Figure 1. Localization of transferrin receptor (TfR) in mouse kidney. TfR expression was detected by immunofluorescence in the cortex (a) and medulla (b) of wild-type (WT) mouse kidney. TfR was highly expressed in the proximal tubule in kidney cortex, especially on the apical membrane of Bowman's capsule (arrow) and proximal tubule (arrowhead), and also was expressed highly on the apical membrane of collecting tubule in medulla (M). There was no staining when primary antibody was omitted (c). Glomeruli in a and c are indicated (G). Paraffin-embedded tissue sections were boiled in a micro oven for 15 min for antigen retrieval after dewaxing and rehydrating. The sections were blocked in 5% normal goat serum in Tris-buffered saline with 0.1% Tween-20, then were incubated with (a and b) or without (c) monoclonal anti-TfR antibody (ZYMED Laboratories, South San Francisco, CA) for 2 h at room temperature, the protein-antibody complex was labeled by CY3-donkey anti-mouse antibody (red), nuclei were labeled by DAPI (blue), and the green color was generated by autofluorescence.

Renal Regulation of Ferritin and TfR Expression: Role of Iron-Responsive Elements and Iron Regulatory Proteins 1 and 2

In general, most cells regulate expression of ferritin, an iron sequestration protein, and TfR to meet their nutritional needs. Iron-regulatory proteins 1 and 2 (IRP1 and IRP2) regulate the expression of both the H and L chains of ferritin, TfR1, and multiple other iron proteins. IRP are cytosolic proteins that sense cytosolic iron levels and bind to RNA stem-loop motifs that are found in the mRNA transcripts of iron metabolism genes. These RNA motifs, known as iron-responsive elements (IRE), consist of conserved sequence and structural elements (Figure 3) (24,25). IRE consist of lower and upper base-paired stems, with an unpaired cytosine separating the upper and lower stems. A six-residue loop, usually with the sequence CAGUGX, where X can be any residue but G, contains a base pair between C1 and G5 of the loop and residues A2, G3, and U4 of the loop are free to move in solution and form contacts with IRP. The IRE structure functions as a molecular ruler that positions two distinct RNA points of contact with IRP: The bulge C that separates the upper and lower stems and the A2, G3, and U4 residues of the loop (26). IRP regulate the expression of IRE-containing transcripts by binding with high affinity to the IRE. When IRP bind to transcripts that contain an IRE in the 5' untranslated region (UTR), they repress its translation, whereas when they bind to the IRE in the 3' UTR of TfR1, they protect the transcript from endonucleolytic cleavage and increase TfR mRNA abundance (reviewed in references [27,28]).

IRP1 and IRP2 share high sequence similarity and exhibit

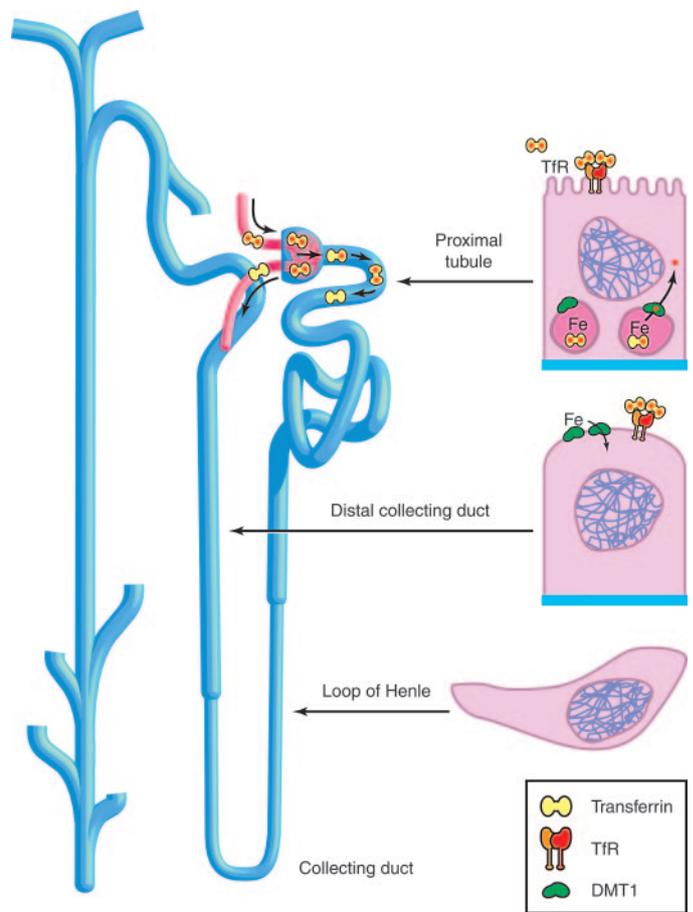


Figure 2. Localization of TfR and divalent metal transporter (DMT-1) in mouse kidney. TfR is localized on the apical membrane of proximal tubule and distal tubule, where it can bind diferric Tf in the glomerular filtrate. The Tf-TfR complex then is internalized into endosomes, whereupon the pH is lowered to approximately 5.5 to facilitate the release of iron from Tf. With the aid of a ferrireductase, the released iron can be exported into cytosol by DMT-1 that is localized at the endosomal/lysosomal membrane in proximal tubule. DMT-1 also is localized on the apical membrane of distal tubule, where it can resorb iron from tubular fluid. Illustration by Josh Gramling—Gramling Medical Illustration.

very similar biochemical activities with respect to their IRE-binding affinity, but their iron-sensing mechanisms are significantly different. IRP1 is a bifunctional protein in which its activity is determined by the presence or absence of an iron-sulfur cluster (Figure 4). When IRP1 contains an iron-sulfur cluster in the active site cleft, it functions as a cytosolic aconitase that interconverts citrate and isocitrate in cytosol (29). When the iron-sulfur cluster is absent, the fourth domain of IRP1 pivots on a flexible hinge linker to open a new binding space that can accommodate the IRE. Disassembly of the iron-sulfur cluster as a result of oxidation by superoxide (30), nitric oxide (31), and other oxidants increases the amount of IRP1 in the IRE-binding form, whereas enzymes that are important in iron-sulfur cluster assembly promote the formation of the cytosolic aconitase form of the protein when there is sufficient

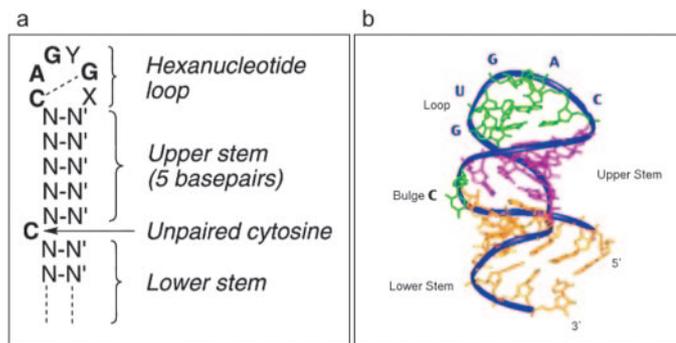


Figure 3. Iron-responsive element (IRE) sequence and secondary structure. (a) The IRE contains a six-residue loop, usually with the sequence CAGYGX, where Y represents U or C and X represents any residue except G. The upper and lower stems are composed of base pairs of variable sequence (N-N') that are separated by an unpaired C. (b) In the nuclear magnetic resonance solution structure of a consensus IRE, a base pair forms between C1 and G5, and A2 stacks on G5 in the conserved loop sequence CAGUGX. The helical upper and lower stems have an A-form conformation, and both the bulge C and the unpaired G residue at position 3 in the loop are disordered in solution. The 5-bp upper stem most likely functions as a molecular ruler that orients and correctly distances the bulge C from residues in the loop, allowing flexible residues to participate in sequence-specific interactions between the IRE and iron-regulatory proteins (IRP). Adapted from reference (11), with permission, courtesy of K. Address.

iron to support iron-sulfur cluster synthesis (Figure 4) (29). When IRP1 is in the cytosolic aconitase form, it does not bind to IRE of the target transcripts, and the translation of H- and L-ferritin proceeds freely, creating a 24-subunit spherical protein that can oxidize and sequester several thousand Fe^{3+} atoms within its core (Figure 4). TfR1 contains five IRE in its 3' UTR, and when IRP are not bound to the 3' UTR, TfR mRNA undergoes iron-dependent degradation and TfR levels decrease accordingly (32).

IRP2 also binds to IRE in iron-depleted cells. However, unlike IRP1, IRP2 undergoes iron-dependent ubiquitination and proteasomal degradation in iron-replete cells (reviewed in references [27,28]). IRP2 is expressed ubiquitously throughout the mouse tissues, including kidney (Figure 5) (33). Animal studies have revealed that IRP2 has a very significant role in regulation of tissue iron metabolism, and complete loss of IRP2 results in microcytic anemia, elevated serum ferritin, and late-onset neurodegeneration (34–36). The anemia results from decreased TfR expression in erythroid precursor cells (34). Interstitial fibroblasts in the kidney respond to hypoxia that results from decreased red cell oxygen-carrying capacity by secreting erythropoietin (37,38), and erythropoietin levels increase three- to five-fold in animals that lack IRP2, as expected in response to anemia (34).

Increased erythropoietin secretion by renal fibroblasts results from activation of hypoxia-inducible factors 1 α and 2 α (HIF-1 α and HIF-2 α) (39). When oxygen levels are normal, HIF undergo hydroxylation by prolyl hydroxylases. Hydroxylated HIF then

An Fe-S Cluster is the Key to Sensing of Iron Levels by IRP1

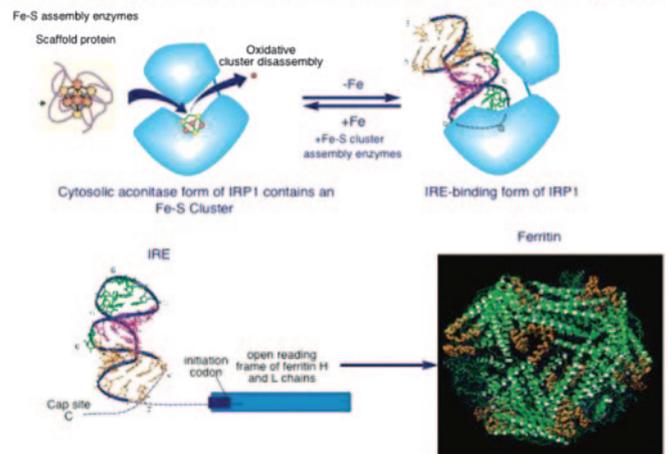


Figure 4. The iron-sulfur switch of IRP1. IRP1 is a bifunctional protein that can exist as a functional cytosolic aconitase when it binds an iron-sulfur cluster, interconverting citrate and isocitrate, or as an apoprotein that can bind IRE to regulate the expression of iron genes. The active site cleft that is responsible for aconitase activity overlaps with the region that binds to IRE, and binding of the iron-sulfur cluster prevents the binding with IRE. Thus, the unstable iron-sulfur prosthetic group may be the key determinant that switches IRP1 from an aconitase to the IRE-binding apoprotein, and numerous factors that can affect the formation or the disassembly of iron-sulfur cluster will regulate the IRP1 function and the expression of iron genes. For example, in iron-replete conditions, IRP1 will have an iron-sulfur cluster and will not bind IRE, and its inhibitory effect on ferritin translation will be absent, resulting in more ferritin synthesis (28).

are ubiquitinated by the Von Hippel-Lindau (VHL) complex and targeted for degradation by the proteasome (40). Prolyl hydroxylases depend on Fe^{2+} for activity, and HIF levels therefore may increase in iron-deficient cells (41). It is interesting that the promoters of both TfR1 (42) (43) and Tf (44) contain HIF response elements, and mRNA levels of both TfR1 and Tf increase in iron-deficient cells.

Kidney is the mouse tissue in which IRP1 is most highly expressed (33). Animals that lack IRP1 are unable to repress ferritin synthesis fully in the kidney under conditions of iron deficiency (33), demonstrating that IRP1 contributes significantly to regulation of iron metabolism in the kidney (Figures 5 and 6). In most other tissues, loss of IRP1-binding activity does not lead to misregulation of iron metabolism, because IRP2 levels increase in compensation (33,45). However, in kidney of IRP1 $^{-/-}$ mice, although IRP2 levels increase in a compensatory manner, as expected, the increase in IRP2 is not sufficient to repress ferritin synthesis completely (Figures 5 and 6). Failure to repress ferritin synthesis appropriately exacerbates iron deficiency, because ferritin competes effectively for available iron in tissue culture cells (46) and in the kidney of intact animals (47). The ability of ferritin overexpression to create relative iron deficiency likely is a major reason that ferritin expression is tightly regulated. As for TfR, there is only a slight

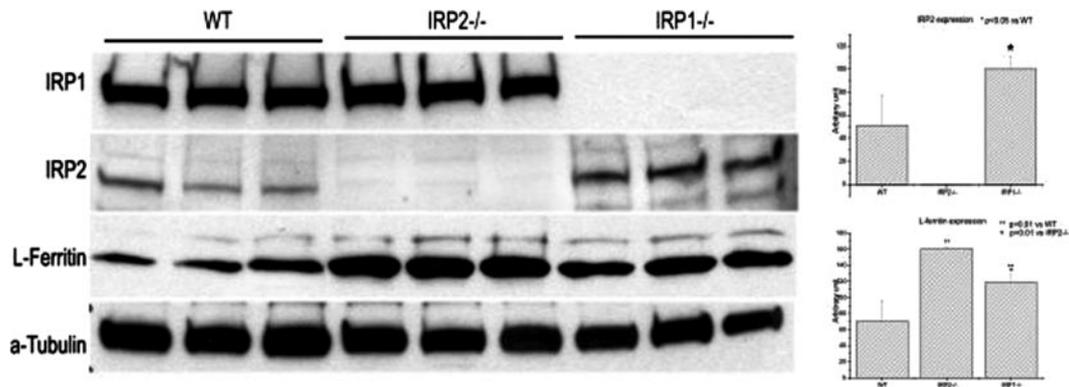


Figure 5. Western blot analysis of the expression of IRP and L-ferritin in mouse kidney. The expression of IRP1 protein was unchanged in IRP2^{-/-} mice compared with WT mice and was completely absent in IRP1^{-/-} mice, as expected. The expression of IRP2 significantly increased in IRP1^{-/-} mice compared with WT mice ($P < 0.05$), and there was no expression in IRP2^{-/-} mice. L-ferritin expression was significantly increased in IRP knockout mice compared with WT mice ($P < 0.01$ for both genotypes), and the expression in IRP2^{-/-} mice was higher than that in IRP1^{-/-} mice ($P < 0.01$). Protein samples (60 μ g for each lane) were separated in 4 to 20% SDS-acrylamide gel. The samples for each group were from three different mice. α -Tubulin was used as loading control. The density of the bands was measured with ImageJ, and the data were analyzed with Microcal Origin 6.0.

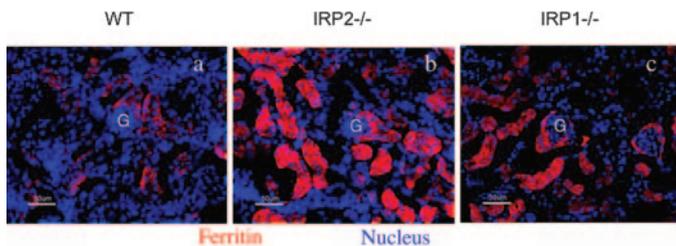


Figure 6. Ferritin expression in mouse kidney. Immunofluorescence showed ferritin expression in the kidney cortex of WT (a), IRP2^{-/-} (b), and IRP1^{-/-} (c) mice. Ferritin was expressed in the proximal tubule of mouse kidney. The expression of ferritin protein was increased in both IRP1^{-/-} and IRP2^{-/-} mice, and the upregulation in IRP2^{-/-} mice was higher than that in IRP1^{-/-} mice. Glomerulus was indicated by G. Paraffin-embedded tissue sections were probed with rabbit anti-ferritin antibody for 2 h at room temperature, then the protein-antibody complex was labeled by CY3-donkey anti-rabbit antibody (red) and nuclei were labeled by DAPI as counterstaining (blue).

decrease in IRP1^{-/-} or IRP2^{-/-} mice compared with wild-type mice (D.Z., unpublished observations). TfR changes may be difficult to assess in kidney lysates because different cell types are mixed together in lysates. TfR mRNA degradation also requires a specific but uncharacterized endonuclease that may be nonabundant in kidney (32) (Figures 1 and 5).

IRP1 Is Highly Expressed in Proximal Tubules

IRP1 is highly expressed in proximal tubules of the kidney (Figure 7). The vast majority of IRP1 is an active aconitase at physiologic oxygen concentrations (33), and the high expression levels of IRP1 in the proximal tubule may be explained by a need for cytosolic aconitase activity at this location. Proximal tubule cells reabsorb 75 to 90% of the citrate that enters the glomerular filtrate (48), and this resorbed citrate likely is me-

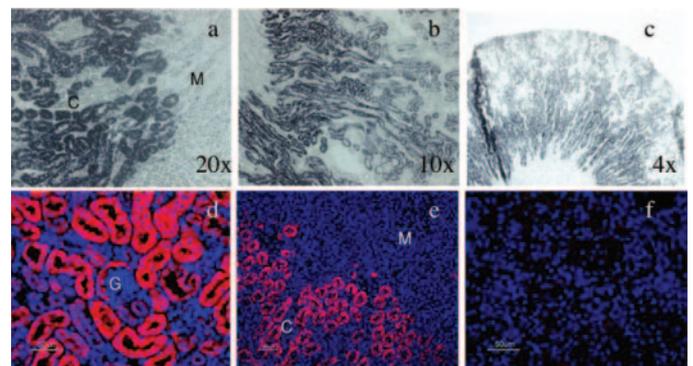


Figure 7. Localization of IRP1 in mouse kidney. *In situ* hybridization showed the IRP1 mRNA expression in sections of WT mouse kidney at various magnifications: $\times 20$ (a), $\times 10$ (b), and $\times 4$ (c) (33). IRP1 mRNA was expressed in the proximal tubule of the kidney cortex (C) of WT mice, whereas there was little, if any, IRP1 mRNA in the kidney medulla (M). Immunofluorescence showed the IRP1 protein expression in mouse kidney (from WT mice [d and e] and from IRP1^{-/-} mice [f]). IRP1 was highly expressed in the proximal tubule in kidney cortex (C), and there was very little expression in kidney medulla (M) and no label in the kidney of IRP1^{-/-} mice. Glomeruli are indicated by G. Paraffin-embedded tissue sections were incubated with rabbit anti-IRP1 antibody for 2 h at room temperature, and the protein-antibody complex then was labeled by CY3-donkey anti-rabbit antibody (red); nuclei were labeled with DAPI (blue) for counterstaining.

tabolized by cytosolic aconitase to yield isocitrate, which in turn can be metabolized by cytosolic isocitrate dehydrogenase to yield 2-oxoglutarate, a carbon source that can replenish the mitochondrial citric acid cycle. Alternatively, 2-oxoglutarate could be transaminated to produce glutamate, which could donate an amino group for urea formation. Kidneys filter and also likely secrete urea, and proximal tubule cells contain the argininosuccinate synthetase and lyase enzymes of the urea

cycle (49), raising the theoretical possibility that proximal renal tubular cells can secrete urea. Glutamate turnover is high in the proximal tubule (50). The striking localization of cytosolic aconitase to proximal tubule cells (Figure 7), together with the fact that most citrate in glomerular filtrate is resorbed at this site, suggests that much of the resorbed citrate must be metabolized to isocitrate for further utilization.

Iron Metabolism and Renal Cancer

In renal cancers in which the oncosuppressor VHL is inactivated, TfR levels increase markedly, perhaps because HIF levels increase TfR transcription (51). Other aspects of iron metabolism have not yet been described in detail in kidney cancers. Notably, iron can function as a renal carcinogen. Exposure of rats to high doses of ferric nitriloacetate predisposes to development of kidney cancer (52), but the mechanism by which iron induces cancer formation is not known.

Conclusion

IRP1 and IRP2 both regulate target transcripts, including ferritin and TfR posttranscriptionally in kidney. Specialized interstitial fibroblasts within the kidney are responsible for sensing hypoxia and releasing erythropoietin. Hypoxia sensing depends on iron-dependent prolyl hydroxylases, which hydroxylate HIF and target HIF for ubiquitination by VHL and proteasomal degradation. This pathway may regulate Tf and TfR transcription. It is likely that expression of TfR on the apical membrane of proximal tubular cells has an important role in retrieving Tf from glomerular filtrate. IRP1, which is expressed in extremely high levels in the kidney, is a bifunctional protein that has an important role as a cytosolic aconitase that converts citrate into isocitrate in proximal tubular cells. Proximal tubular cells resorb citrate, and cytosolic aconitase likely is needed to metabolize citrate. The ultimate fate of resorbed citrate in proximal tubular cells is not known.

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Disclosures

None.

References

- Mims MP, Prchal JT: Divalent metal transporter 1. *Hematology* 10: 339–345, 2005
- Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, Khan Y, Warley A, McCann FE, Hider RC, Frazer DM, Anderson GJ, Vulpe CD, Simpson RJ, McKie AT: Identification of an intestinal heme transporter. *Cell* 122: 789–801, 2005
- Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, Robine S, Andrews NC: The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab* 1: 191–200, 2005
- Wessling-Resnick M: Iron imports. III. Transfer of iron from the mucosa into circulation. *Am J Physiol Gastrointest Liver Physiol* 290: G1–G6, 2006
- Aisen P: Transferrin, the transferrin receptor, and the uptake of iron by cells. *Met Ions Biol Syst* 35: 585–631, 1998
- Harrison PM, Arosio P: The ferritins: Molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1275: 161–203, 1996
- Kidane TZ, Sauble E, Linder MC: Release of iron from ferritin requires lysosomal activity. *Am J Physiol Cell Physiol* 291: C445–C455, 2006
- Jin W, Takagi H, Pancorbo B, Theil EC: “Opening” the ferritin pore for iron release by mutation of conserved amino acids at interhelix and loop sites. *Biochemistry* 40: 7525–7532, 2001
- Aisen P: Transferrin receptor 1. *Int J Biochem Cell Biol* 36: 2137–2143, 2004
- Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, Sharp JJ, Fujiwara Y, Barker JE, Fleming MD: Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nat Genet* 37: 1264–1269, 2005
- Kawabata H, Fleming RE, Gui D, Moon SY, Saitoh T, O’Kelly J, Umehara Y, Wano Y, Said JW, Koeffler HP: Expression of hepcidin is down-regulated in TfR2 mutant mice manifesting a phenotype of hereditary hemochromatosis. *Blood* 105: 376–381, 2005
- Sawazaki K, Yasuda T, Nadano D, Iida R, Kishi K: Transferrin (TF) typing from semen stains using isoelectric focusing and immunoblotting: correlation of TF types among blood, semen, urine, and vaginal secretion. *J Forensic Sci* 37: 1514–1524, 1992
- Norden AG, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FW, Thakker RV, Unwin RJ, Wrong O: Glomerular protein sieving and implications for renal failure in Fanconi syndrome. *Kidney Int* 60: 1885–1892, 2001
- Aisen P: Iron metabolism: An evolutionary perspective. In: *Iron Metabolism in Health and Disease*, edited by Brock JH, Halliday, Pippard MJ, Powell LW, Philadelphia, W.B. Saunders, 1994, pp 1–30
- Kozyraki R, Fyfe J, Verroust PJ, Jacobsen C, Dautry-Varsat A, Gburek J, Willnow TE, Christensen EI, Moestrup SK: Megalin-dependent cubilin-mediated endocytosis is a major pathway for the apical uptake of transferrin in polarized epithelia. *Proc Natl Acad Sci U S A* 98: 12491–12496, 2001
- Canonne-Hergaux F, Gros P: Expression of the iron transporter DMT1 in kidney from normal and anemic mk mice. *Kidney Int* 62: 147–156, 2002
- Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D: Cellular localization of divalent metal transporter DMT-1 in rat kidney. *Am J Physiol Renal Physiol* 280: F803–F814, 2001
- Wareing M, Ferguson CJ, Delannoy M, Cox AG, McMahon RF, Green R, Riccardi D, Smith CP: Altered dietary iron intake is a strong modulator of renal DMT1 expression. *Am J Physiol Renal Physiol* 285: F1050–F1059, 2003
- Abouhamed M, Gburek J, Liu W, Torchalski B, Wilhelm A, Wolff NA, Christensen EI, Thevenod F, Smith CP: Divalent metal transporter 1 in the kidney proximal tubule is expressed in late endosomes/lysosomal membranes: Implications for renal handling of protein-metal complexes. *Am J Physiol Renal Physiol* 290: F1525–F1533, 2006

20. Lu JP, Hayashi K, Awai M: Transferrin receptor expression in normal, iron-deficient and iron-overloaded rats. *Acta Pathol Jpn* 39: 759–764, 1989
21. Gatter KC, Brown G, Trowbridge IS, Woolston RE, Mason DY: Transferrin receptors in human tissues: Their distribution and possible clinical relevance. *J Clin Pathol* 36: 539–545, 1983
22. Ekblom P, Thesleff I, Saxen L, Miettinen A, Timpl R: Transferrin as a fetal growth factor: Acquisition of responsiveness related to embryonic induction. *Proc Natl Acad Sci U S A* 80: 2651–2655, 1983
23. Odorizzi G, Trowbridge IS: Structural requirements for basolateral sorting of the human transferrin receptor in the biosynthetic and endocytic pathways of Madin-Darby canine kidney cells. *J Cell Biol* 137: 1255–1264, 1997
24. Address KJ, Basilion JP, Klausner RD, Rouault TA, Pardi AJ: Structure and dynamics of the iron responsive element RNA: Implications for binding of the RNA by iron regulatory proteins. *J Mol Biol* 274: 72–83, 1997
25. Gdaniec Z, Sierputowska-Gracz H, Theil EC: Iron regulatory element and internal loop/bulge structure for ferritin mRNA studied by cobalt(III) hexamine binding, molecular modeling, and NMR spectroscopy. *Biochemistry* 37: 1505–1512, 1998
26. Selezneva AI, Cavigiolio G, Theil EC, Walden WE, Volz K: Crystallization and preliminary X-ray diffraction analysis of iron regulatory protein 1 in complex with ferritin IRE RNA. *Acta Crystallograph Sect F Struct Biol Cryst Commun* 62: 249–252, 2006
27. Hentze MW, Muckenthaler MU, Andrews NC: Balancing acts: Molecular control of mammalian iron metabolism. *Cell* 117: 285–297, 2004
28. Rouault TA: The role of iron regulatory proteins in mammalian iron homeostasis and disease. *Nat Chem Biol* 2: 406–414, 2006
29. Rouault TA, Tong WH: Opinion: Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis. *Nat Rev Mol Cell Biol* 6: 345–351, 2005
30. Starzynski RR, Lipinski P, Drapier JC, Diet A, Smuda E, Bartlomiejczyk T, Gralak MA, Kruszewski M: Down-regulation of iron regulatory protein 1 activities and expression in superoxide dismutase 1 knock-out mice is not associated with alterations in iron metabolism. *J Biol Chem* 280: 4207–4212, 2005
31. Bouton C, Drapier JC: Iron regulatory proteins as NO signal transducers. *Sci STKE* (182): pe17, 2003
32. Binder R, Horowitz JA, Basilion JP, Koeller DM, Klausner RD, Harford JB: Evidence that the pathway of transferrin receptor mRNA degradation involves an endonucleolytic cleavage within the 3'UTR and does not involve poly(A) tail shortening. *EMBO J* 13: 1969–1980, 1994
33. Meyron-Holtz EG, Ghosh MC, Iwai K, LaVaute T, Brazzolotto X, Berger UV, Land W, Ollivierre-Wilson H, Grinberg A, Love P, Rouault TA: Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis. *EMBO J* 23: 386–395, 2004
34. Cooperman SS, Meyron-Holtz EG, Ollivierre-Wilson H, Ghosh MC, McConnell JP, Rouault TA: Microcytic anemia, erythropoietic protoporphyria, and neurodegeneration in mice with targeted deletion of iron-regulatory protein 2. *Blood* 106: 1084–1091, 2005
35. Galy B, Ferring D, Minana B, Bell O, Janser HG, Muckenthaler M, Schumann K, Hentze MW: Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). *Blood* 106: 2580–2589, 2005
36. LaVaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, Drake SK, Miller G, Abu-Asab M, Tsokos M, Switzer R 3rd, Grinberg A, Love P, Tresser N, Rouault TA: Targeted deletion of iron regulatory protein 2 causes misregulation of iron metabolism and neurodegenerative disease in mice. *Nat Genet* 27: 209–214, 2001
37. Plotkin M, Goligorsky MS: Mesenchymal cells from adult kidney support angiogenesis and differentiate into multiple interstitial cell types including erythropoietin-producing fibroblasts. *Am J Physiol Renal Physiol* 291: F902–F912, 2006
38. Bachmann S, Le Hir M, Eckardt KU: Co-localization of erythropoietin mRNA and ecto-5'-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. *J Histochem Cytochem* 41: 335–341, 1993
39. Schofield CJ, Ratcliffe PJ: Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* 5: 343–354, 2004
40. Haase VH: Hypoxia-inducible factors in the kidney. *Am J Physiol Renal Physiol* 291: F271–F281, 2006
41. Metzzen E, Ratcliffe PJ: HIF hydroxylation and cellular oxygen sensing. *Biol Chem* 385: 223–230, 2004
42. Lok CN, Ponka P: Identification of a hypoxia response element in the transferrin receptor gene. *J Biol Chem* 274: 24147–24152, 1999
43. Tacchini L, Bianchi L, Bernelli-Zazzera A, Cairo G: Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. *J Biol Chem* 274: 24142–24146, 1999
44. Rolfs A, Kvietikova I, Gassmann M, Wenger RH: Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. *J Biol Chem* 272: 20055–20062, 1997
45. Meyron-Holtz EG, Ghosh MC, Rouault TA: Mammalian tissue oxygen levels modulate iron-regulatory protein activities in vivo. *Science* 306: 2087–2090, 2004
46. Cozzi A, Corsi B, Levi S, Santambrogio P, Albertini A, Arosio P: Overexpression of wild type and mutated human ferritin H-chain in HeLa cells: In vivo role of ferritin ferroxidase activity. *J Biol Chem* 275: 25122–25129, 2000
47. Wilkinson JT, Di X, Schonig K, Buss JL, Kock ND, Cline JM, Saunders TL, Bujard H, Torti SV, Torti FM: Tissue-specific expression of ferritin H regulates cellular iron homeostasis in vivo. *Biochem J* 395: 501–507, 2006
48. Moe OW, Preisig PA: Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens* 15: 419–424, 2006
49. Miyataka K, Gotoh T, Nagasaki A, Takeya M, Ozaki M, Iwase K, Takiguchi M, Iyama KI, Tomita K, Mori M: Immunohistochemical localization of arginase II and other enzymes of arginine metabolism in rat kidney and liver. *Histochem J* 30: 741–751, 1998
50. Chauvin MF, Megnin-Chanet F, Martin G, Mispelter J, Baverel G: The rabbit kidney tubule simultaneously degrades and synthesizes glutamate. A ¹³C NMR study. *J Biol Chem* 272: 4705–4716, 1997
51. Alberghini A, Recalcati S, Tacchini L, Santambrogio P, Campanella A, Cairo G: Loss of the von Hippel Lindau tumor suppressor disrupts iron homeostasis in renal carcinoma cells. *J Biol Chem* 280: 30120–30128, 2005
52. Okada S: Prevention of free-radical mediated tissue damage and carcinogenesis induced by low-molecular-weight iron. *Biometals* 16: 99–101, 2003