Expression Study of Genes Involved in Iron Metabolism in Human Tissues

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Iron is required in all organisms for crucial functions, as a number of proteins need iron for activity. Mutations of the genes encoding proteins involved in iron uptake, transport, and utilization result in various human disorders or animal models with very different clinical presentations and organ involvement. However, little is known concerning the expression of iron metabolism genes in various human tissues and their eventual concerted regulation. We therefore examined the expression levels of various genes involved in iron uptake, reduction, and storage, Fe-S protein biogenesis, in mitochondrial electron transport chain, plus the two SOD genes, in human adult tissues by Northern blot analysis. We observed that most of these genes were ubiquitously expressed, but that their transcript showed strongly different levels in the various tissues investigated denoting different mechanisms for iron utilization in various organs. However, surprisingly, no correlation could be made between expression pattern of these genes and the clinical presentation resulting in their mutations.© 2001 Academic Press

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Iron plays a pivotal role in numerous cell functions being required for crucial metabolic pathways including electron transport chain, heme synthesis, Fe/S protein synthesis and DNA repair endonuclease III in all living organisms (1). On the other hand, iron is prone to trigger Fenton chemistry resulting in cellular oxidative stress and devastating peroxidative reactions (2). Accordingly, cell necrosis and/or apoptosis have been reported to result from iron overload under numerous conditions (3). It is therefore not surprising that a large number of proteins for uptake, transport, storage/detoxification and utilization of iron has been identified. Thus abnormalities of iron homeostasis, leading either to iron overload or iron deficiency, can be predicted to have severe clinical consequences. Accordingly, an increasing number of genes encoding proteins involved in iron trafficking have been reported to cause diseases when mutated, namely HFE gene responsible for hemochromatosis (4), ceruloplasmin gene in aceruloplasminemia (5) or L-ferritin in hyperferritinemia-cataract syndrome (6). Most recently, mitochondrial iron overload with generalized deficiency of Fe/S proteins has been reported in Friedreich’s ataxia, the most common hereditary ataxia associated with hypertrophic cardiomyopathy and shown to result from mutations in the frataxin gene (7). Noticeably, neurological and cardiac involvement associated with generalized deficiency of Fe/S proteins resulting in striking phenotypic similarities has been described in frataxin-KO (8) and MnSOD-KO mice (9) suggestive of a complex interaction between iron homeostasis and superoxide control mechanisms. Accordingly, a major feature of mutant yeast strains lacking either frataxin homologue or MnSOD is the occurrence of a mitochondrial iron overload (10, 11).

While investigating patients and animal models with iron metabolism disorders has provided new information on several genes involved in cellular iron trafficking, it has simultaneously raised several questions, particularly on the marked tissue-specificity observed in most of these diseases. Moreover, some of these genes have been disrupted in mice leading to embryological lethality and thus hampering to address this question (H-ferritin, 12, TFRC, 13). Therefore, as a very first step to study this question, we examined the expression levels of various genes involved in iron trafficking in various tissues from human adults by Northern blot study.

MATERIALS AND METHODS

The probes were prepared by RT-PCR of cultured skin fibroblasts using specific primers of each gene. These primers are presented in Table 1. Mouse hephaestin sequence was used for a BLAST searching of the human homologous cDNA and allowed to identified
RESULTS

Iron transporters

In mammals, uptake of iron is mediated either by transferrin receptor (TFRC) endocytosis, which is the principal way of uptake of Fe^{3+} in non-intestinal cells (14), or by non-transferrin receptor endocytosis for intestinal cells that have to deal with diet iron (1). This later iron import pathway requires Nramp2 (15), ferroportin1 (FPN1, 16) and hephaestin (17) that is not an iron transporter per se but is involved in iron redox reactions required for iron uptake as a multicopper oxidase. The stimulator of Fe transport (SFT) facilitates both transferrin and non-transferrin-bound iron uptake (18). We found a ubiquitous expression of TFRC, hephaestin and SFT in adult tissues (Fig. 1; overexposure of X-rays revealed signals in all lanes), whereas Nramp2 and FPN1 transcripts were undetectable in few tissues despite overexposure of X-rays. Interestingly, even when ubiquitously expressed, the transcripts of all these genes show strongly different levels in the various tissues. Hephastin and Nramp2 have a roughly similar expression profile, liver, brain, pancreas, and skeletal muscle presenting the highest level of expression. In contrast, TFRC and FPN1 present low expression levels in the very same tissues. This suggests a concerted action of either TFRC/FPN1 or hephaestin/Nramp2 couple in iron uptake in most tissues. It should also be noted that FPN1 and SFT present a mirror image expression in ovary and testis that might result from hormonal regulation of these two genes, at least in these organs. Comparing TFRC, hephaestin, Nramp2, FPN1 and SFT expression levels revealed that all tissues studied express high level of at least one of these five genes, except leukocytes. At variance with the very low expression of genes involved in iron uptake, high level of H- and L-ferritin transcripts were observed in leukocytes, indicating large capacities for iron storage in these later cells. It can thus be hypothesized that iron import in leukocytes involved either a still unknown protein or a non-protein mediated transport, possibly citrate (19). Interestingly enough, Nramp2, hephaestin and FPN1 are only slightly expressed in small intestine, while Nramp2 is known to mediate iron uptake in the brush border membrane and hephaestin and FPN1 are known to mediate iron export across the basolateral surface of the enterocytes to the circulation (15–17). Finally, FPN1 transcripts are almost undetectable in neurological tissues compared to other tissues.

Ferroxidases

Preliminary reduction of iron at cell surface is often required for its further import and is essentially mediated by plasma membrane associated ferroxidases. We studied the expression of two multicopper ferroxidase genes, namely ceruloplasmin (20) and hephaestin (17). Two transcripts of ceruloplasmin (4.3 and 3.7 kb) are readily detected in liver (Fig. 1). Ceruloplasmin is a multicopper oxidase synthesized in hepatocytes and secreted as a holoprotein. As previously reported in mouse (21) and in human (22), ceruloplasmin is also locally synthesized within the central nervous system in adult tissues but at low level being detectable only when X-rays are overexposed (not shown). Ceruloplasmin transcripts are also detected in small intestine and heart. Human hephaestin also presents 2 transcripts (5 and 2 kb) but with similar expression profile.

Iron storage and Iron Regulatory Proteins

Ferritin is the major intracellular iron storage protein and consists of 24 subunits of H- and L-ferritin (23). All tissues studied present high level of H- and L-ferritin transcripts (overexposure of X-rays also reveals H- and L-ferritin in thymus), the ratio between the two genes depending of the different tissues as previously reported (24). Comparing the levels of storage protein transcripts (H + L-ferritin) and iron carrier transcripts (TFRC + hephaestin + Nramp2 + FPN1 + SFT) did not revealed any correlation (Fig. 2A). This absence of correlation reflects the different
needs and uses of iron by the various tissues. Accordingly, tissues as prostate, testis, heart or placenta with high carrier transcript level and low ferritin transcript level would make immediate use of most cytosolic iron.

H- and L-ferritin, as well as aminolevulinate synthase 1, aconitase 2 and SDH-IP that encode mitochondrial proteins, are regulated by iron regulatory proteins (IRP) which function as sensor of cytosolic iron levels via RNA stem loops sequences, the iron responsive elements (IRE) in the 5' untranslated region of the cDNA (25). IRE are also present on TFRC and Nramp2 mRNA, but in the 3' untranslated region. The IRP1 probe we have used also hybridized to IRP2 transcripts that is 65% homologous to IRP1 in this region. IRP1 and IRP2 are widely expressed in human tissues (spleen and thymus also present IRP1 and IRP2 signal after overexposure of X-rays), but the relative abundance of their transcripts varies between tissues suggesting that these organs may have different mechanisms of iron homeostasis regulation. On the other hand, IRPs expression is not correlated with the expression level of genes that are regulated by IRPs. Thus, spleen, prostate, small intestine and placenta display very low level of IRP1 and IRP2 transcripts while the total amount of IRE-containing transcripts, namely H- and L-ferritin, TFRC, Nramp2, FPN1, ALS1, SDH-IP is elevated (Fig. 2B). Regulation of iron metabolism in these tissues by IRPs should either re-
quire a downstream induction of IRPs or a direct action of iron on IRE-containing transcripts as previously reported in rat liver for ferritin (26). Finally, IRP2 is known to have a higher affinity than IRP1 for ferritin IREs (27) but tissues with high IRP2 transcript levels (testis, liver, kidney) are not those with the highest ferritin transcript levels.

Mitochondrial Iron Metabolism

Most of the iron entering within mitochondria is utilized for biosynthetic purpose, i.e., heme and iron-sulfur clusters synthesis. Interestingly, genes encoding respiratory chain proteins (NDUFV1, SDH-IP, COX6c), aminolevulinate synthase 1 (ALAS1), the terminal enzyme of the heme biosynthesis pathway, NIFS, a regulator of Fe-S cluster synthesis (28), ABC7, a mitochondrial iron transporter (29) and frataxin (7) are highly expressed in mitochondria-rich tissues, namely heart, liver, skeletal muscle, kidney and neurological tissues. This tissue distribution profile may thus roughly reflect the content in mitochondria.

However, no tight correlation exists between the expression levels of different mitochondrial genes, such as ACO2, COX6c or SDH-IP. Indeed, ACO2/COX6c transcript ratio is $\approx 1$ (arbitrary units) in heart while this ratio rises to $\approx 8$ in kidney (Fig. 2C). Moreover, in kidney and liver, SDH-IP is much more expressed than NDUFS1 and COX6c, which paralleled the higher SDH activity in these tissues compared to other respiratory chain complex activities (30). Rhodanese, an enzyme involved in sulfur handling required for Fe-S cluster synthesis, is poorly expressed in neurological tissues, heart and muscle compared to NIFS. Finally, whereas ABC7 is expressed in mitochondria-rich tissues, it should be emphasized that other ABC mitochondrial iron transporters have been reported to have different expression patterns (M-ABC2, 31, MTABC3, 32, 2000, ABC-me, 33). All together, these observations suggest that mitochondrial iron trafficking, heme and Fe-S cluster syntheses and oxidative phosphorylation, all vary in order to cope for tissue-specific requirements.

Antioxidant Defenses

Cytosolic and mitochondrial superoxide-dismutases (SOD1 and SOD2, respectively) show a ubiquitous expression pattern, the higher expression being observed in heart and liver for both genes. Noticeably, leukocytes highly expressed SOD2 gene. Although obviously transcript expression level do not necessary reflect the activity level of a given enzyme, it is interesting to note that the expression levels of SOD1 or SOD2 in human tissues do not correspond with the level of enzyme activity observed in the mouse tissues (9). Indeed, mouse liver, brain and kidney have been reported to display a similar SOD2 activity that is four times less active than in heart, whereas the SOD1 activity proportions in liver, kidney, heart and brain are respectively 4/2/1/1. It is known that 2 to 4% of total oxygen used up by the respiratory chain is diverted to give superoxides normally undergoing dismutation by the SODs. It might be therefore hypothesized that SOD levels, particularly MnSOD, should reflect the activity of the respiratory chain in a given tissue. However, no quantitative relationship between SOD2 and COX6c transcripts could be observed suggesting that, beside
potential post-transcriptional SOD regulation varying superoxide production could be associated with respiratory chain function in various tissues (Fig. 2D).

DISCUSSION

Several of the genes studied here have been shown to be responsible for human genetic diseases or have been disrupted in animal models allowing to further investigate their role. It is often implied that organs involved in a genetic disease or that those affected by the disruption of a gene are organs that actually express the mutant gene. This can be indeed true for genes with tissue-restricted expression. For instance, the causative gene of steroid resistant nephrotic syndrome, NPHS2, encoding the glomerular protein podocin is exclusively expressed in kidney (34). To a certain extent, this can stand true as well for ubiquitously expressed genes. Indeed, SOD2 expression is high in human heart, liver and muscle and disruption of this gene in mouse lead to severe dilated cardiomyopathy, liver enlargement (with normal level of bilirubin and ALT) and lipid deposits in skeletal muscle (9). Neurological tissues also express SOD2, but at a low level and the SOD2−/− mice presented hypotonia.

However, a correlation between clinical involvement and expression pattern might well be rather an exception than a rule. Heart and liver present the highest expression level for almost all the genes studied here. However, there are only few examples where their mutations in human or disruption in animal result into a cardiomyopathy or hepatic dysfunction. Among these, frataxin and SOD2 mutations or disruption of the corresponding genes both result in cardiomyopathy (7–9). In keeping with this, low iron storage transcripts compared to high iron carrier transcripts observed in heart could partially participate to the high sensitivity of this organ to free radical injury. This well established weakness of the heart concerning antioxidant defenses (35). Patients with NDUFV1 mutations present mainly with neurological involvement that is correlated with the expression of the gene in neurological tissues (36). However, NDUFV1 transcripts are extremely abundant in heart, compared to other tissues, but none of the patients presented cardiomyopathy. It appears in this case that a low expression level of the gene in a specific organ results in a high dependency of this organ for this gene. Accordingly, a slight decrease of transcript level, presumably resulting in decreased protein activity in a given tissue induces the clinical involvement of this later tissue. However, numerous other factors can also account for organ specific involvement. The onset of disease in complex I-deficient patients occurs very early in life as the children present symptoms before 1 year of age. The requirement of neurological tissues for NDUFV1 protein in fetus and infant is presently unknown and might strongly differ from what is observed in adult. These genes might well be highly expressed in neurological tissues during fetal life and the expression pattern switched during childhood. Another explanation could be that the requirement for these proteins is specific to few particular cells in neurological tissues and that the estimation of the expression level in the whole tissue is not relevant. Similarly, iron transporter genes, Nramp2, FPN1 and hephaestin, are only slightly expressed in small intestine, whereas these three proteins are known to mediate iron entry in the body from diet (15–17).

This study represents an instant picture of the expression of numerous genes involved in iron metabolism, and, not surprisingly, raises more questions than it solves. Indeed, comparing levels of transcripts for iron import to those involved in iron storage or level of transcripts for antioxidant (SOD) to those involved in pro-oxidant activities (respiratory chain) raises intriguing question. Why mitochondrial SOD2 is highly expressed in leukocytes cells which are known to have a low mitochondria content and where respiratory chain proteins, which potentially produce free radicals, are poorly expressed? Similarly, why lung, which is in direct contact with oxygen, has such a low expression level of key antioxidant enzymes such as superoxide dismutases? How lung or leukocytes accumulate high iron (high ferritin expression but low iron-import gene expression) and why (low expression of iron-handling genes)? Why thymus presents very low transcript levels of all the genes studied, including housekeeping genes? To answer some of these questions can be of importance for understanding the pathogenesis of several iron-related diseases, but this would require the use of alternative approaches complementary to the descriptive longitudinal study as the one reported here.

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REFERENCES


