Expression of the DMT1 (NRAMP2/DCT1) iron transporter in mice with genetic iron overload disorders

François Canonne-Hergaux, Joanne E. Levy, Mark D. Fleming, Lynne K. Montross, Nancy C. Andrews, and Philippe Gros

Iron overload is highly prevalent, but its molecular pathogenesis is poorly understood. Recently, DMT1 was shown to be a major apical iron transporter in absorptive cells of the duodenum. In vivo, it is the only transporter known to be important for the uptake of dietary non-heme iron from the gut lumen. The expression and subcellular localization of DMT1 protein in 3 mouse models of iron overload were examined: hypotransferrinemic (Trf<sup>−/−</sup>) mice, Hfe<sup>−/−</sup> knockout mice, and B2m<sup>−/−</sup> knockout mice. Interestingly, in Trf<sup>−/−</sup> homozygotes, DMT1 expression was strongly induced in the villus brush border when compared to control animals. This suggests that DMT1 expression is increased in response to iron deficiency in the erythron, even in the setting of systemic iron overload. In contrast, no increase was seen in DMT1 expression in animals with iron overload resembling human hemochromatosis. Therefore, it does not appear that changes in DMT1 levels are primarily responsible for iron loading in hemochromatosis. (Blood. 2001;97:1138-1140)

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absorption and to determine their effects on the expression of DMT1, we examined DMT1 protein expression in 3 mouse models of iron overload. One, hypotransferrinemia (Trf<sup>hpx</sup>), results from a spontaneous mutation disrupting a splice donor site in the murine transferrin gene<sup>19</sup> and is directly analogous to human transferrine--spontaneous mutation disrupting a splice donor site in the murine ground) mice were derived from founders purchased from the Jackson laboratories. 20 All strains have been maintained as inbred stocks fed identical diets in the animal facility at Children's Hospital (Boston, MA).

Animals

C57BL/6J (B6) and 129/SvEvBRac mice were purchased from Jackson Laboratory (Bar Harbor, ME) and Taconic Farms (NY) and used as wild-type controls for experiments with the B2m<sup>−/−</sup> and Hfe<sup>−/−</sup> mice, respectively. MK/ReJ-<i>mkl</i>/+, MK/ReJ-<i>mkn</i>/mk, and B2m<sup>−/−</sup> (B6 background) mice were derived from founders purchased from the Jackson Laboratory.<i>Trf<sup>hpx</sup></i> (BALB/cJ background) mice were derived in Boston from founders generously provided by Jerry Kaplan (Salt Lake City, UT).<i>Hfe<sup>−/−</sup></i> mice (129/SvEvBRac background) were generated in one of our laboratories.<sup>20</sup> All strains have been maintained as inbred stocks fed identical diets in the animal facility at Children’s Hospital (Boston, MA). Homozygous <i>Trf<sup>hpx</sup></i> mice were kept alive by weekly intraperitoneal injection of 6 mg human transferrin during weeks 1, 2, and 3 of life as previously described.<sup>17</sup> Heterozygous <i>Trf<sup>hpx</sup></i>/<i>mkl</i> mice and wild-type mice (of either 129Sv or B6 strains, designated <i>Hfe<sup>+</sup></i> and <i>B2m<sup>+</sup></i>), respectively, consistent with earlier findings in wild-type mice.<sup>20</sup> Thus, all control samples gave expected results.

Results and discussion

We first examined levels of expression of DMT1 by Western blotting of membrane fractions from mutant mice and controls (Figure 1). Heterozygous <i>mkl</i>/+ mice, with normal iron stores, and non-transfected CHO cells were used as negative controls. Microcytic <i>mkn/mkn</i> mice, previously shown to overexpress DMT1,16 and DMT1-transfected CHO cells were used as positive controls. The anti-DMT1 antisera detects a mature 90- to 100-kd DMT1 polypeptide, expressed at high levels in duodenal samples from <i>mkl/mkl</i> mice and in CHO–DMT1 transfectants. Little or no DMT1 protein expression was detected in duodenum from <i>mkl</i>/+ mice, <i>Trf<sup>hpx</sup></i>/<i>mkl</i> mice, or wild-type mice (of either 129Sv or B6 strains, designated <i>Hfe<sup>+</sup></i>/<i>mkn</i> and <i>B2m<sup>+</sup></i>/<i>mkn</i>, respectively) with normal iron stores, and <i>Hfe<sup>−/−</sup></i> mice. We have previously shown that <i>Trf<sup>hpx</sup></i>/<i>mkl</i>, <i>Hfe<sup>−/−</sup></i>, and <i>B2m<sup>−/−</sup></i> mutants all have marked tissue iron overload<sup>19,20</sup> (and data not shown).

Cell culture and transfection

The production of Chinese hamster ovary (CHO) cells stably expressing a c-Myc–tagged DMT1 expression plasmid was previously described.<sup>3</sup>

Crude membrane protein extracts

Proximal duodenum was harvested, snap-frozen in liquid nitrogen, and used to prepared crude membrane fractions as previously described.<sup>3</sup> Crude membrane fractions were prepared from cultured CHO cells as previously described.<sup>3</sup>

Production, purification, and use of anti-DMT1 antibody

The preparation of a specific polyclonal antisera recognizing the N-terminal sequence (residues 1 to 73) of DMT1 protein was previously described.<sup>3</sup> Its specificity was established by demonstrating DMT1-specific detection in transfected CHO cell membranes on Western blot<sup>3</sup> and immunofluorescence<sup>22</sup> analyses. This antisera, antisera against transferrin receptor (Trfr), and biliary glycoprotein 1 (Bgp1) were used for immunoblotting and immunohistochemistry as previously described.<sup>3</sup>

Study design

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We next examined DMT1 expression in the 3 mouse models of iron overload. Strong induction of DMT1 expression is noted by Western blotting of the duodenal sample from Tfr$^{hspchop}$ mice (Figure 2A). There is no change in expression of either Trfr or Bgp1 controls in these mice (Figure 1B). This suggests that DMT1 levels respond to the erythroid regulator as the stores regulator must be inactive. The induction of DMT1 was confirmed by immunohistochemistry (Figure 2). No staining of Bgp1 controls in these mice (Figure 1Bi-Ci). This suggests that DMT1 is likely, therefore, to play a role in the iron overload that develops in these animals.

In contrast, neither Hfe$^{-/-}$ nor B2m$^{-/-}$ mice had a measurable induction of DMT1 protein expression as shown by Western blotting (Figure 1) and by immunohistochemistry (Figure 2, for Hfe$^{-/-}$ only; B2m$^{-/-}$ data not shown). Both of these mouse models of hemochromatosis gave results that were not appreciably different from those of controls. In these experiments, slight variations in the low level of basal DMT1 and Trfr expression were observed but were attributed to normal animal-to-animal variation or processing of otherwise identical samples. In contrast to the result seen in Tfr$^{hspchop}$ mice, the lack of induction in Hfe$^{-/-}$ and B2m$^{-/-}$ mice strongly suggests that an increase in DMT1 protein levels is not primarily responsible for accelerated iron absorption in hemochromatosis. Other possibilities, including increased activity of post-translationally modified DMT1 and variation in the reducing step\textsuperscript{23,24} or the basolateral transfer step\textsuperscript{25} may also contribute to the increase of iron uptake in hereditary hemochromatosis.

Other groups have reported increases in DMT1 mRNA levels in a different Hfe knockout mouse\textsuperscript{18} and in human patients with hereditary hemochromatosis.\textsuperscript{26} There are several possible explanations for the discrepancy between their results and ours. First, we analyzed DMT1 protein expression, whereas the other groups examined DMT1 mRNA expression. It is possible that increased mRNA production does not result in increased protein production. We feel this is unlikely, however, because we also examined DMT1 mRNA expression in our Hfe knockout mice, and we could not detect a difference from wild-type mice (data not shown). We feel other explanations are more likely. The Hfe knockout mice studied by Sly et al\textsuperscript{27} did not have a homogeneous genetic background. We believe that their results may be attributable to strain-to-strain variations between mice, as have been reported in the literature. We controlled for this variability in the current study. Similarly, patients reported by Zoller et al\textsuperscript{26} did not have the same genetic background and had been treated with phlebotomy to varying extents. Phlebotomy may activate the erythroid regulator of iron absorption by inducing erythropoiesis. As shown by the analysis of Tfr$^{hspchop}$ mice, the erythroid regulator can increase DMT1 expression even in the setting of tissue iron overload.

This study allows us to begin to distinguish between mechanisms of iron loading in 2 disorders of iron metabolism, transferrinemia and hereditary hemochromatosis. Future work should give additional insight into the molecular regulators of iron balance.

References