A SOLUBLE HFE SPLICE VARIANT SEEMS TO REGULATE THE EXPRESSION OF DUODENAL CYTOCHROME B AND HEPHAESTIN CONTRIBUTING TO IRON METABOLISM REGULATION

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Hereditary Hemochromatosis is an autosomal recessive disorder characterized by excessive intestinal iron absorption and pathological iron deposition in organs such as liver, heart and pancreas. The disease is predominantly caused by homozygosity for the p.C282Y mutation in HFE, which impairs protein association with its chaperone beta-2 microglobulin (B2M) for correct folding and traffic to the cell surface. The role of full length HFE on iron homeostasis remains unclear. It has been postulated that it may contribute to iron metabolism regulation by acting in hepatocytes, activating hepcidin synthesis and in duodenum, regulating the expression of iron metabolism related genes, thus preventing iron overload.

HFE transcripts are widely expressed and the predominant (full length) mRNA identified has ≈4.2 kb in length. In addition, several alternative splicing HFE transcripts have been reported but their functional significance remains elusive.1,2,3

Since we have identified a novel alternative HFE transcript due to the intron 4 inclusion(Fig. 1), we aimed to investigate its tissue-specific expression level, the corresponding protein structure and cellular location, as well as its putative physiological function at duodenal level.

Methods

Results

Conclusions

References

Acknowledgements


Fig. 1. HFE full length and intron 4 inclusion transcripts. A premature stop codon results in the alternative splicing transcript.

1) HFE_intron 4 inclusion transcript expression in different human tissues

RT-qPCR

✓ HFE_intron 4 inclusion transcript has an ubiquitous expression, being its relative expression higher in duodenum and testis and lower in liver.

2) HFE_ivs4 protein variant cellular co-localization in hepatic cells

Immunofluorescence (HepG2)

✓ The HFE_full length protein co-localizes with B2M and TR1, mostly at cell surface but not with calnexin (ER marker).
✓ HFE_ivs4 protein does not localize with TR1 or with calnexin.

Here we show a soluble isoform of the HFE protein (shFE), resulting from a HFE alternative splicing transcript including the intron 4. Due to the presence of a premature termination codon within this intron, it originates a truncated protein which lacks the transmembrane and cytoplasmic domains. However, it seems to be correctly processed, since it surpasses the ER barrier and is secreted to the cell supernatant in association with B2M.

The over expression of the shFE in a duodenal cell line is able to modify de expression of some iron metabolism related genes. We found that it acts by repressing the expression of the duodenal cytochrome b and hephaestin, independently of the iron status, as it happens with the full-length HFE. Consequently, since those are proteins presenting well known functions in iron absorption, we can hypothesize that their decreased expression will be translated to a reduction of dietary iron absorption in the duodenum.

Through this study we might have unveiled the contribution of the shFE splice variant to iron homeostasis. In fact, shFE may be secreted by several tissues into the bloodstream and may act in the regulation of iron absorption in duodenum, reducing its dietary absorption and preventing iron overload.

✓ RT-qPCR using RNAs from 8 human tissues were performed to quantify the HFE intron 4 inclusion splicing transcript as well as the total amount of HFE transcripts.
✓ HFE_cDNA was tagged to the GFP gene in the pEGFP-N1 vector. The hepatocarcinoma cell line HepG2 was transfected with the full length or the intron 4 inclusion construct, in order to obtain the corresponding GFP-tagged protein variants.
✓ Protein cellular localization was detected by immunofluorescence assays raising antibodies against B2M, Calnexin (an endoplasmic reticulum marker; ER) and Transferrin Receptor 1 (TR1).
✓ Cell lysates and cell culture supernatants were submitted to immunoprecipitation assays using mouse anti-GFP antibody. These were subjected to a 12% SDS-PAGE, followed by transfer to a nitrocellulose membrane. Immunodetections were performed with mouse antibody anti-GFP and rabbit anti-B2M.
✓ HuTu-80 cells (human duodenum adenocarcinoma) were transfected with pcDNA3 constructs expressing HFE full length (HFE) and HFE_ivs4 (shFE), followed by an iron stimulus (holo-transferrin 20µM, holo). RNA was extracted from these cells and the expression of iron-related genes TFR2, DMT1, B2M, SLC40A1, CYBRD2 and HEPH were assessed by RT-qPCR.

3) HFE_ivs4 protein variant cellular traffic

Immunoprecipitation (HepG2)

✓ Both HFE_full length and HFE_ivs4 proteins are associated with B2M in cell lysates, however, only the HFE_ivs4 variant if found in the cell culture supernatant in association with B2M.
✓ The HFE_ivs4 protein is a truncated soluble HFE protein variant (shFE) that is secreted by cells maintaining its interaction with B2M.

4) Effect of the shFE variant in iron-metabolism related genes expression in a duodenal cell line (HuTu-80)

✓ As expected, TFR2 expression is inhibited by holo-Tf.
✓ The over expression of HFE_full length (HFE) and HFE_ivs4 (shFE) seems to slightly increase the expression of TFR1.
✓ DMT1 does not vary with HFE or shFE over expression.

✓ The expression of both B2M and SLC40A1 (genes coding for B2M and ferroportin-1 proteins) seems to differ when over expressing HFE or shFE.
✓ SLC40A1 expression seems to be decreased by iron stimulus.

✓ CYBRD1 (duodenal cytochrome b) expression is reduced in about 20% after over expression of both HFE and shFE, independently of iron stimulus.
✓ HEPH (hephaestin) expression is highly down regulated by HFE and shFE, independently of iron status.


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** means p<0.05, *** means p<0.001, unpaired t-test student's t-test.