THE SOLUBLE HFE ISOFORM – A REGULATOR OF IRON ABSORPTION IN THE DUODENUM

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BACKGROUND

The regulation of dietary iron absorption in duodenum is one of the key steps to maintain the body iron homeostasis. Once inside the enterocyte, iron is directed to the basolateral membrane being oxidized by hephaestin which mediates iron efflux towards circulatory transferrin in cooperation with ferroportin.

HFE is a major histocompatibility complex class I-like protein which gene is commonly mutated in Hereditary Hemochromatosis, a disorder characterized by excessive intestinal iron absorption and its deposition in several organs[1]. It has been postulated that HFE may contribute to iron metabolism regulation by activating hepcidin synthesis in hepatocytes and regulating the expression of iron metabolism-related genes in duodenum.

In addition to the full-length *HFE* transcript (≈4.2 kb in length), several alternative splicing *HFE* transcripts have been reported but their functional significance remains elusive[2]. Amongst them two give rise to a truncated and soluble HFE protein isoform (sHFE). The sHFE isoform maintains the ability to associate with β_2M, but not with TfR1, and it is secreted to the extracellular environment in several cell types[3].

AIMS

The main objective of this work was to assess if the sHFE isoform plays a role in the iron absorption regulation. In particular, we aimed:

1) To investigate whether a holo-transferrin stimulus affects the expression levels of *sHFE* transcripts in enterocyte-like and macrophage cell models.

2) To determine the effect of both the endogenous and exogenous sHFE isoform on the expression of several iron metabolism-related genes in a duodenal cell model (HuTu-80).

3) To correlate the peripheral blood iron metabolism biomarkers with *sHFE* levels in duodenum biopsies.

METHODS

✓ HuTu-80 (duodenum adenocarcinoma), PMA-activated THP-1 (monocytic), Caco-2 and HT-29 (colon adenocarcinoma) cells were stimulated with holo-transferrin (holo-Tf; 20µM). RNA was extracted and *TFR1*, total *HFE* and *sHFE* expression were assessed by RT-qPCR.

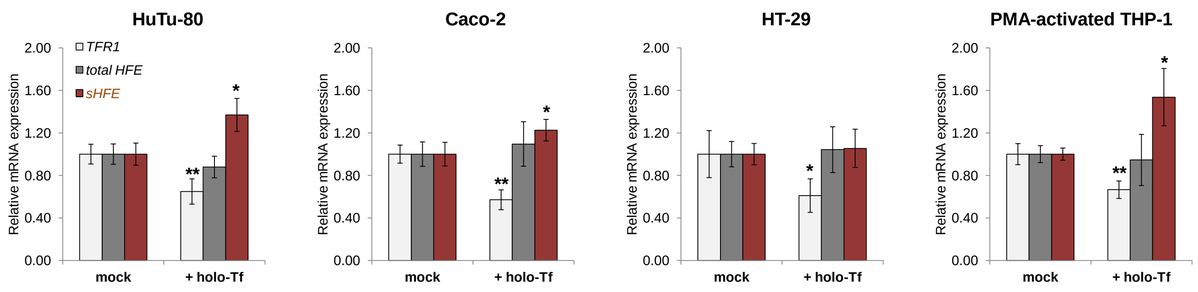
✓HuTu-80 cells were transfected with constructs expressing HFE_full-length (HFE) and HFE_ivs4 (sHFE) tagged to Flag. Cell lysates and cell culture supernatants were submitted to immunoprecipitation assays using mouse anti-Flag antibody. Immunodetections were performed with mouse antibody anti-Flag, mouse anti-TfR1 and rabbit anti-β_2M. The expression of iron-related genes *TFR1*, *SLC11A2*, *SLC40A1*, *CYBRD1* and *HEPH* were assessed by RT-qPCR. *HPRT1* was used as a housekeeping gene.

✓HuTu-80 cells were treated with endocytosis inhibitors (Dynasore, 40µM; Chlorpromazine, 10µg/mL) or transfected with a dominant-negative form of RhoA protein, followed by stimulus with sHFE conditioned medium (obtained by overexpressing sHFE in HEK293 cells). Duodenal cytochrome b and hephaestin expressions were quantified by RT-qPCR.

✓ RNA was extracted from dyspepsia patients duodenum biopsies. The expression levels of *sHFE* were quantified and was investigated the correlation with the peripheral blood iron metabolism biomarkers.

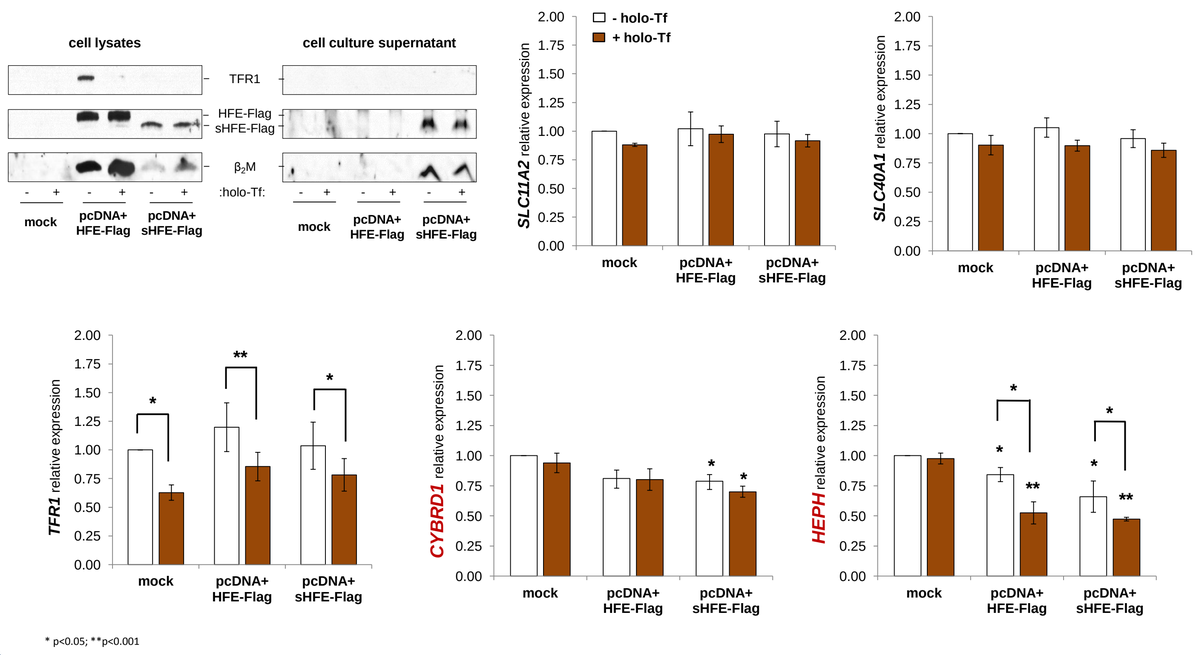
RESULTS

1) The expression of the *sHFE* transcripts is up-regulated by intracellular iron in both enterocyte-like and macrophage cell lines.



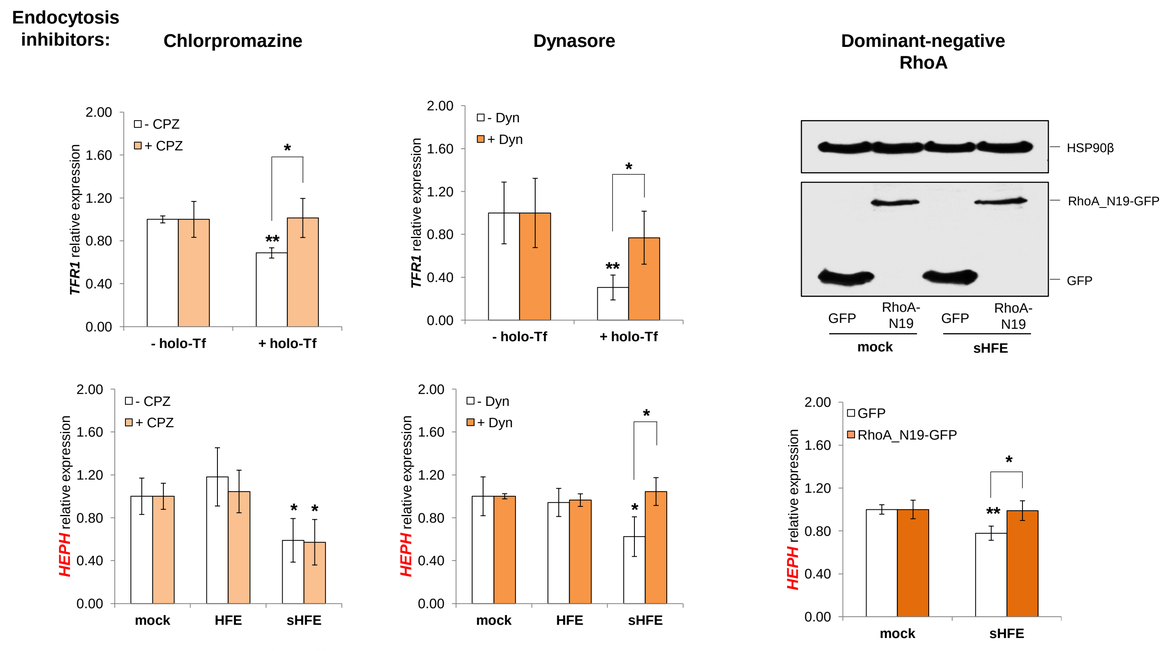
TFR1 was used as an iron responsive control; * p<0.05; **p<0.001

2.1) The endogenous sHFE isoform downregulates the expression of duodenal cytochrome b and hephaestin genes in an enterocyte-like cell model.



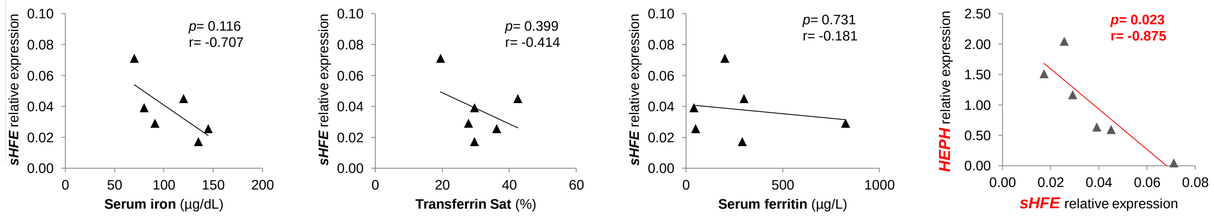
* p<0.05; **p<0.001

2.2) The exogenous sHFE isoform also downregulates hephaestin expression through a clathrin-independent, dynamin-mediated and RhoA-regulated endocytosis mechanism.



TFR1 was used as an iron responsive control ; * p<0.05; **p<0.001

3) Hephaestin and *sHFE* transcript levels present a negative correlation in the duodenum of dyspepsia patients.



CONCLUSIONS

➢ **Through this study we might have unveiled the role of the soluble HFE isoform (resulting from the intron 4 inclusion splicing alternative transcripts)[3] in iron metabolism regulation. The sHFE protein may be secreted by several tissues into the bloodstream, accordingly to the body iron status, and may act in the duodenum by reducing duodenal cytochrome b and hephaestin expression.**

➢**Since duodenal cytochrome b and hephaestin proteins have crucial functions in iron absorption by the duodenum (iron reduction and oxidation at the apical and basolateral membranes of the enterocyte, respectively) we can hypothesize that their decreased expression, modulated by the sHFE isoform, will be translated in a reduction of dietary iron absorption (Fig.1).**

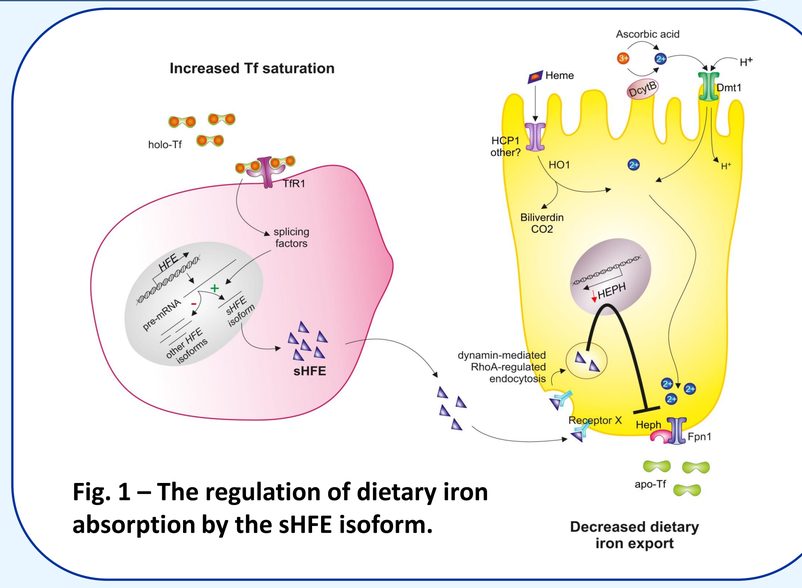


Fig. 1 – The regulation of dietary iron absorption by the sHFE isoform.

References

1. Feder JN *et al* (1996). Nat Genet 13: 399-408.
2. Thenie A *et al* (2000). Blood Cells Mol Dis 26: 155-162.
3. Martins R and Silva B *et al* (2011) Plos One 6(3):e17542.

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