THE EXPRESSION OF THE SOLUBLE HFE CORRESPONDING TRANSCRIPT IS UP-REGULATED BY INTRACELLULAR IRON AND INHIBITS IRON ABSORTPION IN A DUODENAL CELL MODEL

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BACKGROUND

Iron is a crucial element for living beings as it is involved in biological processes such as respiratory and metabolic pathways working as a co-factor of several enzymes[1]. As there are no active mechanisms to excrete iron, and since both iron deficiency and overload can be harmful[2], the human body has developed fine-tuned iron homeostasis regulation mechanisms. Those encompass iron absorption by duodenum enterocytes, storage in liver hepatocytes and recycling by macrophages.

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 deposition in several organs[3].

The role of HFE in iron homeostasis remains unclear. It has been postulated that it may contribute to iron metabolism regulation by activating hepcidin synthesis in hepatocytes and regulating the expression of iron metabolism related genes in duodenum.

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[4]. Amongst the *HFE* mRNA alternative splicing variants, there are two that have been shown to give rise to a truncated soluble HFE protein isoform (sHFE). The sHFE protein maintains the ability to associate with β_2M but not with TfR1 and it has been shown to be secreted to the extracellular environment in several cell types[5].

AIMS

1) To investigate if iron affects the expression levels of *sHFE* transcripts.

2) To correlate the levels of peripheral blood iron metabolism biomarkers with *sHFE* levels in the liver.

3) 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.

METHODS

✓ HuTu-80 (human duodenum adenocarcinoma), PMA-activated THP-1 (monocytic cell line) and HepG2 (liver hepatocellular carcinoma) cells were stimulated with holo-transferrin (holo-Tf; 20µM). RNA was extracted and *TFR1*, total *HFE* and *sHFE* expression assessed by RT-qPCR.

✓ RNA was extracted from HCV patients liver biopsies. The expression levels of *sHFE* and *HAMP* were quantified by RT-qPCR and correlations stablished with the peripheral blood iron metabolism biomarkers and HCV viremia.

✓ HuTu-80 cells (human duodenum adenocarcinoma) were transfected with pcDNA3 constructs expressing HFE_full length (HFE) and HFE_ivs4 (sHFE) tagged to Flag, followed by an iron stimulus (holo-Tf 20µM). Cell lysates and cell culture supernatants were submitted to immunoprecipitation assays using mouse anti-Flag antibody. These were subjected to a 12% SDS-PAGE, followed by transfer to a nitrocellulose membrane. Immunodetections were performed with mouse antibody anti-Flag, mouse anti-TfR1 and rabbit anti-B2M.

✓RNA was extracted from these cells and the expression of iron-related genes *TFR1*, *SLC11A2*, *SLC40A1*, *CYBRD1* and *HEPH* were assessed by RT-qPCR.

✓ HuTu-80 cells were treated with an endocytosis inhibitor (Dynasore, 40µM) and then stimulated with sHFE conditioned medium (obtained by overexpressing sHFE in HEK293 cells). Duodenal cytochrome b and hephaestin expressions were quantified by RT-qPCR.

References
1. Dunn LL et al (2007). Trends Cell Biol 17: 93-100.
2. Cartwright et al (1979). N Eng J Med 301: 175-179.
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4. Thenie A et al (2000). Blood Cells Mol Dis 26: 155-162.
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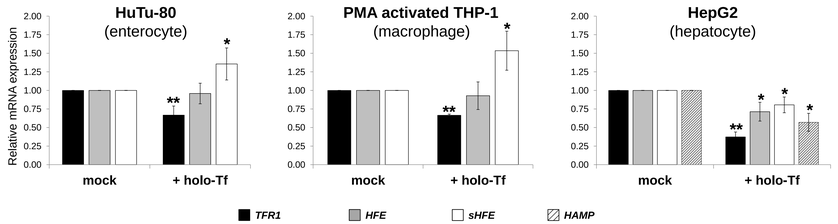
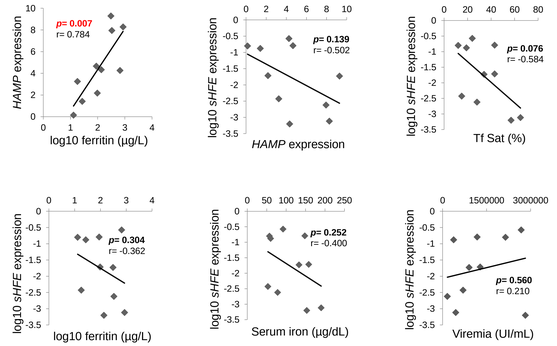
RESULTS

1 – *sHFE* transcript expression after holo-transferrin stimulus in different cell models



- *sHFE* transcript expression increases with iron stimulus in duodenal and macrophage cell lines

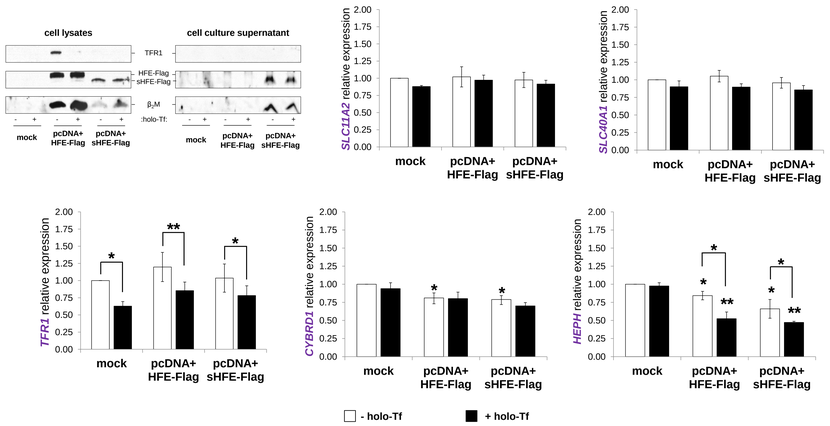
2 – *sHFE* transcript levels correlation with iron parameters in HCV patients liver biopsies



- *HAMP* expression presents a positive correlation with serum ferritin levels

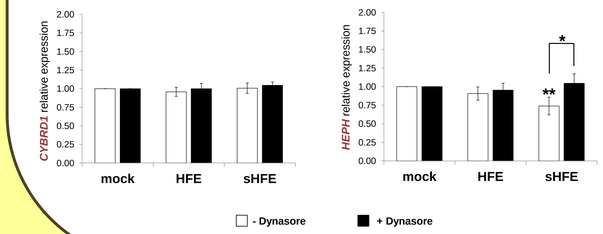
- No significant correlations were achieved between *sHFE* expression and peripheral blood iron metabolism biomarkers

3 – Effect of endogenous sHFE on the expression of iron metabolism-related genes in HuTu-80 cells



- *s*HFE overexpression down-regulates duodenal cytochrome b and hephaestin expressions

4 – Effect of exogenous sHFE endocytosis on *CYBRD1* and *HEPH* expressions



- Exogenous sHFE downregulates hephaestin expression in a endocytosis-dependent manner

CONCLUSIONS

➢ **The expression of sHFE increases with intracelullar iron in both enterocyte and macrophage cell lines, while it decreases in an hepatocyte cell line. However, hepcidin expression also decreases in HepG2 cells, contrarily to what has been previously observed for human primary hepatocytes.**

➢ **The levels of sHFE transcripts in the liver of patients with HCV do not correlate with their peripheral blood iron metabolism biomarkers.**

➢ **The overexpression of the sHFE is able to modify de expression of some iron metabolism related genes. It acts by repressing the expression of duodenal cytochrome b and hephastin.**

➢ **Exogenous sHFE is also able to downregulate the expression of hephaestin through an endocytosis-dependent mechanism.**

➢ **Since duodenal cytochrome b and hephastin are proteins presenting functions in iron absorption (iron reduction and oxidation at the enterocyte, respectively), we can hypothesize that their decrease expression modulated by the sHFE 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. sHFE may be secreted by several tissues into the bloodstream and may act in the regulation of dietary iron absorption in duodenum, reducing iron absorption, preventing iron overload and contributing to iron metabolism regulation.**