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 duodenal biopsies.

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**METHODS**

1. **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 RT-PCR, total HFE and shFE expression were assessed by RT-qPCR.

2. **HuTu-80** cells were transfected with constructs expressing HFE full-length (HFE) and HFE_v54 (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-TR1 and rabbit anti-βM. The expression of iron-related genes TR1, SLC11A2, SLC40A1, CYBB81 and HFE was assessed by RT-qPCR. HPERT1 was used as a housekeeping gene.

3. **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 stimulation with HFE conditioned medium obtained by overexpressing shFE in HEK293 cells. Duodenal cytochrome b and hephaestin expressions were quantified by RT-qPCR.

4. **RNA** was extracted from dyspepsia patients duodenal 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 stimulus in both enterocyte-like and macrophage cell lines**

2. **The endogenous shFE isoform downregulates the expression of duodenal cytochrome b and hephaestin genes in an enterocyte-like cell model (HuTu-80)**

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) in iron absorption 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 decrease of dietary iron absorption (Fig.2).