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In silico strategies and gene expression data indicate EFEMP1 as a potential biomarker for early fibrosis diagnosis in metabolic dysfunction-associated steatotic liver disease

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Background and aims: A quarter of adults worldwide suffer from metabolic dysfunction-associated steatotic liver disease (MASLD). Fibrosis is among the best predictors of liver-related risk in persons with MASLD. Although liver biopsy is still the gold standard for MASLD diagnosis, non-invasive diagnostic tools are crucial in detecting fibrosis, especially in severely obese subjects where non-invasive procedures are not readily available. We aim to identify, using *in silico* methods, circulating biomarkers associated with early fibrosis development and validate their specificity in a MASLD cohort.

Method: RNAseq data GSE125251 was retrieved from the NCBI GEO repository and re-analyzed using the 3DRNAseq analysis pipeline; differentially expressed genes (DEGs) were determined from liver transcriptomes comparison of F0-F1 (early fibrosis, n = 139) vs. F2-F3-F4 (moderate/advanced fibrosis, n = 67) MASLD subjects. Gene-set enrichment analysis was performed to determine DEGs' involvement in molecular pathways. DEGs were filtered, selecting secreted plasma protein-coding genes through an *in-silico* funnel strategy. Gene expression of candidates was analyzed in liver samples of a MASLD biopsy-proven cohort (n = 65) stratified according to minimal (FO/F1) and moderate/advanced fibrosis (F2/F3-F4).

Results: The re-analysis of GSE125251 identified 106 DEGs when comparing moderate vs. early fibrosis. Pathway enrichment analysis demonstrated that most DEGs were associated with pathways involved in fibrogenesis such as extracellular matrix production (ECM) and the inflammatory response. Twenty-two DEGs encoded secreted proteins and were categorized as proteins detectable in plasma using the *in-silico* strategy. Five candidates (*EFEMP1, LTBP2, LUM, DPT, CCL20*) were analyzed at the mRNA level. Among them, *EFEMP1* (EGF-containing fibulin extracellular matrix protein 1) showed the highest change, exhibiting increased mRNA expression levels in F2/F3-F4 fibrotic livers when compared with (F0/F1 -ones (p value <0.005).

Conclusion: Based on this pilot study, EFEMP1 seems to be the best candidate for future validation as a circulating fibrosis biomarker in MASLD. EFEMP1 is noted to be a secreted ECM protein, normally expressed by fibroblasts, and showing an important role in maintaining ECM stability and integrity. Further protein-level analyses are necessary to confirm the utility of EFEMP1 as a biomarker of liver fibrosis in MASLD.

Figure:

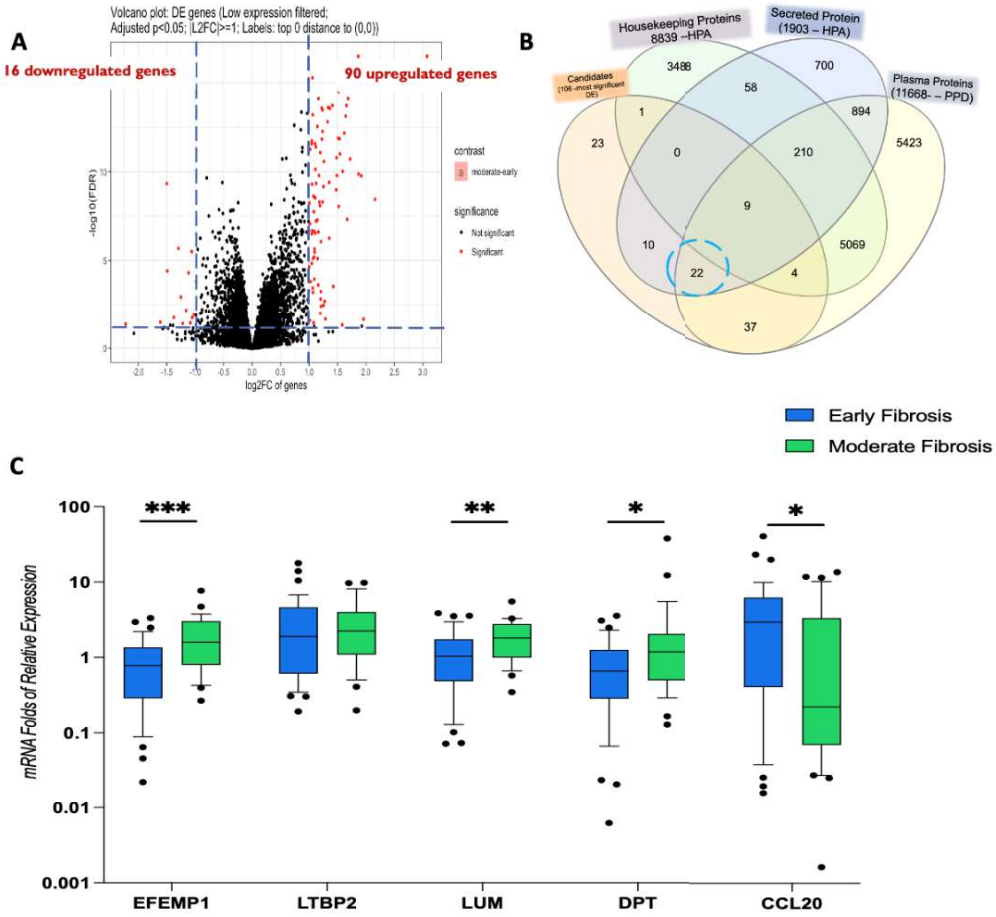

Figure 1: A) Volcano plot illustrating the differentially expressed genes in fibrotic samples of the GSE125251 dataset. B) Venn diagrams showing the number of DEGs identified as candidates by the *in-silico* funnel strategy. C) mRNA liver expression levels of 5 random selected candidates. Data are shown as mean ± SD. Group comparison by Kruskal-Wallis and post hoc Dunn's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.