Proteomics identification of adipocyte proteins involved in the pathogenesis of MAFLD

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Introduction

- Metabolic dysfunction-associated fatty liver disease (MAFLD) is now the most common liver disease globally.
- The prevalence of MAFLD is constantly increasing. In Australia, MAFLD cases are expected to increase 25% from 5.6 million cases in 2019 to 7 million in 2030 cases [1].
- Growing evidence suggests that adipose tissue dysfunction plays a role in hepatic injury in MAFLD [2].
- There is emerging evidence that altered secretion of adipokines secondary to iron accumulation in adipocytes contributes to MAFLD.
- This study determined the effect of iron and hepcidin, a master regulator of iron, on adipocytes and how this interaction might play a role in MAFLD pathogenesis.
- In this study, mass spectrometry proteomics analysis on iron and hepcidin-treated adipocytes was investigated.

Identification of key adipocyte proteins involved in the pathogenesis of MAFLD will further clarify our understanding and provide the opportunity for future mechanistic work.

Methodology

- The fully differentiated 3T3-L1 MBX cells were treated with ferric ammonium citrate (iron) (FAC; 100µM, 48 hours), and hepcidin (2µg/ml, 48 hours).
- Cells were harvested and samples were prepared by centrifuging 10µg of extracted protein in 10 kDA MWCO columns (Cat no UFC901024, Merck Millipore).
- Filter -Aided sample preparation (FASP) was used to process protein samples for proteomics.
- SWATH MS was used for analyses while the mass spectra data for protein identification was processed with ProteinPilot software (ABsciex) and PeakView software (ABsciex).
- Statistics and normalization were performed through MSstats (msstats.org/).
- ShinyGo was used for bioinformatics analysis and STRING v10 database was used to obtain protein-protein interaction networks.

Results

- Proteomics analysis of FAC and hepcidin treated adipocytes showed that 423 and163 proteins, respectively, were differentially expressed (DE) ($p<0.05$) and of these 109 were common to both FAC and hepcidin **[Figure 1A].**
- Out of the 423 DE FAC treated adipocytes, 294 proteins were upregulated while 129 proteins were downregulated **[Figure 1B]**. Out of 163 DE hepcidin treated adipocytes, 66 were upregulated and 98 were downregulated **[Figure 1C]**.

Results (cont'd)

Figure 1. Summary of proteomics analysis of FAC, and hepcidin treated adipocytes.

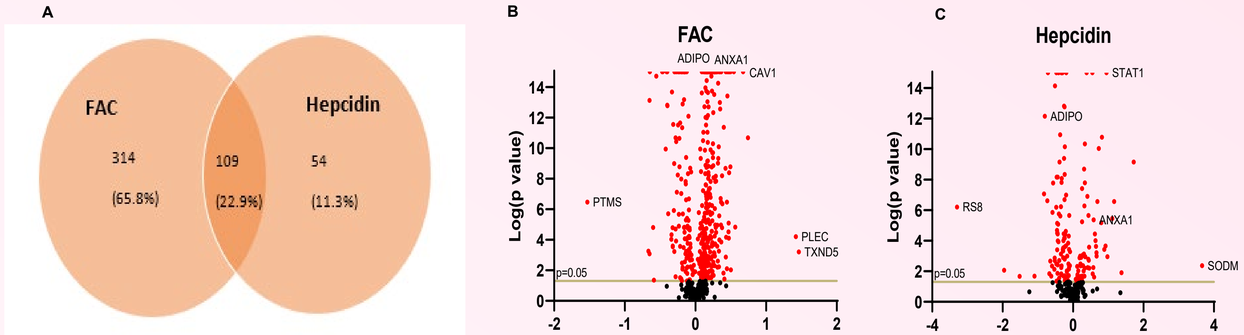
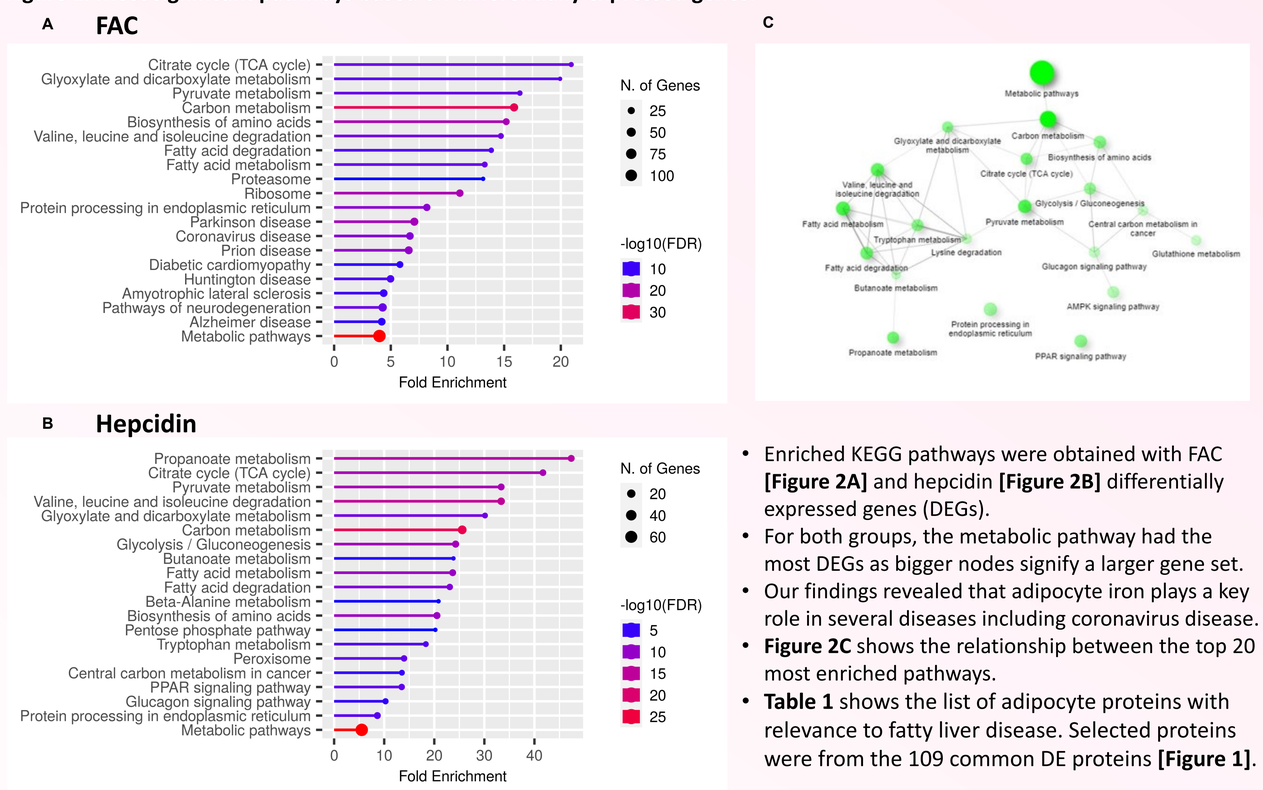


Figure 2. Most significant pathways based on differentially expressed genes.



- Enriched KEGG pathways were obtained with FAC **[Figure 2A]** and hepcidin **[Figure 2B]** differentially expressed genes (DEGs).
- For both groups, the metabolic pathway had the most DEGs as bigger nodes signify a larger gene set.
- Our findings revealed that adipocyte iron plays a key role in several diseases including coronavirus disease.
- **Figure 2C** shows the relationship between the top 20 most enriched pathways.
- **Table 1** shows the list of adipocyte proteins with relevance to fatty liver disease. Selected proteins were from the 109 common DE proteins **[Figure 1]**.

Table 1. List of adipocytes proteins with relevance to MAFLD.

Gene name	Protein name
Cavin1	Caveolae-associated protein 1
Plin4	Perilipin-4
AnxA2	Annexin A2
P4hb	Protein disulfide-isomerase
AnxA1	Annexin A1
FASn	Fatty acid synthase
Capg	Macrophage-capping protein
ACSL1	Long-chain-fatty-acid--CoA ligase 1
Pkm	Pyruvate kinase PKM
Rps8	40S ribosomal protein S8
Slc25a5	ADP/ATP translocase 2
Hspe1	10 kDa heat shock protein, mitochondrial
Prdx1	Peroxiredoxin-1
Echs1	Enoyl-CoA hydratase, mitochondrial
Ldha	L-lactate dehydrogenase A chain
Cnpy2	Protein canopy homolog 2
Acat1	Acetyl-CoA acetyltransferase, mitochondrial
Acads	Short-chain specific acyl-CoA dehydrogenase, mitochondrial
Tagln2	Transgelin-2
Plin1	Perilipin 4
Cav1	Caveolin-1

Conclusion

- This study argued that adipocyte dysfunction plays an important role in the development and progression of fatty liver disease.
- Proteomics analyses on FAC and hepcidin treated adipocytes have identified several adipocyte proteins that could be involved in MAFLD.
- Further mechanistic work on these proteins will further define the effect of iron on adipocytes, this may further clarify the role adipocytes play in MAFLD progression.

References

1. Adams, L.A., et al., *Nonalcoholic fatty liver disease burden: Australia, 2019-2030.* Journal of Gastroenterology and Hepatology, 2020.
2. Marra, F. and C. Bertolani, *Adipokines in liver diseases.* Hepatology, 2009. **50**(3): p. 957-69.