The characterisation of LAT and PAG in lipid rafts in normal and diseased liver tissues.

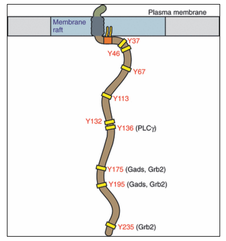
Jennie Parker*, Dr. Ashleigh Herriott, Dr. Helen Reeves,

Northern Institute for Cancer Research, Paul O'Gorman Building, Newcastle University

Jennie Parker ▪ 110058007 ▪ B100 Physiological Sciences ▪ j.parker@ncl.ac.uk

Introduction

- Non-alcoholic fatty liver disease (NAFLD) is a group of diseases characterised by fat accumulation in the liver. It has been proposed that Sulfatase 2 (Sulf2) has a role in steatosis (fat in hepatocytes) and that it is found in lipid rafts.
- LAT and PAG are constitutively expressed in lipid rafts.
- LAT (Linker Activation of T cells) and PAG (Phosphoprotein Associated with Glycosphingolipid microdomains) are both transmembrane adaptor proteins (TRAP). LAT is a 36-38kDa protein which acts as a docking site for SH2 domain containing proteins. PAG is a 68-85 kDa protein.



A model of the LAT molecule
Hořejší, V. et al

AIMS

- **To optimise the methods for the antibodies to LAT and PAG in immunohistochemistry**
- **To investigate the expression of lipid raft proteins LAT and PAG using immunohistochemistry in tissues with varying NASH**

Methods

Optimisation of LAT and PAG

I optimised the methods for immunohistochemistry on two lipid raft marker proteins, LAT and PAG. A range of different conditions were used, varying the retrieval buffer, the retrieval method and the concentration of the antibody. It was found that LAT stained best with an antibody concentration of 1/200, with antigen retrieval using citrate buffer and the antigen retrieval unit. PAG showed the best results with a concentration of 1/250, and using citrate buffer and the microwave to retrieve the antigens.

Expression of LAT and PAG with variable NASH

Immunohistochemistry using the optimised methods was performed on tissues from mice with varied NAFLD activity scores (NAS) from normal control diet mice with little or no fat to mice with simple fat, to compare the characteristic staining.
These were also compared with Sulf2 staining in the same tissues.

Results

Staining of PAG

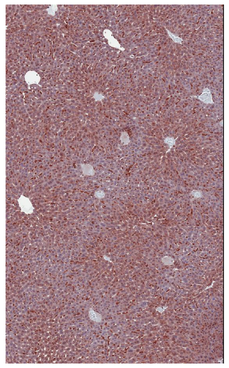


Fig. 1: PAG staining x4

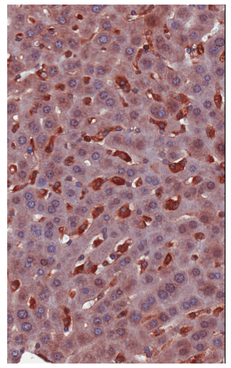


Fig. 2: PAG staining x20

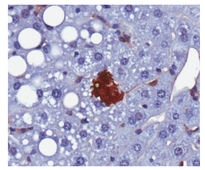


Fig. 3: PAG staining x20
fatty liver

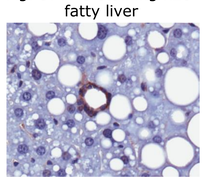


Fig. 4: PAG staining x20
Fatty liver

Features of PAG staining: Fig 1. shows the zonation of PAG in the liver. Fig 2. is a higher magnification (20x) and shows that PAG staining is sinusoidal. Figures 3 and 4 show a microgranuloma and a lipogranuloma respectively on tissues with a NAS score of 4.

Staining of LAT

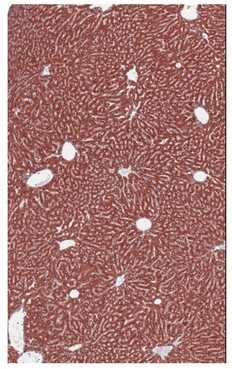


Fig. 5: LAT Staining x5

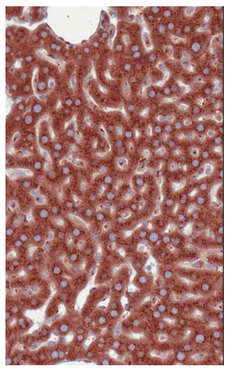


Fig. 6: LAT staining x20

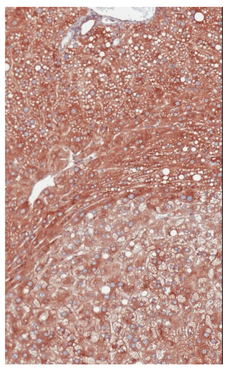


Fig. 7: LAT staining x10

Features of LAT staining: Fig 5. and 6. show LAT staining at different magnifications. Fig 7. shows a well-differentiated HCC (Hepatocellular carcinoma).

Comparison of normal liver (NAS Score 0) with liver with a fatty liver (NAS Score 4)

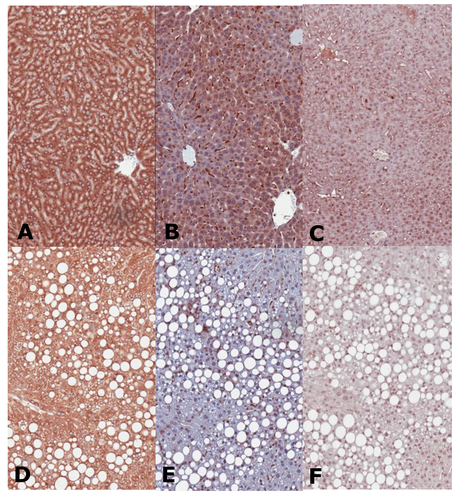


Figure 8: NAS Score 0: A) LAT B) PAG C) Sulf2
NAS Score 4: D) LAT E) PAG F) Sulf2

LAT: A and D have high background non-specific staining, requiring further optimisation. However, more intensely stained areas are seen around hepatocytes swollen with fat around D, likely to represent lipid rafts. PAG is evident in these areas with fat, but it more prominent in inflammatory cells. Sulf 2 staining has a similar appearance to PAG.

Conclusion

- Although LAT and PAG are both markers of lipid rafts, staining appears to be quite different
- PAG shows zonation in the liver. It also clearly showed microgranulomas and lipogranulomas, due to the staining of the clusters of macrophages around these.
- It was possible to identify nodules in all three stains, due to the squashing and distension of some of the cells (Fig. 7)
- PAG appeared to be more sinusoidal, whereas LAT was more cytoplasmic. Sulf2 appears to be found in the sinusoidal epithelium.
- Further optimisation of IHC will be necessary to be confident that some of staining - sinusoidal in particular - is not non-specific.

References

Hořejší, V., Otáhal, P. and Brdička, T. (2010), LAT – an important raft-associated transmembrane adaptor protein. Delivered on 6 July 2009 at the 34th FEBS Congress in Prague, Czech Republic. FEBS Journal, 277: 4383–4397.