

PWE-128

TO STAIN HUH7 CELLS WITH HEPCIDIN USING IMMUNOHISTOCHEMISTRY

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Introduction Hepcidin is a central iron regulator, formed predominantly in the liver. Hepcidin-25 causes hypoferremia by binding to ferroportin channels present on enterocytes, macrophages and hepatocytes. This study was to examine the presence of hepcidin in HuH7 cell line with use of immunohistochemical staining.

Methods Immunohistochemistry was then carried out on HuH7 cells. The cells were cultured. Slides were then incubated with blocking solution, (3% FBS, 1% BSA, 0.05%

Tween in Tris-buffered saline (TBS)) for 30 min, followed by a 4°C overnight incubation with purified rabbit antihepcidin purified primary antibody (1 in 800 dilution). Sections were then incubated for 1 h with secondary antirabbit antibody conjugated with horseradish peroxidase (HRP; Sigma Aldrich). The reaction was visualised by the addition of 3,3'-diaminobenzidine (DAB) substrate (Sigma Aldrich) for 5 min, and the reaction was stopped by washing with laboratory-grade water. Sections were counterstained with haematoxylin (Sigma Aldrich, UK) for 1 min. Slides were then dehydrated in a 70%, 95% and 100% ethanol (2 min each), cleared in xylene for 5 min and then mounted in DPX (Sigma Aldrich).

Results Using rabbit polyclonal antibody against human hepcidin, the authors were able to stain the HuH7 cells with hepcidin and demonstrate a cytoplasmic staining pattern (see figure 1).

Conclusion The findings are in keeping with the current knowledge of cytoplasmic presence of hepcidin. With demonstration of hepcidin in HuH7 cells should pave way for further studies in the pathways of hepcidin regulation.

Competing interests None.

Keywords immunohistochemistry, iron.

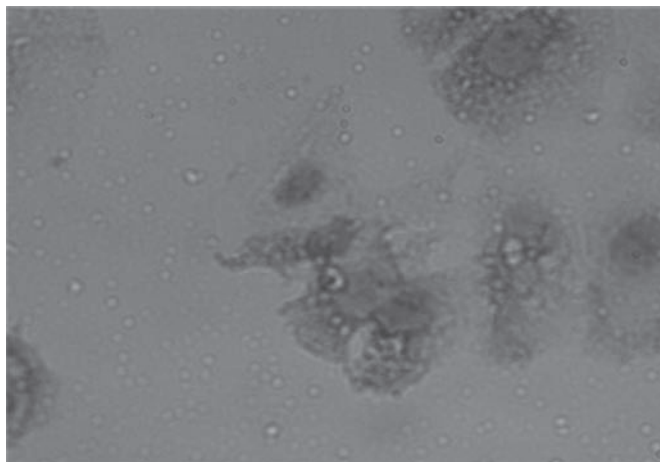


Figure 1 PWE-128

Table 1 PWE-129

	Lower oesophageal sphincter residual pressure	Proximal transition zone break in peristalsis @30 mm Hg	Velocity	Distal wave amplitude	Intrabolus pressure
3 RATERS liquid upright					
ICC (p=)	0.914 (<0.001)	0.651 (<0.001)	0.143 (0.214)	0.963 (<0.001)	0.283 (0.006)
Solid upright					
ICC (p=)	0.524 (0.002)	0.868 (<0.001)	0.078 (0.309)	0.918 (<0.001)	0.685 (<0.001)
2 RATERS liquid supine					
ICC (p=)	0.951 (<0.001)	0.897 (<0.001)	0.766 (0.001)	0.981 (<0.001)	0.848 (<0.001)
Solid supine					
ICC (p=)	0.959 (<0.001)	0.958 (0.001)	0.761 (0.001)	0.972 (<0.001)	0.861 (<0.001)