



Introduction

- Labour is an inflammatory process, mediated via molecules of the innate immune response, including Interleukin-1 β (IL-1 β).
- Premature activation of these inflammatory pathways, following infection for example, is associated with complications of pregnancy including preterm birth (<37 weeks gestation) (1).
- HRPE773 has been proposed to have an antimicrobial function owing to its localisation to the secretory epithelium of several tissues, including the human female reproductive tract (2).
- We therefore hypothesised that HRPE773 expression may be regulated during human labour by inflammatory stimuli.

Methods

- Tissue samples of term human amnion, chorio-decidua, placenta, myometrium & cervix from;
 - i. Labour (spontaneous vaginal delivery >40 weeks gestation)
 - ii. Non-labour (elective caesarean section 39-43 weeks gestation)
 were obtained through the Edinburgh Reproductive Tissue Biobank (ERTBB) with ethical approval of West of Scotland Research Ethics Service.
- Ectocervical (ECT1/E6E7) & endocervical (END1/E6E7) cell lines were treated (24 hrs) with;
 - i. IL-1 β
 - ii. Lipopolysaccharide (LPS)
 - iii. No treatment (control)
- HRPE773 mRNA expression was determined in;
 - i. Labour relative to non-labour samples
 - ii. Treated cervical cell lines relative to an untreated control
 using qRT-PCR (2^{- $\Delta\Delta C_t$} method with 18S RNA as internal control).
- The cell specific localisation of HRPE773 protein in human foetal membranes, placenta, myometrium and cervix was determined using immunohistochemistry.

Results

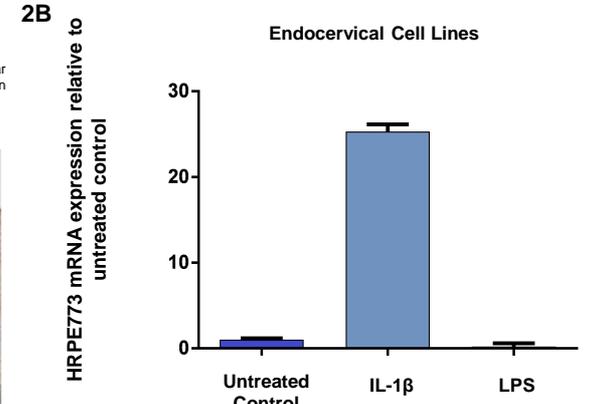
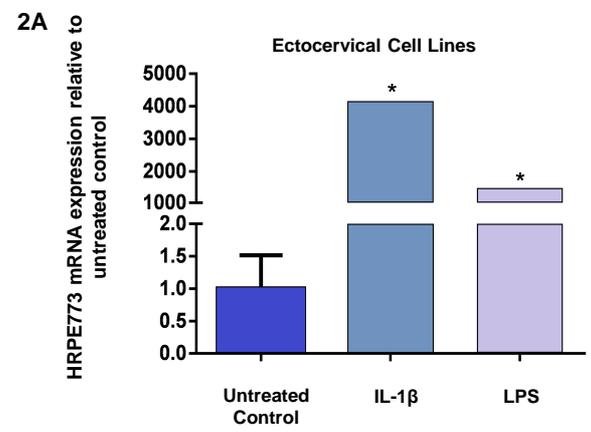
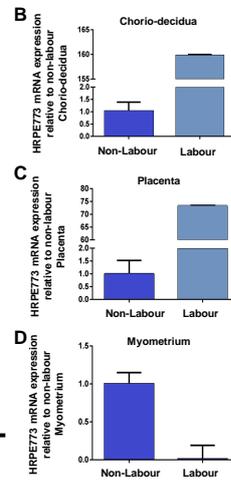
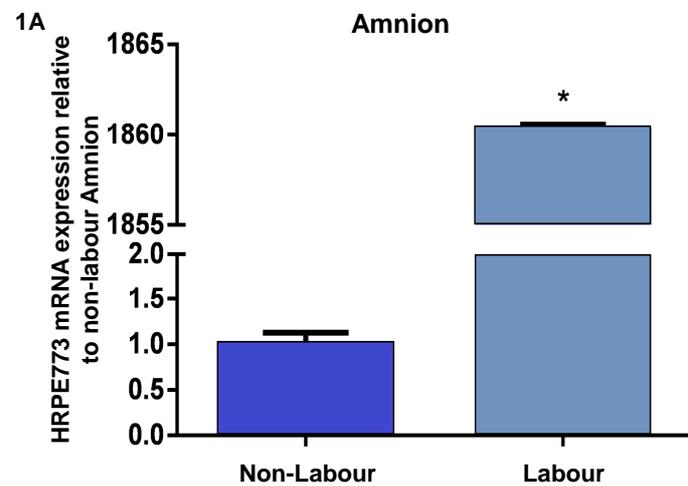


Figure 1. HRPE773 mRNA expression in labour amnion, chorio-decidua, placenta and myometrium relative to non-labour samples. Each bar represents the mean \pm SEM where (n=3). A. Amnion. The asterisk denotes a statistically significant difference in HRPE773 mRNA expression in labour samples relative to non-labour counterparts according to Mann-Whitney test ($p < 0.05$). B. Chorio-decidua. C. Placenta. D. Myometrium.

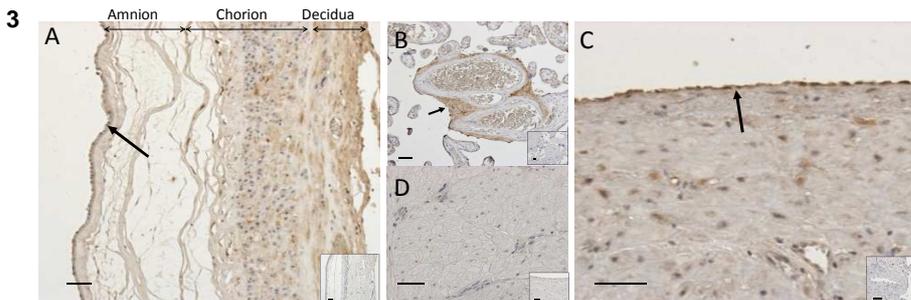


Figure 3. HRPE773 protein localisation in foetal membranes, placenta, myometrium and cervix. Scale bar represents 100 μ m, (n=3). Negative controls, using no primary antibody, are shown in bottom right hand corner. A. Foetal membranes (amnion, chorion & decidua as labelled). Arrow indicates epithelial immunostaining of amnion. B. Placenta. Arrow indicates immunostaining of fibrotic lesion. C. Cervix. Arrow indicates epithelial staining. D. Myometrium. No staining.

Figure 2. HRPE773 mRNA expression in IL-1 β and LPS-treated (A) Ectocervical & (B) Endocervical cell lines (n=1), relative to untreated controls (n=3). Each bar represents mean \pm SEM. Asterisk denotes a significant difference in HRPE773 mRNA expression relative to untreated control according to one-way ANOVA with Tukey's post hoc ($p < 0.05$).

Discussion

- HRPE773 mRNA expression was significantly elevated in labour vs non-labour amnion, but not in other tissues examined (Fig. 1A-D).
- HRPE773 mRNA expression was significantly elevated following treatment with the inflammatory cytokine IL-1 β or LPS bacterial endotoxin in ectocervical, but not endocervical cell lines (Fig. 2A-B).
- HRPE773 protein was largely localised to epithelial surfaces in foetal membranes, placenta and cervix (Figs. 3A-C).

Conclusions

- Elevated HRPE773 mRNA levels in labouring human amnion indicates a role for HRPE773 protein in normal labour.
- Elevated HRPE773 mRNA levels in ectocervical cell lines treated with inflammatory mediators suggests inflammatory regulation in cervix.
- Epithelial localisation is consistent with an innate immune function.
- Further studies using human cervical tissue are required to determine the role of HRPE773 in the cervix during labour.

References

1. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, Yoon BH (2004). Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol.* , 191, 1339-1345.
2. Ng KYB, McDonald SE, Ren X, Mullins JJ, Rae MT, Critchley HOD, Horne AW, Morley SD (2011). HRPE773 (ZG16B) expression is elevated in human endometrium during the early secretory phase and in uterine decidua following miscarriage. *Endocrine Abstracts*, 25 P281.

Acknowledgements

We would like to acknowledge the Edinburgh Reproductive Tissue Biobank (ERTBB) for the provision of tissues and thank all the patients who donated tissues. This work was supported by Small Project Grant awards from the Society for Endocrinology of Great Britain and the Charlie Walters Bequest Fund to SDM.