Identification of a potential biomarker for endometriosis diagnosis

Leah Cooksey[1], Kevin Phillips[2], Isabel Pires[1], Barbara Guinn[1]

[1]Department of Biomedical Sciences, Faculty of Health Sciences, University of Hull, HU6 7RX, [2]Department of Obstetrics and Gynaecology, Hull and East Yorkshire NHS Trust, Anlaby Road, Hull, HU3 2JZ2

contact : L.Cooksey-2014@hull.ac.uk

Introduction

Sampson's theory of retrograde menstruation states that during menstruation, the shed lining flows back through the fallopian tubes[1]. During this process shed cells experience hypoxic stress, contributing to the cellular changes seen in endometriosis[2]. HIF-1α is a key regulator in the cellular response to hypoxia and has been shown to be stabilized in ectopic endometrial tissue[3]. This stabilisation leads to the over-expression of multiple proteins in endometriosis, contributing to increased migration, invasion and proliferation of endometriotic cells[2]. We are therefore investigating the impact of hypoxia and HIF-1α on endometriosis to better understand this disease and to identify novel biomarkers for diagnosis.

Methods

Cell lines human endometrial stromal cells (T-HESC) and human endometriotic endothelial cells (12Z) we used to represent the normal endometrium and endometriosis, respectively.

RNA sequencing analysis of a publicly available data set of the effects of hypoxia on mRNA expression in T-HESC.

Hypoxic conditions were achieved using a hypoxic workstation at 1% O_2. Hypoxic results were compared to normoxic conditions at 21% O_2.

Chemical hypoxia was achieved using DFO

Cell viability was determined using MTS assay

Future directions

EnBi1 has been shown to be involved in cell survival, migration, invasion and EMT in cancer cells. Therefore, we will determine the functional effects of EnBi1 in endometriosis.

In addition to its functional role, EnBi1 has been shown to be excreted in the urine of other diseases, owing to a potential urine biomarker for endometriosis diagnosis. Our clinical trial focuses on EnBi1 detection in patient urine, through ELISA analysis.

Differential gene expression

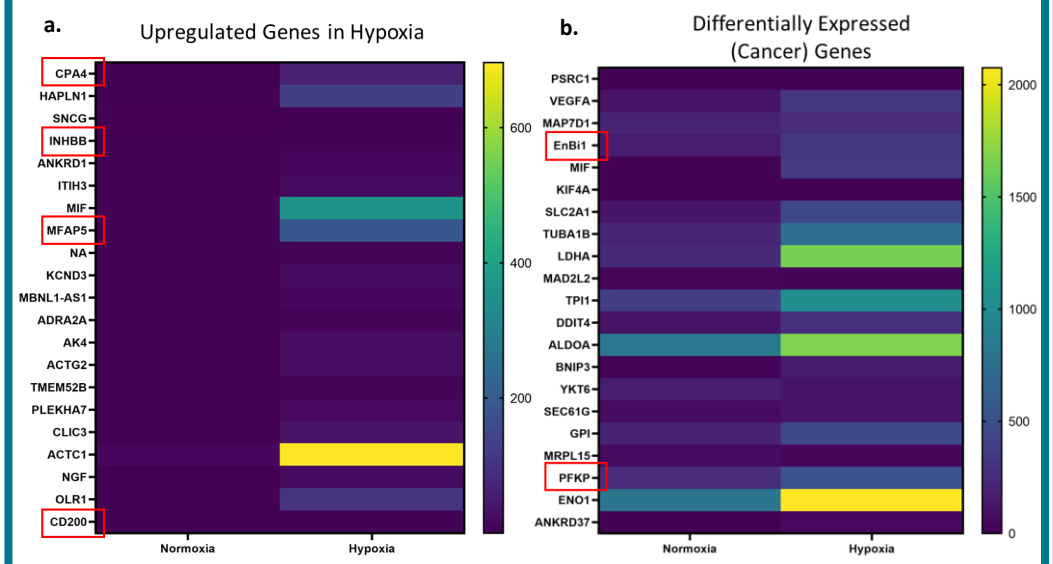

Figure 1 RNA sequencing analysis of previously published data on mRNA levels in T-HESC in hypoxic conditions shows differential expression of 5847 Genes (a). This dataset was compared to a hypoxia cancer metagene (b) to highlight similarities and provide novel targets for endometriosis. Genes were chosen for further investigation based on their overexpression in hypoxia as well as their function in health and disease. 6 genes were chosen, *PFKP, CD200, CPA4, INHBB, MFAP5* and a gene which will be referred to as *EnBi1*.

EnBi1 protein expression

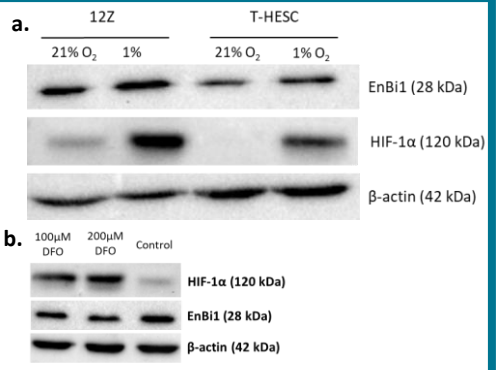

Figure 3 EnBi1 protein expression is greater in endometriotic cells and showed increased expression in hypoxia in both 12Z and T-HESC (a), however chemical induction of HIF-1α with DFO does not increase EnBi1 expression in 12Z (b).

Knockdown of EnBi1

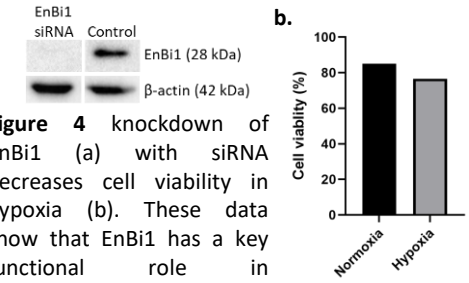

Figure 4 knockdown of EnBi1 (a) with siRNA decreases cell viability in hypoxia (b). These data show that EnBi1 has a key functional role in endometriosis

Hypoxia increases mRNA expression

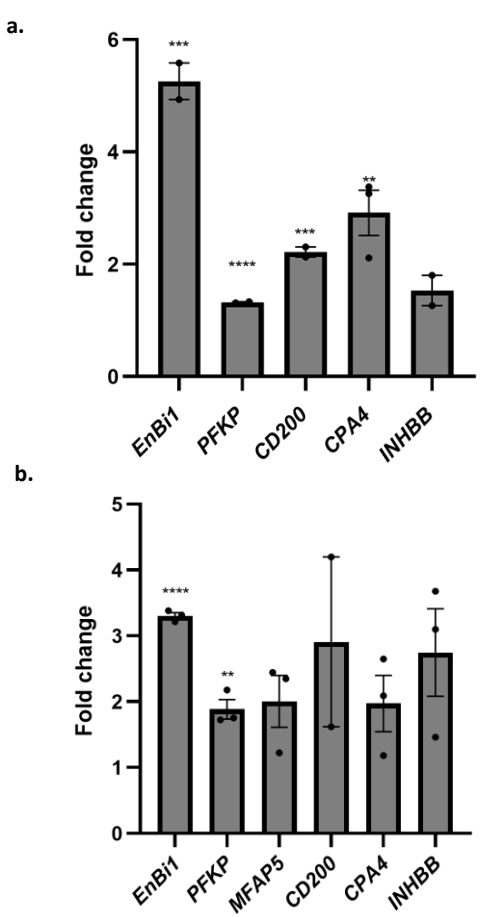

Figure 2 mRNA levels of targets identified through RNAseq show significant expression of multiple genes in hypoxic conditions in both 12Z (a) and T-HESC (b). *EnBi1* has the most significant increase in expression in hypoxia and therefore was chosen for functional analysis.

References

[1]Sampson, J.A., Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation.Am J Pathol, 1927. 3(2): p. 93-110.43.

[2]Wu, M.H., K.Y. Hsiao, and S.J. Tsai, Hypoxia: The force of endometriosis.J Obstet Gynaecol Res, 2019. 45(3): p. 532-541

[3]Zhan, L., et al., Hypoxia-inducible factor-1alpha: A promising therapeutic target in endometriosis.Biochimie, 2016. 123: p. 130-7.

Acknowledgments

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