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ARTICLE

Knockdown of prohibitin expression promotes glucose metabolism in eutopic endometrial stromal cells from women with endometriosis

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Abstract In this in-vitro study, the effect of prohibitin (PHB) on glucose metabolism in eutopic endometrial stromal cells from women with endometriosis was investigated. Endometrial stromal cells were isolated from endometrium in women with endometriosis, in women without endometriosis, or from endometrioma tissues. Glucose metabolic phenotype of stromal cells were examined *in vitro*. Quantitative polymerase chain reaction was used to measure the mRNA expression of glycolysis-related genes. Glucose consumption and lactate production were examined after knockdown of PHB expression in women with endometriosis with siRNA. In endometrioma tissue, significantly increased glucose consumption, lactate production and aberrant expression of glycolysis-related enzymes were found in women with endometriosis compared with women who do not have endometriosis ($P < 0.05$ versus $P < 0.001$). In women

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with endometriosis, PHB mRNA and protein were under-expressed in endometrioma tissue; in women without endometriosis, PHB mRNA and protein were over-expressed. Knockdown of PHB expression in women with endometriosis increased glucose consumption, although it had no effect on lactate production. This study suggests that aberrant expression of glycolysis-related enzymes in endometrioma tissue is associated with enhanced glycolytic metabolism. The malignant-like feature may be partially caused by low-expression of PHB gene in endometriotic stromal cells.

KEYWORDS: endometriosis, glucose metabolism, glycolysis, prohibitin

Introduction

Endometriosis is defined as the presence of active endometrial glands and stroma outside the uterus. Although its exact mechanism is poorly understood, retrograde menstruation is considered a key pathogenic cause (Giudice, 2010). It is believed that enhanced migration, apoptosis escape, and increased invasion and angiogenic activities of endometrial cells contribute to the development of the disease (Moggio et al., 2012; Taylor et al., 2009). Accordingly, endometrial cells from endometriosis may share some features with cancers.

One characteristic feature of cancer is the 'aerobic glycolysis', or 'Warburg effect', first described by Warburg in the early 1930s (Kroemer and Pouyssegur, 2008). Cancer cells preferentially use glycolysis as a main energy source rather than oxidative phosphorylation (OXPHOS) even in the presence of abundant oxygen, which leads to increased glucose consumption and accumulated lactate levels (Vander Heiden et al., 2009). The altered metabolism has been linked to several malignant phenotypes, such as unlimited cell proliferation, apoptosis escape, sustained angiogenesis, tissue invasion and metastasis (Kroemer and Pouyssegur, 2008). Multiple cross-talk signalling pathways and cancer-related mutations are implicated in the alterations in the energy mechanism of cancer cells, including p53, PTEN, MYC, HIF mutations and PI3K, AMP-activated protein kinase pathways (Cairns et al., 2011; Ward and Thompson, 2012).

Although transcriptomic and proteomic profiling allows identification of various gene expression alterations in endometriotic lesions that might be potentially related to energy metabolism (Kobayashi et al., 2009; Mao, 2007), it remains unclear how aberrations in energy metabolism may contribute to the development of endometriosis. Some clinical observational studies have suggested that endometriotic lesions could give positive results on fluorine-18 fluorodeoxyglucose (18F-FDG) positron emission tomography scans, which has been highly accurate in distinguishing malignant lesions from benign tissues, based on an increased uptake of 18F-FDG in malignancies (Fastrez et al., 2011; Jeffry et al., 2004). It has been debated, however, whether the increased uptake of 18F-FDG is caused by the endometriosis itself or by a false-positive scan from inflammatory reactions, which are often associated with the endometriomas (Jeffry et al., 2004). In endometriosis, stromal cells are known to be involved in early menstrual endometrium attachment and be responsible for further growth of glandular epithelial cells, leading to endometrial gland formation at endometriotic lesions (Arnold et al., 2001; Nisolle et al., 2000). In the culture used in the present study, primary glandular epithelial cells were observed as colony growth and showed a lower proliferation than stromal cells (unpublished observations). The hypothesis that the metabolic phenotype of endometrial stromal cells from women with endometriosis is similar to that of cancer cells was tested.

Prohibitin-1 (PHB) is a chaperone protein that is highly conserved evolutionarily and present in different cellular compartments. It has been shown that PHB is differentially expressed in eutopic endometrium from women with endometriosis and those without (Fowler et al., 2007; Mao, 2007). Recent studies have provided strong evidence for an important biological role of PHB in mitochondrial function, cell proliferation and embryo development (Artal-Sanz and Tavernarakis, 2009). It regulates glucose metabolism in RINm5Fβ-cells through insulin receptor activation, PI3K/AKT pathway and its O-GlcNAc modification (Ande et al., 2009; Mishra et al., 2010). Potential involvement of PHB in the pathogenesis of endometriosis, however, has not been investigated. The aim of this study was to evaluate the glucose metabolic phenotype of endometrial stromal cells from endometrium in women with endometriosis, without endometriosis, and endometrial stromal cells from ovarian endometrioma tissues. As an initial attempt to reveal the mechanistic change underlying the observed energy changes associated with endometrisois, the role of PHB on glucose metabolism in eutopic stromal cells from women with endometriosis was also examined.

Materials and methods

Patients and sample collection

Ethical approval was obtained from the First Affiliated Hospital of Nanjing Medical University Ethical Committee on 22 February 2013 (reference number 2013-SRFA-093). Thirty-five patients aged between 23 and 43 years who underwent laparoscopic examination from February 2013 to November 2013 in the First Affiliated Hospital of Nanjing Medical University, donated their endometrium and ovarian endometrioma and provided informed consent. All samples were collected during the proliferative phase of menstrual cycle according to the date of the last menstrual period. Twenty-three of them were histologically confirmed as endometriosis (stromal cells from the endometrium in women with endometriosis $n = 11$); stromal cells isolated from endometrioma tissues: $n = 12$). Endometriosis was staged as III-IV according to the revised American Fertility Society classification. Endometrial tissues obtained from other patients diagnosed with other benign gynaecological disease, including nine cases of hydrosalpinx and three cases of benign ovarian teratomas, were enrolled as normal controls (i.e. stromal cells from endometrium in women without endometriosis: $n = 12$).