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Peiling LV
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Jiezhen Wei
Department of Pathology, Kiangwu Hospital, Macau, Macao, China

Wen-Ian Wong
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Chunke LI
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Haiqi Liang
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Qingye Chen
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Yu Hong
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

Corresponding Author:
Yu Hong
Department of Obstetrics and Gynecology, Kiangwu Hospital, Macau, Macao, China

The difference of HLA-G expression in pathological tissues of endometrial hyperplasia and endometrial cancer

Peiling LV, Jiezhen Wei, Wen-Ian Wong, Chunke LI, Haiqi Liang, Qingye Chen and Yu Hong

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Abstract
Objective: To explore the difference of Human leukocyte antigen-G (HLA-G) expression in pathological tissues of endometrial hyperplasia and endometrial cancer.
Methods: From September 2020 to August 2024, patients who underwent hysteroscopy in the Obstetrics and Gynecology department of Kiang Wu Hospital, Macau were enrolled in this case control study. According to the pathological results, the patients were divided into normal endometrium, endometrial hyperplasia, and endometrial cancer groups. HLA-G expression in pathological tissues was measured by immunohistochemistry in all participants.
Results: Three groups included 65, 37, and 67 patients, respectively. The positive rates of HLA-G expression in the normal group, hyperplasia group, and endometrial cancer group were 12.3%, 29.7%, and 50.7%, respectively, showing an increasing trend. In the endometrial cancer group, 81 patients had endometrioid adenocarcinoma, and seven patients had non-endometrioid cancer with positive rates of HLA-G expression of 49.2% and 66.7%, respectively. The positive rate of HLA-G expression in both endometrial cancer and hyperplasia groups was significantly higher than that in the normal group (p<0.05), and the positive rate of HLA-G expression in the endometrial cancer group was significantly higher than that in the hyperplasia group (p<0.05).
Conclusion: Compared with normal endometrium, the expression of HLA-G was significantly increased in endometrial hyperplasia tissue and even more significantly in endometrial cancer tissue.

Keywords: Human leukocyte antigen G, endometrial hyperplasia, endometrial cancer

Introduction
Human leukocyte antigen-G (HLA-G) is a non-classical major histocompatibility complex (MHC) class I molecule that exists in seven isoforms, including four membrane-bound and three circulating soluble types. It interacts with the corresponding receptors to transmit negative regulatory signals to immune cells such as NK and T cells, exerting immunosuppressive effects. This physiological process plays an important role in maternal fetal tolerance [1]. Early studies have found that under normal physiological conditions, HLA-G is only expressed in a few specific tissues (such as thymus, cornea, and pancreas) except for trophoblast cells. Recent studies have indicated that abnormal HLA-G expression has also been observed under non-physiological conditions such as viral infection, cancer, transplantation, inflammation, and autoimmune diseases. Under these pathological conditions, it may have two different effects: it is beneficial for controlling inflammation and autoimmune diseases; however, it is unfavorable for infections and tumors, which may be related to tumor immune evasion and progression [2, 3]. In developed countries and regions, endometrial cancer has become the most common malignant tumor of female reproductive organs in recent years and is often associated with long-term estrogen stimulation without progesterone antagonism. The precursor lesion is endometrial hyperplasia, including endometrial hyperplasia without atypical and atypical endometrial hyperplasia. Previous studies have found that HLA-G expression is abnormally elevated in patients with endometrial cancer; however, there are currently no relevant reports on endometrial hyperplasia. Therefore, this study aimed to investigate the expression of HLA-G in the pathological tissues of patients with endometrial cancer and hyperplasia at our hospital.

Materials and Methods

Research object

During September 2020 and August 2024, patients, with abnormal uterine bleeding or ultrasound indications of abnormal intrauterine echo, were admitted to the Obstetrics and Gynecology Department of Kiangwu Hospital, Macau. After preoperative examination and evaluation to exclude contraindications, each patient signed an informed consent form and underwent hysteroscopic examination. Based on postoperative pathological results, patients were grouped as follows: 1) normal endometrium (normal group); 2) endometrial hyperplasia (hyperplasia group), including simple, complex, and atypical hyperplasia; and3) endometrial cancer (endometrial cancer group), including endometrioid adenocarcinoma, high-grade serous adenocarcinoma, and clear cell carcinoma. The exclusion criteria were as follows: (1) incomplete clinical data; (2) surgical contraindications; and (3) presence of other tumors or autoimmune diseases. The clinical data of all patients were recorded in detail. This study was approved by the Hospital Ethical Committee (approval number 2022-005).

Hysteroscopy examination process

On the day of surgery, all patients underwent hysteroscopy under intravenous anesthesia using a German Storz hysteroscope and its matching uterine distention instrument, imaging system, and cold light source. A sodium chloride solution (0.9%) was used as the distention medium, and the examination was performed under a distention pressure of 100-120mmHg. The patient was placed in the bladder lithotomy position, and routine sterilization was performed after successful anesthesia. The cervix was gradually dilated to size 7 and a hysteroscope was inserted to observe the walls of the uterine cavity, bilateral uterine horns, and fallopian tube openings in sequence. The following signs were noted: (1) thickening of the endometrium, accompanied by focal or diffuse papillary or polypoid lesions; (2) abnormal vascular morphology; (3) adenocystic changes; and (4) abnormal structural features of the glandular outlet (thickening, irregular glandular density, or dilation). Diagnostic scraping or biopsy is performed by micro-cutting the suspicious lesions. For those with a normal uterine cavity morphology, a small amount of endometrial tissue was scraped for sampling. For solid neoplasms, electro cautery with a capacity of 70-80 W was performed to remove the lesion. For active bleeding, electrocoagulation hemostasis treatment was performed with an electro cautery power of 50-60 W. Morphology and suspected diagnosis results of the hysteroscopy examination were recorded and all specimens were sent to the pathology department for examination.

Histopathological and immunohistochemical analysis

All postoperative specimens from the patients were sent to the pathology department for fixation with 10% formalin solution, paraffin embedding, and routine hematoxylin andeosin (HE) staining. The pathological diagnosis of endometrial tissue was based on the diagnostic criteria of the WHO 2014 [4].

The Immunohistochemistry S-P method was used for the detection of HLA-G in tissues. A mouse antihuman HLA-G antibody [4H84] kit purchased from ABCAM Company (Suite B2304, Cambridge, MA, USA) was used. Specific operating steps were carried out according to the manufacturer's instructions, and the entire process was performed on a fully automated immune machine.

Results: All light yellow, brownish yellow, and dark brown cells in the tissue section were set as positive, and blue cells were seats negative.

Statistical analysis

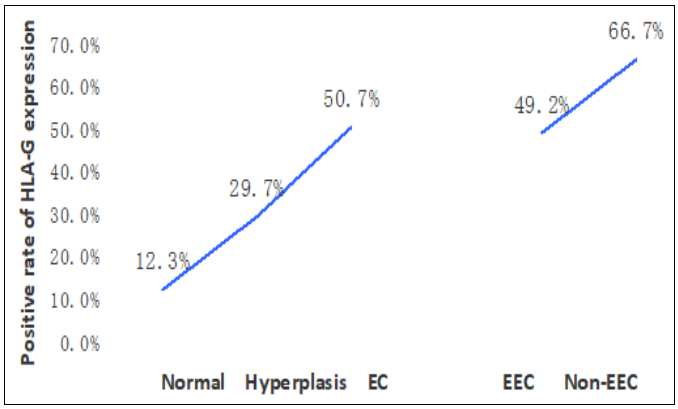
The data were entered into an Excel chart and analyzed using SPSS 22.0. Counting data are expressed in terms of the rate. The rates between the groups were compared using the chi-square test. Statistical significance was set at $p<0.05$.

Results

This study enrolled a total of 67 patients with endometrial cancer (endometrial cancer group), aged 54.3±5.3 years; 37 patients with endometrial hyperplasia (hyperplasia group), aged 47.7±6.3 years; and 65 patients with normal endometrium (normal group), aged 54.1±5.4 years.

Positive rate of HLA-G expression in endometrial tissue of three groups

The positive rates of HLA-G expression in the normal, hyperplasia, and endometrial cancer groups were 12.3%, 29.7%, and 50.7%, respectively, showing an upward trend. In the endometrial cancer group, there were 81 patients had endometrioid adenocarcinoma and 7 patients had non-endometrioid cancer, respectively. The positive rates of HLA-G expression in these tissues were 49.2% and 66.7%, respectively (see Figure 1 for details).



EEC: Endometroid Cancer, Non-EEC: Non-Endometroid Cancer

Fig 1: Positive rate of HLA-G expression in different groups

Comparison of HLA-G expression between different groups

As shown in Table 1, the positive rates of HLA-G expression in the endometrial cancer group and the hyperplasia group were significantly higher than those in the normal group (P<0.05), and the positive rate of HLA-G expression in the endometrial cancer group was significantly higher than that in the hyperplasia group ($p<0.05$).

Table 1: Comparison of HLA-G expression between different groups

	HLA-G +	HLA-G-
Normal group	8	57
Hyperplasia group	11[a]	26
Endometrial cancer group	34[a,b]	33

By $\chi 2$ test: a: Compared to the normal group, $p<0.05$; b: Compared to the hyperplasia group, $p<0.05$

Discussion

In the physiological process of normal pregnancy, how the fetus, as a semi-allogeneic carrier of partial paternal genetic information, successfully survives attacks from the maternal immune system and remains in the body has always been an urgent problem for professionals in fields such as maternal fetal

medicine and immunology. HLA-G, an immune regulatory molecule synthesized by trophoblast cells, is involved in the formation and maintenance of maternal fetal interface immune tolerance, which was first reported in 1990[5]. It functions mainly by inducing natural killer (NK) cell apoptosis through membrane receptors to evade their cell lysis effect In addition, it also acts on antigen-specific regulatory T cells, hindering the proliferation process of normal CD4+T cells, inducing apoptosis of macrophages and activated CD8+T cells, and ultimately reducing maternal immune response activity, while antigen-presenting cells (APCs) in the decidua express high levels of HLA-G, which in turn induces immunoglobulin-like transcripts (ILT4, one of the receptors for HLA-G) on infiltrating immune cells and maintains immune tolerance during pregnancy [2, 6, 7].

In addition to pregnancy, HLA-G is also expressed in the endometrium during normal menstrual cycles [8]. According to previous studies, abnormally high levels of HLA-G expression can be observed in the glandular epithelium of endometriotic lesions in both the endometrium and peritoneum of patients with endometriosis, and similar findings have been observed in both in situ and ectopic endometria of patients with adenomyosis. Therefore, HLA-G may act as a specific antigen that promotes ectopic endometrial cells to evade host immune surveillance [9-12]. In recent years, HLA-G research has expanded to the field of tumors, and it has been found that HLA-G is expressed in tumor cells and the surrounding stroma of various organs, including endometrial cancer [13-15]. Current research suggests that abnormal HLA-G expression is closely related to the immune status of the tumor cell microenvironment, and it's up regulation may inhibit tumor induced immune responses. Therefore, HLA-G may act as an immune checkpoint in cancer, just as it does during pregnancy, protecting tumor cells from immune responses. This may lead to the growth of tumor cells expressing HLA-G. High expression of HLA-G in endometrial cancer cells and patient serum may be associated with prognostic indicators such as clinical stage, pathological tissue type, tumor cell differentiation, myometrial/vascular invasion, and lymph node metastasis. It has the potential to serve as a reference indicator for assisting in the diagnosis and development of treatment strategies for endometrial cancer [16-20]. In this study, the HLA-G expression positivity rate in the endometrial cancer group was significantly higher than that in the normal group, and the HLA-G expression positivity rate in non endometrioid cancer was higher than that in endometrioid cancer. Our previous research group found that HLA-G expression was associated with the depth of uterine muscle infiltration and lymph node metastasis in patients with endometrioid adenocarcinoma [21]. We believe that HLA-G expression is associated with the malignant phenotype and invasion/metastatic ability of endometrial cancer cells, which may contribute to the evaluation of disease severity and prognosis. However, its mechanism of action in immune system regulation in patients requires further research.

Endometrial hyperplasia, a non-physiological and non-invasive abnormal proliferation of the endometrium, is a precursor to endometrial cancer. The pathological manifestation is often diffuse glandular hyperplasia, but it may also be localized, with irregular glandular size and shape and an increased glandular/stromal ratio. Currently, there areno studies, on the relationship between endometrial hyperplasia and HLA-G expression. This study demonstrated that the positivity rate of HLA-G expression in endometrial hyperplasia tissue was between that in normal endometrium and endometrial cancer. Compared to the normal endometrium, HLA-G expression is significantly increased in endometrial hyperplasia, but the mechanism is not yet clear, and further research is needed to reveal this.

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Ethical statement

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any animal studies performed by any of the authors. Informed consent was obtained from all the participants.

Conflict of Interest Statement

The authors have no proprietary, financial, professional, or other personal interest of any nature in any product, service, or company.

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