REVIEWS

Possible therapeutic implication of PD-L1/PD-1 axis in endometrial cancer

Jiezhong Chen[1], Renfu Shao[2], Chen Chen[1]

1. School of Biomedical Sciences, University of Queensland, St Lucia, Australia. 2. GeneCology Research Centre, Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore, Australia.

Correspondence: Dr. Jiezhong Chen or Professor Chen Chen. Address: School of Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia. Email: j.chen4@uq.edu.au or chen.chen@uq.edu.au

Abstract

Endometrial cancer (EC) is a common reproductive system cancer in females and one of the leading causes for cancer-related deaths in women, ranked only after ovarian and cervical cancers. It is classified into two types with type I having better prognosis. At present, surgical removal is the major approach for the treatment of the disease. For those with metastasized cancer, chemotherapy, hormone therapy and radiotherapy are applied. However, the therapeutic efficacy is unsatisfactory and toxicity is severe. Recently, immune system has been recognized as an important factor in both cancer development and treatment. Immunotherapy against PD-1 has been shown to be effective with low side-effects in many cancers. This opens a novel approach for EC treatment as EC has been shown to have increased PD-L1/PD-1 axis. In this review, we summarize the most recent progress in PD-L1/PD-1 axis and prospect that anti PD-L1/PD-1 may be an effective approach for EC treatment.

Key words

1 Introduction

Endometrial cancer (EC) is a common reproductive system cancer in females [1]. EC incidence was estimated to be 32,000 worldwide in 2012 and caused 76,000 deaths in the same year [2]. The common risk factors associated with EC are obesity, diabetes, high blood pressure and excessive estrogen exposure [3-6]. EC incidence is increasing every year in parallel to the increase of obese population [6]. The treatment outcomes of EC depend on cancer types or grades. EC is classified into two types with type I having better prognosis [7]. Type I ECs are low grade endometrioid adenocarcinomas, which are sensitive to hormone therapy as these cancers express both estrogen receptor (ER) and progesterone receptor (PR) [7]. The 5-year survival rate of type I ECs is more than 80% [8]. Type II ECs include high grades of endometrioid adenocarcinoma, serous papillary and clear-cell cancers [9]. Type II ECs are not sensitive to estrogen and progesterone. Type II ECs are poorly differentiated and highly aggressive, resulting in a 5 year survival rate less than 35% [10-13]. Molecular characteristics of high-grade ECs are different from that of low-grade ECs. For example, serous papillary EC has frequent TP53 mutations and decreased ER and PR expression while type I ECs usually have frequent mutations in PTEN, PIK3CA, ARIDIA, Kras and beta-catenin [14, 15]. At present, surgical removal is the main approach for the treatment of ECs in the early stage with high survival rate for low-grade and unmetstasized tumours [16-18]. For those with metastasized cancer, chemotherapy,

hormone therapy and radiotherapy are applied. The therapeutic outcomes, however, are unsatisfactory and toxicity is severe. Thus, new approach is needed to increase treatment efficacy of EC.

Recently, the importance of cancerous immune system is highly recognized. The lack of immunological control is considered as a hallmark for cancer development, i.e. cancer cells are able to evade human immune system during tumour formation [19]. Effective immune system is also necessary for cancer therapy to eliminate cancer cells weakened by chemotherapy or targeted therapy. Thus stimulation of immune system (immunotherapy) has been studied to increase treatment efficacy for EC. There are two types of immune therapies; vaccination and immune checkpoint blockage [20]. Vaccination is based on genetic and epigenetic alterations in cancers, which provide a diverse set of antigens for inducing anti-tumour immunity. At present, sipuleucel-T (Provenge®), which are dendritic cells stimulated by antigen prostatic acid phosphatase, was approved by the Food and Drug Administration for the treatment of metastatic hormone-refractory prostate cancer [21]. However, the effectiveness of vaccination could be reduced by cancer immune escape ability. Cancer can stimulate the inhibition system of immune cells. For example, activation of T-cells requires that antigens are presented by antigen presenting cells (APC) through major histocompatibility complex (MHC) and activation of co-receptor CD28 by cytokines. The activation of T-cells is fine-tuned by inhibition signals through cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or programmed death-1 (PD-1) [20]. Cancer cells can express PD-1 ligand PD-L1 to enhance the inhibition of T-cells. Thus, immunotherapy has been developed to reduce PD-1 to stimulate patients' own immune system and has been shown to be effective with low side-effects in many cancers [22-26]. In melanoma, inhibition of PD-L1/PD-1 caused sustainable tumour-shrinkage effect in 31% patients and was proposed to be used together with targeted therapy against MAPK pathway [26, 27]. The approach also caused 29% and 17% response rate in kidney and lung cancers, respectively [28-30].

Immunotherapy has also been explored in papillary serous EC patients by using patients' dendritic cells which are treated with tumour lysates. The major problem of this method is immunosuppression from cancer cells [31]. Inhibition of PD-L1/PD-1 axis has never been tested in EC. Recent studies show that this axis is increased in EC [32, 33]. This raises a possibility for the treatment of endometrial cancer through inhibition of PD-L1/PD-1 axis. In this review, we summarize the most recent progress in PD-L1/PD-1 axis research and discuss the possible integration of this new approach into EC treatment regime such as combination with chemotherapy, hormone therapy and targeted therapy.

2 PD-L1/PD-1 axis in immune responses

PD-1 was discovered as an immune modulator in 1992, which negatively regulates lymphocyte activity so that the cytotoxic effects of T-cells on self-tissues can be avoided [34]. PD-1 is a 50-55 kDa glycoprotein containing a stalk, a transmembrane domain and an intracellular domain (see Figure 1). PD-1 expresses in many cells including CD4+ and CD8+ T-Cells, B-cells, natural killer cells, macrophages and dendritic cells, indicating its extensive roles in the immune system. PD-1 is able to suppress T-cell proliferation and function to balance activation status, which is stimulated by recognition of antigens through MHC together with co-stimulatory molecules such as CD28 [35]. Loss of PD-1 can lead to over-activation of T-cells and autoimmune diseases. In mice, knockout of PD-1 caused several autoimmune diseases including systemic lupus erythematosus, psoriasis and dilated cardiomyopathy [36, 37]. Blockage of PD-1 by anti-PD-1 antibody in vivo has also been shown to increase experimental autoimmune encephalomyelitis in mice [38].

Two PD-1 ligands are identified including PD-L1 and PD-L2 [35, 39]. PD-L1 and PD-L2 have similar structure but different expression patterns and kinetics. PD-L1 expresses in all cell types and many cancer cells. PD-L2 is only expressed by activated T cells, myeloid dendritic cells and macrophages [40]. Therefore, PD-L1 is more related to cancer immune escape ability. PD-L1 is regulated by many inflammatory factors including IFN-gamma, LPS, GM-CSF, IL-4 and IL-10 through signalling pathways such as MEK and JAK2 [41].

The mechanisms for the inhibitory role of PD-1 in T-cells has been extensively studied. Alterations of signalling pathways by PD-1 are initiated by phosphorylation of SHP-2, which in turn cause phosphorylation of PTEN and decreased PI3K activity [42]. PI3K/Akt plays an important role in T-cell survival, proliferation, migration and function (see Figure 1) [43]. Via Akt PD-1 can decrease the expression of mitochondrial anti-apoptotic molecule Bcl-xL, leading to T-cell apoptosis [44, 45]. Akt downstream target protein mTOR levels are reduced by PD-1, leading to decreased cell size. Cell proliferation is also decreased due to decreased expression of cyclin D and c-myc [46]. PD-1 subunits have been associated with Src members Lck and Lyn [47, 48]. But it is not well studied how these kinases are involved in the regulation of T- and B- cells by PD-1. The inhibitory effect of PD-1 on T-cells can be overcome by co-stimulator CD28 and IL-2, suggesting a regulation balance in activation and inhibition [49].

Figure 1. Negative regulation of PD-1 on lymphocytes.

Note. TCR can activate PI3K/Akt pathway to promote T-cell survival, proliferation, cell growth and CTL ability. PD-1 increases SHP-2 phosphorylation which in turn phosphorylates PTEN, leading to a decrease in PI3K activity.

Abbreviation: TCR: T-cell receptor; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; Akt: protein kinase B; PTEN: Phosphatase and tensin homolog; CTL: Cytotoxic T Lymphocytes.
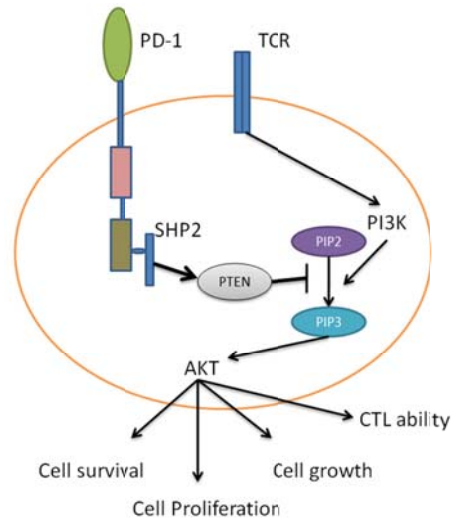
Except expression on cell surface, there are many soluble forms of PD-1 caused by RNA splicing [35, 50, 51]. PD-1 is encoded by several exons. Splicing of RNAs results in various PD-1s including several soluble forms [51]. Soluble PD-1 (sPD-1) has been involved in regulation of PD-L1/PD-1 axis. sPD-1 containing exon-2 can bind to PD-L1, leading to disruption of PD-L1/PD-1 interaction and thus reducing PD-1 inhibitory effect on T-cells.

3 Expression of PD-L1 in EC

The expression of PD-L1 in ECs has been studied. Vanderstraeten et al assessed the expression of PD-L1, PDL2 in EC patient samples [32]. PD-L1 was found at high levels in 92% of ECs while PD-L2 expressed at very low levels in these tumors. In this study, other immune related molecules including B7-H4, indoleamine 2,3-dioxygenase (IDO), galectin-1,galectin-3 and arginase-1 were also examined. B7-H4, which also negatively regulates T-cells, was expressed in 90% of ECs [32, 52]. However, IDO was only expressed in 21% of ECs and expression of glectin-1 and 3 in tumor lysates was not different from benign tissues. Overall, this study suggested that the PD-L1/PD-1 interaction and B7-H4 might be important for ECs to escape from immune responses and could be targets for inhibition for treating EC patients.

EC has been shown to be associated with inflammatory status [53]. Increased CD8 can increase IFN-gamma which can stimulate PD-L1 [54]. It has been shown that serous papillary EC has IFN-gamma receptor and thus PD-L1 is stimulated in these cells [33]. The mechanism is mediated by Stat1 [33]. Indeed, Stat1 is highly elevated in serous papillary EC cells. Stat1 can also up-regulate other genes to increase EC proliferation and metastasis. Another study showed that IFN-gamma increased PD-L1 expression via another signalling pathway PKD2, suggesting multiple signalling pathways are involved in regulation of PD-L1 (see Figure 2) [55].

Hypoxia status is a major feature of cancer. It has been shown that hypoxia upregulates PD-L1 via HIF-1α but not HIF -2α [56]. Barsoum et al showed that hypoxia for 24 hours resulted in an increase in HIF-1α and PD-L1 in human MDA-MB-231 breast cancer cells, human DU145 prostatic carcinoma cells, human Jurkat leukemia T cells and mouse B16-F10 melanoma and 4T1 mammary carcinoma cells [57]. Glyceryl trinitrate (GTN), an agonist of nitric oxide (NO), which is a blocker of HIF-1α accumulation in hypoxic cells, prevented hypoxia-induced PD-L1 expression. Therefore, HIF-1α is a major mediator of hypoxia-induced induction of PD-L1 (see Figure 2) [57]. PD-L1 induced T cell apoptosis was blocked by disruption of PD-L1/PD-1 interaction. This is applicable in EC which associated with hypoxia [58-60]. For example, EC is highly associated with obesity which induces hypoxia [61]. Obesity-induced hypoxia in microenvironments of tumour has been well documented [62, 63]. However, whether this is linked to PD-L1 increase is not studied.
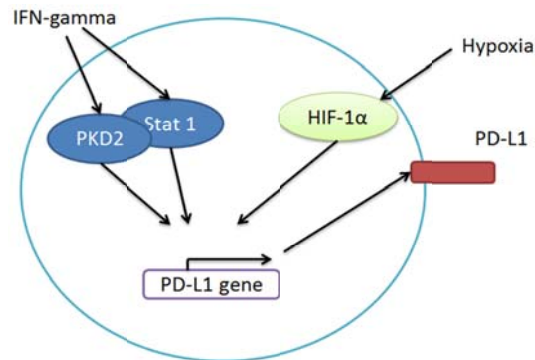

Figure 2. Increased expression of PD-L1 in endometrial cancer cells

IFN-gamma increases PD-L1 via Stat1 and PKD2. Hypoxia upregulates PD-L1 via HIF-1α. Abbreviations: IFN-gamma; interferon gamma, PKD2; protein kinase D isoform 2, Stat1; Signal transducer and transcription activator, HIF-1α; hypoxia-induced factor 1α.

4 Therapeutic implications

As PD-L1/PD-1 axis may play a key role in immune suppression in endometrial cancer, inhibition of this axis could be effective in increasing immune responses against cancer cells. Three approaches may be applied to achieve suppression of PD-L1/PD-1 axis including using anti-PD-1 or anti-PD-L1 antibodies, RNA interference against PD-1 or PD-L1 and supplementation of soluble PD-1.

Anti-PD-1 antibodies include Nivolumab, Pembrolizumab and Pidilizumab. Anti-PD-L1 antibodies are BMS-936559, MPDL3280A and MEDI-4736. These antibodies have been used in clinical trials and showed effectiveness and low toxicities in several solid tumours[64]. Therefore, it is possible to use them for the treatment of ECs. The advantage for the application of these antibodies in ECs is topical usage, which may increase treatment efficacy.

RNA interference has been used to knock out important genes for the treatment of cancer. This method has also been applied for manipulation of PD-1/PD-L1 axis. Li et al (2012) used siRNA to silence PD-L1 and demonstrated that it decreased cancer cell growth in lymphoma [65]. Iwamura et al (2011) showed siRNA effect in lung cancer cell line [66]. Borkner et al (2010) used siRNA against PD-1 to increase CD8 function in melanoma cells [67].

In addition, soluble PD-1 may be used to dilute PD-L1 thus block the interaction of increased PD-L1 with PD-1 on lymphocytes. Over-expression of sPD-1 or sPD-L1 has been shown to increase cell immunity against cancer cells [68]. Song et al (2011) used sPD-1 DNA to enhance CD8 T-cells and DC cells [69]. Qiu et al (2009) used sPD-1 peptide to increase anti-tumour activity [70]. sPD-1 is also effective in hepatoma [71, 72].

5 Combination therapy

Combination of anti-PD-L1/PD-1 and other common therapies in ECs increases treatment efficacy and reduces side-effects reduction. Common chemotherapeutic agents used in ECs included paclitaxel, doxorubicin and carbop-

latin [73]. High-dose of the administration of these drugs has been shown to increase patient survival rate [73]. These drugs, however, cause side-effects including hair loss, low neutrophil levels and gastrointestinal problems. Combination of chemotherapy with anti-PD-L1/PD-1 may reduce the dosage of these drugs and thus decrease side-effects.

Many signalling pathways have been shown to be increased in endometrial cancer such as PI3K and Wnt, VEGF and EGFR [74-77]. These signalling pathways promote cell proliferation and decrease apoptosis and thus are important for cancer maintenance and progression. Activation of signalling pathways could be caused by hormones, viruses and gene mutations [78-82]. Inhibitors have been developed to target key molecules in these pathways for the treatment of EC. Among them, PI3K/Akt pathway is the most studied pathway [77]. Akt can regulate mitochondrial apoptotic pathway to increase EC cell survival and proliferation. Akt down-stream mitochondrial anti-apoptotic protein Bcl-2 is increased in ECs and correlated with disease stages [83]. Cisplatin-induced Bcl-2 increase through Akt activation is associated with drug resistance [84]. Dual PI3K/mTOR inhibitor GDC-0941 and mTOR inhibitor (temsirolimus) are effective to EC cell lines with PIK3CA or PTEN mutations [85]. It is possible to combine targeted therapy with anti-PD-L1/PD-1 therapy. Cancer cells weakened by targeted therapy could be eliminated further by activated T-cells.

6 Conclusions

PD-L1/PD-1 inhibition is effective in many cancers and has been in Phase III clinical trials. Recent studies showed that PD-L1 is also increased in endometrial cancer. This raises an opportunity for the treatment of EC by manipulating this axis. Inhibition of PD-L1/PD-1 will lead to activation of immune cells, especially T-cells, which can produce cytotoxic effect on cancer cells. The anti-PD-L1 /PD-1 therapy may be combined with other therapies such as chemotherapy, targeted therapy, etc.

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