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Review Article

Tissue Infiltrating Immune Cells and Endometrial Cancer Prognosis

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ABSTRACT

The number of patients diagnosed with endometrial cancer surpasses that of any other gynaecological cancer. This disease is usually detected early after disease onset and with current therapy 80 percent of patients with early-stage disease reach a five-year survival milestone. However, patients with advanced or recurrent disease have a grim outcome and the five-year survival rate for these patients is only about 16 percent. In several cancer types there is accumulating evidence that immune cells play a crucial role in the initiation, progression and outcome of disease. In order to provide novel and effective immunotherapeutic treatments for advanced disease endometrial cancer, an understanding of the relevance of immune cells needs to be addressed. This review briefly discusses current knowledge in the area of immune cells and how they may alter the course of endometrial cancer, as well as the implications of these cells for novel therapy and outcome.

Introduction

Endometrial cancer (uterine corpus endometrial cancer; EC) is the predominantly occurring female gynaecologic cancer in the Western world. In the United States it is expected that there will be 66,570 new cases of EC in 2021, and about 12, 940 deaths from this disease [1]. Risk factors for this disease include co-morbidities such as obesity, hypertension and diabetes [2, 3]. Additionally, EC is primarily found in postmenopausal women and in patients who are advanced in age. Conventional and successful treatment for early-stage EC consists of surgery, radiation and/or chemotherapy. This results in more than 80% of patients presented with early-stage EC reaching a 5-year overall survival rate. On the contrary, a small percentage of EC patients are usually diagnosed at the stage of advanced /recurrent disease, and such patients have a five-year survival rate as low as 16% [1]. There is potential for this poor outcome to be improved by the use of conventional therapy combined with targeted therapy or immunotherapy [4].

EC has been classified by different characterization standards, two of which we will discuss here. This disease has been grouped by the International Federation of Gynaecology and Obstetrics (FIGO) as type 1 and type 2. Type 1 EC consists of FIGO grade 1 and grade 2 EC and comprise 80% of EC cases. Type 2 EC consists of 10-20% EC and include FIGO grade 3 more aggressive disease [5, 6]. Like most histological classifications of cancer, type I and type 2 EC may sometimes overlap and this classification system may not be as objective as needed. The Cancer Genome Atlas classification of EC has proved to be a good indicator of EC prognosis [7]. The four molecular groupings of this characterization are: Polymerase ε (POLE)-mutant ultramutated, microsatellite instability-high (MSI-H, hypermutated), copy number low and copy number high [7]. The MSI-H hypermutated group has the highest number of MMR defects and is most responsive to immunotherapeutic agents [7, 8]. The MMR pathway acts to repair single strand breaks, mispairings, as well as small insertions or deletions which occur during DNA replication. Germline MMR deficiencies may be associated with Lynch syndrome, which affects between 2 to 6 percent of endometrial cancer patients; however, most of the MMR pathways deficiencies are due to somatic mutations [9-11].

Much emphasis has been placed on the molecular groupings of EC as prime predictors of disease prognosis. Yet, it is important to understand

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the immune nature of the EC tumor microenvironment (TME) and how this cellular environment can regulate disease, so that we can better determine patients who will be most responsive to novel targeted and immune therapies. The immunosuppressive EC TME consists of tumor cells, immune cells, fibroblasts, cytokines, chemokines and other soluble molecules, cell surface receptors and other molecular targets. Even though the sum total of these parameters predisposes to an immune-suppressive environment, which fosters tumor immune evasion, immune cell subsets in the TME can fill dichotomous roles and induce pro-tumor mechanisms or anti-tumor immunity. This review provides an understanding of the primary cell types in the EC TME and briefly discusses how novel treatments may potentially modulate these cells to improve survival.

Opposing Roles of T Cell Subsets in the Regulation of Endometrial Cancer

T cells play a critical role in most of the cancer types as they are the ultimate effector mechanism in the response to therapy [12]. CD8+ T cells activate tumor cell killing by a cytotoxic T cell (CTL) dependent apoptosis mechanism [13, 14]. The beneficial role of CD4+ and CD8+ T cells in the TME on survival has been documented for several cancer types, however T cells in the EC TME has been less studied than that of most malignancies [15, 16]. In a study of 35 malignant and 23 non-malignant endometrial samples and peripheral blood samples, it was found that the numbers of CD8+ T cells were lower in the endometrium of EC patients than in corresponding controls. These workers also found that CD8 expression was downregulated in EC [17]. It has also been reported that in EC, increased CD8+ T cells at the tumor invasive margin were found in tumors of low grade and was associated with prolonged overall survival (O/S) [18]. Other groups found that the higher CD8+ T cell infiltrate was correlated with prolonged survival in EC patients (Figure 1) [19, 20]. The molecular characterization groups POLE-ultramutated and MSI/ MMR deficiency (MMRd) tumors often have high CD3+ and high cytotoxic CD8+ T cells, and generally have the best outcome of the four EC groups [21-23].

In a meta-analysis of 17 studies, subgroup analysis according to the localization of CD8+ T cells, showed that patients with a high density of CD8+ T cells in the intraepithelial region of the tumor were more likely to have a good progression free survival (PF/S) than those with a high density of CD8+ T cells in the tumor stroma [24]. In the two studies of this meta-analysis where CD45RO+ memory T cells were studied there was a strong correlation between a high density of CD45RO+ T cells and O/S [24]. CD45RO+ T cells are associated with improved survival in several tumors [25, 26]. The purported role of CD8+ T cells on EC outcome is encouraging as it strongly suggests that this cancer may be highly amenable to immunotherapy by the manipulation of CD8+ cytotoxicity potential.

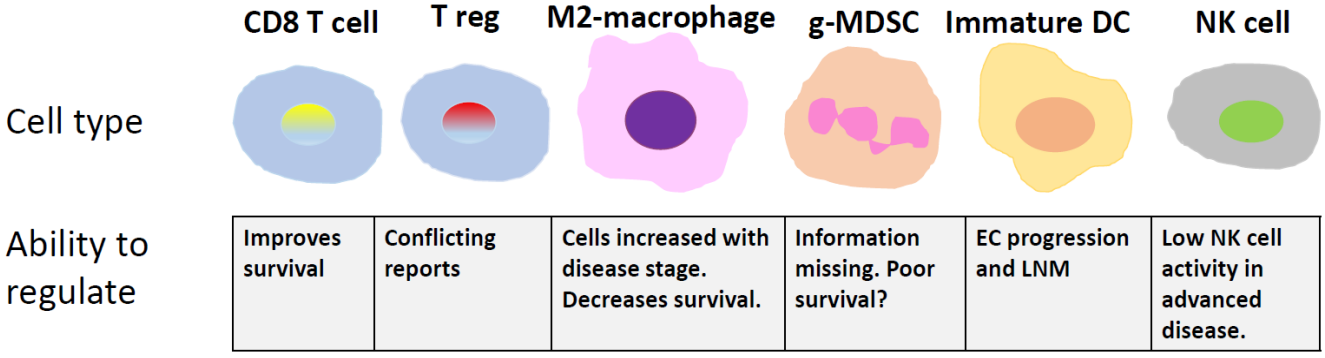

Figure 1: Primary immune modulatory cells in the endometrial cancer tumor microenvironment. The tumor microenvironment of EC consists of a variety of cell types, many of which favour disease progression and poor survival.
EC: Endometrial Cancer; DC: Dendritic Cell; g-MDSC: granulocytic Myeloid Derived Suppressor Cell; LNM: Lymph Node Metastasis; NK cell: Natural Killer cell; T reg cell: T regulatory cell.

The CD4+CD25+FoxP3+ T cell (T regs) subset has often been referred to as cells which reduce anti-tumor responses and contribute to poor survival [27]. In a study cohort of 57 EC patients, CD4+CD25+ T cells were elevated in tumor infiltrating lymphocytes (TILs) in comparison with their presence in peripheral blood lymphocytes (PBLs). This parameter had a strong positive correlation with high tumor grade, myometrium invasion, tumor stage, LNM and in general poor prognosis [28, 29]. Of note, T regs in the EC TME, but not in the PBLs expressed cytotoxic molecules granzyme B and perforin, molecules which are not usually expressed in T regs. Others have shown that T regs from the tumor environment could induce natural killer (NK) and CD8+ T cell death in a granzyme B and perforin-dependent fashion [30]. Granzyme B and perforin are both important for the ability of NK cells and CD8+ T cells to perform the function of killing their targets. Therefore, regulatory T cells may use the perforin-granzyme pathway as a mechanism to suppress the function of immune cells through suppression of NK cells and CD8+ T cells, preventing them from clearing tumors [30]. It is reported that activated murine regulatory T cells suppressed CD4+ CD25+ T effector cells by a granzyme B-dependent mechanism [29, 31]. It remains to be investigated whether the secretion of granzyme B and perforin in the EC TME by T regs is a relevant mechanism of suppressing NK cell and/or CD8+ T cell function in this disease.

In contrast to the above reports, others have published different relationships concerning FoxP3 T regs and EC prognosis. For example, analysis of 4 studies in which FoxP3 T cells were investigated as part of a meta-analysis, concluded that FoxP3 T regs were not significantly associated with EC prognosis [24]. Another study reported that a higher fraction of infiltrating T regs was associated with better survival in EC

patients [20]. This latter finding is similar to some reports in pancreatic cancer, colorectal, bladder and esophageal cancers [20, 32-34]. Indeed, in pancreatic cancer, Zhang and colleagues found that T regs depletion resulted in tumor progression due to a reprogramming of the fibroblast population with loss of tumor-restraining, smooth muscle actin-expressing fibroblasts, as well as other critical immune-suppressive events [34]. Overall, these studies indicate the complexity of understanding the TME of each cancer to predict how different cell subsets may influence survival.

In EC, other critical immune regulators expressed on T cells include immune checkpoint molecules such as cytotoxic T lymphocyte associated protein-4 (CTLA-4) and programmed death-1 (PD-1), among others, which contribute to T cell exhaustion and pro-tumor immune responses [35-39]. As we shall discuss in the subsequent text, therapy blocking the ligation of PD-1 to PD-L1 shows great promise in improving survival predominantly among MMRd and MSI-H EC patients [40, 41].

Messages from Macrophages in the Endometrial Cancer Tumor Microenvironment

Diverse myeloid cell subsets have been characterized in the TME of cancers with macrophages comprising about 10 percent of the cell population. Tumor associated macrophages (TAMs) may consist of the M1 (induce anti-tumor immunity) or M2 (pro-tumor) immunosuppressive polarization macrophages or myeloid derived suppressor cells [42-45]. Th1 cytokines such as IL-12 and IL-18 or Toll-like receptors induce M1 cells. These cells are efficient in killing tumor cells via secreting reactive oxygen or nitrogen species (ROS/ RNS), cytokines IL-6, IL-1β, or tumor necrosis factor-α (TNF-α) [46]. M2 macrophage polarization is favoured by cytokines IL-4, IL-10, IL-13 and transforming growth factor-β (TGF-β), which predispose to tumor development [46].

In EC, like most cancers, tumor associated macrophages (TAMS) generally favour an immunosuppressive M2 pro-tumor environment and more severe disease [47, 48]. CD68+ macrophages have been identified in high density in the epithelial and stromal areas of EC, when compared with benign tumors [49, 50]. In another study, high numbers of CD68+ macrophages in the intra-tumoral border was associated with worse PF/S and O/S than in patients with low macrophage frequency [51]. Furthermore, in a cohort of type I EC patients, a positive correlation was shown between the frequency of TAMS, and a higher tumor grade, more advanced disease and lymph node metastasis (LNM) [52].

Natural suppressor myeloid cells were described in tumor bearing mice as early as 1964 [53, 54]. MDSC inhibit the proliferation of T cells and NK cells, stimulate tumor cell proliferation, cancer metastasis and angiogenesis [55-58]. MDSC are renowned for their involvement in cancer. These cells have been extensively studied in several cancer types and attributed to play a prime role in disease progression and resistance to conventional and novel therapies [58, 59]. Granulocytic MDSC (g-MDSC) have morphological features of granulocytes, and monocytic MDSC (m-MDSC) look like monocytes. In EC patients, however, these cells have not been well studied. In this disease it is reported that g-MDSC (CD11b+CD33+CD15+CD14-) are the primary occurring subset in contrast to m-MDSC (CD11b+CD33+CD14+CD15-) [60, 61]. Many of the intricate networks controlling MDSC immune down-regulatory functions are mediated by arginase-1 (Arg-1), nitric oxide (NO) and signal transducer and activator of transcription-3 (STAT-3) [62-64].

Because TAMs and MDSC in particular are major contributors to cancer progression, targeting of TAMS is an excellent strategy for advanced/ metastatic EC management [55, 60]. There are ongoing clinical trials targeting these cells in malignancies such as lung cancer, head and neck cancer and pancreatic cancer, but clinical trials are urgently needed in this treatment direction for other diseases such as EC [60]. Agents used to inhibit TAMs may decrease macrophage differentiation, prevent TAM recruitment or increase the efficacy of immune checkpoint blockade. CCL2 and CCL5 recruit macrophages, and pharmacologic inhibitors which block these chemokines limit TAM accumulation. Trabectedin reduces TAM in the TME, and Bevacizumab neutralizes vascular endothelial growth factor (VEGF) which is secreted by TAMS [65]. Several clinical trials blocking TAM parameters in single and combination studies in cancers are described elsewhere [60, 66].

Dendritic Cells and Endometrial Cancer

Dendritic cells (DC) are superb antigen presenting cells (APC) which capture, process and present antigen as peptides to the immune system [67]. These cells play critical roles in both health and disease [68, 69]. They are a heterogeneous group of myeloid cells which infiltrate tumors and present tumor derived antigen to antigen-specific T cells, priming these cells and eliciting anti-tumor immunity. However, the anti-tumor function of DC can be diminished by suppressive mechanisms present in the TME [70, 71]. Additionally, immature DC can be recruited to tumors and contribute to tumor progression. The expression of DC markers S100 and HLA-DR were found to correlate with EC progression and LNM [72]. Abnormal DC function is associated with EC progression. Co-stimulatory molecules CD80, CD86 and CD40 were significantly more highly expressed on DC in normal human endometrium, in comparison with the expression of these markers on DC in endometrial adenocarcinoma [73]. In the TME diminished DC co-stimulatory molecules can reduce the potency of immune responses and create an immune-suppressive environment. Several therapeutic inventions have been employed to enhance DC function in various cancer types, but to date the success of this therapy in most cancers needs to be improved to prolong survival in a significant percentage of patients [74].

Impaired Natural Killer Cell Activity in Endometrial Cancer Patients

NK cells are effector cells which bestow cytotoxic anti-tumor immunity and function in anti-viral immune responses. There is generally a reduction in the function and/or numbers of NK cells in most cancers [75, 76]. Investigators found that NK cells were diminished in the EC tumor infiltrate. Tumor resident CD56+CD103+ NK cells expressed higher levels of immune checkpoint inhibitor molecule T cell immunoglobulin and mucin domain-containing protein-3 (TIM-3) (a parameter of exhausted T cells) in comparison with recruited CD56+CD103- NK cells [77]. Further study showed that TIM-3 expression on tumor NK cells from EC patients with lymph node (LN) invasion was higher in these patients with worse disease, than in those

without LN invasion [77]. Differential levels of critical soluble molecules capable of regulating NK cell function were found in healthy and diseased tissue. For example, CCL27, an NK cell chemoattractant molecule, and CCL21 which recruits immune cells to tumors to establish cellular immune responses, were significantly reduced in tumor tissue compared to adjacent healthy tissue. In contrast, the cytokine IL-6, a negative regulator of NK cell function and promoter of tumorigenesis was increased in the tumor. In this same study, cytolytic function was assessed by studying mediators granzyme B, CD107 (degranulation marker) and IFN-γ. Tumor NK cells produced less IFN-γ, TNF-α, granzyme B and CD107, than those of adjacent healthy tissue [77]. These parameters all support the idea of a less efficient NK cell in EC progression.

In patients with stage I EC, NK cell activity was lower with increased nuclear grade [78]. The human leukocyte antigen (HLA) -class 1 system comprises of the HLA-A, -B and -C, as well as the non-classical HLA-E, -F and - G antigens. The receptor NKG2A is selectively expressed on cytotoxic lymphocytes, such as NK cells and CD8+ T cells. NKG2A binds to its ligand HLA-E which is commonly detected in human cancers [79]. In one study, using tissue microarrays (TMAs) of endometrial tumors, investigators reported that upregulation of HLA-E predicted PF/S and O/S in EC patients. The number of NK cells were associated with survival when HLA-E expression was upregulated, however these cells were associated with worse outcome when HLA-E expression was normal. Thus, in this study, it appears that the prognostic benefit of NK cells is influenced by HLA-E expression in EC [80]. Other cell types which are not well investigated but may be important in EC include cells such as neutrophils. In a study of 510 EC patients, it was determined that increased neutrophil to lymphocyte ratio was associated with advanced stage (P= 0.039), higher histological grade (P= 0.005) and lymph node metastasis (P= 0.041) [81].

Regulation of Endometrial Cancer by Cell Surface Receptors and Soluble Molecules

Immune checkpoint molecule PD-1 (CD279) is predominantly expressed on T cells, and programmed death-1 ligand (PD-L1; CD274) on antigen presenting cells (APC), immunosuppressive macrophages and tumor cells. These molecules are generally overexpressed in tumors. Linkage of PD-1 to PD-L1 is a potent immune-suppressive mechanism in the TME. In cancers, ligation of PD-1 to PD-L1 is generally consistent with a TME abundant in MDSC with increased activity, CD8+ T cells with low cytotoxic potential, and other immunosuppressive parameters which favour tumor progression [82-84].

POLE-ultramutated and MSI-H EC tumors have overexpression of programmed death-1 ligand (PD-L1). Additionally, POLE-ultramutated and MSI/ MMR deficiency (MMRd) tumors generally have high CD3+ and high cytotoxic CD8+ T cells, correlating with the best outcome to immune checkpoint inhibitor therapy of the four EC groups described [21, 23, 85-88]. With the appropriate selection of EC patients, blocking the PD-1/ PD-L1 interaction axis with monotherapy treatment may lead to an ORR as high as 57 % [41]. This is a very successful outcome for an immunotherapy regimen. There are now several FDA approved antibodies such as Pembrolizumab targeting the PD-1 axis, many of which are in single and combination therapy clinical trials for endometrial cancer and other malignancies [89-91].

In a cohort of 486 EC patients investigators reported the loss of the classical HLA-class 1 (HLA-A and/or -B/-C) molecules was found in 41.3% patients, and more frequently in high grade EC than in low grade cancers [92]. Inhibition of these HLA-class 1 molecules may be an immune escape mechanism whereby cancer cells evade the immune response.

Several soluble molecules are also associated with EC stage or EC prognosis [93]. Levels of interleukin-6 (IL-6, a pro-inflammatory cytokine) mRNA expression and of IL-6 were significantly elevated in uterine serous papillary carcinoma [94].

Indoleamine 2, 3- dioxygenase (IDO) is an enzyme which catalyzes the metabolism of the amino acid tryptophan in the initial stages of the kynurenine pathway [95]. IDO enhances tumor angiogenesis and spread and downregulates tumor infiltrating lymphocyte (TIL) proliferation and other anti-tumor functions. In EC patients, high levels of IDO were found in patients who had shorter P/FS and O/S, when compared with EC patients with low IDO expression. This parameter of high IDO expression also correlated with low TIL density and low NK cell frequency [96]. Targeting IDO may be a useful strategy for some EC patients.

Cyclooxygenase-2 (COX-2) regulates the synthesis of prostaglandins. In EC patients COX-2 overexpression was positively associated with EC stage [97]. There was an inverse relationship between COX-2 expression and the frequency of CD8+ T cells in tumors. COX-2 levels were a good predictor of EC recurrence [98]. The relevance of COX-2 in EC is further supported by a study which showed that MSI positive EC and high COX-2 expression patients had worse outcome than MSI positive EC patients with low COX-2 expression [99]. The use of COX-2 inhibitors may hold promise as an EC therapy option.

Conclusion

Even though there are a limited number of studies in the literature characterizing the TME of EC patients, it is evident that immune-suppression in EC is multifaceted, and that clues for improved and novel treatments for this disease will result after a better understanding of the immune cells, receptors, soluble molecules and molecular targets in the TME of EC patients. Furthermore, it needs to be determined in preclinical studies which mechanisms of tumor evasion are the most critical to EC progression and poor prognosis so that we can focus primarily on developing treatments which will be most effective.

The modulation of cells of the TME with immunotherapeutic and targeted agents provides a basis for increased use of these agents in combination in clinical practice to improve the dismal survival rate in advanced/metastatic EC. So far, immune checkpoint inhibitors have resulted in good outcome in some subgroups of EC patients. It is however unlikely that any single agent will be highly effective in any large cohort of EC patients. Thus, moving forward, we must consider a combination therapy approach with immune checkpoint inhibitors, vaccines, targeted therapy and conventional treatment agents, to

simultaneously address the diverse nature of immune-suppression in the EC TME. It is hopeful that the outcome of ongoing clinical trials and the design of novel clinical trials with combination treatment regimens in EC patients will significantly advance the field of EC therapy, and soon provide much needed translational options for patients with advanced/metastatic EC, resulting in improved survival.

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Conflicts of Interest

None.

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