Review Article

Future directions in the field of endometrial cancer research: the need to investigate the tumor microenvironment

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Summary

Endometrial cancer is the most commonly diagnosed gynecologic malignancy in the United States. In 2008, approximately 40,000 cases were newly diagnosed. Although the majority of these cancers are curable by means of hysterectomy and radiotherapy, a subset of endometrial tumors exhibits an aggressive phenotype characterized by lymphovascular invasion, high histological grade, and myometrial invasion, leading to poor prognosis. The mechanisms involved in this aggressive transformation are largely unknown, however, interactions between the primary tumor mass and the surrounding stroma likely play a role in this transformation. Despite the fact that research in other common malignancies has elucidated important associations between stromal protein expression and invasion, these mechanisms have been poorly explored in the area of endometrial cancer. In fact, few investigations have been conducted in the area of tumor microenvironment for endometrial tumors. Invasion and metastasis are two primary reasons for treatment failure related to endometrial cancer. Expression of stromal-derived proteins can potentially serve as biomarkers of aggressive disease as well as biomarkers for remission monitoring. In order to study how expression of these proteins relates to the prognosis of endometrial cancer, these proteins need to be explored in large sets of existing data and/or tissue banks. In this paper, we briefly review the role of three stromal related pathways, SDF-1alpha/CXCR4, HGF/c-Met, and VEGF-A in endometrial cancer prognosis as an overview of the literature. We report that the role of SDF-1alpha/CXCR4 and HGF/c-Met in endometrial cancer prognosis remains unclear, whereas the evidence pertaining to VEGF indicates that overexpression is involved in tumor growth and metastasis. Finally, we would like to highlight the need to explore stromal proteins as a potential tool for the detection of aggressive endometrial tumors and explore some of the molecular approaches that can be utilized in the exploration of the tumor environment.

Key words: Microenvironment; Tumor markers; Endometrial carcinoma.

Introduction

An increasing body of research indicates that stroma surrounding cancer cells plays an important role in the development and subsequent behavior of tumors [1]. Evidence shows that the interaction between neoplastic cells and the stroma is a critical factor in solid tumor growth [2]. The tumor microenvironment has been poorly investigated in endometrial cancer, the most common gynecologic malignancy in the US, affecting over 40,000 women annually. The Epidemiology and Genetics Research Program (EGRP) at the National Cancer Institute (NCI) recognized endometrial cancer as an under-investigated cancer at their 2005 workshop. Specifically, this group identified the lack of biomarkers for endometrial cancer development and progression as key challenges in the field [3].

In terms of prognosis, between 75 and 80% of endometrial cancer patients presenting with low-stage disease are successfully treated, however a subset of patients have a biologically aggressive disease characterized by lymphovascular invasion, high histological grade, and myometrial invasion [4]. Patients with these characteristics are at increased risk of recurrence following hysterectomy and signify a therapeutic challenge. The mechanisms that allow an aggressive endometrial cancer phenotype are largely unknown, although recent studies suggest the tumor microenvironment plays a role in this process. Research on the tumor microenvironment has been conducted for other malignancies, such as breast, prostate, and lung, however the endometrial cancer literature has lagged behind in this topic of research [5-7]. For example, the number of articles focusing on breast cancer and stroma returns more than 600 articles in Pubmed, whereas the same search for endometrial cancer produces only 68 articles. In the area of NIH funding, 45 breast cancer grants specifically studying tumor stroma are currently funded while no stroma-specific endometrial cancer projects were identified in the Computer Retrieval of Information on Scientific Projects database (CRISP) [8].

Furthermore, within the endometrial cancer literature, most of the research has focused on cancer initiating mutations, i.e. those involving oncogenic and tumor suppressor genes. Indeed, mutations in PTEN, k-ras, β-catenin, microsatellite instability, HER2/*neu*, and p53 comprise the majority of research related to endometrial cancer biology and prognosis. Using the keywords "endometrial cancer", "oncogene", and "tumor suppressor" yields over 500 journal articles whereas the keywords "endometrial cancer" and "stroma" yielded 68 journal articles. Hence,

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the research related to this field has only recently acknowledged the stromal microenvironment and its contribution to endometrial cancer progression.

The tumor microenvironment includes both non-cellular and cellular components, namely the extracellular matrix (ECM) and stromal cells, respectively [9]. While the ECM provides structural support to the cell, stromal cells, including fibroblasts, endothelial cells, and inflammatory cells comprise a vast network of cells that supply the epithelium with paracrine factors which can enhance the progression of endometrial cancer. As endometrial epithelial cells continually acquire mutations, the ability of the local microenvironment to regulate cell growth becomes disrupted and results in an activated stroma, characterized by increased quantities of collagens, proteoglycans, and glycosaminoglycans [10]. Consequently, the activated stroma recruits additional inflammatory cells and fibroblasts which support the survival and proliferation of carcinoma cells due to abnormal paracrine signaling [11]. The reciprocal relationship between tumor cells and stromal cells allows for the continued growth and invasion of the primary tumor mass. The tumor microenvironment can also limit the access of therapeutics to the tumor, alter drug metabolism, and contribute to the development of drug resistance. Because of their role in all the stages of tumor development, stromal elements represent attractive therapeutic targets. Manipulating host-tumor interactions may be important in preventing or reverting malignant conversion, and re-establishing normal control mechanisms [2].

In this publication, we provide a brief overview of the keys cells of the stroma microenvironment related to endometrial cancer and highlight the importance of investigating this area in the future research studies. Moreover, the role of a few important pathways within each cellular context is presented. Finally, we briefly summarize molecular tools used in studying the stromal microenvironment in endometrial tumors.

Cells of the microenvironment

Fibroblasts

Fibroblastic cells are responsible for the remodeling of the ECM as well as producing paracrine growth factors that control cellular proliferation, survival, and death [12]. Importantly, fibroblasts are the predominant cell type in the stroma [13]. During the carcinogenic process fibroblasts migrate to the neoplastic lesion and begin to proliferate, increase collagen production, and express alpha-smooth muscle actin. These changes are collectively termed the desmoplastic response which is a hallmark of carcinoma-associated fibroblasts (CAF) [14]. Importantly, these changes are often accompanied by the recruitment of inflammatory cells which further promotes the dysregulated programming of tissues [12].

Fibroblastic-derived ligands and their cognate receptors have been studied in endometrial cancer, however the impact of these proteins on prognosis remains unclear. An important ligand/receptor pair is hepatocyte growth factor (HGF) and c-Met. This fibroblast-derived growth factor has mitogenic and motogenic effects on various cell types, yet, few studies have examined the prognostic role of these proteins in endometrial cancer. The association between overexpression of c-Met and poor prognosis has been reported in ovarian, breast, pancreatic, renal cell, and prostate cancers [15-19].

The only study to characterize this pathway in endometrial cancer patients was performed by Wagatsuma and colleagues [20]. Diffuse staining, defined as more than one-third of cancer cells showing positive staining of c-Met was significantly correlated with FIGO Stages III and IV and poorly differentiated histology compared to focal, or less than one-third of cancer cells showing positive c-Met staining. In terms of survival, diffuse c-Met expression was not indicative of worse survival, independent of FIGO stage, grade, myometrial invasion, and microvessel count. The importance of this pathway in other epithelial cancers suggests potential for these proteins to be involved in endometrial cancer progression. As only one study has analyzed this pathway in relation to endometrial cancer prognosis, more studies are needed to clarify this relationship. Additionally, the potential for these proteins to serve as therapeutic targets warrants further investigation into this system.

Inflammatory cells

The link between inflammation and cancer has been suggested frequently by epidemiology, basic sciences, and pathology disciplines. Although normal inflammation is essential to the host, perturbations in this system produce a microenvironment rich in cytokines and growth factors that promote cancer invasion [21, 22]. Inflammatory cells include macrophages, natural killer cells, dendritic cells, mast cells, and lymphocytes. In response to tissue injury, a network of chemical signals initiates the host response which is intended to heal the wounded tissue [22]. The initial step in the cascade of inflammatory events is the recruitment of leukocytes from the venous system, which is regulated by chemokines [22]. Following wound stimulation, chemokines are secreted by many cell types [23]. Leukocytes that express the appropriate receptors for chemokine ligands are attracted to high concentration areas of chemokines [24].

The main chemokines studied in endometrial cancer are SDF-1alpha (CXCL12) and its receptor, CXCR4. Four studies have studied the association between overexpression of SDF-1alpha/CXCR4 and prognosis, with contradictory findings. Using immunohistochemistry (IHC) Tsukamoto *et al*. reported that CXCR4 expression was significantly higher in tumors that invaded deep into the muscle layer of the endometrium compared to those tumors with superficial invasion. Muscular infiltration is an important prognostic factor in endometrial cancer as regional node metastases and distant organ metastases are significantly more likely to occur as the depth of muscular invasion increases [25].

On the contrary, Mizokami and colleagues reported that SDF-1alpha and CXCR4 expression in human endometrial cancer tissues was inversely related to histological grade, another established prognostic factor in endometrial cancer [26]. Similarly, Kodama *et al*. reported CXCR4 expression to be significantly lower in patients with endometrial tumors of high grade. Additionally, survival rates were significantly better in patients with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed in high-grade endometrial tumors. Most recently, Gelmini *et al*. examined protein expression of CXCR4 in 41 patients who underwent hysterectomy for the treatment of endometrial cancer and reported no association between CXCR4 expression and prognosis [25]. Carefully designed immunohistochemical studies with reliable information on tumor pathology are needed to clarify whether SDF-1alpha and CXCR4 are associated with characteristics indicative of advanced endometrial cancer. The potential for these proteins to serve in endometrial cancer therapy is significant.

Endothelial cells

Endothelial cells maintain tissue homeostasis during tissue repair and growth and are activated during carcinogenesis [12]. The formation of new blood vessels from the preexisting vasculature is necessary for invasive growth and metastasis of the primary tumor to distant sites, as blood vessels deliver nutrients and oxygen to tumor cells and provide a means of gas exchange and waste disposal [21, 27]. Endothelial cells secrete a number of soluble proangiogenic factors in response to cytokine production, growth factor secretion, and local conditions such as hypoxia. Cytokines and growth factors that induce angiogenic factor expression in tumor cells include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-1beta (IL-1beta), and tumor necrosis factor-alpha (TNF-alpha) [28].

Vascular endothelial growth factor (VEGF) is among the most studied angiogenic factors in human cancers [29]. VEGF is responsible for increasing permeability of endothelial cells, thereby promoting the degradation of the basement membrane which is usually followed by endothelial cell proliferation [29]. Kamat *et al*. studied the association between VEGF-A, an isomer of the VEGF family, in 111 patients with endometrioid adenocarcinoma (type 1) by means of IHC [30]. High expression of VEGF-A in endometrial tumors was significantly associated with high FIGO stage [30]. Disease specific survival following endometrial cancer treatment was significantly lower in the univariate analyses among patients classified as high VEGF-A expressers; the relative risk of death was 19 times higher for high VEGF-A expressers compared to low expressers. When adjusted for known prognostic factors such as FIGO stage, grade, depth of myometrial invasion, high VEGF-A levels remained a significant prognostic factor of disease specific survival ($p < 0.05$).

Likewise, Hirai *et al*. reported an association between VEGF-A expression and established prognostic factors in postmenopausal endometrial cancer patients [31]. Specifically, positive VEGF-A expression was significantly associated with vascular invasion, myometrial invasion, lymphatic vessel invasion, and lymph node metastasis. Despite being associated with these risk factors, positive VEGF-A expression was not associated with 5-year disease-free survival or 10-year disease-free survival. Finally, in a population-based series of endometrial cancer cases (N = 316) with complete follow-up, Stefansson *et al*. reported that patients with a high expression of VEGF-A had significantly worse survival compared to those with low expression. Additionally, high VEGF-A expression was associated with the serous/clear cell histology, grade 3 tumors, and the presence of tumor necrosis [32]. The cumulative evidence related to VEGF-A in endometrial cancer suggests that this protein plays a significant role in aggressive endometrial cancers.

The need for further studies in the area of estrogen receptors

Exposures that increase circulating levels of estradiol-17β (E2) are known to increase the risk of developing type 1 endometrial tumors [33]. The molecular mechanisms of E2 signaling in endometrial cancer have not been fully clarified; however E2 is known to act with estrogen receptor (ER) to influence uterine growth and development [34, 35].

In addition to E2 stimulation of ER, stromal cells contribute to the activation of ER through two important mechanisms. In the first mechanism, stromal-derived pathways such as SDF-1alpha/CXCR4 and HGF/c-Met activate downstream kinases, notably mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT, which subsequently phosphorylate ER on the transcriptional activation function domain, AF-1 [36-39]. Ligand-independent stimulation of ER by MAPK and PI3K/Akt results in conformational changes in ER, recruitment of co-activators, and activation of target gene transcription, similar to estrogen activation of the receptor [40].

In the second mechanism, stromal cells surrounding the primary tumor cells contribute directly to the biosynthesis of estrogen. Estrogen metabolizing enzymes such aromatase and the 17β-hydroxysteroid dehydrogenases (17β-HSDs) are abundantly expressed in stromal cells and convert androgen precursors and inactive estrogens into the metabolically active E2. Consequently, the intratumoral concentration of E2 increases which may further promote endometrial cancer progression through ER activation [35].

As ER interacts with stromal cells, this emphasizes the need to further investigate the endometrial tumor microenvironment, utilizing a broad spectrum of existing technologies for this research. Moreover, the role of stromal cells in ER-activation may be particularly important for patients with aromatase-positive stromal cells, as these patients have significantly worse survival compared

to aromatase-negative stromal cells [41]. Aromatase inhibitors, although used infrequently for the adjuvant treatment of endometrial cancer, could potentially improve the outcomes for this subpopulation of patients.

Approaches for studying the microenvironment

Popular molecular techniques used for the detection of proteins in tissue and serum include immunohistochemistry (IHC), multianalyte technology, and gene expression profiling. IHC refers to the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. Although IHC is crucial in complementing the information collected by histopathology, lack of reproducibility and standardization of IHC are major barriers to the widespread clinical application of this method in endometrial cancer. Moreover, the semi-quantitative nature of IHC does not lend itself to making informative predictions for survival and prognosis [42].

Another technique used for the detection of molecular abnormalities in cancer patients is multiplexed bead-based immunoassays, which can screen for hundreds of biomarkers simultaneously. In the area of endometrial cancer, this technology is used mainly in the context of serum, although fresh frozen tissue can also be used. At present, no serum biomarkers for the early detection of endometrial cancer or recurrence monitoring are routinely screened. A recent study performed at the University of Pittsburgh Cancer Institute distinguished prolactin as a potential marker for early detection of endometrial cancer based on its ability to differentiate endometrial cancer cases from normal controls [43]. Further studies utilizing multiplexed technology can potentially distinguish biomarkers that can predict recurrence following primary surgery, however the rationale for choosing biomarkers to study should be informed by biologically plausible mechanisms.

Finally, gene expression profiling is a powerful tool for distinguishing genes that are differentially expressed in normal vs neoplastic tissue. Few studies have implemented this approach in endometrial cancer, however this type of profiling can significantly add to the detection of abnormally expressed genes. Salvesen *et al*. recently investigated the genomic profile of aggressive endometrial cancers [44]. Their findings suggest aggressive endometrial cancers share a distinct transcriptional signature which can ultimately illuminate chemotherapy targets.

The major barriers to implementation of any molecular test are cost, availability of samples, and standardized protocols for the analysis of samples. Tissues that are collected and banked in tissue repositories are not routinely checked for many of the markers that could be of great diagnostic and prognostic value. Moreover, collection and banking of blood samples prior to treatment is seldom performed. The lack of standard collection of specimens has hindered the development of screening protocols in endometrial cancer.

Conclusion

Investigating the endometrial cancer microenvironment is very important, as it potentially facilitates the selective survival and growth of transformed cells. Furthermore, an improved understanding of stromal signaling pathways is likely to identify additional therapeutic targets for endometrial cancer, therefore it is critical to study the tumor microenvironment. To our knowledge, few studies have examined the endometrial cancer microenvironment. Factors such as tumor grade, FIGO stage, and histologic type comprise the traditional panel for determining the prognosis of endometrial cancer following hysterectomy, however these clinicopathologic features cannot reliably indicate which therapies are needed to prevent cancer recurrence. Adjuvant chemotherapy following hysterectomy may be necessary to prevent recurrence, but this knowledge relies on ascertaining the molecular abnormalities present in each individual case. Investigating the tumor microenvironment can potentially provide useful information for choosing the appropriate treatment regimen and for improving survival of patients. In endometrial cancer, no routine panel of molecular markers is examined following surgery yet this would greatly inform treatment protocols. Categorizing patients into meaningful risk strata would preclude over-treatment in low-risk patients while aggressive tumors would be treated with individualized therapies.

In this publication, three stromal-related pathways in the context of fibroblast, inflammatory, and endothelial cells have been reviewed. Although this paper is not an exhaustive review of all stromal markers and their significance in endometrial cancer, we have presented three pathways in order to highlight the importance of each pathway in endometrial cancer progression and characterize the approach for studying these proteins in endometrial cancers. The role of HGF and c-Met expression on endometrial cancer prognosis has only been examined in one study which argues for the need future investigations. In the case of SDF-1alpha and CXCR4, the prognostic function of these proteins is unclear; the few studies that have examined this pathway present conflicting data. On the other hand, the evidence pertaining to VEGF indicates that overexpression is involved in tumor growth and metastasis and poorer prognosis in endometrial cancer.

Several challenges in studying endometrial cancer were identified by the EGRP report; namely, lack of endometrial cancer consortia prohibits researchers from examining risk factors and biomarkers in large cohorts of patients [3]. In the area of biomarkers, validation and replication of findings requires large datasets of cases with available tissue specimens. To overcome this limitation, partnering with established cancer consortia, for example, the Breast Cancer Family Registry would guide endometrial cancer investigators to setting up successful collaborative groups. Finally, the report identified a need for an interdisciplinary approach to studying endometrial cancer. Building collaborative networks among epidemiologists, physicians and other medical professionals has

great potential to develop scientifically feasible, well-designed studies that investigate the interplay of various factors involved in endometrial cancer [3]. Finally, future investigations need to consider finding newer cost effective approaches for analyzing large numbers of samples, as high cost of these analyses and the need for highly specialized facilities is one of the key challenges to endometrial cancer investigation.

Summary

Better insight into molecular pathways involved in endometrial cancer may lead to the identification of novel biomarkers and targets for the development of diagnostic and therapeutic approaches for prevention and treatment of endometrial cancer. The tumor microenvironment is an under-studied area that could explain the differences in poor outcome following initial treatment. Obstacles in the area of biomarker development in endometrial cancer include the lack of standard protocols for sample collection at the time of surgery as well as cost. Developing a panel of markers to be immunohistochemically screened at the time of surgery would be advantageous for improving the current survival rates for endometrial cancer survivors. Moreover, developing serum biomarkers will be useful for screening women at risk of endometrial cancer.

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