Abstract: Human ovarian cancer is the most lethal gynecological cancer in the western world, causing enormous physical and mental suffering as well as financial hardship to the patients and their families. However, the etiology of the human ovarian cancer is not well understood. To date, multiple etiological factors, including but not limited to Slit/Robo family, TGF-β family, sex hormones, and angiogenic factors, have been found involved in the pathological process of human ovarian cancer. The involvement of these factors in the human ovarian cancer makes them potential targets for treating human ovarian cancer. Given that human ovarian cancer is highly heterogeneous at both the cellular and molecular levels, the better understanding of actions of these factors and underlying cellular mechanisms in each subtype of human ovarian cancer cells will facilitate the personalized medicine of the lethal disease on the basis of individual characteristics at the cellular and molecular levels.

Key words: Human ovarian cancer; Slit/Robo Family; TGF-β family; Sex hormones; Angiogenesis; AhR, therapy.

Introduction

Ovarian cancer is still the most lethal gynecological cancer in the western world, although great improvements have been made in diagnosis and treatments of human ovarian cancer over the past two decades (1, 2). The high morbidity and mortality of ovarian cancer are primarily due to the fact that majority of ovarian cancer is diagnosed at the advanced stage and chemoresistance of reoccurring cancer cells, as well as the high degree of heterogeneity at the cellular and molecular level of human ovarian cancer (1-3); thereby, understanding of the underlying mechanisms of pathology of human ovarian cancer is essential for future therapies of the lethal disease.

It is believed that almost 90% of human ovarian malignant neoplasms originate from epithelium, with the rest originating from granulosa cells, stroma or germ cells (4). However, etiological factors involved in ovarian cancer is still poorly understand (5). It could be either with sporadic ovarian cancers or familial cases (1). At the molecular and cellular levels, there are multiple genetic and epigenetic abnormalities have been identified in human ovarian cancer, which includes mutations of TP53, BRCA1/2, and PTEN, or promoter methylation of ARHI, SAPK1, etc. (1). In addition to the gene mutation, many other factors such as signaling pathways, sex steroids and peptide growth factors also participate in the pathological process of human ovarian cancer (1, 2, 5). In this review, we mainly focus on discussing the roles of Slit/Robo family, TGF-β family, sex hormones, and angiogenic factors in the development of human ovarian cancer.

Slit/Robo Family

Slit glycoproteins (Slit) and their Roundabout (Robo) receptors, as axon guidance molecules, are important in regulation of many physiological processes including axon guidance, cell proliferation, cell proliferation, cell motility and angiogenesis (6). In the Slit/Robo protein family, there are three members of Slit, Slit1, Slit2, Slit3, as well as four member of Robo, Robo1, Robo2, Robo3, Robo4, those member expressed in different species including elegans, mouse, and human (6). Recently, the Slit/Robo pathway also has been reported to involve in tumorigenesis and cancer progression (6). Slit/Robo functions as a tumor suppressor in most of cancer by suppressing cell invasion and migration. Therefore, stimulation or suppression of the Slit/Robo pathway is associated with the development of progression of various cancer (6-8). For example, mutation of Robo1 has been found in lung and breast tumor cell lines (6, 9, 10). In lung cancer, expression of Slit2 is suppressed as compared with the normal tissue (6).

In regard to its roles in human ovarian cancer, Dai et al. demonstrated that Slit2/3 and Robo1 were immunolocalized primarily in stromal cells in human normal ovaries and in cancer cells in many histotypes of ovarian cancer tissues including disgerminoma, yolk sac tumor, mucinous adenocarcinoma, low grade serous adenocarcinoma, and high grade serous adenocarcinoma (3). Protein expression of Slit2/3 and Robo1/4 was also identified in OVCAR-3 and SKOV-3 cells, two human ovarian cancer cell lines (3). However, recombinant human Slit2 neither significantly affected SKOV-3 cell migration, and OVCAR-3 as well as SKOV-3 cell proliferation, nor induced ERK1/2 and AKT1 phosphorylation in OVCAR-3 and SKOV-3 cells (3). This study indicates that three major members (Slit2/3 and Robo1) of Slit/Robo family are widely expressed in the human normal and malignant ovarian tissues and in OVCAR-3 and SKOV-3 cells (3). However, Slit2/Robo signaling may not play an important role in regulating human ovarian cancer cell proliferation.
and migration (3). In contrast, Dickinson et al. reported that exogenous cortisol reduce expression of Slit2, 3 and Robo1, 2, 4 in normal ovarian surface epithelial cells and PEO-14 cell, a human ovarian cancer cell line, and blocking Slit/Robo activity suppress apoptosis in PEO-14 and SKOV-3 cell lines (6, 11).

**TGF-β family**

Transforming growth factor beta (TGF-β) is a type of cytokine which control cell proliferation, cellular differentiation, etc. (4, 12). There are many members of TGF-β family protein including TGF-β1, TGF-β2, TGF-β3, activin, and inhibitin (12). TGF-β1, TGF-β2, TGF-β3 and their receptors are all present in both human ovarian surface epithelium and ovarian surface stroma (13), and they have been identified as important modulators of neoplastic human ovarian epithelial cell function (4, 12). TGF-β is well known for its roles of inhibiting proliferation of epithelial cells (12). Biswas et al. reported that attenuation of TGF-β signaling promotes tumor progression of a mesenchymal-like mammary tumor cell line in a syngeneic murine model (14). However, the role of TGF-β in human ovarian cancer is controversial. For example, TGF-β increases apoptosis of human ovarian cancer cells in early stage of tumors by suppressing bcl-2, an anti-apoptotic factor, while TGF-β promotes its growth in late stage (4, 12, 15). Activin and inhibitin, another two member of TGF-β protein family and produced locally in the ovary, may play important role in regulating FSH secretion and menstrual cycle possibility through the autocrine/paracrine mechanisms (4, 12). Activin can be secreted by ovarian surface epithelial cells in vitro, while inhibitin can functionally counteract with activin through competing activating receptors (4, 12).

**Sex hormones**

There are a variety type of sex hormones in human, among which many of them have been reported to play important roles in human ovarian cancer (4, 5). Epidemiological data has suggested roles of many sex hormones including but not limited to estrogen, androstenedione, testosterone, and progesterin in the pathogenesis of ovarian cancer (4, 5). Specially, the postmenopausal usage of estrogens as hormone replacement therapies is associated with the increased risk of incidence of human ovarian cancer (5, 16-21). In contrast, ascorbic acid (AA), a naturally compound with antioxidant properties and one form of Vitamin C, has been implicated in preventing and treating cancers (22). The exact roles of estrogen and its active metabolites in the development of ovarian cancer is still controversial (1, 23, 24), the role of AA in cancer prevention and treatment is still not conclusive (23). In one of studies, Li et al. examined the roles of estrogen and its active metabolites in the ovarian cancer progression (23). Li et al. firstly found that the expression of cytochrome P450, family 1, subfamily A (CYP1A1) and B (CYP1B1), polypeptide 1, and catechol-O-methyltransferase (COMT), ERα, and ERβ in most cell lines tested including human ovarian surface epithelial (IOSE-385) and cancer cell lines (OVCA-3, SKOV-3, and OVCA-432) (23). Active metabolites of estradiol 17β (E2β, a steroid and estrogen sex hormone) including 2-hydroxyestradiol (2OHE2), 4-hydroxyestradiol (4OHE2), 2-methoxyestradiol (2ME2), and 4-methoxyestradiol (4ME2). Those active E2β metabolites were synthesized by CYP1A1, CYP1B1, or COMT (23). They further found that treating those cells at physiological concentrations of E2β and its metabolites promoted proliferation of IOSE-385 and OVCAR-3 (23), and antagonist of ER suppressed E2β induced-proliferation of IOSE-385 and OVCAR-3, but not its metabolites induced proliferation of these two cell lines (23). AA could suppress OVCAR-3 cell proliferation induced by serum, but its effects may be counteracted by E2β and its metabolites, suggesting that blockade of estrogen and its metabolites receptor may enhance AA inhibitory effects on growth of ovarian cancer (23).

**Angiogenesis in human ovarian cancer**

Angiogenesis of ovarian cancer is an essential event for propagation of ovarian cancer growth and metastasis (1, 2, 25, 26). Many factors including angiogenic factors such as vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2); interleukins IL-6, IL-8; Lysophosphatidic acid all contribute to angiogenesis in human ovarian cancer (27, 28). Hypoxia plays an essential role in the development and progression of tumor (29-31), solid tumors outgrow beyond several cubic millimeters with aberrant blood vessel formation will result in hypoxia, since the diffusion of oxygen and nutrients from blood vessels is limited to support the outgrowing solid tumors (29-31). Jiang et al. have found that hypoxia further promotes FGF2- and VEGFA-induced endothelial cell growth (32, 33). However, hypoxia-inducible factors (HIF1α), which mediates the effects of hypoxia on the cell, may not modulate FGF2 and VEGFA induced-cell proliferation and migration in endothelial cells under long term low O2 (34). In addition, the role of HIF1α in ovarian cancer is also controversial, the role of HIF1α is related to the malignant degree, FIGO stage, histological grade, metastasis of epithelial ovarian cancer while not associated with histological types (29). Therefore, the hypoxia environment of vascular in ovarian cancer makes it more aggressive (29).

**Treatment of ovarian cancer**

Since human ovarian cancer is characterized by its high degree of heterogeneity at the cellular and molecular levels, understanding individual type of ovarian cancer is critical to develop an efficacious, but low side-effect cancer therapy (1, 2). Currently, the therapy regimen of human ovarian cancer is debulking surgery followed by chemotherapy of platinum-paclitaxel combination (1, 2). However, most of these patients will eventually relapse (2). Another challenge of ovarian cancer treatment is chemoresistance, in which DrrAB (drug transporter) may play an important role, which can transport at least seven types of anti-cancer drugs out of cancer cells (35-38).

Wang et al. found a new potential drug (ITE) for treatment of human epithelial ovarian cancer (39). ITE, an endogenous aryl hydrocarbon receptor (AhR) ligand, is first isolated from porcine lung tissue (40, 41, 42). ITE has no typical dioxin-induced toxicity like cleft palate and hydrenephrosis (43). First, Wang et al. found AhR was widely expressed in various histological subtype of human...
ovarian cancer tissues including disgerminoma, teratoma malignant change, yolk sac tumor, mucinous adenocarcinoma, low grade serous adenocarcinoma, and High grade serous adenocarcinoma and two ovarian cancer cell lines SKOV-3 and OVCAR-3, as well as one normal ovarian epithelial cell line (IOSE-385) (39). Wang et al. found that ITE inhibit the cell proliferation and migration of two ovarian cancer cell lines SKOV-3 and OVCAR-3, while has no effect on the normal ovarian epithelial cells (IOSE-385) (39). These ITE-inhibited growth of ovarian cancer cells is via activation of AhR, since knockdown of AhR block its effects (39). Those characteristics of ITE make it a great potential drug of developing an optimal chemotherapy regimen with high efficient and low side effects.

In addition to ITE, another AhR ligand TCDD also has been reported to suppress the growth of ovarian cancer cell lines SKOV-3 and OVCAR-3, while it has no effect on the growth of normal ovarian epithelial cells (IOSE-385) (44). The different response of the two ovarian cancer cell line may due to the fact that OVCAR-3, but not SKOV-3 expresses CA125 (a major ovarian cancer biomarker) and mutant of ERα in SKOV3, although both cell lines are p53 defective (1, 2, 5). Those findings indicates that although TCDD is an environmental dioxin (45), but in regard to ovarian cancer, it inhibits the growth of ovarian cancer while has no adverse effect on the normal ovarian epithelial cells. Following TCDD treatment, protein kinase C-delta (PKCδ) is activated in ovarian surface epithelial cancer cells, suggesting a potential intracellular role for PKCδ as an effector molecule for TCDD-mediated biological events in this ovarian cancer (46). PKCδ and its downstream target have been identified as critical factors mediating vascular injury response, such as intimal hyperplasia and aneurysm (47-51). Ren and colleagues reported that activated PKCδ increases production of pro-inflammatory chemokines through cytosolic interaction with the NF-κB subunit p65 (50). Therefore, it raises the possibility that PKCδ-upregulated chemokines are involved in the inflammatory process and mediate immune responses that inhibit tumor progression.

Antioangiogenic cancer therapies in early-phase ovarian cancer showed efficacy in clinical trials (2). Bevacizumab, humanized anti-VEGF monodal antibody, has been reported to have significant effect in treatment of human ovarian cancer (52).

Concluding Remarks

Although there are already huge improvements in the surgical techniques and chemotherapy in the treatment of human ovarian cancer, it is still the most lethal reproductive cancer in the western world, which render numerous women to physical and mental suffering as well as financial hardship. Multiple etiological factors, including but not limiting to Slit/Robo family, TNF-β family, sex hormones, and angiogenic factor, and the characteristics of high degrees of heterogeneity at both the cellular and molecular level impose major challenges to the treatment of this cancer. Therefore, the future treatment strategies of human ovarian cancer may focus on the personalized medicine on the basis of individual characteristics at cellular and molecular levels.

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