Anti-Metastatic Gene Therapy in Patients with Advanced Epithelial Ovarian Cancer (EOC)

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Abstract

Ovarian cancer is the foremost cause of death from gynecological cancer in the developed world. In the USA 27,000 new cases of ovarian cancer and 14,000 deaths are reported in 2010. About 80% of patients with ovarian cancer present with metastatic disease. The overall 5-year survival rate for women with cancer is 30%. The epithelial cells of the ovary constitute 1% of the total ovarian mass but constitute 90% of the ovarian neoplasms. Epithelial ovarian cancer (EOC) spreads initially by direct extensions into adjacent organs, especially the fallopian tubes, uterus and contralateral adnexa and occasionally the rectum, bladder and pelvic side wall. After direct extension, epithelial ovarian cancer frequently disseminates via transcocelomic route, with 70% of patients having peritoneal metastases at staging laparotomy. The correlation between molecular profiles and metastatic spread varies depending on tumor type and metastatic site and is combination of 2 models. First, tumors are genetically heterogeneous and that metastases arise from clones with a genetically acquired metastatic phenotype and that the clonal genotype determines the final site of metastases. The second model is that metastatic cells are not a genetically primary tumor, instead they arise as stochastic event, with a low but finite probability from tumor cell clones distinct from the primary tumor. Several cofactors, such as MMP-2/-9 inhibitor, TNF, lymphotoxin a, Fas Ligand Fas L, APO3L, TRAIL, interleukin -8 and P38 MAPK regulating ovarian cancer cells attachment to omentum and /or peritoneum have been identified and would have noticeable clinical inhibition of the metastatic process, by enabling the identification of cellular or molecular targets that therapeutically viable. That would be able to block the steps necessary for ovarian cancer metastasis within the peritoneal cavity.

Introduction

Ovarian cancer is the most common cause of death from gynecological cancer in the developed world. In the USA 27,000 new cases of ovarian cancer and 14,000 deaths are reported in 2010. About 80% of patients with ovarian cancer present with metastatic disease. The mortality rate for ovarian cancer is high despite intensive debulking surgery and chemotherapy. It is, therefore, imperative that research into ovarian cancer focuses on better treatments for all stages of this disease and identifying markers of disease progression. The initial Epithelial Ovarian Cancer (EOC) dissemination is intra-abdominal involving local invasion of pelvic and abdominal organs [1]. Malignant cells of the primary tumor are shed into the peritoneal cavity where they are disseminated throughout the abdominal cavity. These malignant cells often aggregate and form spheroid-like structures [2]. These structures represent an impediment to efficient treatment of late stage EOC where the vascular-independent growth restrictions may restrict the ability of therapies to eradicate the disease. It has been shown that spheroids, are capable of tumorgenesis in vivo and has a diminished response to chemotherapeutic agents in vitro [3,4].

Progression of Ovarian Cancer

It is accepted that there are three main cellular origins of ovarian cancer, the epithelium (90%), germ cells (5%) and stromal cells (5%) (OvCa Patient information, 2007, http://www.ovca.org.au). Early in progression of ovarian cancer, the subtypes are differentiated, in both histologic presentation and molecular profile. There are three subtypes of EOC, Serous carcinomas, accounting for 50–65% of EOC, while endometroid and mucinous carcinomas account for 8–19% and 3–11% respectively [5,6]. The Federation Internationale de Gynecologie et d’Obstetrique (FIGO) has divided clinical progression of ovarian cancer into 4 stages. In early stage ovarian cancer (FIGO stage I) the disease is confined to one or both of the ovaries. In stage II the disease has begun spreading, with localized extensions into the adjacent pelvic tissues and organs. In stage III the tumor has spread to the organs or tissues in the upper abdominal cavity and/or involves the lymph nodes, while at the final stage (FIGO stage IV) metastatic tumor is present at distant, extra-peritoneal sites [7,8]. The primary mode of distant metastasis involves the shedding of cells from the primary tumor, into the abdominal cavity, followed by implantation on the mesothelial lining of the peritoneum.

A Model of Ovarian Cancer Metastasis

A current model for epithelial ovarian cancer metastasis is illustrated in Figure 1 [9]. The development of peritoneal metastases in EOC is regulated, by the ability of shed ovarian tumor cells to survive and subsequently attach to and infiltrate the mesothelial lining of the abdominal cavity. Cells shed then settle onto the surface of the peritoneum where disaggregation and metastatic outgrowth may occur. Successful metastasis requires the remodeling of cell–cell adhesion molecules (cadherins) as the spheroids disaggregate on the mesothelium of the peritoneum, while cell-extracellular matrix...
(ECM) adhesion molecules (integrins) anchor the spheroid to the sub-mesothelial ECM [10]. Invasion of tissues and/or organs requires additional protease activation (matrix metalloproteases, MMPs) for the degradation of the basement membrane and establishment of a metastatic lesion.

**Molecular Factors Involved in Metastatic Ovarian Cancer Process and its Potential Therapeutic Value**

**MMPs**

Lewis (y) antigen is a difucosylated oligosaccharide containing two fucoses and is carried by glycoconjugates (glycoproteins and glycolipids) on the plasma membrane [11]. Lewis (y) antigen is expressed during embryogenesis. Its expression in adults is restricted to the surface of granulocytes and epithelium. However, overexpression of Lewis (y) antigen is frequently found in human cancers and has been shown to be associated with poor prognosis [12]. Studies on α1,2-FUT transfected ovarian cancer cell line RMG-1-hFUT have shown that Lewis (y) antigen plays a positive role in the process of invasion and metastasis of ovarian cancer cells [13]. Metastasis is a complex process consisting of a series of steps, including the organized breakdown of the extracellular matrix (ECM) by matrix metalloproteases (MMPs) [14]. MMPs belong to a family of structurally related endopeptidases capable of degrading all ECM components. Each ECM element is cleaved by a specific MMP or MMP group [15]. MMP-2 and MMP-9 play vital roles in the degradation of the ECM and high level expression of MMP-2 and MMP-9 has been frequently correlated with increased tumor invasion and poor prognosis in various types of human cancer [16]. The activity of MMPs is inhibited by specific tissue inhibitors of MMPs known as TIMPs. There are four different TIMPs (TIMP-1, -2, -3 and -4) have been identified in humans. TIMP-1 is more specific for MMP-9 and TIMP-2 regulates the activity of MMP-2 and MMP-9 in a concentration dependent fashion and it is accepted that

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**Figure 1:** A working model of ovarian cancer progression. During ovarian cancer progression, epithelial ovarian cancer cells growing on the surface of the ovary undergo EMT to attain motile functions required for cancer metastasis. Rupture of the ovarian tumor result in shedding of tumor cells into the peritoneum where they survive as cellular aggregates/spheroids. These spheroids undergo changes into invasive mesenchymal phenotype to sustain survival and motility. Cancer spheroids and the surrounding mesothelial and infiltrating blood cells secrete cytokines and growth factors (e.g., VEGF, TNF-α, IL-6, IL-8, bFGF, lysophosphatidic acid, etc.) in the form of as cites in the peritoneum. The secreted factors form an autocrine/paracrine loop that initiate and sustain EMT to facilitate the invasiveness of carcinoma spheroids until they find a secondary attachment site. Growth on the omentum however, requires MET to sustain cancer growth.

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the degradation of ECM, increased invasion capacity and metastatic potential of tumor cells results from the imbalance between the activities of these proteases and their inhibitors. It was demonstrated that RMG-1-hFUT cells exhibited increased proliferation, invasion capacity and showed significant tolerance to common chemotherapy drugs for ovarian cancer, such as carboplatin, 5-fluorouracil and taxol [17]. There is a strong evidence to suggest that the epidermal growth factor receptor pathway and PI3K/Akt signal transduction pathway are key regulators of TIMP/MMP balance [18].

**E1A CR2- deleted adenovirus d/922-947**

Oncolytic viruses are shown to be novel treatments for cancer. These viruses multiply within cancer cells and cause death with release of mature viral particles. E1A CR2-deleted adenovirus d/922-947 can replicate in human ovarian cancer cells and has greater efficacy than E1A wild-type adenoviruses and the E1B-55K deletion mutant dl520 (Onyx-015) [19]. Significant inflammatory responses have been reported in gene-therapy trials involving replicating adenoviral vectors. These responses are characterized by the induction of several cytokines and chemokines, such as tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6), IL-1a and b. Multiple signaling pathways are activated by adenoviruses upon binding to Cow-sackie Adenovirus Receptor (CAR) and interactions with a B3 integrins. These include NF-kB and MAP kinases ERK and p38 [22], which promote cytokine and chemokine induction and inhibition of NF-kB may increase the oncolytic efficacy [23]. Early production of TNF-a is prominent in the initiation of a complex cascade involving cytokines, chemokines and endothelial adhesions, which results in the activation of neutrophils, macrophages and lymphocytes at sites of damage and infection. Autocrine production of TNF-a by ovarian carcinoma cells stimulates a network of other cytokines, angiogenic factors and chemokines that may act in an autocrine/paracrine manner to promote peri toneal growth and spread [24]. Recent clinical trials have suggested that inhibition of TNF-a may have therapeutic potential in ovarian cancer [25]. It has been shown that TNF-a expression is induced in ovarian carcinoma cells following adeno viral infection and that its suppression augments cytotoxicity.

This results from a decrease in expression of cellular inhibitor of apoptosis-1 (cIAP1) and cellular inhibitor of apoptosis-2 (cIAP2) with a consequent increase in apoptosis and adenoviruses induce expression of TNF-a in ovarian cancer cells and that TNF-a act as a survival factor in infected cells to decrease viral efficacy. Also, it was demonstrated that induction of IL-6 and IL-8 generates and sustains other inflammatory mediators [26]. In addition, the suppression of TNF-a using RNA interference or inhibitory antibodies augmented oncolytic activity both in vitro and in vivo. Moreover, it has been reported that adenoviral mutants induce a novel mode of programmed cell death in ovarian cancer cells with evidence of significant apoptosis [27]. It was noted, that some of the inflammatory responses in gene-therapy clinical trials have been severe, so inhibition of TNF-a may reduce systemic toxicity and allow greater doses to be delivered.

**APO3L**

Apoptosis is a process playing an essential role in controlling cell differentiation, tissue homeostasis and eliminating cells that undergo uncontrolled cellular proliferation [28,29]. Caspases, a family of cysteine proteinases, are key elements in apoptosis and, at least 10 capsizes have been reported [30], each encoding sequences essential for proteolytic activity and induction of apoptosis. Activation of the caspase cascade is triggered by various death signals, subsequently leading to apoptotic cell death. Cell death receptors such as APO-1/ FAS or functional tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors exhibit an intracellular sequence denoted as death domain (DD). Fas-associated death domain protein (FADD) is recruited to the death receptors by homotypic DD interaction. Ligation of death receptors by their cognate ligands leads to recruitment of FADD, leading to activation of caspase-8, resulting in an assembly of the death-inducing signaling complex (DISC) initiating the apoptotic cascade [31]. The apoptosis signaling pathway induced by death receptors is regulated by inhibitor proteins such as the cellular Fas-associated death domain-like-interleukin-1β-converting enzyme (FLICE)-like inhibitory protein (c-FLIP). c-FLIP interferes with activation of pro-caspase-8 proximal to the plasma membrane [32]. The removal of c-FLIP, from TRAIL-resistant OC cells inhibits tumor growth in vitro and in vivo. It has been shown that c-FLIP prevents NK cells and CTLs-mediated immunosurveillance, resulting in aggressive tumor growth in vivo [33]. The expression level of c-FLIP, was found to be upregulated in various ovarian tumors (endometrioid, serous, mucinous and clear cell carcinomas) when compared to normal ovarian tissue samples [34,35]. It was noted that in the presence of soluble TRAIL, c-FLIP depletion completely inhibited the migratory behavior of ovarian cancer cells and inhibited the invasive behavior of ovarian cancer cells following adenoviral infection and that its suppression augments cytotoxicity. This results from a decrease in expression of cellular inhibitor of apoptosis-1 (cIAP1) and cellular inhibitor of apoptosis-2 (cIAP2) with a consequent increase in apoptosis and adenoviruses induce expression of TNF-a in ovarian cancer cells and that TNF-a act as a survival factor in infected cells to decrease viral efficacy. Also, it was demonstrated that induction of IL-6 and IL-8 generates and sustains other inflammatory mediators [26]. In addition, the suppression of TNF-a using RNA interference or inhibitory antibodies augmented oncolytic activity both in vitro and in vivo. Moreover, it has been reported that adenoviral mutants induce a novel mode of programmed cell death in ovarian cancer cells with evidence of significant apoptosis [27]. It was noted, that some of the inflammatory responses in gene-therapy clinical trials have been severe, so inhibition of TNF-a may reduce systemic toxicity and allow greater doses to be delivered.

**Interleukin-8 (IL8)**

Chronic neurobehavioral stress can promote tumor growth [39] secondary to sustained activation of the sympathetic nervous system (SNS). This results in elevated levels of catecholamines, especially norepinephrine (NE) and epinephrine (Epi). These catecholamines have been shown to increase tumor cell proliferation [40], adhesion [41], migration [42] and invasion [43]. Among the genes showing the greatest up-regulation in this process is interleukin-8 (IL8). IL8 is an 8-kDa molecule, which is a potent proangiogenic cytokine. It is highly expressed in the majority of human cancers, including ovarian carcinoma [44]. IL8 has also been shown to promote angiogenesis, tumor growth and metastasis in murine carcinoma models [45], including ovarian carcinoma [46]. Stress hormones NE and Epi can enhance IL8 expression and thereby mediate effects of stress on growth and metastasis of ovarian cancer. Chronic stress has been shown to increase NE and Epi levels leading to augmented tumor growth and metastasis [39]. High levels of these catecholamines cause increased production of IL8, which is a potent proangiogenic cytokine overexpressed in most human cancers, including ovarian carcinoma [44]. More recently, IL8 gene silencing with liposomal siRNA incorporated in DOPC has
shown decreased tumor growth and angiogenesis in ovarian cancer. FosB is a member of the Fos gene family (Fos, FosB, FosL1 and FosL2). The Fos family of proteins form heterodimers with Jun family members and make up a variety of AP1 complexes. These AP1 complexes bind to the 12-O-tetradecanoylphorbol-13-acetate-response elements in the promoter and enhancer regions of their target genes [46], thus critically regulating many different cellular and biological processes [47]. IL8 has been shown to modulate matrix metalloproteinase expression in tumor and endothelial cells, thereby regulating angiogenic activity [48]. These findings point to a prominent role for increased sympathetic nervous system activity in promoting tumor growth and metastasis via FosB-mediated production of IL8. Specific interventions targeting the pathways involved in neuroendocrine function may represent novel strategies for helping individuals counteract the effects of stress on tumor progression.

Ras homologous (Rho) GTPases

It is a branch of the Ras superfamily of small GTPases. Rac1, Cdc42 and RhoA are well characterized [49]. Rho GTPases are important regulators of actin cytoskeleton organization, cell polarity and migration, cell cycle progression and gene expression. Rho GTPases are not directly mutated, but their functions are deregulated indirectly through the altered expression of Rho regulatory proteins. One of the best characterized mechanisms involve inappropriate activation of guanine nucleotide exchange factors (RhoGEFs) that promote formation of the activated, GTP-bound form of Rho GTPases and loss of expression of GTPase activating proteins (RhoGAPs) that accelerate GTP hydrolysis and formation of inactive GDP-bound Rho GTPases. The involvement of a third class of regulators, Rho GDP dissociation inhibitors (RhoGDI)s as a third class regulators are considered as possible therapeutic targets for cancer therapy [50]. The three human RhoGDI isoforms, RhoGDI1, RhoGDI2 and RhoGDI3, are considered to function as negative regulators of Rho GTPase activity. RhoGDI1 protein expression was identified via proteomic analyses to be overexpressed in three invasive ovarian tumors when compared with three low malignant potential ovarian tumors [51] consistent with a role for RhoGDI1 in promotion of invasion and metastasis. Jones and Tapper et al. [52,53] in a gene array study of six serous cystadenocarcinoma and one cystadenoma, found upregulation of RHOGDI2 transcription was associated with carcinoma when compared with the benign adenoma tissue. In another microarray study to identify gene expression changes associated with paclitaxel resistance in ovarian cancers, they found that RhoGD12 overexpression correlated with resistance. Their immunohistochemical analyses of serous ovarian cancer tissues from patients who received paclitaxel-based chemotherapy found that RhoGD12 protein overexpression was not correlated with stage or histological grade, but was observed more frequently in non-responders (four of five cases) than in responders (two of 16 cases). They concluded that RhoGD12 expression may be a predictive marker of paclitaxel resistance not only in paclitaxel-resistant cell lines, but also in patient samples. It has been demonstrated that RhoGD12 protein expression varied in a panel ovarian cancer cell lines and ovarian tumors and additionally, was elevated in Ras-transformed human ovarian surface epithelial cells, suggesting that RhoGD12 overexpression may promote tumor growth. RhoGD12 was significantly overexpressed in high-grade compared with low-grade ovarian cancers. It was noted that interfering RNA suppression of RhoGD12 in the HeyA8 ovarian carcinoma cell line increased Matrigel invasion and increased lung colonization in a tail-vein lung metastatic assay. Utilizing IHC analyses found that RhoGD12 was overexpressed in high-grade compared with low-grade ovarian cancers, which RhoGD12 expression correlated with histological subtype of cancer and no statistically significant association with survival. It has been suggested that RhoGD12 may function as a tumor suppressor in HeyA8 ovarian cancer cells and RhoGD12 expression is higher in more advanced ovarian cancer patient tissues [53]. RhoGD12 may function as an invasion and metastasis suppressor. It is possible to assume that RhoGD12 expression may still provide a marker for drug response. RhoGD12 function as a negative regulator of Rho GTPase function and appears to preferentially regulate Rac1 [54]. P38 and JNK activation has been shown to inhibit ovarian cancer metastasis [55]; this suggests that the reduced activity of these Rac-associated signaling pathways contributes to the enhanced growth properties of RhoGD12-depleted HeyA8 cells. In addition, the functions of RhoGD12 in cancer may also involve functions independent of Rho GTPase regulation.

MAPK

Patients with late-stage ovarian cancer often incur peritoneal invasion of tumor cells, contributing directly to their death. Because of the relatively confined space it occupies and its ready accessibility for therapeutic intervention, the peritoneal cavity also represents a model for investigatory gene therapy. Human ovarian cancer cell line Hey8 to establish ovarian tumor xenografts in the peritoneal cavity has been used for evaluation of potentially useful oncolytic viruses [56]. That lead to the formation of (1 or 2) tumor nodules in the peritoneal cavity which led to widespread dissemination of relatively small tumor nodules across the pelvic and abdominal surfaces. Two injections of Fsu-On-H2 at a moderate dose into the peritoneal cavity produced a clear antitumor effect, leading to complete eradication of metastases in over 87% of the animals. These results indicate that Fsu-On-H2 is an effective oncolytic agent for the treatment of metastatic human ovarian cancer in the peritoneal cavity [57]. It appears that HSV-2 is probably inherently more fusogenic than HSV-1 in this human ovarian cancer cell line and that the mutagenesis procedure for the deletion of the PK domain enhanced the fusogenic property of the virus. It has been shown that incorporation of cell membrane fusion activity into an oncolytic virus can significantly enhance its antitumor effect [58]. This potent antitumor activity of Fsu-On-H2 in this model is due to the presence of a fusogenic property related to deletion of the PK domain from the parental HSV-2 virus. The replication capacity of Fsu-On-H2 depends on the activation status of the Ras signaling pathway. Ras gene mutations in ovarian cancer cells is frequently activated through other mechanisms, such as phosphoinositide-3 kinase or protein kinase B upregulation, [59] making ovarian cancer a target for Fsu-On-H2 treatment. By exploiting both Ras signaling and cell-cycle status for its oncolytic activity, Fsu-On-H2 would be expected to show enhanced tumor cell specificity and thus increased safety in patients with ovarian cancer. All the above findings would indicate that Fsu-On-H2 is severely attenuated and likely retains the safety profile of a conventional oncolytic HSV.

MMK4

The mitogen-activated protein (MAP) kinase signaling pathways are important mediators of cellular responses to extracellular signals that include growth factors, hormones, cytokines and environmental stresses. These pathways feature a triple kinase cascade comprised of the MAP kinase which is phosphorylated and activated by a MAP kinase kinase (MKK), which itself is phosphorylated and activated by a MAP kinase kinase kinase (MKKK). In mammals, four distinct MAP kinase pathways have been identified that lead to the activation of the extracellular signal-regulated kinase (ERK), ERK5, c-Jun N-terminal kinases 1 and 2 (JNK1/2) and p38 mitogen-activated protein kinase (p38 MAPK). These pathways are involved in a wide range of cellular processes, including cell growth, differentiation, apoptosis, and stress responses. In cancer, these pathways are often dysregulated, leading to uncontrolled cell proliferation and survival. Understanding the regulation and function of these pathways in cancer is essential for developing targeted therapeutic strategies.

References


