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

Samir A. Farghaly*

Joan and Sanford I. Weill Medical College/ The Graduate School of Medical Sciences and The New York Presbyterian Hospital – Weill Cornell Medical Center, Cornell University, USA

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 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].

Introduction

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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 endometrioid 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

*Corresponding author: Samir A. Farghaly, MD, PhD, Joan and Sanford I. Weill Medical College/ The Graduate School of Medical Sciences and The New York Presbyterian Hospital – Weill Cornell Medical Center, Cornell University, New York, USA; E-mail: samirfarghaly@yahoo.com

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(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 stable 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 metalloprotease (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

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 ascites 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].

EIA 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. EIA CR2-deleted adenovirus d/922-947 can replicate in human ovarian cancer cells and has greater efficacy than EIA wild-type adenoviruses and the E1B-55K deletion mutant d/55K (Onyx-015) [19]. Significant inflammatory responses have been reported in gene-therapy trials involving replicating [20] and nonreplicating (Onyx-015) [19]. Significant inflammatory responses have been reported in gene-therapy trials involving replicating [20] and nonreplicating (Onyx-015) [19].

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 induced the invasive behavior in vitro. This observation indicates that c-FLIP depletion induces apoptosis in OC cells, which might be the reason that these cells do not go through the peritoneal cavity and therefore inhibit their invasive behavior. Additionally, c-FLIP has been shown to regulate ERK and NF-κB pathways [36], which could explain why c-FLIP induces OC invasion. Notably, OC in humans metastasizes in most cases into the free peritoneal cavity and is restricted to the peritoneal cavity. Several small molecules have been shown to inhibit c-FLIP, expression and, sensitize resistant tumor cells to apoptosis. These molecules include actinomycin D, cycloheximide, Trichostatin A, as well as inhibitors of several kinases (MEK1/2, PKC and PI3K) [37]. Notably, the crystal structure for v-FLIP has been developed [38]. Soluble TRAIL or agonistic antibodies to its functional receptors (DR4 or DR5) might be a potential target for metastatic ovarian cancer therapy.

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 and invasion [42]. 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 (RhoGDIs) 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 RhoGD21 overexpression correlated with resistance. Their immunohistochemical analyses of serous ovarian cancer tissues from patients who received paclitaxel-based chemotherapy found that RhoGD21 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 RhoGD21 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 RhoGD21 protein expression varied in a panel ovarian carcinoma cell lines and ovarian tumors and additionally, was elevated in Ras-transformed human ovarian surface epithelial cells, suggesting that RhoGD21 overexpression may promote tumor growth. RhoGD21 was significantly overexpressed in high-grade compared with low-grade ovarian cancers. It was noted that interfering RNA suppression of RhoGD21 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 RhoGD21 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 functions as a negative regulator of Rho GTPTase 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 GTPTase 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 investigative 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 FusOn-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 FusOn-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 FusOn-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 FusOn-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 FusOn-H2 treatment. By exploiting both Ras signaling and cell-cycle status for its oncolytic activity, FusOn-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 FusOn-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
kinase (JNK) and p38 [60]. The JNK and p38 MAP kinases are referred to as stress-activated MAP kinases. They are activated in response to a variety of environmental stresses and pro-inflammatory cytokines and also play important roles in cancer development and progression. A number of MKKs can phosphorylate and activate JNK and p38. MKK3 and MKK6 activate p38, MKK7 activates JNK, whereas MKK4 can activate both JNK and p38 [61]. MKK4 protein expression is reduced in ovarian metastatic tissues compared to normal ovarian epithelial cells [62]. To demonstrate a potential role for MKK4 in metastasis suppression in ovarian cancer, a similar complementation approach was used as that described above for examining prostate cancer metastasis. MKK4 was ectopically expressed in the human ovarian cell line SKOV3ip.1, which lacks endogenous MKK4 expression and injected into SCID mice. This led to a significant decrease in overt metastatic implants on a number of tissues and organs compared to parental cells and increased the life span of the mice by 70% [63]. In contrast to the prostate metastasis model, the ectopic expression of the p38 activator MKK6 also suppressed metastasis whereas expression of MKK7 did not, suggesting that p38, rather than JNK, may be the relevant MKK4 target in ovarian cancer [64]. The JNK and p38 signaling pathways contribute to tumor suppression via several mechanisms. (a) JNK promotes Bax activation and apoptosis via direct phosphorylation and also through the phosphorylation of the proapoptotic Bcl2 family members Bim and Bmf. JNK also phosphorylates and inactivates the antiapoptotic family members Bcl2, Bcl-XL and Mcl-1 and inhibiting cytoplasmic sequestration of Bax by 14-3-3. In addition, JNK inhibits TGF-β1 gene transcription via c-Jun. (b) p38 negatively regulates cyclin D1 both by reducing gene transcription and by promoting protein instability and thereby blocking the G1/S transition. The p38 pathway, via MAPKAPK2, also downregulates the activity of CDC25 family members thereby inhibiting cell cycle progression. The tumor suppressor p53 is a direct target of p38 phosphorylation, which promotes its stability and transcriptional activation leading to cell cycle arrest and apoptosis. It has been proposed that JNK may act as a tumor suppressor by regulating the autocrine expression of TGF-β. Increased expression levels of TGF-β1 have been previously reported to contribute to cancer progression and the inhibition of TGF-β1 signaling blocks tumor growth in mice injected with Ras transformed cells [65]. This effect was attributable to a distal promoter region in the TGF-β1 gene that binds to c-Jun and represses transcription by recruiting the histone deacetylase HDAC3 in wild-type cells, but has reduced binding to c-Jun and HDAC3 in JNK-null cells.

References


