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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 transcoelmic 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, lypmphotoxin 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 endometrioid and mucinous carcinomas account for 8–19% and 3–11% respectively [5,6]. The Federation Internationale de Ginecologie 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

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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 metalloproteinases (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

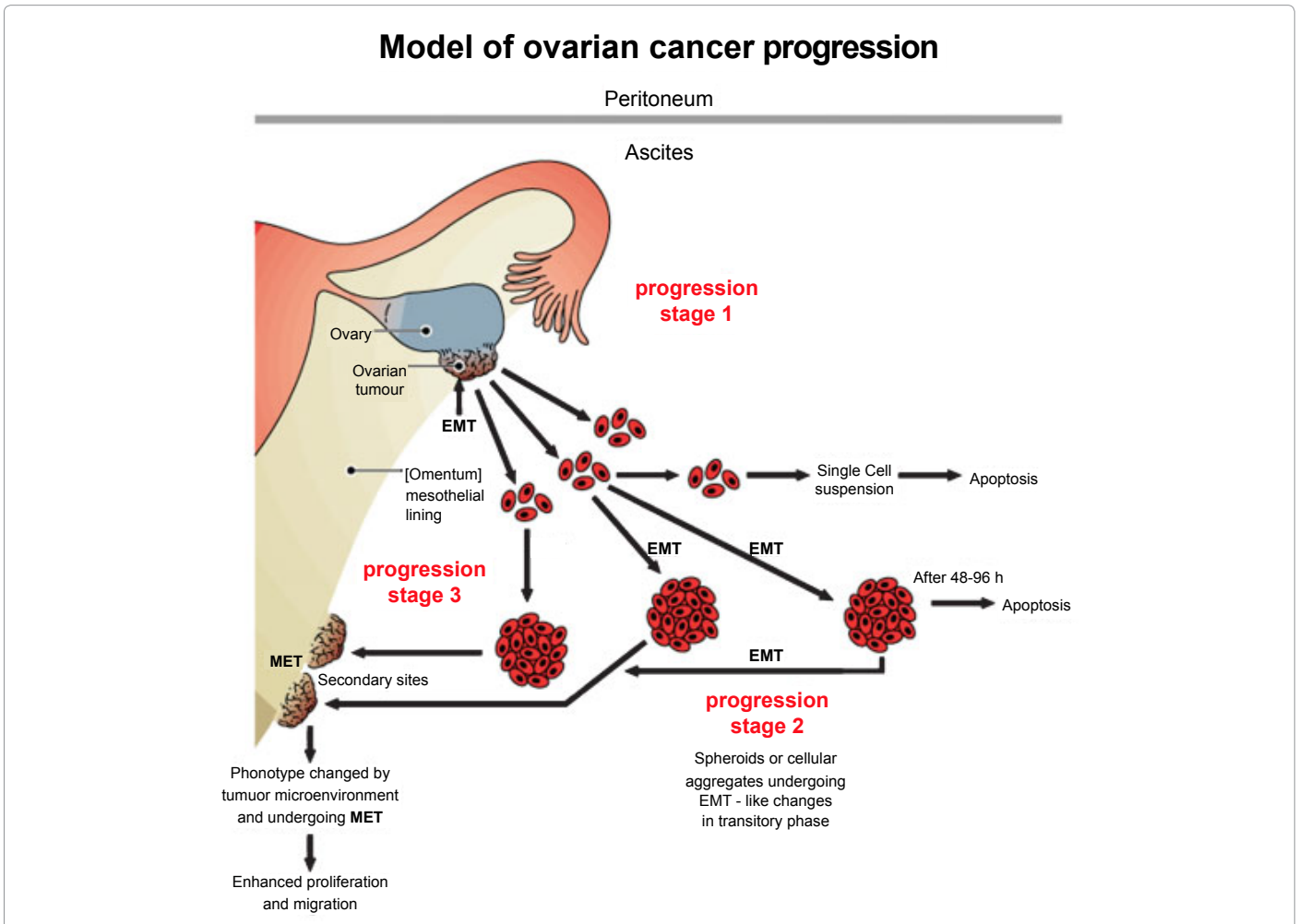

Figure 1: A working model of ovarian cancer progression. During ovarian cancer progression, epithelial ovarian cancer cells growing on the surface of the ovary undergo EMT to attain motile functions required for cancer metastasis. Rupture of the ovarian tumor result in shedding of tumor cells into the 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 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 significanttolerance 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 *dl*922-947 can replicate in human ovarian cancer cells and has greater efficacy than E1A wild-type adenoviruses and the E1B-55K deletion mutant *dl*1520 (Onyx-015) [19]. Significant inflammatory responses have been reported in gene-therapy trials involving replicating [20] and nonreplicating [21] adenoviral vectors. These responses are characterized by the induction of several cytokines and chemokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), IL-1α and β. Multiple signaling pathways are activated by adenoviruses upon binding to Coxsackie Adenovirus Receptor (CAR) and interactions with $\alpha_v\beta_{3/5}$ integrins. These include NF-κB and MAP kinases ERK and p38 [22], which promote cytokine and chemokine induction and inhibition of NF-κB may increase the oncolytic efficacy [23]. Early production of TNF-α 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-α by ovarian carcinoma cells stimulates a network of other cytokines, angiogenic factors and chemokines that may act in an autocrine/paracrine manner to promote peritoneal growth and spread [24]. Recent clinical trials have suggested that inhibition of TNF-α may have therapeutic potential in ovarian cancer [25]. It has been shown that TNF-α expression is induced in ovarian carcinoma 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-α in ovarian cancer cells and that TNF-α act as a survival factor in infected cells to decrease viral efficacy. Also, it was demonstratedthat induction of IL-6 and IL-8 generates and sustains other inflammatory mediators [26]. In addition, the suppression of TNF-α 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-α 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, leads 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-$FLIP_L$ from TRAIL-resistant OC cells inhibits tumor growth *in vitro* and *in vivo*. It has been shown thatc-FLIP prevents NK cells and CTLs-mediated immunosurveillance, resulting in aggressive tumor growth *in vivo* [33]. The expression level of c-$FLIP_L$ 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-$FLIP_L$ depletion completely inhibited the migratory behavior of ovarian cancer cellsand inhibited the invasive behavior *in vivo*. This observation indicates that c-$FLIP_L$ depletion induces apoptosis in OC cells, which might be the reason that these cells do not go through the peritoneal cavity and therefore inhibits their invasive behavior. Additionally, c-$FLIP_L$ has been shown to regulate ERK and NF-κB pathways [36], which could explain why c-$FLIP_L$ 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-$FLIP_L$ 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 attractive therapeutic combination partners for these agents to prevent ovarian cancermetastasis. It is reasonable to suggest thatc-$FLIP_L$ could 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 [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 (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 RhoGDI2 overexpression correlated with resistance. Their immunohistochemical analyses of serous ovarian cancer tissues from patients who received paclitaxel-based chemotherapy found that RhoGDI2 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 RhoGDI2 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 RhoGDI2 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 RhoGDI2 overexpression may promote tumor growth. RhoGDI2 was significantly overexpressed in high-grade compared with low-grade ovarian cancers. It was noted that interfering RNA suppression of RhoGDI2 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 RhoGDI2 was overexpressed in high-grade compared with low-grade ovarian cancers, which RhoGDI2 expression correlated with histological subtype of cancer and no statistically significant association with survival. It has been suggested that RhoGDI2 may function as a tumor suppressor in HeyA8 ovarian cancer cells and RhoGDI2 expression is higher in more advanced ovarian cancer patient tissues [53]. RhoGDI2 may function as an invasion and metastasis suppressor. It is possible to assume that RhoGDI2 expression may still provide a marker for drug response. RhoGDI2 functionsas 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 RhoGDI2-depleted HeyA8 cells. In addition, the functions of RhoGDI2 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 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 developmentand 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 of 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- β1 Increased expression levels of TGF-β1 have previously been reported to contribute to cancer progression and the inhibition of TGF- β1 signaling blocks tumor growth in mice injected with Ras transformed cells (JNK-null fibroblasts display increased TGF-β1 levels compared with wild-type cells and this correlates with increased invasive behavior and proliferation [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

1. Naora H, Montell DJ (2005) Ovarian cancer metastasis: integrating insights from disparate model organisms. Nat Rev Cancer 5: 355-366.
2. Allen HJ, Porter C, Gamarra M, Piver MS, Johnson EA (1987) Isolation and morphologic characterization of human ovarian carcinoma cell clu sters present in effusions. Exp Cell Biol 55: 194-208.
3. L'Espérance S, Bachvarova M, Tetu B, Mes-Masson AM, Bachvarov D (2008) Global gene expression analysis of early response to chemotherapy treatment in ovarian cancer spheroids. BMC Genomics 9: 99.
4. Shield K, Riley C, Quinn MA, Rice GE, Ackland ML, et al. (2007) Alpha2beta1 integrin affects metastatic potential of ovarian carcinoma spheroids by supporting disaggregation and proteolysis. J Carcinog 6: 11.
5. Seidman JD, Horkayne-Szakaly I, Haiba M, Boice CR, Kurman RJ, et al. (2004) The histologic type and stage distribution of ovari an carcinomas of surface epithelial origin. Int J Gynecol Pathol 23: 41-44.
6. Naora H (2007) The heterogeneity of epithelial ovarian cancers: reconciling old and new paradigms. Expert Rev Mol Med 9: 1-12.
7. Colombo N, Van Gorp T, Parma G, Amant F, Gatta G, et al. (2006) Ovarian cancer. Crit Rev Oncol Hematol 60: 159-179.
8. Shepherd JH (1989) Revised FIGO staging for gynaecological cancer. Br J Obstet Gynaecol 96: 889-892.
9. Ahmed N, Thompson EW, Quinn MA (2007) Epithelial-mesenchymal interconversions in normal ovarian surface epithelium and ovarian carcinomas: an exception to the norm. J Cell Physiol 213: 581-588.
10. Burleson KM, Hansen LK, Skubitz AP (2004) Ovarian carcinoma spheroids disaggregate on type I collagen and invade live human mesothelial cell monolayers. Clin Exp Metastasis 21: 685-697.
11. Kitamura K, Stockert E, Garin-Chesa P, Welt S, Lloyd KO, et al. (1994) Specificity analysis of blood group Lewis-y (Le(y)) antibodies generated against synthetic and natural Le(y) determinants. Proc Natl Acad Sci USA 91: 12957-12961.
12. Madjd Z, Parsons T, Watson NF, Spendlove I, Ellis I, et al. (2005) High expression of Lewis y/b antigens is associated with decreased survival in lymph node negative breast carcinomas. Breast Cancer Res 7: R780-R787.
13. Zhu LC, Lin B, Hao YY, Li FF, Diao B, et al. (2008) Impact of alpha1,2-fucosyl transferase gene transfection on cancer-related gene expression profile of human ovarian cancer cell line RMG-1. Ai Zheng 27: 934-941.
14. Vernon AE, LaBonne C (2004) Tumor metastasis: a new twist on epithelial-mesenchymal transitions. Curr Biol 14: R719-R721.
15. Visse R, Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92: 827-839.
16. Pellikainen JM, Ropponen KM, Kataja VV, Kellokoski JK, Eskelinen MJ (2004) Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. Clin Cancer Res 10: 7621-7628.
17. Chang C, Werb Z (2001) The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol 11: S37-S43.
18. Liu JJ, Lin B, Hao YY, Li FF, Liu DW, et al. (2010) Lewis(y) antigen stimulates the growth of ovarian cancer cells via regulation of the epidermal growth factor receptor pathway. Oncol Rep 23: 833-841.
19. Choi YA, Lim HK, Kim JR, Lee CH, Kim YJ, et al. (2004) Group IB secretory phospholipase A2 promotes matrix metalloproteinase-2-mediated cell migration via the phosphatidylinositol 3-kinase and Akt pathway. J Biol Chem 279: 36579-36585.
20. Lockley M, Fernandez M, Wang Y, Li NF, Conroy S, et al. (2006) Activity of the adenoviral E1A deletion mutant dl922-947 in ovarian cancer: comparison with E1A wild-type viruses, bioluminescence monitoring, and intraperitoneal delivery in icodextrin. Cancer Res 66: 989-998.
21. Reid T, Galanis E, Abbruzzese J, Sze D, Wein LM, et al. (2002) Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. Cancer Res 62: 6070-6079.
22. Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, et al. (2003). Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 80: 148-158.
23. Bowen GP, Borgland SL, Lam M, Libermann TA, Wong NC (2002) Adenovirus vector-induced inflammation: capsid-dependent induction of the C-C chemokine RANTES requires NF-kappa B. Hum Gene Ther 13: 367-379.
24. Palmer DH, Chen MJ, Searle PF, Kerr DJ, Young LS (2005) Inhibition of NF-kappaB enhances the cytotoxicity of virus-directed enzyme prodrug therapy and oncolytic adenovirus cancer gene therapy. Gene Ther 12: 1187-1197.
25. Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, et al. (2007) The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. Cancer Res 67: 585-592.
26. Brown ER, Charles KA, Hoare SA, Rye RL, Jodrell DI, et al. (2008) clinical

study assessing the tolerability and biological effects of infliximab, a TNF-alpha inhibitor, in patients with advanced cancer. Ann Oncol 19: 1340-1346.

27. Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, et al. (2007) The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. Cancer Res 67: 585-592.

28. Baird SK, Aerts JL, Eddaoudi A, Lockley M, Lemoine NR, et al. (2008) Oncolytic adenoviral mutants induce a novel mode of programmed cell death in ovarian cancer. Oncogene 27: 3081-3090.

29. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407: 770-776.

30. Vaux DL, Strasser A (1996) The molecular biology of apoptosis. Proc Natl Acad Sci USA 93: 2239-2244.

31. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, et al. (1996) Human ICE/CED-3 protease nomenclature. Cell 87: 171.

32. Ashkenazi A (2002) Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2: 420-430.

33. Inohara N, Koseki T, Hu Y, Chen S, Núñez G (1997) CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. Proc Natl Acad Sci USA 94: 10717-10722.

34. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, et al. (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388: 190-195.

35. Medema JP, de Jong J, van Hall T, Melief CJ, Offringa R (1999) Immune escape of tumors in vivo by expression of cellular FLICE-inhibitory protein. J Exp Med 190: 1033-1038.

36. Lu KH, Patterson AP, Wang L, Marquez RT, Atkinson EN, et al. (2004) Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res 10: 3291-3300.

37. Kataoka T, Budd RC, Holler N, Thome M, Martinon F, et al. (2000) The caspase-8 inhibitor FLIP promotes activation of NF-kappaB and Erk signaling pathways. Curr Biol 10: 640-648.

38. Kataoka T (2005) The caspase-8 modulator c-FLIP. Crit Rev Immunol 25: 31-58.

39. Li FY, Jeffrey PD, Yu JW, Shi Y (2006) Crystal structure of a viral FLIP: insights into FLIP-mediated inhibition of death receptor signaling. J BiolChem 281: 2960-2968.

40. Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R, et al. (2006) Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 12: 939-944.

41. Badino GR, Novelli A, Girardi C, Di Carlo F (1996) Evidence of functional bets-adrenoceptor subtypes in cG-5 breast cancer cell. Pharmacol Res 33: 255-260.

42. Rangarajan S, Enserink JM, Kuiperij HB, de Rooij J, Price LS, et al. (2003) Cyclic AMP induces integrin-mediated cell adhesion through Epac and Rap1 upon stimulation of the beta 2-adrenergic receptor. Cell Biol 160: 487-493.

43. Masur K, Niggemann B, Zanker KS, Entschladen F (2001) Norepinephrine-induced migration of SW 480 colon carcinoma cells is inhibited by beta-blockers. Cancer Res 61: 2866-2869.

44. Sood AK, Bhatty R, Kamat AA, Landen CN, Han L, et al. (2006) Stress hormone-mediated invasion of ovarian cancer cells. Clin Cancer Res 12: 369-375.

45. Kassim SK, El-Salahy EM, Fayed ST, Helal SA, Helal T, et al. (2004) Vascular endothelial growth factor and interleukin-8 are associated with poor prognosis in epithelial ovarian cancer patients. Clin Biochem 7: 363-369.

46. Karashima T, Sweeney P, Kamat A, Huang S, Kim SJ, et al. (2003) Nuclear factor-kappaB mediates angiogenesis and metastasis of human bladder cancer through the regulation of interleukin-8. Clin Cancer Res 9: 2786-2797.

47. Lamph WW, Wamsley P, Sassone-Corsi P, Verma IM (1988) Induction of proto-oncogene JUN/AP-1 by serum and TPA. Nature 334: 629-631.

48. Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072: 129-157.

49. Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, et al. (1998) Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst 90: 447-454.

50. Vega FM, Ridley AJ (2008) Rho GTPases in cancer cell biology. FEBS Lett 582: 2093-2101.

51. Harding MA, Theodorescu D (2010) RhoGDI signaling provides targets for cancer therapy. Eur J Cancer 46: 1252-1259.

52. Jones MB, Krutzsch H, Shu H, Zhao Y, Liotta LA, et al. (2002) Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. Proteomics 2: 76-84.

53. Tapper J, Kettunen E, El-Rifai W, Seppälä M, Andersson LC, et al. (2001) Changes in gene expression during progression of ovarian carcinoma. Cancer Genet Cytogenet 128: 1-6.

54. Stevens EV, Banet N, Onesto C, Plachco A, Alan JK, et al. (2011) RhoGDI2 antagonizes ovarian carcinoma growth, invasion and metastasis. Small Gtpases 2: 202-210.

55. DerMardirossian C, Bokoch GM (2005) GDIs: central regulatory molecules in Rho GTPase activation.Trends Cell Biol 15: 356-363.

56. Hickson JA, Huo D, Vander Griend DJ, Lin A, Rinker-Schaeffer CW, et al. (2006) The p38 kinases MKK4 and MKK6 suppress metastatic colonization in human ovarian carcinoma. Cancer Res 66: 2 264-2270.

57. Nakamori M, Fu X, Meng F, Jin A, Tao L, et al. (2003) Effective therapy of metastatic ovarian cancer with an oncolytic herpes simplex virus incorporating two membrane fusion mechanisms. Clin Cancer Res 9**:** 2727-2733.

58. Fu X, Tao L, Zhang X (2007) An oncolytic virus derived from type 2 herpes simplex virus has potent therapeutic effect against metastatic ovarian cancer. Cancer Gene Ther 14: 480-487.

59. Ebert O, Shinozaki K, Kournioti C, Park MS, García-Sastre A, et al. (2004) Syncytia induction enhances the oncolytic potential of vesicular stomatitis virus in virotherapy for cancer. Cancer Res 64**:** 3265-3270.

60. Patton SE, Martin ML, Nelsen LL, Fang X, Mills GB, et al. (1998) Activation of the ras-mitogen-activated protein kinase pathway and phosphorylation of ets-2 at position threonine 72 in human ovarian cancer cell lines. Cancer Res 58**:** 2253-2259.

61. Chang L, Karin M (2001) Mammalian MAP kinase signalling cascades. Nature 410: 37-40.

62. Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 81: 807-869.

63. Yamada SD, Hickson JA, Hrobowski Y, Vander Griend DJ, Benson D, et al. (2002) Mitogen-activated protein kinase kinase 4 (MKK4) acts as a metastasis suppressor gene in human ovarian carcinoma. Cancer Res 62: 6717-6723.

64. Hickson JA, Huo D, Vander Griend DJ, Lin A, Rinker-Schaeffer CW, et al. (2006) The p38 kinases MKK4 and MKK6 suppress metastatic colonization in human ovarian carcinoma. Cancer Res 66: 2264-2270.

65. Ventura JJ, Kennedy NJ, Flavell RA, Davis RJ (2004) JNK regulates autocrine expression of TGF-beta1. Mol Cell 15: 269-278.