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Review

New determinates of disease progression and outcome in metastatic ovarian carcinoma

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Summary. Ovarian cancer is the most lethal gynecologic malignancy. This is attributed to frequent presentation at late stage, when the tumor has metastasized, as well as to development of chemotherapy resistance along tumor progression. Patients with advanced-stage ovarian carcinoma have widespread intraperitoneal metastases, including the formation of malignant serous effusions within the peritoneal cavity. Pleural effusions constitute the most frequent site of distant metastasis (FIGO stage IV disease). Unlike the majority of solid tumors, particularly at the primary site, cancer cells in effusions are not amenable to surgical removal, and failure in their eradication is one of the main causes of treatment failure. Our research in recent years has demonstrated that a large number of cancer-associated molecules are differentially expressed in effusions compared to primary carcinomas and solid metastases. We have additionally observed that expression of several of these molecules differs between primary diagnosis (pre-chemotherapy) and disease recurrence (post-chemotherapy) specimens, and that they are significantly associated with response to chemotherapy and patient survival. These observations are thought to be related to disease progression, as well as to the unique microenvironment of effusions, and may have impact on the selection of targeted therapy in this cancer. This review discusses our recent observations with respect to the biology of ovarian carcinoma cells in effusions, and focuses on the clinical role of tumor-associated molecules at this anatomic site.

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Introduction

Ovarian carcinoma (OC) is the most lethal gynecologic cancer and currently ranks fifth in causing cancer-related deaths among women (Jemal et al., 2009). OC is treated by combined surgery and chemotherapy, the latter with platinum compounds and paclitaxel as choice agents. Despite moderate improvement in the 5-year survival rate in recent years, the majority of patients still die of their disease. This is mainly attributed to the frequent presentation at advanced stage (FIGO stage III-IV) and to primary or acquired drug resistance (Holschneider and Berek, 2000; Agarwal and Kaye, 2003; Cannistra, 2004).

OC and the closely-related primary peritoneal carcinoma (PPC) and fallopian tube carcinoma have a strong predilection towards metastasis within the serosal cavities, disseminating in the form of solid metastases and peritoneal accumulation of effusion fluid (ascites). Solid metastases are commonly found in the walls of the peritoneal cavity, as well as in intra-abdominal structures, including the omentum, intestines, genital organs, spleen and urinary bladder. This manifests clinically as abdominal discomfort or pain, swelling or heaviness, and is the most common presentation of this disease. The pleural space is frequently involved, either at diagnosis or, more commonly, at a later stage, and the pleural cavity is the most common anatomic site defining stage IV disease (Curtin et al., 1997; Bonnefoi et al., 1999; Akahira et al., 2001). Lymph node metastases and dissemination to distant organs occur, but are distinctly less common (Tsuruchi et al., 1993;

Cormio et al., 1995; Sakai et al., 1997).

The accumulation of effusion fluid within the pleural, pericardial and peritoneal cavities is a complex process that may occur in the presence of relatively few or no cancer cells within the fluid itself. It is believed to occur due do the combined effect of lymphatic obstruction by metastatic cancer cells, increased production of peritoneal fluid by mesothelial cells lining the peritoneal cavity and increased vascular permeability (Hirabayashi and Graham, 1970; Feldman et al., 1972; Nagy et al., 1995).

Tumor cells in effusions differ from those in the primary organ or in solid metastases in several respects. Lacking tissue anchorage, they most frequently assume a tri-dimensional spheroid morphology, which is most pronounced in serous carcinoma, the most common histological type of OC, PPC and tubal carcinoma. Another central difference is the changing population of host cells, creating a unique microenvironment at this anatomic site. In solid lesions, the synthetic capacity of tumor cells is complemented by that of stromal myofibroblasts, endothelial cells and leukocytes, predominantly macrophages (Elenbaas and Weinberg, 2001). In addition, tumor cells are able to induce the formation of new leaky vessels (angiogenesis) and obtain nutrients and oxygen, in addition to gaining access to the circulation. In contrast, tumor cells in effusions have cross-talk mainly with mesothelial cells, which constitute the native resident cell population of the serosal cavities, highly adaptable cells that are able to synthesize many of the proteins that positively regulate tumor growth at this site, as well as assume epithelial or mesenchymal characteristics through the process of epithelial-to-mesenchymal transition (EMT; van der Wal and Jeekel, 2007; Witowski et al., 2008; Yung and Chan, 2009). The ability of cancer cells to survive and proliferate in effusions attests to their resistance to cell death induced by loss of anchorage (anoikis) and to apoptosis, suggesting the presence of strong pro-survival signals.

As the detection of cancer cells in effusion marks the presence of metastatic disease, the majority of OC patients with this manifestation of advanced-stage disease have little chance of achieving cure, even with complete response to chemotherapy at diagnosis. The inability of chemotherapy to eradicate OC has led in recent years to growing efforts to inhibit tumor growth using targeted therapy (Blagden and Gabra, 2009; Barrena Medel et al., 2010; Campos and Ghosh, 2010). Several classes of drugs targeting growth factors and their receptors, DNA repair systems, signaling molecules, and mitotic regulators are currently being evaluated for their role in inhibiting angiogenesis, cell survival, and proliferation, and thereby tumor progression. While the characterization and validation of new molecular targets in the primary tumor provides useful insights into the biology of OC, it is crucial to direct efforts to the molecular characterization of the true site of disease recurrence and treatment failure, i.e. metastases. Failure to do so is likely to hamper our ability to improve patient outcome through the use of targeted therapy.

In recent years, our group has focused on defining molecular differences between OC cells in primary tumors, effusions and solid metastases. We were able to identify multiple molecules that are differentially expressed at these anatomic sites, and that have different prognostic role as function of their specific expression along tumor progression. Further highlighting the importance of tumor progression in this setting are the profound differences between primary vs. recurrent OC cells in effusions (Davidson, 2004, 2007). Using genome-based technology, we additionally characterized new molecular targets that are overexpressed in high grade serous OC and are associated with chemoresistance and patient survival (Shih and Davidson, 2009). The present review summarizes some of new data generated by our group in recent years, with focus on effusion biology.

EMT

EMT is a process by which mesenchymal cells are formed during embryogenesis, in a process that facilitates migration through the extracellular environment (ECM) and settlement in areas of new organ formation (Thiery, 2002). In recent years, EMT-like processes have been investigated for their potential role as determinants of cancer cell invasion and metastasis. The current hypothesis is that carcinoma cells lose their epithelial characteristics and acquire certain mesenchymal properties that promote ECM invasion and distant metastasis in an EMT-like process. This involves the same transcription factors used during the embryonic EMT, including Snail, Slug, Twist1, Zeb1 and SIP1 (Batlle et al., 2000; Comijn et al., 2001; Hajra et al., 2002; Bolós et al., 2003; Rosivatz et al., 2002; Vernon and LaBonne, 2004; Aigner et al., 2007). However, tumor cells differ from embryonic cells by having lost specific target recognition, as well as by the ability to activate autocrine loops of growth signals, evade apoptosis, and elicit angiogenesis (Gotzmann et al., 2004).

Transcription factors that mediate EMT negatively regulate E-cadherin, the prototype member of the classical cadherin family, which maintains cell polarity and normal epithelial structure (Pecina-Slaus, 2003; Cavallaro and Christofori, 2004). In most carcinomas, E-cadherin-mediated intercellular adhesion is lost during malignant transformation and tumor progression (Hanahan and Weinberg, 2000). In addition to E-cadherin transcriptional inhibition (Poser et al., 2001; Yokoyama et al., 2001; Jiao et al., 2002), Snail has been shown to indirectly upregulate the expression of matrix metalloproteinases (MMP), the most important family of proteases mediating invasion, metastasis and angiogenesis (Yokoyama et al., 2003; Miyoshi et al., 2005), as well as the mesenchymal marker vimentin, and

to downregulate cytokeratin 18, desmoplakin, MUC1, claudin and occludin (Cano et al., 2000; Ikenouchi et al., 2003). The transcriptional repressor activity of Snail is modulated by its expression level and intracellular localization.

p21-activated kinase (Pak1) is a serine/threonine protein kinase, first isolated as a Rac-binding protein in the rat brain (Manser et al., 1994). Pak1 contains phosphorylation sites by Akt, cdc2/cdk5 and PDK1 (Bokoch, 2003), and binds to and is activated by several GTPases, including Rac1, rac2, Rac3 and Cdc42, as well as by the SH3-containing adapter proteins Nck and Grb2. Extracellular Pak activators include growth factors (EGF, Heregulin, PDGF), ECM proteins (via integrins), lipids, interleukins and other cytokines (Bokoch, 2003). Pak substrates include cytoskeletal proteins (e.g., myosin, caldesmon, desmin), kinases (Raf-1, MEK-1), and various other molecules, including the pro-apoptotic molecule Bad and Estrogen receptor (ER). Pak1 additionally regulates Snail through phosphorylation at Ser^{246}, resulting in Snail accumulation in the nucleus, thereby modulating its activity (Yang et al., 2005). Pak1 activation thus affects major cellular processes, including regulation of the cytoskeleton and motility, neurogenesis, angiogenesis, metastasis, apoptosis and cell survival (Bokoch, 2003).

Recent research has generated data regarding the expression, biological role and clinical relevance of EMT regulators in OC. In experimental models, Snail was shown to mediate E-cadherin downregulation induced by hypoxia (Imai et al., 2003), promote tumor growth and metastasis (Jin et al., 2010), induce EMT (Theriault et al., 2007; Pon et al., 2008), and mediate invasion (Kurrey et al., 2005). Slug was similarly shown to be involved in EMT and invasion (Kurrey et al., 2005; Thériault et al., 2007). Snail expression is increased in OC compared to borderline tumors (Blechschmidt et al., 2008) and is associated with poor survival (Tuhkanen et al., 2009).

Twist expression is related to Paclitaxel resistance *in vitro* and to peritoneal metastasis (Wang et al., 2004; Terauchi et al., 2007). In patient material, Twist expression is associated with shorter survival (Hosono et al., 2007). Snail, Slug, Twist1, and SIP1 expression is higher in OC compared to the ovarian surface epithelium, and benign and borderline tumors (Yoshida et al., 2009). Snail expression additionally correlated with poor survival in this study (Yoshida et al., 2009). Finally, reduced expression of Zeb transcription factors in OC compared to the ovarian surface epithelium was recently reported (Bendoraite et al., 2010).

Our group has previously shown that E-cadherin protein expression is upregulated in OC effusions compared to corresponding (patient-matched) primary carcinomas (Davidson et al., 2000), and reported on co-expression of E-cadherin and the EMT-associated N-cadherin in OC cells in effusions (Sivertsen et al., 2006). *Snail*, *Slug* and *SIP1* mRNA was frequently found in OC effusions (Fig. 1A), and higher *E-cadherin* mRNA expression was associated with longer progression-free survival, whereas high *SIP1*/*E-cadherin* ratio was associated with poor overall survival (Elloul et al., 2005). However, Snail and Slug protein expression was significantly lower in effusions compared to primary tumors (Elloul et al., 2006). In the latter study, we additionally observed that Snail localizes to the cytoplasm rather than the nucleus in uncultured OC cells from effusions, suggesting that it is not functional in these cells (Elloul et al., 2006). These data suggest that OC does not fully follow the classical model of EMT, and that OC cells in effusions probably undergo at least partially the reverse process of mesenchymal-to-epithelial transition.

Recently, we observed that E-cadherin interferes with spheroid formation in OC cells *in vitro*, and that Snail silencing using Small Hairpin RNA reduces invasion and MMP-2 activity in ES-2 cells. Dominant negative (DN) Pak1 ES-2 and OVCAR-3 clones had reduced attachment to ECM proteins, invasion and MMP-2 activity compared to constitutively active (CA) and wild-type cells. DN Pak1 ES-2 cells additionally had reduced binding to LP9 cells, generated from benign mesothelium (Elloul et al., 2010). These data suggest that the re-expression of E-cadherin in OC effusions is regulated by Pak1, and that this may affect dissemination within the serosal cavities. This postulated role of Pak1 is of interest in view of our previous observation that the *Pak1* gene is overexpressed in a subset of the OC specimens using digital karyotyping (Shih et al., 2005). Additionally, our observation that *Pak1* protein expression in primary diagnosis pre-chemotherapy effusions is associated with longer survival whereas the opposite is true for disease recurrence post-chemotherapy effusions (Davidson et al., 2008a) suggests altered cellular function along disease progression and merits further research. Of note, Siu et al. recently reported association between Pak1 and p-Pak1 protein expression in primary OC and poor overall and disease-free survival (PFS; Siu et al., 2010).

Analysis of clinical specimens showed significantly higher *Twist1, Zeb1 and Vimentin* mRNA expression in solid metastases compared to primary carcinomas and effusions (Elloul et at., 2010), further attesting to the dynamic nature of EMT in OC as function of anatomic site.

The insulin-like growth factor (IGF) system

The IGF system consists of the peptide hormones Insulin, IGF-I and IGF-II; the cell surface receptors insulin receptor (IR), IGF-1R, IGF-2R and hybrid IGF-1R/IR receptors; and a family of circulating IGF-binding proteins (IGFBP) (Denley et al, 2005; Guvakova, 2007; Sachdev and Yee, 2007). IGF-I and IGF-II are 7.6 kDa and 7.5 kDa peptides that share about 50% homology with proinsulin and 62% among themselves (Guvakova, 2007). These three ligands bind with different affinity to IGF system receptors, of which all except IGF-2R

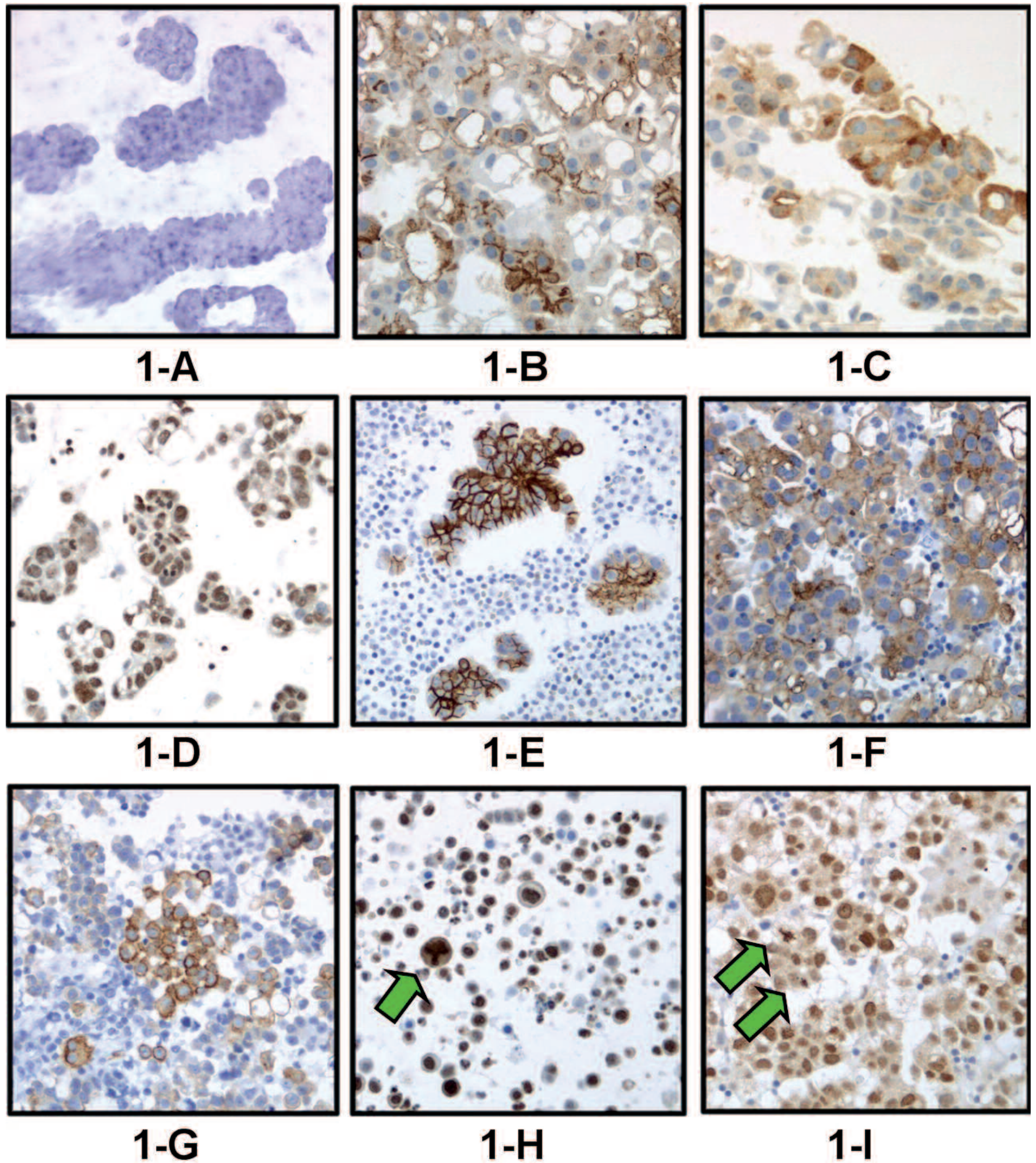

Fig. 1. Expression of cancer-associated molecules in ovarian carcinoma effusions. **A:** Snail mRNA; **B:** IGFBP3; **C:** IGF-II; **D:** NF-κB p65; **E:** claudin-1; **F:** claudin-7; **G:** phospho-mTOR (p-mTOR); **H:** p-AKT; **I:** Survivin. Note mitotic figures in **H** and **I** (arrows). All figures except 1-A are using immunohistochemistry, 1-A is by mRNA *in situ* hybridization (NBT-BCIP staining, counterstained with nuclear fast red).

possess tyrosine kinase activity (Sachdev and Yee, 2007). Activation of IGF receptors leads to phosphorylation of adaptor proteins of the IRS family or SHC, with subsequent activation of the MAPK family member ERK. IRS proteins additionally activate the PI3K signaling pathway via AKT and mTOR. The net effect of MAPK and PI3K activation is cell proliferation and survival, adhesion, migration and metastasis (Denley et al, 2005; Guvakova, 2007; Sachdev and Yee, 2007).

The six IGFBP members have a size of 23-31kDa and contain three domains, the N- and C-domains and the linker (L-) domain. The N- and C-domains are the ones mediating IGF binding. Multiple other interactions are mediated via the C-domain, including binding of extracellular matrix proteins such as fibronectin, collagen I, osteopontin and vitronectin, as well as cellular proteins including integrins and caveolin. IGFBP members are present in the serum, extracellularly and in the circulation. In the circulation, they normally bind 99% of the IGF present, mainly by IGFBP3, the most abundant family member at this site. IGFBP positively or negatively regulate the biologic activity of IGF-I and IGF-II through control of their availability for ligand-receptor binding. IGF is released by proteolysis of IGFBP at its L-domain, a process mediated by multiple proteases, including plasmin, thrombin and members of the MMP family, or by binding of IGFBP to the extracellular matrix. Regulation of the IGFBP affinity to IGF is also achieved via phosphorylation of the former (Firth and Baxter, 2002; Bach et al., 2005).

The IGF system is under investigation for its possible role as a target for molecular therapy in cancer, including OC (reviewed in Beauchamp et al., 2010). Previous work by other investigators has been inconclusive with respect to the prognostic role of IGFBP3 in primary OC (Katsaros et al., 2001; Lu et al., 2006; Wiley et al., 2006; Walker et al., 2007), whereas high IGF-II levels have been associated with poor survival (Sayer et al., 2005; Lu et al., 2006). IGF-II is part of a panel of 6 serum markers suggested for detection of early OC (Kim et al., 2009).

We previously reported on the overexpression of the *IGFBP3* and *IGF-II* genes in OC/PPC compared to diffuse malignant peritoneal mesothelioma (DMPM), a tumor that shares many of the clinical, morphological and molecular characteristics of the former cancer (Davidson et al., 2006a). We subsequently analyzed the diagnostic and clinical role of these proteins in effusions (Slipicevic et al., 2009). Immunostaining of 327 effusions, including 205 OC, showed significantly higher expression of both proteins in carcinomas compared to mesotheliomas (Fig. 1B,C). IGFBP3 was found in the effusion supernatant of all OC, as well as in breast carcinomas and 16 mesotheliomas using ELISA. High IGFBP3 expression in pre-chemotherapy and high IGF-II expression in post-chemotherapy OC effusions correlated with poor overall survival, and IGF-II expression in post-chemotherapy effusions was an independent prognostic factor in Cox multivariate analysis. This study shows that IGF-II and IGFBP3 are more frequently expressed in metastatic carcinomas compared to mesothelioma in effusions, and that IGF-II and IGFBP3 may be novel prognostic markers in metastatic OC.

The NF-κB transcription factor

The NF-κB family consists of five proteins, named RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2) that form homodimers or heterodiomers. NF-κB is localized in the cytoplasm in complex with inhibitors of NF-κB (IκBs), i.e. IκBα, IκB and IκBε. Cell stimulation results in IκB phosphorylation in a site-specific manner by activated IκB kinase (IKK) complexes, the most common of includes IKKα, IKKß and a regulatory IKKγ subunit, also named NEMO (NF-κB essential modulator) (Neumann and Naumann, 2007; Perkins, 2007).

The 'canonical' NFκB pathway is activated by TNF-α, IL-1 and other stimuli, and involves IκBα phosphorylation at ser32 and ser36 predominantly by IKKß. IκBα is subsequently ubiquitinated and degraded in the 26S proteasome, thereby releasing the NF-κB p65/p50 heterodimer to translocate into the nucleus (Neumann and Naumann, 2007; Perkins 2007). Other NF-κB activation pathways have been described, and their diversity, in addition to the existence of hundreds of NF-κB activators and target genes (Pahl, 1999), results in a myriad of cellular functions and effects (Perkins and Gilmore, 2006). The binding of NF-κB transcription factors to κB elements in promoters and enhancers can therefore both induce and repress gene expression, affecting cell proliferation and survival, inflammation, the immune response, and programmed cell death (reviewed in Perkins, 2007). Pro-survival NF-κB signaling is activated by many oncogenes, e.g. HER-2 and HRAS, and the PI3K/AKT pathway and involves increased proliferation via cyclin D1 activation, suppression of the JNK cascade, and induction of angiogenesis, invasion and metastasis, mediated through upregulation of molecules such as VEGF, VCAM-1 and MMPs (Madrid et al., 2000; Pianetti et al., 2001; Bassères and Baldwin, 2006; Sethi et al., 2008). NF-κB-mediated resistance to cell death occurs through upregulated expression of anti-apoptotic molecules, including the inhibitor of caspase activation cFLIP, the inhibitor of apoptosis (IAP) family members cIAP1, cIAP2 and XIAP, and the Bcl-2 family members A1/Bfl1 and Bcl-XL (Wang et al., 1999; Chen et al., 2000; Tang et al., 2001; Xiao et al., 2003).

Studies using *in vitro and in vivo* OC models have shown that inhibition of NF-κB enhances the anti-tumor efficacy of cisplatin and paclitaxel (Mabuchi et al., 2004a,b; Liu et al., 2006). The microRNA miR-199A and MEKK3, part of the mitogen-activated protein kinase (MAPK) pathway, were recently shown to regulate IIKß and NF-κB activation (Chen et al., 2008; Samanta et al., 2009). NF-κB p65 protein expression

was reported to be higher in OC compared to borderline and benign tumors and to normal ovaries, and was associated with high histological grade, advanced stage and shorter survival (Guo et al., 2008).

We investigated the role of NF-κB in OC in three studies. In the first one, we compared NF-κB p65 and IκBα protein expression in OC effusions to that of corresponding primary carcinomas and solid metastases (Kleinberg et al., 2009). Nuclear NF-κB p65 expression, indicating NF-κB activation, was observed in the majority of tumors irrespective of anatomic site, with less frequent cytoplasmic NF-κB p65 and IκB expression (Fig. 1D). However, the percentage of cells expressing NF-κB p65 (nuclear or cytoplasmic) and IκBα was significantly higher in solid lesions compared to effusions. NF-κB p65 phosphorylation at Ser536 was found in 94% of the effusions using Western blotting. In effusions, high (>25% of cells) nuclear NF-κB p65 expression was significantly associated with larger volume of residual disease and poor response to chemotherapy at disease recurrence. Nuclear NF-κB p65 expression correlated with poor PFS in univariate survival analysis and was an independent predictor of shorter PFS in Cox analysis. These data suggest that NF-κB p65 is frequently expressed and activated in advanced-stage OC. The association between nuclear NF-κB p65 expression in effusions and poor chemoresponse and PFS supports its involvement in cancer cell survival.

In a recent study, we analyzed the protein expression and clinical role of IKKα, IKKß, IKKε, NF-κB p50, NF-κB p65, RelB, and MMP-9 using immunohistochemistry in solid OC specimens obtained at diagnosis from a cohort of 33 patients subsequently treated with paclitaxel, cisplatin, and cyclophosphamide (Annunziata et al., 2010). NF-κB p65 and RelB were co-expressed with IKKα, supporting their existence as a tri-molecular regulatory complex. A significant association of NF-κB p50 with poor overall survival was found (p=0.02), whereas MMP-9 expression was associated with better prognosis (p=0.01). This study demonstrates that deregulation of NF-κB activity may influence outcome in women treated with standard therapy for advanced OC, and suggests that modification of this pathway may improve outcome in this cancer.

In the third paper (Hernandez et al., 2010), IKKß expression by immunohistochemistry was studied in 119 tumors comprised of 42 primary carcinomas and 77 solid metastases. IKKß was expressed in 89 of 119 tumors, with comparable immunostaining in primary carcinomas and metastases. High expression (>25% of cells) of IKKß in primary carcinomas was associated with poor overall survival.

Treatment of the OC cell line CAOV3 with the IKKß small molecule inhibitor MLN120b and RNA interference with a short hairpin RNA (shRNA) directed against IKKß identified set of 9 genes, of which 8 were downregulated and 1 upregulated, including *CLD1*, coding for the gap junction protein claudin-1, *IL8*, coding for the angiogenic molecule Interleukin-8, and the chemokine genes *CXCL1* and *CXCL2*. Inhibition of IKKß *in vitro* suppressed growth, invasiveness, adhesion to endothelial cells and IL-8 secretion to a variable degree in OC cell lines.

These data provide further evidence supporting the role of the NF-κB pathway in general, and IKKß in particular, in OC biology.

Claudins

Claudins are a family of more than 20 tight junction-specific integral membrane proteins. Tight junctions are located in the apical aspect of epithelial or endothelial cells, where they maintain cell polarity and regulate the paracellular transport of solutes and the diffusion of proteins and lipids (Tsukita et al., 2001; González-Mariscal et al., 2003; Van Itallie and Anderson, 2006). Claudins contain intracellular amino and carboxy termini, four transmembrane domains, and two extracellular loops mediating intercellular interactions between claudins (Tsukita et al., 2001; González-Mariscal et al., 2003; Van Itallie and Anderson, 2006). The second extracellular loop serves as a binding site for *Clostridium perfringens* enterotoxin in claudin-3 and -4 (Morita et al., 1999). The carboxy terminus of most claudins contains potential serine and/or thereonine phosphorylation sites and a PDZ-binding motif, to which the tight junction cytoplasmic scaffolding proteins ZO-1, -2 and -3 bind (Morita et al., 1999).

The expression patterns of claudins in normal tissue, benign tumors and cancer are complex and organ-dependent (Morin, 2005; Swisshelm et al., 2005; Hewitt et al., 2006). Higher expression of claudin-3 (Hough et al., 2000; Lu et al., 2004; Santin et al., 2004; Bignotti et al., 2006), claudin-4 (Hough et al., 2000; Hibbs et al., 2004; Lu et al., 2004; Santin et al., 2004; Bignotti et al., 2006), claudin-7 (Bignotti et al., 2006; Tassi et al., 2008) and claudin-10 (Bignotti et al., 2006) has been reported in OC compared to normal ovarian surface epithelium, with less conclusive data in comparative analyses of OC with cystadenomas or borderline tumors (Hough et al., 2000; Rangel et al., 2003; Heinzelmann-Schwarz et al., 2004; Soini and Talvensaari-Mattila, 2006; Zhu et al., 2006; Choi et al., 2007). In one of these studies (Tassi et al., 2008), claudin-7 was found to be expressed in OC cells in ascites, whereas it was absent from leukocytes and reactive mesothelial cells. Recently, claudin-4 exosomes were found in the plasma of 32/63 OC patients compared to 1/50 healthy volunteers (Li et al., 2009).

Only few studies have investigated the prognostic role of claudins in OC. Low claudin-3 protein expression (undefined cut-off) using immunhistochemistry was associated with a trend for poor survival in an analysis of 115 primary carcinomas (Heinzelmann-Schwarz et al., 2004). In contrast, Choi et al. found claudin-3 and -4 protein expression in 81% and 85.7% of 84 serous adenocarcinomas, respectively, and high claudin-3

expression was associated with shorter survival in both univariate and multivariate analysis (Choi et al., 2007). Despite the inconclusive prognostic role of claudins in OC, their overexpression in tumor cells compared to normal epithelium and other host cells has led to growing interest in targeting claudin-3 and -4 as a therapeutic approach in OC (Romani et al., 2009; Saeki et al., 2009; Suzuki et al., 2009; Yuan et al., 2009).

In our comparative gene expression array analysis of OC/PPC and DMPM effusions, we found significantly higher gene expression of claudin-3, claudin-4 and claudin-6 in OC compared to DMPM and validated these findings for claudin-3 and claudin-4 using quantitative RT-PCR and immunohistochemistry (Davidson et al., 2006a). We subsequently analyzed the diagnostic role of claudin-1, claudin-3 and claudin-7 in 325 effusions, and reported on the higher expression of claudins in OC compared to adenocarcinomas of other origin, malignant mesotheliomas and benign reactive mesothelial cells (Kleinberg et al., 2007a).

In a second study (Kleinberg et al., 2008), we analyzed the anatomic site-related expression and prognostic role of claudins in OC. Effusions (n=218), corresponding primary tumors (n=81) and solid metastases (n=164) (total=463 tumors) were immunostained for claudin-1, -3, -4 and -7. All four claudins were expressed in the majority of tumors at all anatomic sites (Fig. 1E,F). However, the percentage of immunostained cells was significantly higher in effusions compared to primary carcinomas and solid metastases for claudin-1, -3, and -7. In univariate survival analysis of the entire cohort, higher claudin-3 and -7 expression in effusions correlated with shorter overall survival, with similar results for claudin-7 with respect to progression-free survival. In survival analysis for patients with primary diagnosis pre-chemotherapy effusions, higher claudin-7 expression correlated with shorter overall survival, whereas claudin-1 and -3 expression was associated with poor overall survival for patients with disease recurrence post-chemotherapy effusions. In multivariate survival analysis of the entire cohort, claudin-7 expression was an independent predictor of poor progression-free survival, whereas claudin-3 independently predicted poor overall survival for patients with post-chemotherapy effusions. These two studies demonstrate that claudins are widely expressed in OC, with significant upregulation for some family members in effusions. Claudin expression in OC effusions has both diagnostic and prognostic value, and their cancer-specific expression suggest that they may be a therapeutic target in this disease.

The PI3K/AKT/mTOR signaling pathway

Dysregulation of the PI3K/AKT/mTOR signaling pathway is a major characteristic of multiple types of cancer. Following activation by receptor tyrosine kinases, PI3K converts phosphatidylinositol (4,5) bisphosphate (PIP_2) to phosphatidylinositol (3,4,5) triphosphate (PIP_3) which then acts as a second messenger activating downstream pathways involving AKT, mTOR and other proteins (Vivanco and Sawyers, 2002). The tumor suppressor phosphatase with tensin homology (PTEN) negatively regulates PI3K activity, and is mutated or deleted in multiple cancer types (Chow and Baker, 2006; Cully et al., 2006). AKT, a serine/threonine kinase, comprises three homologous family members (AKT1, AKT2 and AKT3) which are broadly expressed, although there are some isoform-specific features (Bellacosa et al., 2005). Activation involves a dual mechanism requiring PIP_3 to recruit the PH-domain of the AKT protein to the plasma membrane altering its conformation to allow subsequent phosphorylation at either Thr308 alone, or for maximal activation, additional phosphorylation at Ser473 (Bellacosa et al., 2005; Song et al., 2005). Phosphorylated AKT (p-AKT) promotes cell growth, protein translation and cell survival, antagonizes cell cycle arrest, impacts glucose metabolism, and regulates angiogenesis and invasion (Thompson and Thompson, 2004; Bellacosa et al., 2005; Song et al., 2005).

A major target in the downstream cascade of AKT activity is mTOR, a protein residing in two functionally distinct complexes, mTORC1 and mTORC2. mTORC1 phosphorylates 4E-BP1 (eukaryotic initiation factor 4E binding protein-1) and p70S6K (ribosomal p70S6 kinase), both regulators of mRNA translation and cell growth (Guertin and Sabatini, 2007). p70S6K indirectly exerts negative feedback on PI3K (Guertin and Sabatini, 2007; Yap et al., 2008), while the mTORC2 complex directly phosphorylates AKT, making mTOR an upstream as well as a downstream effector in the signaling pathway (Sarbassov et al., 2005).

Several lines of evidence point to a biological role for the PI3K/AKT/mTOR signaling pathway in OC progression, and blocking of this pathway is under active investigation as a treatment modality in OC (reviewed in Blagden and Gabra, 2009; Trinh et al., 2009; Campos and Ghosh, 2010). Tranfection of OC cells with AKT2 results in increased adhesion and invasion *in vitro* and increased metastatic potential *in vivo* (Arboleda et al., 2003). Constitutive AKT1 activity or AKT2 gene amplification in OC cell lines confers resistance to paclitaxel (Page et al., 2000), and treatment of OC cell lines with high AKT levels with the PI3K inhibitor LY294002 sensitizes the cells to cisplatin-induced apoptosis (Altomare et al., 2004). Combination of LY294002 and paclitaxel increases chemotherapy efficacy on tumor growth and reduces ascites volume *in vivo* (Hu et al., 2002). Treatment with the mTOR inhibitor RAD001 inhibits cell proliferation and enhanced cisplatin-induced apoptosis in OC cell lines with high AKT activity, with reduced phosphorylation of 4E-BP1 and p70S6 kinase (Mabuchi et al., 2007). Combined therapy with rapamycin and the VEGF inhibitor bevacizumab in SCID mice has been shown to inhibit intraperitoneal OC cell growth *in vivo* (Huynh et al., 2007). Overexpression of PTEN in SHIN-3 OC cells

leads to reduced VEGF secretion and intraperitoneal tumor growth in mice (Takei et al., 2008). miR-214 induces cell survival and resistance to cisplatin in OC cells in vitro through targeting of PTEN (Yang et al., 2008).

The *AKT2* gene is amplified in OC (Bellacosa et al., 1995; Thompson et al., 1996), and activating mutations of the *PIK3CA* gene were recently reported to be frequent (50% in fresh frozen material) in OC (Kuo et al., 2009).

Data regarding the prognostic role of AKT and mTOR in OC are to date inconclusive. Two studies have shown an association between p-AKT expression in primary OC and poor survival in univariate, but not in multivariate analysis (Oda et al., 2007; Guo et al., 2008). A trend towards higher mortality was found for patients with *AKT2* gene amplification (Bellacosa et al., 1995). However, increased p-AKT expression did not significantly correlate with survival in two other studies (Castellvi et al., 2006; Woenckhaus et al., 2007). Increased expression of mTOR was associated with better OS in univariate analysis, but not in multivariate analysis (Noske et al., 2008). Association between reduced PTEN expression in primary OC and shorter disease-free survival was recently reported (Lee and Park, 2009).

We investigated the expression and clinical role of the PI3K/AKT/mTOR pathway in effusions in three studies. Using proteomics, we observed a trend for shorter survival for patients with disease recurrence post-chemotherapy effusions with high AKT activation level (Davidson et al., 2006b).

In a subsequent study (Davidson et al., 2008b), we analyzed the expression of PTEN and its inhibitor DJ-1, an oncogene overexpressed in different cancers (Kim et al., 2005). DJ-1 mRNA was expressed in >80% of analyzed effusions (n=72), primary carcinomas (n=42) and solid metastases (n=15), with comparable levels at all anatomic sites. DJ-1 expression was positively associated with that of its transcriptional regulators Sp1 and Sp3. DJ-1 expression was significantly higher in post-chemotherapy compared to pre-chemotherapy effusions, and predicted shorter progression-free survival in univariate analysis for patients with post-chemotherapy effusions. PTEN protein expression by immunohistochemistry was low in effusions (n=210) and solid tumors (n=92; 23% and 13%, respectively), and PTEN expression in effusions was unrelated to DJ-1 levels or patient survival.

This study showed that DJ-1 is frequently expressed in advanced-stage OC irrespective of anatomic site, and that DJ-1 is co-expressed with its transcriptional regulators Sp1 and Sp3. PTEN expression is infrequent in OC, and analyzing PTEN status in effusions does not provide prognostic information.

In a recent study, we analyzed p-AKT and p-mTOR protein expression by immunohistochemistry in a large cohort of patients diagnosed with serous OC, with focus on effusions and solid metastases, and evaluated their clinical relevance (Fig. 1G,H). We additionally compared the phosphorylation pattern of AKT at Thr308 and Ser473 by immunoblotting, and studied the relationship between expression of p-AKT, mTOR and the PTEN inhibitor DJ-1 in serous OC effusions using flow cytometry (Bunkholt Elstrand et al., 2010).

p-AKT expression in effusions was higher in grade 3 vs. 1-2 tumors. Flow cytometry analysis showed significant co-expression of AKT, mTOR and DJ-1 expression in effusions (Fig. 2A). Higher p-AKT Thr308/pan-AKT ratio by Western blotting was associated with more advanced FIGO stage and a trend for poor response to chemotherapy at first disease recurrence. Higher p-mTOR protein expression (>25% of cells) in effusions by immunohistochemistry was associated with poor progression-free survival for patients with post-chemotherapy effusions in univariate and multivariate analysis.

The data in these three studies support a clinical role for the PI3K/AKT/mTOR pathway in OC and reinforces the validity of this signaling pathway as a potential therapeutic target in metastatic OC.

Folate receptors

Folate (vitamin B9) is involved in one-carbon transfer reactions that are essential for RNA and DNA synthesis. Folate is also involved in the remethylation of homocysteine to methionine, an important step in the biosynthesis of S-adenosyl methionine, which provides methyl groups for methylation of DNA, RNA, proteins and phospholipids. Cellular folate uptake is mediated by several molecules, including the folate receptor (FR) family, which consists of four family members, termed FR-α, -ß, -γ and –δ. The genes coding for *FR, FOLR1-4*, are located on the long arm of chromosome 11, and have about 70% sequence homology. *FOLR1* and *FOLR2* code for membrane-bound glycoproteins bound to glycosyl phosphatidylinositol (GPI) anchor, whereas *FOLR3* codes for two forms of secreted protein, FR-γ and FR-γ', the latter of which is a mutated form. The protein product of *FOLR4* has not been identified to date (reviewed in Kelemen, 2006; Salazar and Ratnam, 2007).

FR members are differentially expressed in normal and tumor tissues. FR-α, the most extensively studied family member, is expressed in urogenital organs, the female genital tract, salivary and bronchial glands, the choroid plexus, retinal pigment cells and the placenta. High FR-α levels have been detected in different carcinomas, including non-mucinous ovarian carcinoma (OC) (Hough et al., 2001; Parker et al., 2005; Kalli et al., 2008), endometrial carcinoma and cervix carcinoma, as well as in non-genital tumors. FR-ß is expressed by hematopoietic cells and the placenta, as well as by leukemia and lymphoma cells. FR-γ has been detected in normal and malignant hematopoietic cells, as well as carcinomas of the ovary, endometrium and cervix (reviewed in Kelemen, 2006; Salazar and Ratnam,

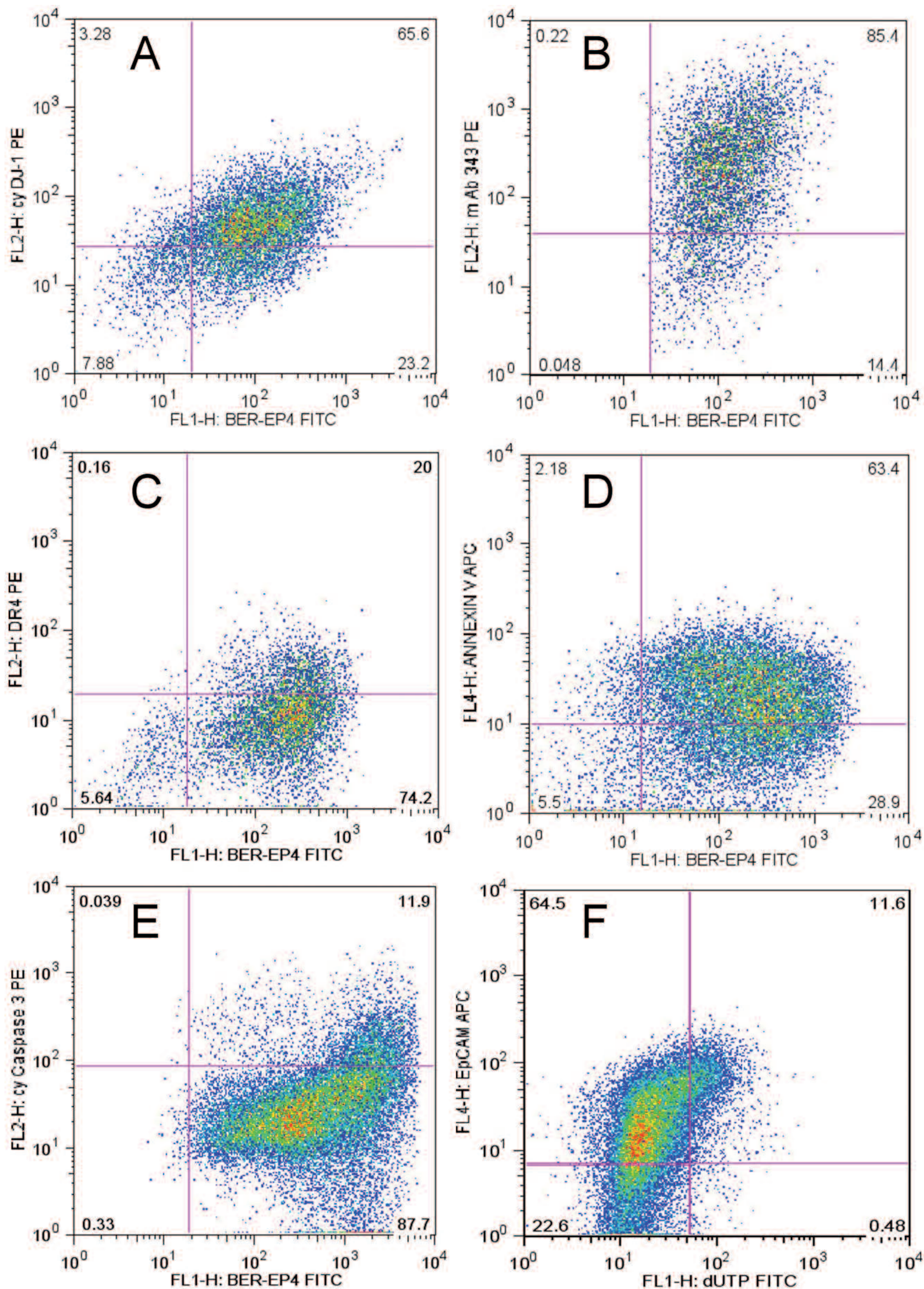

Fig. 2. Expression of survival- and apoptosis-associated molecules in ovarian carcinoma effusions by flow cytometry. **A.** DJ-1, expressed in 65.6% of tumor cells (upper right quadrant); **B.** Folate receptor-α (FRα), expressed in 85.4% of tumor cells; **C.** Death receptor-4 (DR4), expressed in 20% of tumor cells; **D-F.** Annexin-V, cleaved caspase-3 and dUTP incorporation in the same effusion. Annexin-V labeling is seen in 63.4% of cells **(D)**, compared to 11.9% and 11.6% for cleaved caspase-3 **(E)** and dUTP incorporation **(F)**, respectively.

2007).

FR-α has been investigated for its potential role as a target for molecular therapy, as modulator of the immune system, and as a diagnostic marker in imaging (Salazar and Ratnam, 2007). Therapeutic approaches for blocking FR-α include the use of antibodies or folic acid conjugates, and vaccines targeting this protein (Campos and Ghosh, 2010). Farletuzumab (MORAb-003), a monoclonal antibody to α-FR that activates antibody-dependent cell-mediated cytotoxicity and complement-mediated toxicity is currently investigated for its therapeutic role in OC (Spannuth et al., 2010).

FOLR1 and *FOLR3* were identified as genes that are overexpressed OC/PPC compared to DMPM in our previously-discussed gene expression array study. In a follow-up study (Yuan et al., 2009), we validated this finding at the mRNA and protein level. Ninety-one effusions (71 OC, 10 breast carcinomas, 10 malignant mesotheliomas) were assayed for *FOLR1* and *FOLR3* gene expression using qPCR. FR-α expression was analyzed in 40 OC/PPC, 10 breast carcinomas and 9 nesotheliomas using flow cytometry. qPCR analysis showed significantly higher *FOLR1* and *FOLR3* mRNA levels in OC compared to both other cancers, and the levels of these two genes were directly interrelated in OC effusions. FR-α protein was similarly more highly expressed in OC compared to breast carcinoma and mesothelioma effusions (Fig. 2B). *FOLR1*and *FOLR3* mRNA and FR-α protein expression in OC effusions was unrelated to clinical parameters or survival.

This study confirmed the role of folate receptors in the differential diagnosis between OC/PPC and other cancers affecting the serosal cavities and showed that *FOLR1and FOLR3* are co-expressed in this tumor. Whereas the high expression of folate receptors in OC effusions supports their validity as molecular therapeutic

Table 1. The prognostic role of cancer-associated molecules in ovarian carcinoma effusions.

Ref.	Molecule	Method	Effusion type	No.[a]	Univariate	Multivariate	Prognosis
Elloul et al., 2005	E-cadherin	RT-PCR	All	70	p=0.023 (PFS)[b]	NP[c]	Good
Elloul et al., 2005	Snail	RT-PCR	All	70	NS[d]	NS	-
Elloul et al., 2005	Slug	RT-PCR	All	70	NS	NS	-
Elloul et al., 2005	SIP1	RT-PCR	All	70	NS[e]	NS	-
Davidson et al., 2008a	Pak1	IHC[f]	Pre-chemotherapy	83	p=0.024 (OS)[g] / p=0.015 (PFS)[h]	NS (OS) / p=0.016 (PFS)	Good
Davidson et al., 2008a	Pak1	IHC	Post- chemotherapy	64	p=0.044 (OS)	p=0.049 (OS)	Poor
Slipicevic et al., 2009	IGFBP3[i]	IHC	Pre-chemotherapy	92	p=0.031 (OS)	NS	Poor
Slipicevic et al., 2009	IGF-II	IHC	Post- chemotherapy	73	p=0.024 (OS)	p=0.04 (OS)	Poor
Kleinber et al., 2009	NF-κB p65	IHC	All	164	p=0.048 (PFS)[j]	NS	Poor
Kleinber et al., 2009	IκBα	IHC	All	164	NS	NS	-
Kleinber et al., 2009	dUTP	FCM	All	52	NS	NS	-
Kleinber et al., 2009	Caspase-3	FCM	All	52	p=0.024 (OS) / p=0.046 (PFS)[k]	NS	Good
Kleinber et al., 2009	Caspase-8	FCM	All	52	NS	NS	-
Kleinber et al., 2008	claudin-1	IHC	All	180	NS	NS	-
Kleinber et al., 2008	claudin-3	IHC	All	180	p=0.038 (OS)	NS	Poor
Kleinber et al., 2008	claudin-7	IHC	All	180	p=0.035 (OS) / p=0.026 (PFS)[l]	NS (OS) / p=0.017 (PFS)	Poor
Davidson et al., 2008b	PTEN	IHC	All	163	NS	NS	-
Davidson et al., 2008b	DJ-1	RT-PCR	Post- chemotherapy	30	p=0.027 (PFS)	NS	Poor
Bunkholt Elstrand et al., 2010	p-AKT	IHC	Post- chemotherapy[m]	56	NS	NS	-
Bunkholt Elstrand et al., 2010	p-mTOR	IHC	Post- chemotherapy	56	p=0.005 (PFS)	p=0.03 (PFS)	Poor
Yuan et al., 2009	FR-α	qRT-PCR	All	68	NS	NS	-
Yuan et al., 2009	FR-γ	qRT-PCR	All	68	NS	NS	-
Davidson et al., 2003	P85-PARP	IHC	All	80	p=0.017 (OS)	NP	-
Dong et al., 2008a	TNFR1	FCM[n]	All	76	NS	NS	-
Dong et al., 2008a	TNFR2	FCM	All	76	NS	NS	-
Dong et al., 2008a	FAS	FCM	All	76	NS	NS	-
Dong et al., 2008a	DR4	FCM	All	76	p=0.035 (OS) / p=0.041 (PFS)	p=0.008 (OS) / p=0.003 (PFS)	Poor
Dong et al., 2008a	DR5	FCM	All	76	NS	NS	-
Kleinberg et al., 2007b	XIAP	IHC	Pre-chemotherapy	101	NS	NS	-
Kleinberg et al., 2007b	Survivin[o]	IHC	Pre-chemotherapy[p]	101	p=0.002 (OS) / p<0.001 (PFS)	NS (OS) / p=0.004 (PFS)	Good
Dong et al., 2009	Annexin-V	FCM	Post-chemotherapy	27	p=0.005 (OS) / p=0.013 (PFS)	NP	Poor

[a]No.: patient number; [b]PFS: progression-free survival. In some studies, the terms relapse-free survival or disease-free survival are used. [c]NP: not performed; [d]NS: not significant; [e]: Higher SIP1/E-cadherin ratio correlated with worse overall survival (p=0.018); [f]IHC: immunohistochemistry; [g]OS: overall survival; [h]: p=0.013 for staining intensity, p=0.015 for staining extent); [i]: Additionally associated with poor OS in primary carcinomas (p=0.016); p=0.025 for patients with post-chemotherapy effusions; [k]: Additionally associated with poor OS (p=0.042) and PFS (p=0.036) for patients with post-chemotherapy effusions; [l]: Claudin-7 additionally associated with poor OS in pre-chemotherapy effusions (p=0.045), claudin-1 (p=0.018) and claudin-3 (p=0.009) associated with poor OS in post-chemotherapy effusions; [m]: No association with survival for p-AKT or p-mTOR in the entire cohort; [n]FCM: Flow cytometry; [o]: nuclear localization; [p]: No association with survival for XIAP or Survivin in the entire cohort.

targets in this disease, their levels do not provide prognostic information.

Apoptosis

Apoptosis, or programmed cell death, is characterized morphologically by cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation (Lowe and Lin, 2000; Taylor et al., 2008). This process regulates cell death following irreparable DNA damage and is also important for controlling cell number during normal development (reviewed in Igney and Krammer, 2002).

The distinct morphological and biochemical changes that define apoptosis are mediated by caspases, a family of cysteinyl aspartate-specific proteases. Two major pathways leading to caspase activation have been characterized.

The extrinsic pathway is initiated by ligation of transmembrane death receptors including Fas/CD95, Tumor necrosis factor receptor (TNFR) and TNF-related apoptosis-inducing ligand receptor (TRAILR) to activate membrane-proximal caspases (caspase-8 and -10). These in turn cleave and activate caspase-3, -6 and -7 or B-cell chronic lymphocytic leukemia/lymphoma 2 (Bcl-2) proteins, depending on the cell type (Lowe and Lin, 2000; Igney and Krammer, 2002; Taylor et al., 2008).

Many stimuli, including chemotherapy, kinase inhibitors, hypoxia, growth factor withdrawal and radiation, trigger apoptotic signaling through the intrinsic pathway. Following the disruption of the mitochondrial membrane, mitochondrial proteins that regulate apoptosis, primarily cytochrome c, are released into the cytoplasm. The released cytochrome c forms a complex with the adaptor protein apoptotic protease-activating factor 1 (APAF1) and pro-caspase-9, in which pro-caspase-9 is activated. Caspase-9 downstream activates effector caspases, most notably caspase-3, resulting in substrate cleavage and the appearance of apoptotic-related morphological changes. Considerable crosstalk exists between the extrinsic and the intrinsic pathways (Wang, 2001). Additionally, growing evidence shows that other intracellular compartments and organelles such as the nucleus (Norbury and Zhivotovsky, 2004), the endoplasmic reticulum (Orrenius et al., 2003) and the lysosomes (Guicciardi et al., 2004) participate in apoptotic signaling.

Apoptosis is a highly regulated process. A large number of molecules, including p53, RAS, heat shock proteins, cyclins, PI3K pathway members, caspase-8 and FADD-like apoptosis regulator (cFLIP), Second mitochondria-derived activator of caspase/ Direct-IAP binding protein with low pI (Smac/DIABLO), Bcl-2 proteins, Inhibitor of apoptosis proteins (IAPs), and NF-κB modulate apoptosis (Igney and Krammer, 2002; Johnstone et al., 2002; Maddika et al., 2007; Riedl and Shi, 2004). Bcl-2 family proteins are key regulators of mitochondrial membrane permeabilization, and include both pro-apoptotic members (e.g. Bax, Bak, Bad, Bcl-XS, Bid, Bim) that facilitate release of mitochondrial cytochrome c, and anti-apoptotic members (e.g. Bcl-2, Bcl-X_L) that block the process (Igney and Krammer, 2002; Maddika et al., 2007; Taylor et al., 2008). The functions of caspases are modulated by IAPs, which upon binding with caspases sterically hinder caspase-substrate binding (Igney and Krammer, 2002; Riedl and Shi, 2004).

Cancer cells are characterized by resistance to apoptosis and the presence of overriding survival signals initiated by autocrine and paracrine pathways, and defects in various molecules mediating apoptosis or its regulation have been shown to contribute to tumor initiation, progression and primary or acquired treatment resistance (Lowe and Lin, 2000; Igney and Krammer, 2002; Johnstone et al., 2002). For example, in OC cell lines, decreased activity of caspase-3, deficiency in APAF1 and overexpression of cFLIP, Bcl-2 and Bcl-X_L have been associated with chemoresistance (Liu et al., 2002; Abedini et al., 2004; Yang et al., 2004).

A large number of methods are available for measuring apoptosis, including transmission electron microscopy, and measurement of DNA fragmentation, caspase activity, membrane alterations, mitochondrial changes or phosphatidylserine emergence on the outer plasma membrane using the Annexin-V assay (Kerr et al., 1972; Darzynkiewicz et al., 1997; Vermes et al., 2000; Otsuki et al., 2003; Pozarowski et al., 2004; Kaufmann et al., 2008). The use of more than one assay for any given test sample is strongly recommended due to the limited sensitivity (positive identification of apoptotic cells) and specificity (detection of apoptotic vs. necrotic cells) of current assays (Darzynkiewicz et al., 1997, 2001).

Little is known regarding the degree and clinical relevance of apoptosis or expression of anti-apoptotic proteins in metastatic OC. Our group studied this aspect of cancer biology in several studies.

In the first one, we observed infrequent and focal expression of the 85kDa cleaved fragment of Poly(ADP-ribose) polymerase (p85-PARP) using immunohistochemistry, with only 6/80 effusions showing immunostaining in >5% of tumor cells, suggesting that OC cells in effusions undergo little apoptosis. Notably, p85-PARP expression, irrespective of the percentage of cells, was significantly associated with longer overall survival (Davidson et al., 2003).

Death receptors (DRs) are members of the tumor necrosis factor receptor (TNFR) superfamily that are able to induce the extrinsic apoptosis signaling pathway upon ligand binding. In addition to exhibiting the cysteine-rich extracellular domain typical of the TNFR family, DRs are characterized by a conserved cytoplasmic domain of approximately 80 amino acids, the death domain (DD), which is essential for transduction of the apoptotic signal. The mechanism of signal transduction is best described for Fas (CD95/Apo-1), TNFR1, TRAILR1 (DR4) and TRAILR2 (DR5). Their respective ligands include FasL (CD95L/Apo-1L),

TNF and TRAIL which can bind to both DR4 and DR5. Three other receptors, TRAILR3 (DcR1), TRAILR4 (DcR2) and the soluble receptor osteoprotegerin, lack functional cytoplasmic domains and do not transmit the apoptotic signal following binding to TRAIL (reviewed in Ashkenazi, 2002; Wajant et al., 2003; Takeda et al., 2007).

We investigated the presence of the death receptors DR4, DR5, Fas and tumor necrosis factor receptor-1 and -2 (TNFR1 and TNFR2) in 95 OC effusions using flow cytometry (Dong et al., 2008). DR4, DR5 and Fas were expressed by the majority of specimens, with less frequent expression of TNFR1 and TNFR2 (Fig. 2C). Surprisingly, DR4 and TNFR2 expression was higher in FIGO stage IV compared to stage III tumors, and higher DR4, DR5 and Fas expression in OC cells was significantly associated with poor response to chemotherapy administered at disease recurrence. In addition, higher DR4 expression correlated with poor overall and progression-free survival in univariate and multivariate survival analysis.

The results of this study suggest that death receptors are linked to aggressive clinical course in metastatic OC, presumably through activation of AKT, ERK and NF-κB, signaling molecules that have been reported to be downstream targets for these receptors (Secchiero et al., 2003; Imamura et al., 2004; Morel et al., 2005). These data do not support the rationale of activating death receptor signaling, a widely investigated approach for treating cancer (Reed, 2006), as a treatment modality for OC.

The IAP family consists of eight members in humans: cIAP1, cIAP2, NAIP, Survivin, XIAP, Apollon, ILP2, and Livin (reviewed in Liston et al., 2003; Schimmer, 2004). IAPs inhibit apoptosis induced by a variety of stimuli by direct binding and steric inhibition of caspase activity (Roy et al., 1997; Takahashi et al., 1998; Sun et al., 1999; Shiozaki et al., 2003), and several family members, including XIAP and Livin additionally regulate apoptosis in a caspase-independent manner through activation of the mitogen-activated protein JNK1 signal transduction pathway (Sanna et al., 2002). NF-kB activation by cIAP2 and XIAP (Chu et al., 1997; Hofer-Warbinke et al., 2000) may further contribute to the pro-survival effect of IAPs.

A unique property of Survivin is a cell cycle-dependent expression in the G2/M phase with localization to components of the mitotic apparatus, where it is thought to facilitate cell division. Survivin therefore regulates both cell division and apoptosis (Li et al., 1998; Altieri, 2003).

We studied the expression of three members of the IAP family, Survivin, XIAP and Livin, in OC (Kleinberg et al., 2007b). Western blotting analysis of 106 effusions showed XIAP and Survivin expression in the majority of specimens, with no expression of Livin.

Effusions, corresponding primary tumors and solid metastases were further immunohistochemically analyzed for XIAP and Survivin expression. Both proteins were expressed in the majority of specimens at all anatomic sites (Fig. 1I). However, XIAP and cytoplasmic Survivin expression was highest in effusions and solid metastases, respectively, whereas nuclear Survivin expression was comparable. Nuclear Survivin was significantly co-expressed with the proliferation marker Ki-67 in effusions. In univariate survival analysis for patients with pre-chemotherapy effusions, higher nuclear Survivin expression by immunohistochemistry correlated with better progression-free and overall survival, and the former correlation was an independent prognostic factor in multivariate analysis.

This study demonstrated that XIAP and Survivin, but not Livin, are frequently expressed in OC, with upregulated XIAP and cytoplasmic Survivin expression in metastatic lesions. We hypothesized that the association between higher nuclear Survivin expression and longer survival may be due to the sensitivity of proliferating cancer cells to chemotherapy.

In our next study, we established a protocol for the detection of epithelial cells in effusions combined with quantification of apoptosis by flow cytometry (Dong et al., 2008b). OC cells in effusions, detected by expression of the cell surface markers Ber-EP4 and EpCAM and lack of CD45 expression, were analyzed for the expression of intracellular/nuclear markers of apoptosis (cleaved caspase-3 and caspase-8 and incorporated dUTP).

We subsequently applied this method to a study of 76 OC effusions (Kleinberg et al., 2009). Caspase cleavage and dUTP incorporation were limited to <10% of cells in the majority of effusions. Strong direct association was seen between cleaved caspase-3 and caspase-8 levels, as well as between levels of both cleaved caspases and dUTP incorporation. Levels of the above apoptotic markers were comparable in pre- and post-chemotherapy effusions. Higher than median cleaved caspase-3 levels correlated with improved OS and PFS.

The data in this study confirmed our earlier observations that cancer cells in effusions generally undergo little apoptosis, even following chemotherapy, and that when present, apoptosis is associated with improved patient survival.

In a recent study, we applied the Annexin-V assay to the same series of 76 OC effusions analyzed for caspase cleavage and dUTP incorporation with the aim of comparing these methods (Dong et al., 2009). Annexin-V was expressed in all specimens and was more frequently detected compared to cleaved caspases and dUTP incorporation (Fig. 2D,F). Annexin-V expression was higher in grade 3 vs. 1-2 tumors, and higher percentage of Annexin-V-expressing cells in post-chemotherapy specimens was associated with poor progression-free and overall survival.

This study showed that measurement of apoptosis using the Annexin-V assay in OC effusions does not correlate with the caspase cleavage and dUTP

incorporation assays. This discrepancy and the association between higher Annexin-V expression and high-grade disease, as well as poor patient survival, suggest a role in cell survival rather than apoptosis in effusions.

Future perspectives

Designing new therapeutic strategies against cancer requires understanding of the biological mechanisms which sustain and promote tumor cell proliferation and survival. In particular, our understanding of the cellular mechanisms mediating resistance to chemotherapy is crucial for overcoming this major obstacle in treating cancer patients. While precious knowledge has been and is currently gained through *in vitro* and animal studies, studies of patient material from large and well-characterized cohorts are the ultimate test of relevance and are the ones to decide the clinical role of new molecules considered as possible candidates for targeted therapy. In OC, such studies should inevitably include analyses of metastatic lesions, as these are present in the majority of cases at diagnosis and are the ones responsible for the morbidity and mortality of this disease.

Our studies in clinical material suggest that many cancer-associated molecules are differentially expressed in primary tumors, effusions and solid metastases. They further demonstrate that pre- and post-chemotherapy effusions have different expression patterns and that a given molecule may have a different predictive and prognostic role at diagnosis vs. disease recurrence, which we regard as evidence of disease progression. This emphasizes the need to obtain new specimens at each disease recurrence in order to test them for the presence of molecules that may be therapeutically targeted. Our group continues to investigate molecules that promote cell survival, metastasis and chemo-resistance in metastatic OC. We increasingly focus on the mechanisms which regulate gene and protein expression (e.g., microRNA) in effusions, as understanding of these processes may help in developing new agents for gene therapy that may be the basis for individualized treatment and slow or reverse disease progression in OC. It is hoped that additional investigators will focus on validating the expression and clinical role of cancer-associated molecules in effusions.

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References

Abedini M.R., Qiu Q., Yan X. and Tsang B.K. (2004). Possible role of FLICE-like inhibitory protein (FLIP) in chemoresistant ovarian cancer cells in vitro. Oncogene 23, 6997-7004.

Agarwal R. and Kaye S.B. (2003). Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat. Rev. Cancer 3, 502-516.

Aigner K., Dampier B., Descovich. L., Mikula M., Sultan A., Schreiber M., Mikulits W., Brabletz T., Strand D., Obrist P., Sommergruber W., Schweifer N., Wernitznig A., Beug H., Foisner R. and Eger A. (2007). The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. Oncogene 2, 6979-6988.

Akahira J.I., Yoshikawa H., Shimizu Y., Tsunematsu R., Hirakawa T., Kuramoto H., Shiromizu K., Kuzuya K., Kamura T., Kikuchi Y., Kodama S., Yamamoto K. and Sato S. (2001). Prognostic factors of stage IV epithelial ovarian cancer: A multicenter retrospective study. Gynecol. Oncol. 81, 398-403.

Altieri D.C. (2003). Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 22, 8581-8589.

Altomare D.A., Wang H.Q., Skele K.L., De Rienzo A., Klein-Szanto A.J., Godwin A.K. and Testa J.R. (2004). AKT and mTOR phosphorylation is frequently detected in ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. Oncogene 23, 5853-5857.

Annunziata C.M., Tuft Stavnes H., Kleinberg L., Berner A., Hernandez L.F., Birrer M.J., Steinberg S.M., Davidson B. and Kohn E.C. (2010). NF-κB transcription factors are co-expressed and convey poor outcome in ovarian cancer. Cancer 116, 3276-3284.

Arboleda M.J., Lyons J.F., Kabbinavar F.F., Bray M.R., Snow B.E., Ayala R., Danino M., Karlan B.Y. and Slamon D.J. (2003). Overexpression of AKT2/protein kinase Bß leads to up-regulation of ß1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. Cancer Res. 63, 196-206.

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

Bach L.A., Headey S.J. and Norton R.S. (2005). IGF-binding proteins--the pieces are falling into place. Trends Endocrinol. Metab. 16, 228-234.

Barrena Medel N.I., Wright J.D. and Herzog T.J. (2010). Targeted therapies in epithelial ovarian cancer. J. Oncol. 2010, 314326.

Bassères D.S. and Baldwin A.S. (2006). Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene 25, 6817-6830.

Batlle E., Sancho E., Francí C., Domínguez D., Monfar M., Baulida J. and García De Herreros A. (2000). The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat. Cell Biol. 2, 84-89.

Beauchamp M.C., Yasmeen A., Knafo A. and Gotlieb W.H. (2010). Targeting insulin and insulin-like growth factor pathways in epithelial ovarian cancer. J. Oncol. 2010, 257058.

Bellacosa A., de Feo D., Godwin A.K., Bell D.W., Cheng J.Q., Altomare D.A., Wan M., Dubeau L., Scambia G., Masciullo V., Ferrandina G., Benedetti Panici P., Mancuso S., Neri G. and Testa J.R. (1995). Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int. J. Cancer 64, 280-285.

Bellacosa A., Kumar C.C., Di Cristofano A. and Testa J.R. (2005). Activation of AKT kinases in cancer: Implications for therapeutic targeting. Adv. Cancer Res. 94, 29-86.

Bendoraite A., Knouf E.C., Garg K.S., Parkin R.K., Kroh E.M., O'Briant K.C., Ventura A.P., Godwin A.K., Karlan B.Y., Drescher C.W., Urban N., Knudsen B.S. and Tewari M. (2010). Regulation of miR-200

family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. Gynecol. Oncol. 116, 117-125.

Bignotti E., Tassi R.A., Calza S., Ravaggi A., Romani C., Rossi E., Falchetti M., Odicino F.E., Pecorelli S. and Santin A.D. (2006). Differential gene expression profiles between tumor biopsies and short-term primary cultures of ovarian serous carcinomas: identification of novel molecular biomarkers for early diagnosis and therapy. Gynecol. Oncol. 103, 405-416.

Blagden S. and Gabra H. (2009). Promising molecular targets in ovarian cancer. Curr. Opin. Oncol. 21, 412-419.

Blechschmidt K., Sassen S., Schmalfeldt B., Schuster T., Höfler H. and Becker K.F. (2008). The E-cadherin repressor Snail is associated with lower overall survival of ovarian cancer patients. Br. J. Cancer 98, 489-495.

Bokoch G.M. (2003). Biology of the p21-activated kinases. Annu. Rev. Biochem. 72, 743-781.

Bolós V., Peinado H., Pérez-Moreno M.A., Fraga M.F., Esteller M. and Cano A. (2003). The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. J. Cell Sci. 116, 499-511.

Bonnefoi H., A'Hern R.P., Fisher C., Macfarlane V., Barton D., Blake P., Shepherd J.H. and Gore M.E. (1999). Natural history of stage IV epithelial ovarian cancer. J. Clin. Oncol. 17, 765-775.

Bunkholt Elstrand M., Dong H.P., Ødegaard E., Holth A., Elloul S., Reich R., Tropé C.G. and Davidson B. (2010). Mammalian target of rapamycin is a biomarker of poor survival in metastatic ovarian carcinoma. Hum. Pathol. 41, 794-804.

Campos S.M. and Ghosh S. (2010). A current review of targeted therapeutics for ovarian cancer. J. Oncol. 2010, 149362.

Cannistra S.A. (2004). Cancer of the ovary. N. Engl. J. Med. 351, 2519-2529.

Cano A., Pérez-Moreno M.A., Rodrigo I., Locascio A., Blanco M.J., del Barrio M.G., Portillo F. and Nieto M.A. (2000). The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat. Cell Biol. 2, 76-83.

Castellvi J., Garcia A., Rojo F., Ruiz-Marcellan C., Gil A., Baselga J. and Ramon y Cajal S. (2006). Phosphorylated 4E binding protein 1: a hallmark of cell signaling that correlates with survival in ovarian cancer. Cancer 107, 1801-1811.

Cavallaro U. and Christofori G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer 4, 118-132.

Chen C., Edelstein L.C. and Gélinas C. (2000). The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol. Cell Biol. 20, 2687-2695.

Chen R., Alvero A.B., Silasi D.A., Kelly M.G., Fest S., Visintin I., Leiser A., Schwartz P.E., Rutherford T. and Mor G. (2008). Regulation of IKKbeta by miR-199a affects NF-kappaB activity in ovarian cancer cells. Oncogene 27, 4712-4723.

Choi Y.L., Kim J., Kwon M.J., Choi J.S., Kim T.J., Bae D.S., Koh S.S., In Y.H., Park Y.W., Kim S.H., Ahn G. and Shin Y.K. (2007). Expression profile of tight junction protein claudin 3 and claudin 4 in ovarian serous adenocarcinoma with prognostic correlation. Histol. Histopathol. 22, 1185-1195.

Chow L.M. and Baker S.J. (2006). PTEN function in normal and neoplastic growth. Cancer Lett. 241, 184-196.

Chu Z.L., Mckinsey T.A., Liu L., Gentry J.J., Malim M.H. and Ballard D.W. (1997). Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappaB control. Proc. Natl. Acad. Sci. USA 94, 10057-10062.

Comijn J., Berx G., Vermassen P., Verschueren K., van Grunsven L., Bruyneel E., Mareel M., Huylebroeck D. and van Roy F. (2001). The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol. Cell 7, 1267-1278.

Cormio G., Maneo A., Parma G., Pittelli M.R., Miceli M.D. and Bonazzi C. (1995). Central nervous system metastases in patients with ovarian carcinoma. A report of 23 cases and literature review. Ann. Oncol. 6, 571-574.

Cully M., You H., Levine A.J. and Mak T.W. (2006). Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat. Rev. Cancer 6, 184-192.

Curtin J.P., Malik R., Venkatraman E.S., Barakat R.R. and Hoskins W.J. (1997). Stage IV ovarian cancer: impact of surgical debulking. Gynecol. Oncol. 64, 9-12.

Darzynkiewicz Z., Juan G., Li X., Gorczyca W., Murakami T. and Traganos F. (1997). Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis). Cytometry 27, 1-20.

Darzynkiewicz Z., Bedner E. and Traganos F. (2001). Difficulties and pitfalls in analysis of apoptosis. Methods Cell. Biol. 63, 527-546.

Davidson B. (2004). Malignant effusions: from diagnosis to biology. Diagn. Cytopathol. 31, 246-254.

Davidson B. (2007). The biological characteristics of cancers involving the serosal cavities. Crit. Rev. Oncog. 13, 189-227.

Davidson B., Berner A., Nesland J.M., Risberg B., Berner H.S., Tropé C.G., Kristensen G.B., Bryne M. and Flørenes V.A. (2000). E-cadherin and alpha-, beta-, and gamma-catenin protein expression is up-regulated in ovarian carcinoma cells in serous effusions. J. Pathol. 192, 460-469.

Davidson B., Reich R., Lazarovici P., Nesland J.M., Risberg B., Tropé C.G. and Flørenes V.A. (2003). Expression and activation of the nerve growth factor receptor TrkA in serous ovarian carcinoma. Clin. Cancer Res. 9, 2248-2259.

Davidson B., Zhang Z., Kleinberg L., Li M., Flørenes V.A., Wang T.L. and Shih Ie.M. (2006a). Gene expression signatures differentiate ovarian/peritoneal serous carcinoma from diffuse malignant peritoneal mesothelioma. Clin. Cancer Res. 12, 5944-5950.

Davidson B., Espina V., Flørenes V.A., Liotta L.A., Kristensen G.B., Tropé C.G., Berner A. and Kohn E.C. (2006b) Proteomic profiling of malignant ovarian cancer effusions: survival and injury pathways discriminate clinical outcome. Clin. Cancer Res. 12, 791-799.

Davidson B., Shih I.M. and Wang T.L. (2008a). Different clinical roles for p21-activated kinase-1 in primary and recurrent ovarian carcinoma. Hum. Pathol. 39, 1630-1636.

Davidson B., Hadar R., Schlossberg A., Sternlicht T., Slipicevic A., Skrede M., Risberg B., Flørenes V.A., Kopolovic J. and Reich R. (2008b). Expression and clinical role of DJ-1, a negative regulator of PTEN, in ovarian carcinoma. Hum. Pathol. 39, 87-95.

Denley A., Cosgrove L.J., Booker G.W., Wallace J.C. and Forbes B.E. (2005). Molecular interactions of the IGF system. Cytokine Growth Factor Rev. 16, 421-439.

Dong H.P., Kleinberg L., Silins I., Flørenes V.A., Trope' C.G., Risberg B., Nesland J.M. and Davidson B. (2008a). Death receptor expression is associated with poor response to chemotherapy and shorter survival in metastatic ovarian carcinoma. Cancer 112, 84-93.

Dong H.P., Kleinberg L., Davidson B. and Risberg B. (2008b). Methods for simultaneous measurement of apoptosis and cell surface phenotype of epithelial cells in effusions by flow cytometry. Nat.

Protoc. 3, 955-964.
Dong H.P., Holth A., Kleinberg L., Ruud M.G., Elstrand M.B., Tropé C.G., Davidson B. and Risberg B. (2009). Evaluation of cell surface expression of phosphatidylserine in ovarian carcinoma effusions using the Annexin-V/7-AAD assay - Clinical relevance and comparison to other apoptosis parameters. Am. J. Clin. Pathol. 132, 756-762.
Elenbaas B. and Weinberg R.A. (2001). Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. Exp. Cell Res. 264, 169-184.
Elloul S., Elstrand M.B., Nesland J.M., Tropé C.G., Kvalheim G., Goldberg I., Reich R. and Davidson B. (2005). Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. Cancer 103, 1631-1643.
Elloul S., Silins I., Tropé C.G., Benshushan A., Davidson B. and Reich R. (2006). Expression of E-cadherin transcriptional regulators in ovarian carcinoma. Virchows Arch. 449, 520-528.
Elloul S., Vaksman O., Tuft Stavnes H., Tropé C.G., Davidson B. and Reich R. (2010). Mesenchymal-to-Epithelial transition determinants as characteristics of ovarian carcinoma effusions. Clin. Exp. Metastasis 27, 161-172.
Feldman G.B., Knapp R.C., Order S.E. and Hellman S. (1972). The role of lymphatic obstruction in the formation of ascites in a murine ovarian carcinoma. Cancer Res. 32, 1663-1666.
Firth S.M. and Baxter R.C. (2002). Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 23, 824-854.
González-Mariscal L., Betanzos A., Nava P. and Jaramillo B.E. (2003) Tight junction proteins. Prog. Biophys. Mol. Biol. 81, 1-44.
Gotzmann J., Mikula M., Eger A., Schulte-Hermann R., Foisner R., Beug H. and Mikulits W. (2004). Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. Mutat. Res. 566, 9-20.
Guertin D.A. and Sabatini D.M. (2007). Defining the role of mTOR in cancer. Cancer Cell 12, 9-22.
Guicciardi M.E., Leist M. and Gores G.J. (2004). Lysosomes in cell death. Oncogene 23, 2881-2890.
Guo R.X., Qiao Y.H., Zhou Y., Li L.X., Shi H.R. and Chen K.S. (2008). Increased staining for phosphorylated AKT and nuclear factor-kappaB p65 and their relationship with prognosis in epithelial ovarian cancer. Pathol. Int. 58, 749-756.
Guvakova M.A. (2007). Insulin-like growth factors control cell migration in health and disease. Int. J. Biochem. Cell Biol. 39, 890-909.
Hajra K.M., Chen D.Y. and Fearon E.R. (2002). The SLUG zinc-finger protein represses E-cadherin in breast cancer. Cancer Res. 62, 1613-1618.
Hanahan D. and Weinberg R.A. (2000) The hallmarks of cancer. Cell 100, 57-70.
Heinzelmann-Schwarz V.A., Gardiner-Garden M., Henshall S.M., Scurry J., Scolyer R.A., Davies M.J., Heinzelmann M., Kalish L.H., Bali A., Kench J.G., Edwards L.S., Vanden Bergh P.M., Hacker N.F., Sutherland R.L. and O'Brien P.M. (2004). Overexpression of the cell adhesion molecules DDR1, Claudin 3, and Ep-CAM in metaplastic ovarian epithelium and ovarian cancer. Clin. Cancer Res. 10, 4427-4436.
Hernandez L., Hsu S., Davidson B., Birrer M.J., Kohn E.C. and Annunziata C.M. (2010). Activation of NF-κB signaling by IKKb increases aggressiveness of ovarian cancer. Cancer Res. 70, 405-414.
Hewitt K.J., Agarwal R. and Morin P.J. (2006). The claudin gene family: expression in normal and neoplastic tissues. BMC Cancer 6, 186.
Hibbs K., Skubitz K.M., Pambuccian S.E., Casey R.C., Burleson K.M., Oegema T.R. Jr, Thiele J.J., Grindle S.M., Bliss R.L. and Skubitz A.P. (2004). Differential gene expression in ovarian carcinoma: identification of potential biomarkers. Am. J. Pathol. 165, 397-414.
Hirabayashi K. and Graham J. (1970). The genesis of ascites in ovarian cancer. Am. J. Obstet. Gynecol. 106, 492-497.
Hofer-Warbinek R., Schmid J.A., Stehlik C., Binder B.R., Lipp J. and de Martin R. (2000). Activation of NF-kappa B by XIAP, the X chromosome-linked inhibitor of apoptosis, in endothelial cells involves TAK1. J. Biol. Chem. 275, 22064-22068.
Holschneider C.H. and Berek J.S. (2000). Ovarian cancer: epidemiology, biology, and prognostic factors. Semin. Surg. Oncol. 19, 3-10.
Hosono S., Kajiyama H., Terauchi M., Shibata K., Ino K., Nawa A. and Kikkawa F. (2007). Expression of Twist increases the risk for recurrence and for poor survival in epithelial ovarian carcinoma patients. Br. J. Cancer 96, 314-320.
Hough C.D., Sherman-Baust C.A., Pizer E.S., Montz F.J., Im D.D., Rosenshein N.B., Cho K.R., Riggins G.J. and Morin P.J. (2000). Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. Cancer Res. 60, 6281-6287.
Hough C.D., Cho K.R., Zonderman A.B., Schwartz D.R. and Morin P.J. (2001). Coordinately up-regulated genes in ovarian cancer. Cancer Res. 61, 3869-3876.
Hu L., Hofmann J., Lu Y., Mills G.B. and Jaffe R.B. (2002). Inhibition of phosphatidylinositol 3'-kinase increases efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. Cancer Res. 62, 1087-1092.
Huynh H., Teo C.C. and Soo K.C. (2007). Bevacizumab and rapamycin inhibit tumor growth in peritoneal model of human ovarian cancer. Mol. Cancer Ther. 6, 2959-2966.
Igney F.H. and Krammer P.H. (2002) Death and anti-death: tumour resistance to apoptosis. Nat. Rev. Cancer 2, 277-288.
Ikenouchi J., Matsuda M., Furuse M. and Tsukita S. (2003). Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. J. Cell Sci. 116, 1959-1967.
Imai T., Horiuchi A., Wang C., Oka K., Ohira S., Nikaido T. and Konishi I. (2003). Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells. Am. J. Pathol. 163, 1437-1447.
Imamura R., Konaka K., Matsumoto N., Hasegawa M., Fukui M., Mukaida N., Kinoshita T. and Suda T. (2004). Fas ligand induces cell-autonomous NF-κB activation and interleukin-8 production by a mechanism distinct from that of tumor necrosis factor-α. J. Biol. Chem. 279, 46415-46423.
Jemal A., Siegel R., Ward E., Hao Y., Xu J. and Thun M.J. (2009). Cancer statistics, 2009. CA Cancer J. Clin. 59, 225-249.
Jiao W., Miyazaki K. and Kitajima Y. (2002) Inverse correlation between E-cadherin and Snail expression in hepatocellular carcinoma cell lines in vitro and in vivo. Br. J. Cancer 86, 98-101.
Jin H., Yu Y., Zhang T., Zhou X., Zhou J., Jia L., Wu Y., Zhou B.P. and Feng Y. (2010). Snail is critical for tumor growth and metastasis of ovarian carcinoma. Int. J. Cancer 126, 2102-2111.
Johnstone R.W., Ruefli A.A. and Lowe S.W. (2002). Apoptosis: A link between cancer genetics and chemotherapy. Cell 108, 153-164.

Kalli K.R., Oberg A.L., Keeney G.L., Christianson T.J., Low P.S., Knutson K.L. and Hartmann L.C. (2008). Folate receptor alpha as a tumor target in epithelial ovarian cancer. Gynecol. Oncol. 108, 619-626.

Katsaros D., Yu H., Levesque M.A., Danese S., Genta F., Richiardi G., Fracchioli S., Khosravi M.J., Diamandi A., Gordini G., Diamandis E.P. and Massobrio M. (2001). IGFBP-3 in epithelial ovarian carcinoma and its association with clinico-pathological features and patient survival. Eur. J. Cancer 37, 478-485.

Kaufmann S.H., Lee S.H., Meng X.W., Loegering D.A., Kottke T.J., Henzing A.J., Ruchaud S., Samejima K. and Earnshaw W.C. (2008). Apoptosis-associated caspase activation assays. Methods 44, 262-272.

Kelemen L.E. (2006). The role of folate receptor α in cancer development, progression and treatment: Cause, consequence or innocent bystander? Int. J. Cancer 119, 243-250.

Kerr J.F., Wyllie A.H. and Currie A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239-257.

Kim K., Visintin I., Alvero A.B. and Mor G. (2009). Development and validation of a protein-based signature for the detection of ovarian cancer. Clin. Lab. Med. 29, 47-55.

Kim R.H., Peters M., Jang Y., Shi W., Pintilie M., Fletcher G.C., DeLuca C., Liepa J., Zhou L., Snow B., Binari R.C., Manoukian A.S., Bray M.R., Liu F.F., Tsao M.S. and Mak T.W. (2005). DJ-1, a novel regulator of the tumor suppressor PTEN. Cancer Cell 7, 263-273.

Kleinberg L., Holth A., Fridman E., Schwartz I., Shih Ie-M. and Davidson B. (2007a). The diagnostic role of claudins in serous effusions. Am. J. Clin. Pathol. 127, 928-937.

Kleinberg L., Flørenes V.A., Silins I., Haug K., Trope' C.G., Nesland J.M. and Davidson B. (2007b). Nuclear expression of Survivin is associated with improved survival in metastatic ovarian carcinoma. Cancer 109, 228-238.

Kleinberg L., Holth A., Trope' C.G., Reich R. and Davidson B. (2008). Claudin upregulation in ovarian carcinoma effusions is associated with poor survival. Hum. Pathol. 39, 747-757.

Kleinberg L., Dong H.P., Holth A., Risberg B., Trope' C.G., Nesland J.M., Flørenes V.A. and Davidson B. (2009). Cleaved caspases and NF-κB p65 are prognostic factors in metastatic ovarian carcinoma. Hum. Pathol. 40, 795-806.

Kuo K.T., Mao T.L., Jones S., Veras E., Ayhan A., Wang T.L., Glas R., Slamon D., Velculescu V.E., Kuman R.J. and Shih Ie-M. (2009). Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am. J. Pathol. 174, 1597-1601.

Kurrey N.K., K A. and Bapat S.A. (2005). Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol. Oncol. 97, 155-165.

Lee Y.K. and Park N.H. (2009). Prognostic value and clinicopathological significance of p53 and PTEN in epithelial ovarian cancers. Gynecol. Oncol. 112, 475-480.

Li F., Ambrosini G., Chu E.Y., Plescia J., Tognin S., Marchisio P.C. and Altieri DC. (1998). Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 396, 580-584.

Li J., Sherman-Baust C.A., Tsai-Turton M., Bristow R.E., Roden R.B. and Morin P.J. (2009). Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. BMC Cancer 9, 244.

Liston P., Fong W.G. and Korneluk R.G. (2003). The inhibitors of apoptosis: there is more to life than Bcl2. Oncogene 22, 8568-8580.

Liu G.H., Wang S.R., Wang B. and Kong B.H. (2006). Inhibition of nuclear factor-kappaB by an antioxidant enhances paclitaxel sensitivity in ovarian carcinoma cell line. Int. J. Gynecol. Cancer 16, 1777-1782.

Liu J.R., Opipari A.W., Tan L., Jiang Y., Zhang Y., Tang H. and Nuñez G. (2002). Dysfunctional apoptosome activation in ovarian cancer: implications for chemoresistance. Cancer Res. 62, 924-931.

Lowe S.W. and Lin A.W. (2000). Apoptosis in cancer. Carcinogenesis 21, 485-495.

Lu K.H., Patterson A.P, Wang L., Marquez R.T., Atkinson E.N., Baggerly K.A., Ramoth L.R., Rosen D.G., Liu J., Hellstrom I., Smith D., Hartmann L., Fishman D., Berchuck A., Schmandt R., Whitaker R., Gershenson D.M., Mills G.B. and Bast R.C. Jr (2004). Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin. Cancer Res. 10, 3291-3300.

Lu L., Katsaros D., Wiley A., Rigault de la Longrais I.A., Risch H.A., Puopolo M. and Yu H. (2006). The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-alpha expression to disease progression in epithelial ovarian cancer. Clin. Cancer Res. 12, 1208-1214.

Madrid L.V., Wang C.Y., Guttridge D.C., Schottelius A.J., Baldwin A.S. Jr and Mayo M.W. (2000). Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-kappaB. Mol. Cell Biol. 20, 1626-1638.

Mabuchi S., Ohmichi M., Nishio Y., Hayasaka T., Kimura A., Ohta T., Saito M., Kawagoe J., Takahashi K., Yada-Hashimoto N., Sakata M., Motoyama T., Kurachi H., Tasaka K. and Murata Y. (2004). Inhibition of NFkappaB increases the efficacy of cisplatin in *in vitro* and *in vivo* ovarian cancer models. J. Biol. Chem. 279, 23477-23485.

Mabuchi S., Ohmichi M., Nishio Y., Hayasaka T, Kimura A., Ohta T., Kawagoe J., Takahashi K., Yada-Hashimoto N., Seino-Noda H., Sakata M., Motoyama T., Kurachi H., Testa J.R., Tasaka K. and Murata Y. (2004). Inhibition of inhibitor of nuclear factor-kappaB phosphorylation increases the efficacy of paclitaxel in in vitro and in vivo ovarian cancer models. Clin. Cancer Res. 10, 7645-7654.

Mabuchi S., Altomare D.A., Cheung M., Zhang L., Poulikakos P.I., Hensley H.H., Schilder R.J., Ozols R.F. and Testa J.R. (2007). RAD001 inhibits human ovarian cancer cell proliferation, enhances cisplatin-induced apoptosis, and prolongs survival in an ovarian cancer model. Clin. Cancer Res. 13, 4261-4270.

Maddika S., Ande S.R., Panigrahi S., Paranjothy T., Weglarczyk K., Zuse A., Eshraghi M., Manda K.D., Wiechec E. and Los M. (2007). Cell survival, cell death and cell cycle pathways are interconnected: implications for cancer therapy. Drug Resist. Updat. 10, 13-29.

Manser E., Leung T., Salihuddin H., Shao Z.S. and Lim L. (1994). A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367, 40-46.

Miyoshi A., Kitajima Y., Kido S. Shimonishi T., Matsuyama S., Kitahara K. and Miyazaki K. (2005). Snail accelerates cancer invasion by upregulating MMP expression and is associated with poor prognosis of hepatocellular carcinoma. Br. J. Cancer 92, 252-258.

Morel J., Audo R., Hahne M. and Combe B. (2005). Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) induces rheumatoid arthritis synovial fibroblast proliferation through mitogen- activated protein kinases and phosphatidylinositol 3-kinase/Akt. J. Biol. Chem. 280, 15709-15718.

Morin P.J. (2005). Claudin proteins in human cancer: promising new

targets for diagnosis and therapy. Cancer Res. 65, 9603-9606.
Morita K., Furuse M., Fujimoto K. and Tsukita S. (1999). Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc. Natl. Acad. Sci. USA 96, 511-516.
Nagy J.A., Masse E.M., Herzberg K.T., Meyers M.S., Yeo K.T., Yeo T.K., Sioussat T.M. and Dvorak H.F. (1995). Pathogenesis of ascites tumor growth: vascular permeability factor, vascular hyperpermeability, and ascites tumor accumulation. Cancer Res. 55, 360-368.
Neumann M. and Naumann M. (2007). Beyond IkappaBs: alternative regulation of NF-kappaB activity. FASEB J. 21, 2642-2654.
Norbury C.J. and Zhivotovsky B. (2004). DNA damage-induced apoptosis. Oncogene 23, 2797-2808.
Noske A., Lindenberg J.L., Darb-Esfahani S., Weichert W., Buckendahl A.C., Röske A., Sehouli J., Dietel M. and Denkert C. (2008). Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis. Oncol. Rep. 20, 1409-1417.
Oda Y., Ohishi Y., Basaki Y., Kobayashi H., Hirakawa T., Wake N., Ono M., Nishio K., Kuwano M. and Tsuneyoshi M. (2007). Prognostic implications of the nuclear localization of Y-box-binding protein-1 and CXCR4 expression in ovarian cancer: their correlation with activated Akt, LRP/MVP and P-glycoprotein expression. Cancer Sci. 98, 1020-1026.
Orrenius S., Zhivotovsky B. and Nicotera P. (2003). Regulation of cell death: The calcium apoptosis link. Nat. Rev. Mol. Cell Biol. 4, 552-565.
Otsuki Y., Li Z. and Shibata M.A. (2003). Apoptotic detection methods--from morphology to gene. Prog. Histochem. Cytochem. 38, 275-339.
Page C., Lin H.J., Jin Y., Castle V.P., Nunez G., Huang M. and Lin J. (2000). Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. Anticancer Res. 20, 407-416.
Pahl H.L. (1999). Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853-6866.
Parker N., Turk M.J., Westrick E., Lewis J.D., Low P.S. and Leamon C.P. (2005). Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. Anal. Biochem. 338, 284-293
Pecina-Slaus N. (2003). Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell Int. 3, 17.
Perkins N.D. (2007). Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat. Rev. Mol. Cell Biol. 8, 49-62.
Perkins N.D. and Gilmore T.D. (2006). Good cop, bad cop: the different faces of NF-kappaB. Cell Death Differ. 13, 759-772.
Pianetti S., Arsura M., Romieu-Mourez R., Coffey R.J. and Sonenshein GE. (2001). Her-2/neu overexpression induces NF-kappaB via a PI3-kinase/Akt pathway involving calpain-mediated degradation of IkappaB-alpha that can be inhibited by the tumor suppressor PTEN. Oncogene 20, 1287-1299.
Pon Y.L., Zhou H.Y., Cheung A.N., Ngan H.Y. and Wong A.S. (2008). p70 S6 kinase promotes epithelial to mesenchymal transition through snail induction in ovarian cancer cells. Cancer Res. 68, 6524-6532.
Poser I., Domínguez D., de Herreros A.G., Varnai A., Buettner R. and Bosserhoff A.K. (2001). Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. J. Biol. Chem. 276, 24661-24666.
Pozarowski P., Grabarek J. and Darzynkiewicz Z. (2004). Flow cytometry of apoptosis. Curr. Protoc. Cell Biol. Chapter 18, Unit 18.8.
Rangel L.B., Agarwal R., D'Souza T., Pizer E.S., Alò P.L., Lancaster W.D., Gregoire L., Schwartz D.R., Cho K.R. and Morin P.J. (2003). Tight junction proteins claudin-3 and claudin-4 are frequently overexpressed in ovarian cancer but not in ovarian cystadenomas. Clin. Cancer Res. 9, 2567-2575.
Reed J.C. (2006). Drug insight: cancer therapy strategies based on restoration of endogenous cell death mechanisms. Nat. Clin. Pract. Oncol. 3, 388-398.
Riedl S.J. and Shi Y. (2004). Molecular mechanisms of caspase regulation during apoptosis. Nat. Rev. Mol. Cell Biol. 5, 897-907.
Romani C., Comper F., Bandiera E., Ravaggi A., Bignotti E., Tassi R.A., Pecorelli S. and Santin A.D. (2009). Development and characterization of a human single-chain antibody fragment against claudin-3: a novel therapeutic target in ovarian and uterine carcinomas. Am. J. Obstet. Gynecol. 201, 70.e1-9.
Rosivatz E., Becker I., Specht K. Fricke E., Luber B., Busch R., Höfler H. and Becker K.F. (2002). Differential expression of the epithelial-mesenchymal transition regulators snail, SIP1, and twist in gastric cancer. Am. J. Pathol. 161, 1881-1891.
Roy N., Deveraux Q.L., Takahashi R., Salvesen G.S. and Reed J.C. (1997). The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J. 16, 6914-6925.
Sanna M.G., da Silva Correia J., Ducrey O., Lee J., Nomoto K., Schrantz N., Deveraux Q.L. and Ulevitch R.J. (2002). IAP suppression of apoptosis involves distinct mechanisms: the TAK1/JNK1 signaling cascade and caspase inhibition. Mol. Cell Biol. 22, 1754-1766.
Sachdev D. and Yee D. (2007). Disrupting insulin-like growth factor signaling as a potential cancer therapy. Mol. Cancer Ther. 6, 1-12.
Saeki R., Kondoh M., Kakutani H., Tsunoda S., Mochizuki Y., Hamakubo T., Tsutsumi Y., Horiguchi Y. and Yagi K. (2009). A novel tumor-targeted therapy using a claudin-4-targeting molecule. Mol. Pharmacol. 76, 918-926.
Sakai K., Kamura T., Hirakawa T., Saito T., Kaku T. and Nakano H. (1997). Relationship between pelvic lymph node involvement and other disease sites in patients with ovarian cancer. Gynecol. Oncol. 65, 164-168.
Salazar M.D. and Ratnam M. (2007). The folate receptor: What does it promise in tissue-targeted therapeutics? Cancer Metastasis Rev. 26, 141-152.
Samanta A.K., Huang H.J., Le X.F., Mao W., Lu K.H., Bast R.C. Jr and Liao W.S. (2009). MEKK3 expression correlates with nuclear factor kappa B activity and with expression of antiapoptotic genes in serous ovarian carcinoma. Cancer 115, 3897-3908.
Santin A.D., Zhan F., Bellone S., Palmieri M., Cane S., Bignotti E., Anfossi S., Gokden M., Dunn D., Roman J.J., O'Brien T.J., Tian E., Cannon M.J., Shaughnessy J. Jr and Pecorelli S. (2004). Gene expression profiles in primary ovarian serous papillary tumors and normal ovarian epithelium: identification of candidate molecular markers for ovarian cancer diagnosis and therapy. Int. J. Cancer 112, 14-25.
Sarbassov D.D., Guertin D.A., Ali S.M. and Sabatini D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101.
Sayer R.A., Lancaster J.M., Pittman J., Gray J., Whitaker R., Marks J.R. and Berchuck A. (2005). High insulin-like growth factor-2 (IGF-2) gene expression is an independent predictor of poor survival for patients with advanced stage serous epithelial ovarian cancer.

Gynecol. Oncol. 96, 355-361.

Schimmer A.D. (2004). Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. Cancer Res 64, 7183-7190.

Secchiero P., Gonelli A., Carnevale E., Milani D., Pandolfi A., Zella D. and Zauli G. (2003). TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. Circulation 107, 2250-2256.

Sethi G., Sung B. and Aggarwal B.B. (2008). Nuclear factor-kappaB activation: from bench to bedside. Exp. Biol. Med. (Maywood) 233, 21-31.

Shih Ie.M., Sheu J.J., Santillan A., Nakayama K., Yen M.J., Bristow R.E., Vang R., Parmigiani G., Kurman R.J., Trope C.G., Davidson B. and Wang T.L. (2005). Amplification of a chromatin remodeling gene, Rsf-1/HBXAP, in ovarian carcinoma. Proc. Natl. Acad. Sci. USA 102, 14004-14009.

Shih Ie-M. and Davidson B. (2009). Tumor-associated genes in ovarian cancer. Future Oncol. 5, 1641-1657.

Siu M.K., Wong E.S., Chan H.Y., Kong D.S., Woo N.W., Tam K.F., Ngan H.Y., Chan Q.K., Chan D.C., Chan K.Y. and Cheung A.N. (2010). Differential expression and phosphorylation of Pak1 and Pak2 in ovarian cancer: effects on prognosis and cell invasion. Int. J. Cancer 99, 431-433.

Sivertsen S., Berner A., Michael C.W., Bedrossian C. and Davidson B. (2006). Cadherin expression in ovarian carcinoma and malignant mesothelioma cell effusions. Acta Cytol. 50, 603-607.

Slipicevic A., Øy G.F., Askildt I.C., Holth A., Hellesylt E., Flørenes V.A. and Davidson B. (2009). The diagnostic and prognostic role of the insulin growth factor pathway members IGF-II and IGFBP3 in serous effusions. Hum. Pathol. 40, 527-537.

Shiozaki E.N., Chai J., Rigotti D.J., Riedl S.J., Li P., Srinivasula S.M., Alnemri E.S., Fairman R. and Shi Y. (2003). Mechanism of XIAP-mediated inhibition of caspase-9. Mol. Cell 11, 519-527.

Soini Y. and Talvensaari-Mattila A. (2006). Expression of claudins 1, 4, 5, and 7 in ovarian tumors of diverse types. Int. J. Gynecol. Pathol. 25, 330-335.

Song G., Ouyang G. and Bao S. (2005). The activation of Akt/PKB signaling pathway and cell survival. J. Cell Mol. Med. 9, 59-71.

Spannuth W.A., Sood A.K. and Coleman R.L. (2010). Farletuzumab in epithelial ovarian carcinoma. Expert Opin. Biol. Ther. 10, 431-437.

Sun C., Cai M., Gunasekera A.H., Meadows R.P., Wang H., Chen J., Zhang H., Wu W., Xu N., Ng S.C. and Fesik S.W. (1999). NMR structure and mutagenesis of the inhibitor-of -apoptosis protein XIAP. Nature 401, 818-822.

Suzuki M., Kato-Nakano M., Kawamoto S., Furuya A., Abe Y., Misaka H., Kimoto N., Nakamura K., Ohta S. and Ando H. (2009). Therapeutic antitumor efficacy of monoclonal antibody against Claudin-4 for pancreatic and ovarian cancers. Cancer Sci. 100, 1623-1630.

Swisshelm K., Macek R. and Kubbies M. (2005). Role of claudins in tumorigenesis. Adv. Drug Deliv. Rev. 57, 919-928.

Takahashi R., Deveraux Q., Tamm I., Welsh K., Assa-Munt N., Salvesen G.S. and Reed J.C. (1998). A single BIR domain of XIAP sufficient for inhibiting caspases. J. Biol. Chem. 273, 7787-7790.

Takeda K., Stagg J., Yagita H., Okumura K. and Smyth M.J. (2007). Targeting death-inducing receptors in cancer therapy. Oncogene 26, 3745-3757.

Takei Y., Saga Y., Mizukami H., Takayama T., Ohwada M., Ozawa K. and Suzuki M. (2008). Overexpression of PTEN in ovarian cancer cells suppresses i.p. dissemination and extends survival in mice. Mol. Cancer Ther. 7, 704-711.

Tang G., Minemoto Y., Dibling B., Purcell N.H., Li Z., Karin M. and Lin A. (2001). Inhibition of JNK activation through NF-kappaB target genes. Nature 414, 313-317.

Tassi R.A., Bignotti E., Falchetti M., Ravanini M., Calza S., Ravaggi A., Bandiera E., Facchetti F., Pecorelli S. and Santin A.D. (2008). Claudin-7 expression in human epithelial ovarian cancer. Int. J. Gynecol. Cancer 18, 1262-1271.

Taylor R.C., Cullen S.P. and Martin S.J. (2008). Apoptosis: controlled demolition at the cellular level. Nat. Rev. Mol. Cell Biol. 9, 231-241.

Terauchi M., Kajiyama H., Yamashita M., Kato M., Tsukamoto H., Umezu T., Hosono S., Yamamoto E., Shibata K., Ino K., Nawa A., Nagasaka T. and Kikkawa F. (2007). Possible involvement of TWIST in enhanced peritoneal metastasis of epithelial ovarian carcinoma. Clin. Exp. Metastasis 24, 329-339.

Thériault B.L., Shepherd T.G., Mujoomdar M.L. and Nachtigal M.W. (2007). BMP4 induces EMT and Rho GTPase activation in human ovarian cancer cells. Carcinogenesis 28, 1153-1162.

Thiery J.P. (2002). Epithelial-mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2, 442-454.

Thompson F.H., Nelson M.A., Trent J.M., Guan X.Y., Liu Y., Yang J.M., Emerson J., Adair L., Wymer J., Balfour C., Massey K., Weinstein R., Alberts D.S. and Taetle R. (1996). Amplification of 19q13.1-q13.2 sequences in ovarian cancer. G-band, FISH, and molecular studies. Cancer Genet. Cytogenet. 87, 55-62.

Thompson J.E. and Thompson C.B. (2004). Putting the rap on Akt. J. Clin. Oncol. 22, 4217-4226.

Trinh X.B., van Dam P.A., Dirix L.Y., Vermeulen P.B. and Tjalma W.A. (2009). The rationale for mTOR inhibition in epithelial ovarian cancer. Expert Opin. Investig. Drugs. 18, 1885-1891.

Tsukita S., Furuse M. and Itoh M. (2001). Multifunctional strands in tight junctions. Nat. Rev. Mol. Cell Biol. 2, 285-293.

Tsuruchi N., Kamura T., Tsukamoto N., Akazawa K., Saito T., Kaku T., To N. and Nakano H. (1993). Relationship between paraaortic lymph node involvement and intraperitoneal spread in patients with ovarian cancer--a multivariate analysis. Gynecol. Oncol. 49, 51-55.

Tuhkanen H., Soini Y., Kosma V.M., Anttila M., Sironen R., Hämäläinen K., Kukkonen L., Virtanen I. and Mannermaa A. (2009). Nuclear expression of Snail1 in borderline and malignant epithelial ovarian tumours is associated with tumour progression. BMC Cancer 9, 289.

van der Wal J.B. and Jeekel J. (2007). Biology of the peritoneum in normal homeostasis and after surgical trauma. Colorectal Dis. 9 Suppl 2, 9-13.

Van Itallie C.M. and Anderson J.M. (2006). Claudins and epithelial paracellular transport. Annu. Rev. Physiol. 68, 403-429.

Vermes I., Haanen C. and Reutelingsperger C. (2000). Flow cytometry of apoptotic cell death. J. Immunol. Methods 243, 167-190.

Vernon A.E. and LaBonne C. (2004). Tumor metastasis: a new twist on epithelial-mesenchymal transitions. Curr. Biol. 14, R719-721.

Vivanco I. and Sawyers C. (2002). The phosphatidylinositol 3-kinase-Akt pathway in human cancer. Nat. Rev. Cancer 2, 489-501.

Wajant H., Pfizenmaier K. and Scheurich P. (2003). Tumor necrosis factor signaling. Cell Death Differ. 10, 45-65.

Walker G., MacLeod K., Williams A.R., Cameron D.A., Smyth J.F. and Langdon S.P. (2007). Insulin-like growth factor binding proteins IGFBP3, IGFBP4, and IGFBP5 predict endocrine responsiveness in patients with ovarian cancer. Clin. Cancer Res. 13, 1438-1444.

Wang C.Y., Guttridge D.C., Mayo M.W., Baldwin A.S. Jr (1999). NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to

preferentially suppress chemotherapy-induced apoptosis. Mol. Cell Biol. 19, 5923-5929.
Wang X. (2001). The expanding role of mitochondria in apoptosis. Genes Dev. 15, 2922-2933.
Wang X., Ling M.T., Guan X.Y., Tsao S.W., Cheung H.W., Lee D.T. and Wong Y.C. (2004). Identification of a novel function of TWIST, a bHLH protein, in the development of acquired taxol resistance in human cancer cells. Oncogene 23, 474-482.
Wiley A., Katsaros D., Fracchioli S. and Yu H. (2006). Methylation of the insulin-like growth factor binding protein-3 gene and prognosis of epithelial ovarian cancer. Int. J. Gynecol. Cancer 16, 210-218.
Witowski J., Ksiazek K. and Jorres A. (2008). New insights into the biology of peritoneal mesothelial cells: the roles of epithelial-to-mesenchymal transition and cellular senescence. Nephron Exp. Nephrol. 108, e69-73.
Woenckhaus J., Steger K., Sturm K., Münstedt K., Franke F.E. and Fenic I. (2007). Prognostic value of PIK3CA and phosphorylated AKT expression in ovarian cancer. Virchows Arch. 450, 387-395.
Xiao C.W., Yan X., Li Y., Reddy S.A. and Tsang B.K. (2003). Resistance of human ovarian cancer cells to tumor necrosis factor alpha is a consequence of nuclear factor kappaB-mediated induction of Fas-associated death domain-like interleukin-1beta-converting enzyme-like inhibitory protein. Endocrinology 144, 623-630.
Yang H., Kong W., He L., Zhao J.J., O'Donnell J.D., Wang J., Wenham R.M., Coppola D., Kruk P.A., Nicosia S.V. and Cheng J.Q. (2008). MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 68, 425-433.
Yang X., Zheng F., Xing H., Gao Q., Wei W., Lu Y., Wang S., Zhou J., Hu W. and Ma D. (2004). Resistance to chemotherapy-induced apoptosis via decreased caspase-3 activity and overexpression of antiapoptotic proteins in ovarian cancer. J. Cancer Res. Clin. Oncol. 130, 423-428.
Yang Z., Rayala S., Nguyen D., Vadlamudi R.K., Chen S. and Kumar R. (2005). Pak1 phosphorylation of snail, a master regulator of epithelial-to-mesenchyme transition, modulates snail's subcellular localization and functions. Cancer Res. 65, 3179-3184.
Yap T.A., Garrett M.D., Walton M.I., Raynaud F., de Bono J.S. and Workman P. (2008). Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. Curr. Opin. Pharmacol. 8, 393-412.
Yokoyama K., Kamata N., Hayashi E., Hoteiya T., Ueda N., Fujimoto R. and Nagayama M. (2001). Reverse correlation of E-cadherin and snail expression in oral squamous cell carcinoma cells in vitro. Oral Oncol. 37, 65-71.
Yokoyama K., Kamata N., Fujimoto R., Tsutsumi S., Tomonari M., Taki M., Hosokawa H. and Nagayama M. (2003). Increased invasion and matrix metalloproteinase-2 expression by Snail-induced mesenchymal transition in squamous cell carcinomas. Int. J. Oncol. 22, 891-898.
Yoshida J., Horiuchi A., Kikuchi N., Hayashi A., Osada R., Ohira S., Shiozawa T. and Konishi I. (2009). Changes in the expression of E-cadherin repressors, Snail, Slug, SIP1, and Twist, in the development and progression of ovarian carcinoma: the important role of Snail in ovarian tumorigenesis and progression. Med. Mol. Morphol. 42, 82-91.
Yuan X., Lin X., Manorek G., Kanatani I., Cheung L.H., Rosenblum M.G. and Howell S.B. (2009). Recombinant CPE fused to tumor necrosis factor targets human ovarian cancer cells expressing the claudin-3 and claudin-4 receptors. Mol. Cancer Ther. 8, 1906-1915.
Yuan Y., Nymoen D.A., Dong H.P., Bjørang O., Shih Ie-M., Low P.S., Trope' C.G. and Davidson B. (2009). Expression of the folate receptor genes FOLR1 and FOLR3 differentiates ovarian carcinoma from breast carcinoma and malignant mesothelioma in serous effusions. Hum. Pathol. 40, 1453-1460.
Yung S. and Chan T.M. (2009). Intrinsic cells: mesothelial cells -- central players in regulating inflammation and resolution. Perit. Dial. Int. 29 Suppl 2, S21-7.
Zhu Y., Brannstrom M., Janson P.O. and Sundfeldt K. (2006). Differences in expression patterns of the tight junction proteins, claudin 1, 3, 4 and 5, in human ovarian surface epithelium as compared to epithelia in inclusion cysts and epithelial ovarian tumours. Int. J. Cancer 118, 1884-1891.

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