Histol Histopathol (2012) 27: 707-719
DOI: 10.14670/HH-27.707

**http://www.hh.um.es**

**Histology and Histopathology**
*Cellular and Molecular Biology*

## *Review*

# Ovarian cancer: insights into genetics and pathogeny

**Ludmila Liliac, Cornelia Amălinei, Raluca Balan, Adriana Grigoraș and Irina-Draga Căruntu**
Department of Histology, "Gr. T. Popa" University of Medicine and Pharmacy, Iași, Romania

**Summary.** Starting from the information on ovarian cancer provided by the mainstream publications, we construct a review focusing on the following issues: (i) the genetic profile, (ii) the role of the epithelial-mesenchymal transition in the acquirement of malignant features, (iii) the controversial hypothesis regarding the origin, and (iv) the involvement of the immune system in the tumoral microenvironment. Advances in the decipherment at the genetic level in the pathogenic mechanisms progressively lead to the idea of a genetic signature for the ovarian cancer. Moreover, the complementary approaches oriented towards the decryption of the intrinsic structure of the expressed molecules and, implicitly, the development of proteomics open new perspectives for an early diagnosis and an appropriate treatment. The research on the epithelial-mesenchymal transition (mainly those exploring the signaling pathways responsible for the switch between the loss of the epithelial characteristics and the gain of a mesenchymal cell phenotype, with results in the amplification of differentiation, motility and tumoral invasion) allow a deeper understanding of the complex pathogenic mechanism which governs ovarian carcinogenesis. The classic conception of ovarian cancer pathogeny, based on the role of the ovarian surface epithelium, is currently reconsidered, and a novel hypothesis is formulated, which supports direct involvement of the Fallopian tubes for the serous type. Although recent research suggests the implication of immune/inflammatory cells by specific mechanisms in ovarian cancer pathogenesis, there is yet reliable evidence concerning their modality of direct action and/or modulation of tumoral growth. Thus, ovarian carcinogenesis remains a research challenge, due to still numerous unknown factors involved in the malignant transformation sequences, originating from the genetic-molecular alterations and reflected by cellular and tissue expression patterns.

**Key words:** Ovarian cancer, Pathogeny, Genetics, Proteomics, Epithelial-mesenchymal transition

*Offprint requests to:* Irina-Draga Căruntu, "Gr. T. Popa" University of Medicine and Pharmacy, 16, University Street, Iași, 700115, Romania. e-mail: irinadragacaruntu@gmail.com, irina_caruntu@yahoo.com

### Introduction

Ovarian cancer (OC) is the ninth most frequently occurring type of cancer and stands as the fifth cause of cancer-related death among women (American Cancer Society, 2009). It is considered as the most aggressive, with a number of deaths that can be equaled with the sum of those caused by all the other female gynecologic malignancies (Kurman and Shih, 2010). Approximately 190,000 new cases are recorded each year (Gómez-Raposo et al., 2010), and the prevalence increases with patients' age, with reported ranges between 0.7% in women younger than 20 years and reaching 55.5% in patients aged 74 or more (Ricciardelli and Oehler, 2009). Five year survival rates are less than 30% in 70% of patients with advanced disease. Despite the initial response to chemotherapy in about 80% of cases, an important percentage is recurrent (Ricciardelli and Oehler, 2009).

Although significant progress was achieved in understanding the biology of OC, the diagnosis is frequently late, in advanced stages (III-IV), when intraperitoneal carcinomatosis is already apparent and the disease is disseminated (Vergara et al., 2010). It is almost impossible to make an early detection (Ricciardelli and Oehler, 2009), due to the lack of specificity of clinical symptoms and the absence of effective screening programs, which should rely on an appropriate diagnosis tool, including specific biomarkers, and on a health policy targeting also the ovary, not only the breast, colon and cervix.

The most important risk factors involved in OC pathogeny are age, genetic profile (expressed by a strong family history of ovarian and breast cancer, mainly supported by mutations in the BRCA1 and BRCA2

tumor suppressor genes), and the hormonal profile which determines specific reproductive characteristics (early onset of menses, high number of ovulatory cycles, late menopause, nulliparity / low parity, infertility, endometriosis) (Gates et al., 2010; Jelovac and Armstrong, 2011; Lalwani et al., 2011). On the other hand, the hormonal - reproductive axis may intervene in a protective way by late menarche, a reduced number of ovulatory cycles, early menopause, multiple pregnancies, prolonged lactation, over 5 years long use of oral contraceptives and special medical conditions such as tubal ligation and hysterectomy (Lalwani et al., 2011). The implication of the environmental factors as risk factors is still in debate; despite research focused on the role of diet, obesity, smoking, asbestosis, talc powder exposure, the results are controversial (Sueblinvong and Carney, 2009). However, the differences between the incidence of OC in various geographical areas could be explained by the intervention of some of these environmental factors (i.e. diet) (Jelovac and Armstrong, 2011), or by racial and ethnic variation (correlated with the genetic inheritance - e.g. the Ashkenazi Jews) (Saunders et al., 2011).

Considering the total amount of OCs, approximately 5-10% are hereditary, all the rest being sporadic (Jelovac and Armstrong, 2011).

## Genetic profile correlated to ovarian carcinogenesis

### *Landmarks*

The identification of the genetic pathways and mechanisms involved in the pathogeny of OC represents an essential stage in the understanding of the malignant transformation process undergone by the normal ovarian epithelium (Pejovic et al., 2009). Nevertheless, although it is unanimously accepted that an important number of genetic and epigenetic abnormalities is associated with OC, the modality of their appearance is still incompletely elucidated. Within this framework, it can be asserted that even though we can identify a wide range of genetic anomalies, we do not know yet who and how the occurrence, the inheritance or the acquisition of these changes is governed by.

The genetic polymorphism of OC is based on high genetic instability, causing unscheduled alterations in the genome, as a result of the activation or inactivation of certain genes (Bast et al., 2009). The activation is performed either through genic amplification (RAB25, PRKCI, EVI1 and PIK3CA, FGF1, MYC, PIK3R1 and AKT2, AURKA), mutation (KRAS, BRAF, CITNNB1, CDKN2A, APC, PIK3CA, KIT, SMAD4) or hyper-methylation (IGF2, SAT2). Genetic inactivation involves the deletion of numerous chromosomial regions, loss of heterozygosity at specific loci (BRCA1, BRCA2, PTEN, TP53, PEG3, PLAGL1, ARHI), mutations (BRCA1, BRCA2, PTEN, TP53) and promoter methylation (MUC2, PEG3, MLH1, ICAM1, PLAGL1, ARH1) (Bast et al., 2009).

According to the literature, within this wide genetic panel several main categories of genes can be identified, as follows: tumor suppressor genes (a total of 16: ARHI, RASSF1A, DLEC1, SPARC, DAB2, PLAGL1, RPS6KA2, PTEN, OPCML, BRCA2, ARL11, WWOX, TP53, DPH1, BRCA1, PEG3), oncogenes (a total of 15: RAB25, EVI1, EIF5A2, PRKCI, PIK3CA, MYK, EGFR, NOTCH3, KRAS, ERBB2, PIK3R1, CCNE1, AKT2, AURKA) and imprinted tumor suppressor genes (ARH1, PLAGL1 and PEG3) (Bast et al., 2009), which deserve a special remark. The imprinting process - characterized by the downregulation of the functional allele - is achieved through the loss of heterozygosity, hypermethylation and transcriptional regulation and represents a marker for a reserved prognosis. On the other hand, the reexpression of these genes contributes to an improvement of OC prognosis, due to their ability to inhibit tumor cell proliferation, motility and angiogenesis.

In the pathogeny of OC, in over 50% of cases, the sequence of events includes the activation of several signaling pathways, as follows: the Lysophosphatidic Acid (LPA) pathway, the Phosphatidylinositol 3-Kinases (PI3K) pathway, the Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κB) pathway, the Interleukin-6 - Interleukin-6 Receptor (IL-6 - IL-6R) or Janus Kinase 2 - Signal Transducer and Activator of Transcription 3 (Jak-STAT 3) pathway, the Mitogen-Activated Protein Kinase (MAPK) pathway, the Proto-Oncogene Tyrosine-Protein Kinase Src pathway, the Mullerian Inhibitory Substance Receptor pathway (Bast et al., 2009).

The signaling pathways consist of several molecules with diverse biochemical structure which interact and consequently intervene in cell metabolism by a critical regulatory role in cell proliferation, cell differentiation, cell movement and cell death.

Table 1 summarizes the most investigated pathways, highlighting the main steps of their mechanism, in relationship with the possible involvement in the therapeutic approach of OC.

### *Hereditary ovarian cancer versus sporadic ovarian cancer*

Research on OC leads to the identification and characterization of the two primary types: hereditary (type I) and sporadic (type II). In 90% of the cases of hereditary type the major responsibility is attributed to the germline mutations in BRCA1 and BRCA2 genes. However, the germline mutations in BRCA1 and BRCA2 genes are not exclusively associated with the hereditary type. Though uncommon, they can be involved in the pathogeny of the sporadic type, and there is increasing evidence that the downregulation of BRCA1 protein expression may play a role in these tumor types (Geisler et al., 2002; Hilton et al., 2002; Jazaeri, 2009). As counterparts to the hereditary type, the sporadic OC associated with BRCA1 and BRCA2

**Table 1.** Signaling pathways in ovarian cancer pathogenesis.

| WHY IS IT IMPORTANT? | MECHANISM | THERAPEUTIC POTENTIAL |
|---|---|---|
| LPA PATHWAY | | |
| • role in regulation of gene expression in normal and neoplastic cells (Oyesanya et al., 2008)<br>• evidence for involvement in pathogeny of 90% cases of OC: initiation, progression and metastasis (including associated ascites) (Bast et al., 2009; Song et al., 2009) | LIGAND: LPA<br>RECEPTORS: LPA1/Edg-2, LPA2/Edg-4, LPA3/Edg 7<br>ACTIVATION: LPAR+LPA through unsaturated fatty acyl chains<br>DOWNSTREAM EFFECTS:<br>• cell proliferation (by cyclin D1)<br>• tumor angiogenesis (VEGF and IL-8)<br>• MMP activation<br>• Cox-2 expression | • inhibition of carcinogenesis, by LPAR inhibitors<br>• research in preclinical phase (Hennessy et al., 2009) |
| PI3K PATHWAY | | |
| • role in cell cycle progression, survival, motility, angiogenesis, immune surveillance<br>• evidence for involvement in pathogeny of 70% of cases of OC (histological types: clear cell, endometrioid, TCG) (Bast et al., 2009; Kuo et al., 2009)<br>• evidence for role in acquired chemoresistance in OC (Yang et al., 2006; Markman et al., 2010) | LIGANDS: growth factors (BMP, EGF, HGF, TGFα, LT, CD 20L)<br>RECEPTORS: EGFR, c-kit, IGFR-1<br>ACTIVATION:<br>• amplification of PIK/AKT2 gene<br>• inactivating mutations in PTEN gene<br>• activating mutations in PI3CA gene<br>• signals transmitted through RTK of growth factors<br>DOWNSTREAM EFFECTS:<br>• PI3 dimerisation /autophosphorylation → PIP2 → PIP3 → AKT →proliferation by inactivating cell cycle inhibitors ($p27^{Kip1}$, p21), inhibition of pro-apoptotic genes (FasL, Bim, BAD), degradation of p53 | • inhibition of proliferative activity by specific developed drugs (PI3K inhibitors)<br>• research in 1/2 phase of clinical trial (Hennessy et al., 2009; Markman et al., 2010; Rho et al., 2011) |
| NF-kB PATHWAY | | |
| • role in regulation of genes that participate in cell survival, angiogenesis, and metastasis<br>• evidence for involvement in pathogeny of more than 50% of cases of OC (Bast et al., 2009; Hernandez et al., 2010; Annunziata et al., 2010) | LIGANDS: pro-inflammatory cytokines (IL-1, TNF-α), growth factors<br>RECEPTOR: EGFR<br>ACTIVATION:<br>• direct, through mutations of genes for NF-κB/ IKβ<br>• indirect, through other pathways: MEKK3/MAPK → (+) IKK, and PI3K<br>DOWNSTREAM EFFECTS:<br>• CK → (+) IKK → (P) IKβ → (+) NF-κB → (-) apoptosis (CEFLAR genes), (+) proliferation, (+) angiogenesis, (+) inflammation (cytokine secretion IL-6), (+) activation of antioxidant proteins | • inhibition of signal transduction pathway by specific developed drugs (proteasome inhibitors)<br>• research in phase 1 of clinical trial (Aghajanian et al., 2005; Annunziata et al., 2010) |
| IL-6-IL-6R / Jak/STAT 3 PATHWAY | | |
| • contribution to neovascularization of OC, by induction of endothelial cell proliferation<br>• evidence for involvement in pathogeny of 70% cases of ovarian cancer (Bast et al., 2009; Hennessy et al, 2009) | LIGAND: IL-6<br>RECEPTORS: IL-6Rα (CD126) gp 80 and a signal transducer gp 130 (CD 130)<br>ACTIVATION: NF-κB, LPA, Ras<br>DOWNSTREAM EFFECTS:<br>• IL-6+IL-6R → Jak/STAT 3 Ras/MAPK → translocation of focal adhesion complexes, up-regulation of genes that (+) proliferation and motility, (-) apoptosis and induce angiogenesis | • inhibition of angiogenesis, by specific developed drugs (antibodies anti-IL-6)<br>• research in preclinical / experimental phase (Coward et al., 2011) |
| MAPK PATHWAY | | |
| • role in transmission of a signal from a cell surface receptor to nuclear DNA and, consequently, role in cell growth, differentiation, survival and apoptosis<br>• evidence for involvement in pathogeny of less that 50% of cases of OC (Bast et al., 2009), low-grade: MPSC, SBC (Cho and Shih, 2009) | LIGAND: EGF, Trk A / B<br>RECEPTOR: EGFR<br>ACTIVATION: oncogenic mutation KRAS, BRAF, ERBB2, other mutation<br>DOWNSTREAM EFFECTS:<br>• GRB2 (SH2) → SOS → SOS + GRB2 (EGFR !) → SOS ! → SOS + RAS (H / K) GTP → RAS ! → RAS + RAF (P) → MEK ! → MAPK ! → up-regulation of CCND1, COBRA 1, transglutaminase 2 → uncontrolled proliferation | • inhibition of carcinogenesis, by specific developed drugs (MAPK inhibitors)<br>• research in phase 2 of clinical trials (Hennessy et al., 2009) |

LPAR: Lysophosphatidic Acid Receptor; PI3CA: PI3K Catalytic Subunit-α; PTEN: Phosphatase and Tensin Homolog; AKT: Protein Kinase B (PKB), EGFR: Epidermal Growth Factor Receptor; MEKK3 (MAP3K/MEKK): MAP Kinase Kinase Kinase 3; IKK: IκB kinase; CK: Cytokines; IKbeta: Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-cells Inhibitor; RAS: Ras subfamily; GRB2: Growth Factor Receptor-Bound Protein 2; SH2: Src Homology 2 domain; MEK: Mitogen/Extracellular Signal-Regulated Kinase; P: phosphorylation; !: activation; +: stimulation; -: inhibition; MPSC: Micropapillary Serous Carcinoma of Ovary; SBC: Serous Borderline Carcinoma

are named BRCA1-like and BRCA2-like (Jazaeri, 2009).

There are several other overlaps in the genetic characterization of the two OC types. One of the examples is the tumor suppressor gene TP53: its mutations, although present in up to 80% of cases diagnosed, on histopathologic criteria, as type II / sporadic OC, are also reported in hereditary OC cases (Press et al., 2008).

In the following tables we present a synopsis of the main genetic aspects for hereditary OC (Table 2), as well as a characterization of the relationship between BRCA1 / BRCA2, and hereditary / sporadic OC (Table 3).

The intrinsic value of achieving a deeper understanding of OC genetic profile derives from the possibility of translating this information into a general characterization, through the definition of the various genetic and histologic subtypes, prediction of evolution, individualization of therapy and validation of screening and diagnosis markers (Gómez-Raposo et al., 2010).

### *From the gene signature to the proteomic signature: perspectives for early diagnosis and appropriate treatment*

Advances in the decipherment at the genetic level in the pathogenic mechanisms progressively lead to the idea of a genetic signature for OC. Unlike breast cancer, already in the stage of tests for a gene signature in randomized clinical trials, in the case of OC, the implementation of the concept of gene-expression profiling or gene signature is younger.

Since 2005 the number of studies focused on this issue is rising, despite the fact that OCs are genetically unstable, which makes the detection of genetic alterations more difficult (Despierre et al., 2010). Hence, the results differ considerably between the reported studies, only few genes being similar in signatures (Despierre et al., 2010; Braem et al., 2011). Nevertheless, we cannot deny the fact that the recorded progress is fast, due to the development of new sets of genomic technologies (such as Comparative Genomic Hybridization - CGH, microarray expression profiling, or serial analysis of gene expression, linked to well designed bioinformatics methods) (Tainsky, 2009). Within this context, we believe the presentation of some examples in the dynamics of their publishing not only necessary, but also eloquent.

One of the first studies in this area shows that OC is associated with the increased expression of some already known genes (claudin 3 - CLDN3, WAP four-disulfide core domain 2 - WFDC2/HE4, folate receptor 1 - FOLR1, collagen type XVIII alpha1 - COL8A1, cyclin D1 - CCND1) and of newly discovered ones as well (FLJ12988) (Peters et al., 2005). Later, the occurrence of a genetic overlap between serous borderline tumors and low-grade papillary serous tumors was demonstrated (Bonome et al., 2005), as well as major differences between these two types and high-grade papillary serous tumors (Bonome et al., 2005; Meinhold-Heerlein et al., 2005), on the basis of the genes coding proteins involved in STAT1, STAT3 and JAK1/2 pathways. In ovarian clear cell carcinoma, the gene signature includes Hepatocyte Nuclear Factor-1ß - HNF-1ß and versican - VCAN, along with other genes responsible for oxidative stress (Yamaguchi et al., 2010).

Two completely separate profiles are identified for the endometriosis-associated endometrioid OC (genes for cytokines and TDGF/1) and endometriosis-independent endometrioid OC (genes for proteins involved in cellular interaction, differentiation and proliferation) (Banz et al., 2010).

As a result of the Cancer Genome Atlas project, initiated in 2006 by the National Cancer Institute and National Human Genome Research Institute, a deeper genomic characterization of high-grade serous ovarian adenocarcinomas was achieved (Cancer Genome Atlas Research Network, 2011). The recently published data differentiates four transcriptional subtypes, three microRNA subtypes, and four promoter methylation subtypes. From the survival point of view, the authors discuss the existence of a transcriptional signature, correlated with the length of survival, as well as the influence of BRCA1/2 and CCNE1 genetic aberrations on disease outcome.

Unfortunately, the scientific community has to accept that only a small number of genetic variants are now associated, through valuable evidence, with OC (Braem et al., 2011). However, the research focused on the identification of gene signature(s) opens large perspectives for an individualized approach to OC, mainly through the possibilities (i) of improving the ability to diagnose earlier (Zhang et al., 2011), (ii) of developing a personal treatment (Sabatier et al., 2009), and (iii) of providing more accurate evaluation of prognosis and survival (Sabatier et al., 2009). Thus, the definition of a genetic profile widely surpasses the classic clinical and histologic features background - which dictated, for decades, patient assessment.

Moreover, the genetic level in the carcinogenesis mechanisms is not the final frontier of knowledge. At the same time as the development of research strategies envisaging the genetic territory, at the end of the last decade of the 20$^{th}$ century, a complementary direction

**Table 2.** Genes involved in hereditary ovarian cancer pathogenesis.

| OVARIAN CANCER - HEREDITARY TYPE TUMOR SUPPRESSOR GENE | | |
|---|---|---|
| HIGH PENETRANCE | | LOW PENETRANCE |
| BRCA1 | MLH1 | GSTM1 (Glutathione S Tranferase M1)<br>Steroid-5-alpha-Reductase |
| BRCA2 | MSH2 | Alpha Polypeptide 2<br>Progesterone Receptor |

BRCA1/2: Breast Cancer type I/type II; MLH 1: Human mutL Homolog 1; MSH2: Human mutS Homolog 2

was initiated, oriented towards the decryption of the intrinsic structure of the expressed molecules, from the genome to the organism. The introduction of proteomics was an instant success in tumoral pathology - and implicitly for OC. The focus on the definition of a gene's signature was doubled by the interest to identify specific proteins as tumoral biomarkers, in order to define a proteomic signature (Kim et al., 2009; Zhang et al., 2010, 2011; Sinclair et al., 2011). This endeavour is still extremely difficult, because gene levels are not always linked directly to protein levels (Zhang et al., 2011). Furthermore, a direct relationship between the specificity of a certain protein and a specific tumoral type is hard to prove.

The proteomic studies required the implementation of high technology, relying on the principles of mass spectrometry, which ensures, by Matrix Assisted Laser Desorption and Ionization - MALDI-TOF and Surface Enhanced Laser Desorption and Ionization - SELDI-TOF, the precise characterization of the proteins (Plebani, 2005; Zhang et al., 2010), or, more recently, on the new techniques named Reverse Phase Protein Arrays - RPPA (Annunziata et al., 2008).

Considerable breakthroughs are recorded mainly in the field of early diagnosis (Husseinzadeh, 2011) through studies dedicated to the discovery and validation of new biomarkers, which could be used in a screening based on biologic liquids (serum (Petricoin et al., 2002), urine (Petri et al., 2009) and ascites (Kuk et al., 2009)) or tissue samples. At the same time, the research groups take into account the translation of their applicability from early diagnosis to the choice of an adequate treatment, through the discovery of new molecular targets, and to prognostic assessment and follow-up (Hays et al., 2010; Husseinzadeh, 2011).

A recent review on the most important biomarkers that have been proposed as specific biomarkers for OC (Husseinzadeh, 2011) includes the following main molecules, organized into 6 classes on the basis of their biochemical structure: glycoproteins (CA-125 (mucin 16), OVX1, WFDC2/HE4, mesothelin), hepatic and acute phase proteins (haptoglobin-α, bikunin, C-reactive protein), cytokines and growth factors (Vascular Endothelial Growth Factor - VEGF, Insulin-Like Growth Factor II - ILGF II, IL-6, IL-10, Macrophage-Colony Stimulating Factor - M-CSF, osteopontin, macrophage inhibitory factor), serin proteases (kallikrein, prostasin), protein hormones (leptin, prolactin) and other protein-like structures (such as transthyretin - TTR, transferrin - TR, ß-hemoglobin, apolipoprotein A1 - Apo-A1, vitamin E-binding plasma protein).

Thirty years after the first report (Bast et al., 1981), CA-125 definitely remains the most widely used biomarker for the management of epithelial OC (Moore et al., 2010), but the relationship between the overexpression of CA-125 and the detection of early disease remains controversial. CA-125 alone is not a valid marker for screening. Its specificity is low, the reference value (50-60% positivity in early stages) published by Woolas et al., 1993 having yet to be surpassed. Furthermore, CA-125 may have a false positive expression in various medical conditions, benign gynecologic lesions or other malignant tumors - endometrial or mammary cancer (Moore et al., 2010).

**Table 3.** BRCA genes in hereditary and sporadic ovarian cancer - a comparative approach.

| BRCA1 chromosom location: 17q21 | | BRCA2 chromosom location: 13q12-13 |
|---|---|---|
| high penetrance: a single allelic mutation initiates carcinogenesis | | |
| incidence in general population: 1/500 - 1/1000 | | incidence in general population: still imprecisely delimited |
| HEREDITARY TYPE | SPORADIC TYPE | HEREDITARY TYPE |
| Mechanism: genetic → mutation<br>• risk correlated with location of mutation: high, for central position (nucleotides 2401-4190) | Mechanism: epigenetic and somatic alterations:<br>→ hypermethylation promotion<br>→ loss of heterozygosity<br>→ haploinsufficiency<br>AKT pathway<br>PTEN pathway<br>WNT2, SFP4 pathways | Mechanism: genetic → mutation<br>• risk correlated with location of mutation: high, for ovarian cancer cluster region - OCCR (nucleotides 3059-6629) |
| risk for carriers: 40-50% | no carriers | risk for carriers: 20-30% |
| age of diagnosis: 50 years | age of diagnosis: 60 years | age of diagnosis: 60 years |
| genetic syndromes<br>• ovarian cancer syndrome<br>• hereditary breast-ovarian cancer syndrome | no genetic syndromes | genetic syndromes<br>• ovarian cancer syndrome<br>• hereditary breast-ovarian cancer syndrome |
| histology: type II<br>• high-grade serous OC<br>• high-grade endometrioid OC<br>• mixed malignant mesodermal tumors<br>• carcinosarcomas<br>• undifferentiated OC | histology<br>"silent" BRCA1 profile can be correlated with a histologic subtype | histology: type II<br>• high-grade serous OC<br>• high-grade endometrioid OC<br>• mixed malignant mesodermal tumors<br>• carcinosarcomas<br>• undifferentiated OC |

The experience accumulated in biomarker research recommends, for the increase of the sensitivity and reproducibility, the simultaneous usage of several markers. The panels proposed include CA-125 together with Apo-A1, TTR and HE4 (Zhang et al., 2004), ß-hemoglobin, Apo-A1, TF (Kozak et al., 2005), leptin, prolactin, osteopontin, ILGF II, macrophage inhibitory factor (Visintin et al., 2008), Apo-A1, TTR, TF (Nosov et al., 2009), mesothelin, osteopontin and HE4 (Moore et al., 2010). The results obtained from the comparison between the detection sensitivity of a marker *versus* a panel (Husseinzadeh, 2011) indicate as an optimal combination the triplet CA-125, HE4 and mesothelin (Anderson et al., 2010).

A special mention has to be made for the autoantibodies reactive with wild-type TP53, which, in association with CA-125, are extremely useful in two situations. The first is the detection of patients with "type 2" high grade serous OC, with p53 mutations, before the occurrence of metastases (Moore et al., 2010). The second is the early diagnosis of the BRCA patients who seem to be susceptible to tumors originating in the Fallopian tubes (Anderson et al., 2010).

Until now, none of these proposed biomarkers is validated as an ideal marker for the early diagnosis, because none of them fulfills the requirement of a positive predict value of 10% (Zhang et al., 2011), and the reproducibility of the studies is either very low or is lacking completely (Cadron et al., 2009). It is possible that the clinical trials recently opened, having primary proteomic endpoints, to solve these problems (Zhang et al., 2010). Moreover, these developing clinical trials have to meet high expectations regarding the connection between the biomarkers and targeted therapeutic agents, such as the ones for the colon and mammary cancer (Hays et al., 2010; Lee et al., 2011). The establishment of such correlations may surpass the current limit in the proteomic approach to OC, which is the absence of a component regarding the development of a targeted therapy.

## Epithelial-mesenchymal transition correlated to ovarian carcinogenesis

Based on the mechanisms at the intracellular and intercellular level, epithelial-mesenchymal transition (EMT) is defined as a biological process which enables epithelial cells to undergo a series of complex reprogrammed cellular events, including multiple biochemical modifications, resulting in the loss of epithelial characteristics and the possibility of assuming *de novo* a mesenchymal cell phenotype, which includes an increase in migration abilities, invasion, apoptosis resistance and production of extracellular matrix elements (Kalluri and Weinberg, 2009). Introduced for the first time in 1968, the initial term “transformation” was later replaced with that of “transition” (Acloque et al., 2009).

### *EMT - classification*

EMT is subdivided into three different biological subtypes, on the basis of the biological context of their occurrence (Kalluri and Weinberg, 2009). EMT type 1, characterized as transitory, is associated with nidation, embryogenesis and organogenesis. It is responsible for the generation of the main cell types, through its capacity to translate the epithelial phenotype into the mesenchymal phenotype and, later, of the mesenchymal phenotype into a new epithelial phenotype, sometimes different from the starting one. EMT type 2, present in the context of inflammation, is associated with wound healing, tissue regeneration and organ fibrosis. EMT type 3 is the result of genetic and epigenetic modifications of the oncogenes and of tumor suppressor genes intervening in the regulation circuits of the EMT.

In the transformation of a phenotype from the normal to the malignant type cells undergo a multistage process with three main phases (Sabbah et al., 2008; Acloque et al., 2009). The first phase consists of the elimination of the epithelial features and is characterized by loss of polarity (and consequent loss of junctions), reorganization of the cytoskeleton, partial disintegration of the basal membrane, and changes in the interaction with the extracellular matrix, due to damage in the cell-to-cell and cell-to-matrix relationships, normally ensured by the adhesion molecules. The second phase is represented by the accumulation of mesenchymal features (motility, migration into the blood stream, extravasations, and colonization at a distance) and the reshaping of the extracellular matrix (through the intervention of matrix metalloproteinases - MMPs and their specific inhibitors - TIMPs). The third phase implies the regaining of epithelial features. The cancer cells may only go through certain sequences of the EMT, not necessarily through the entire process. This explains either the partial preservation of the epithelial properties, or the complete mesenchymal transformation, with the accumulation of the corresponding characteristics (Kalluri and Weinberg, 2009).

### *EMT in ovarian cancer*

EMT in OC, as a component of the complex pathogenic mechanism which governs carcinogenesis, is a focus element for research groups in this field (Vergara et al., 2010; Strauss et al., 2011). Recent studies investigate the signals triggering the EMT process that result either from specific genetic modifications or as a consequence of processes such as hypoxia, fibrosis, necrosis and apoptosis, characteristic for the cells associated with the tumor microenvironment (Sabbah et al., 2008). These signals include a wide range of gene products, cytokines, growth factors and matrix molecules which act independently or are integrated into specific signaling pathways (Sabbah et al., 2008; Acloque et al., 2009). Consequently, the switch between

the loss and the gain of some cell morphology features takes place, at a microscopic level (translated mainly through shape and placement) and also at a molecular one (translated through specific markers, epithelial *versus* mesenchymal).

Short presentation of the signaling pathways

According to the literature on OC, there are six molecules involved in EMT (namely Transforming Growth Factor ß - TGFß, Epidermal Growth Factor - EGF, Hepatocyte Growth Factor - HGF, Endothelin 1 - ET-1, Estrogen Receptor - ER and Bone Morphogenic Protein 4 - BMP 4) (Vergara et al., 2010). Each of these molecules centers on a distinct pathogenic pathway.

Transforming Growth Factor ß

The role of TGF-ß in regulation of the proliferation, migration and apoptosis processes is widely recognized, through its capacity to intervene in the modification of the cell shape and of the anchorage structures (Roberts et al., 1981). The transduction of the TGF-ß signal is achieved by activation, at a nuclear level, of the Smad pathway or of some non-Smad pathways (GTP-ase Rho - like, PI3K/Akt pathway and MAPK pathway). TGF-ß, usually absent from the normal ovarian surface epithelium, is present in OCs, both in the tumor cells and in the adjacent stroma, at the same time as the overexpression of the specific receptors (Inan et al., 2006). Numerous *in vitro* studies, using different cell lineages, focused on the identification of the exact mechanism by which TGF-ß influences ovarian carcinogenesis (Yamada et al., 1999). The controversial results may be explained by the heterogeneous characteristics of the different ovarian tumor cell lineages. Despite the inconsistencies in the results, it is accepted that TGF-ß has a double role in tumor progression, stimulating cell death in the early stages of carcinogenesis and acting as a promoter in the late stages (Vergara et al., 2010).

The normal antiproliferative effect of TGF-ß is countered by the tumor cells through changes in the expression of the genes regulating the activity of TGF-ß. Thus, the literature indicates the presence of seven genes with aberrant expressions: 3 genes that inhibit TGF-ß signaling (DACH1, EVI1, and BMP7) which are upregulated, and 4 genes that enhance TGF-ß signaling (TGFBRII, SMAD4, TFE3 and PCAF) which are downregulated (Sunde et al., 2006).

Evident proof in favor of the role of TGF-ß in EMT was obtained experimentally, several studies on murine ovarian cell lineages revealing the fact that TGF decreases the expression of the epithelial markers (E-cadherin, ZO-1, desmoplakins I / II), modifies the cytoskeleton through the activation of numerous proteins (ß-actin, cofilin-1, moesin, filamin A and B, heat-shock protein ß-1, trangelin-2, calgizarrin, calpactin, and profilin-1), and increases the expression of fibronectin, a mesenchymal marker, and also the abilities to migrate and invade (Vergara et al., 2010).

Epidermal Growth Factor

EGF induces EMT in the ovarian surface epithelium and in cancer cells (Vergara et al., 2010). However, its intervention in EMT occurs only after the coupling with its specific receptor, EGFR, expressed in 70% of malignant ovarian tumors and correlated with serous histologic type, a reserved prognosis and chemo-resistance (Hegymegi-Barakonyi et al., 2009; Vergara et al., 2010). This binding determines activation through phosphorylation of the JAK/STAT3, MAPK and ILK pathways (Ahmed et al., 2006, Vergara et al., 2010).

At the level of the ovarian surface epithelium, the role of EGF appears to be a beneficial and positive one, by the decrease of the malignant potential, preventing the formation of epithelial inclusion cysts, in the context of the transformation of the epithelial cells into fibroblast-like cells (Vergara et al., 2010).

EGF intervenes in EMT through the inhibition of keratin expression, increased mobility, enhancement of the expression of pro-MMP-2/9 and induction of the activation of ERK and ILK pathways (Ahmed et al., 2006).

The participation of EGF in EMT associated with metastasis was experimentally investigated using cell lineages. The results indicate the increase of cell motility and the transformation of the epithelial morphology into fibroblast-like morphology, coupled with a decrease in the expression of some molecular products or the increase in the expression of others ($\alpha 2$, $\alpha 6$, ß1 integrin subunits, leukaemia inhibitory factor - LIF and IL-6) (Vergara et al., 2010).

Estrogen receptor

The role of ER$\alpha$ in the promotion of EMT was also experimentally demonstrated on ovarian cell lineages which acquire phenotypic and molecular features specific for EMT as a consequence of exposure to 17ß-estradiol (Ding et al., 2006; Park et al., 2008). At the same time, ERß had an inverse/antagonist role with ER$\alpha$.

The estrogen-signaling pathway supports phenotypic plasticity in the OC, targeting E-cadherin, Snail, and Slug (Gallo et al., 2010). Through the intervention of ER$\alpha$, E-cadherin expression is inhibited, and the transcription factors Snail and Slug were significantly up-regulated through gene transcription.

Hepatocyte Growth Factor

HGF and its specific tyrosin-kinase receptor - cMET play a role in EMT through the activation of the transduction pathways MAPK, MEK-ERK1/2, PI3K,

AKT, Snail (Vergara et al., 2010).

Endothelin-1 (ET-1)

ET-1 (Endothelin-1) acts by its specific receptors $ET_A$ and $ET_B$ binded to two transmembranar G proteins (Grant et al., 2003). ET-1 is expressed in 90% of primary tumors and 100% metastatic OCs; its RNA was significantly higher in tumors than in normal ovarian tissues. The correlations between the overexpressed ET-1/$ET_A$R axis and the EMT mechanism are demonstrated, based on the activation of MAPK, PI3K/Akt and ILK pathways, with a consecutive inhibition of GSK-3 beta (Rosanò et al., 2005, 2011; Bagnato and Rosanò, 2007).

The invasive behavior of OC, in the EMT context, is sustained by evidence proving the downregulation of E-cadherin (with a resulting decrease in E-cadherin expression and suppression of its promoter activity) and an increase in ß-catenin levels, through the up-regulation of the transcriptional factors Snail, Slug and Twist; concomitantly, there occurs an increase in the expression of mesenchymal markers, such as N-cadherin and vimentin (Rosanò et al., 2011).

Bone Morphogenic Protein 4

A member of the TGF family, BMP - a ligand protein - is expressed both in several normal ovarian cell types (mainly in the ovarian surface epithelium) and in the malignant ones (Shepherd and Nachtigal, 2003). BMP acts on specific genes, namely ID1 and ID3, members of the ID gene family (inhibitor of differentiation/DNA binding), increasing their expression - which indicates a reserved prognosis (Schindl et al., 2003).

Experimentally, it was demonstrated that BMP-4 induces the EMT in primary OCs through the activation of Snail, Slug and GTPase Rho-like pathways. The intervention of BMP-4 causes the reorganization of actin fibers, a decrease of E-cadherin expression and an increase of integrin-like receptors expression, extracellular matrix proteins and focal adhesion proteins (Thériault et al., 2007).

The significance of changes in the E-cadherin expression

Recent data in the mainstream publications support the thesis that an essential role in EMT is played by the transformation of the E-cadherin profile into an N-cadherin one (Voulgari and Pintzas, 2009; Vergara et al., 2010). E-cadherin, a member of the integrin family, is responsible for the preservation of intercellular adhesion and of cell-matrix adhesion (Auersperg et al., 1999; Wu et al., 2008), thus inhibiting metastasis (Kuwabara et al., 2008). Consequently, the decrease in expression or the absence of E-cadherin facilitates cell migration in the tumor context and the increase of their invasion potential. The mechanism by which the expression of E-cadherin is changed involves the intervention of repressors (mainly from the SNAIL family), regulated through EGFR/FAK/ERK-MAPK pathways (Cavallaro and Christofori, 2004; Yoshida et al., 2009). The overexpression of N-cadherin consequent to the loss of E-cadherin determines an increase in the adherence capacities of tumor cells and enables their interaction with stromal and endothelial components (Cavallaro and Christofori, 2004).

## Controversial aspects regarding the origin of an epithelial component in ovarian cancer: from classical to recent hypotheses

The classic conception of OC pathogeny, based on the role of the ovarian surface epithelium, is currently being reconsidered (Kurman and Shih, 2010). According to the theory, widely accepted until recently, the ovarian surface epithelium, due to repetitive injuries caused by ovulation, can change its configuration by invagination in the adjacent stroma. Therefore, subcortical inclusion cysts are formed. Their lining epithelium reacts to different local stimuli (such as hormones and/or molecules produced in inflammatory context) and may alter its normal genetic profile accordingly, thus triggering the sequences of carcinogenesis (Kurman and Shih, 2010).

The first step in this sequence is metaplasia, the ovarian surface epithelium turning into serous, endometrioid, clear, transitional (Brenner) or mucinous cell types, similar to various epithelial locations: Fallopian tubes, endometrium, endocervix, gastrointestinal or urinary tracts. Later, these cell types determine, by malignant transformation, the development of some tumoral entities, with morphological features which cannot be found in the normal structure of the ovary (Jelovac and Armstrong, 2011). In order to explain this sequence of events, embryological data was used. Thus, the ovary is developed from the mesodermic epithelium of the urogenital crest separated from the Mullerian ducts, while the Fallopian tubes, the endometrium and the cervix are developed from the Mullerian ducts themselves. Consequently, a new hypothesis was formulated, which supports, for ovarian tumors with Mullerian phenotype, an origin in the Mullerian tissues and not in the ovarian surface epithelium (Dubeau, 2008).

In favor of this hypothesis, supplementary arguments were brought, based on the comparison between the genetic profiles of epithelium derived from Mullerian ducts, of normal surface epithelium and of ovarian tumors (Hennessy et al., 2009; Kurman and Shih, 2010). The differentiation of the Fallopian tubes, endometrium and cervix from the Mullerian ducts is regulated by HOXA9, HOXA10 and HOXA11 genes, and the epithelial cells express these genes (HOXA9 in the Fallopian tube, HOXA10 in the endometrium and HOXA11 in the cervix) (Hennessy et al., 2009).

Although the surface ovarian epithelium does not normally express HOX genes, they were identified in experimental studies, in ovarian tumors with serous, endometrioid or mucinous morphology. Their presence may be explained by the fact that the cells on the surface of the ovary, under the influence of steroid hormones regulating HOX genes, may begin to express (in a pathologic manner) these genes, thus their genotype turns towards the Mullerian lineage (Hennessy et al., 2009).

Hence, according to the new hypothesis, the origin of OC cannot be attributed to the surface ovarian epithelium, because the ovary is believed to be secondarily involved (Kurman and Shih, 2010).

It is interesting that this "new" hypothesis was foreseen several decades ago (Lauchlan, 1972), through the observations on the secondary Mullerian system, formed by paratubal and paraovarian cysts, lined with Mullerian epithelium. This data suggested that the significant expansion (associated with the malignant transformation) of these tumors adjacent to the ovary, with their subsequent penetration into the ovary, leads to their assessment as tumors of the ovary.

However, research on the pathogenesis of OC suggests the possible direct involvement of the Fallopian tubes (Kurman and Shih, 2010). The starting point of this hypothesis is the morphologic and genetic similarities between ovarian and tubal carcinomas. The results reported in the literature after the parallel investigation of malignant ovary and corresponding Fallopian tubes indicate that almost 70% of sporadic ovarian tumors are associated with serous tubal intraepithelial carcinomas in the tubal mucosa (Kindelberger et al., 2007). Several mechanisms are proposed for ovarian tumoral "implantation", in correspondence with the various histologic subtypes of OC, the crucial event which determines the secondary involvement of the ovary being the rupture of the ovarian surface epithelium at the moment of ovulation.

The serous subtype occurs by the "migration" of the epithelium with small foci of malignant transformation, from the terminal end of the Fallopian tubes towards and into the ovary. Thus, serous tubal intraepithelial carcinomas are considered as a source of high-grade serous OCs, with a genetic substrate shared both by tubal and ovarian carcinoma, through mutations in the BRCA genes (Kurman and Shih, 2010). The endometrioid and clear cell subtypes develop within the same context of intraovarian penetration from the endometrioid foci, as a result of retrograde menses. The mucinous and Brenner cell subtypes appear from the transitional epithelium located at the junction between the mesothelium and the Fallopian tubes, where the fimbria comes in close contact with the peritoneum (Kurman and Shih, 2010).

Apart from these mentioned possible mechanisms, we must stress the fact that repeated ovulations, endometriosis and pelvic inflammatory disease may contribute to OC pathogenesis by tissue injury itself. This injury is always associated with an inflammatory response that causes the release of nitric oxide (NO) free radicals which have the ability to directly destroy DNA, to stimulate cytokine and prostaglandin production, associated with progression and tumor invasion (Lurie et al., 2009). Moreover, the tissue repair process is also associated with the development of genetic mutations.

### Immune cell involvement in ovarian carcinogenesis

Recent research (Curiel et al., 2004; Nelson, 2008) suggests the implication of immune/inflammatory cells by specific mechanisms in OC pathogenesis.

Ovarian tumors frequently include lymphocytes and macrophages - key mediators of specific tolerance, which supports the role of anti-tumoral immunity of the host organism in tumor development and metastasis. Moreover, the lymphocytes and macrophages, together with the numerous cytokines and chemokines released, are also present in the ascites associated with ovarian metastases (Milliken et al., 2002).

T lymphocytes are mainly located in solid tumors and less in the cystic ones (Zhang et al., 2003). The papers focused on tumor-infiltrating lymphocytes (TILs) report, as intra or peritumoral subtypes, T-regulatory lymphocytes ($T_{reg}$), CD8, CD4, CD25 and FoxP3 positive lymphocytes (Barnett et al., 2010; Kandalaft et al., 2011). At the same time, macrophages and dendritic cells are also present. Unfortunately, the published results are inconsistent.

As a result of immune cell intervention, the immune response becomes operational through the expression of cytokines, chemokines, antigens, MHC molecules and other stimulating molecules. The initiation of antitumoral immunity may involve disruption, by $T_{reg}$ lymphocytes, of the mediated peripheral tolerance induced by tumor-associated antigens. A significant number of T lymphocytes (especially CD8 positive) is correlated with a five year survival rate of 38%, as opposed to 4.5% for patients that do not have T lymphocytes in the tumoral infiltrates (Sato et al., 2005).

A possible explanation is the fact that the interferons produced by activated T cells prevent tumor proliferation, most probably through the reduction of angiogenesis, and intervene in the adjustment of the immune recognition process, by the decrease of IL-8 secretion and stimulation of MHC molecule expression (Bast et al., 2009).

The presence of macrophages, in association with lymphocytes, is caused by the synthesis of M-CSF and other chemokines, both by tumoral cells and T lymphocytes. The exact role of activated macrophages is still incompletely known, because they develop the ability to produce molecules involved in the stimulation of carcinogenesis (IL-1, IL-6 and Tumor Necrosis Factorα - TNFα), as well as in its inhibition (NO and TNFα), together with a significant decrease of the phagocytic and antibody-dependent cell-mediated

cytotoxicity functions (Bast et al., 2009).

## Final remarks

In the ovarian cancer research of the last decade, the study of the mechanisms involved in initiation, development and evolution focuses on several pathogenic pathways that can explain the carcinogenesis process on the basis of biochemical changes at cellular level, and the biochemical signals between cells. Moreover, gene and protein expression analysis have been introduced extensively (in order to reveal a gene-proteomic-signature that may yield the identification of several profiles, with different therapy responses and prognosis). The development and implementation of advanced investigation technologies lead to a substantial change of research objectives and, consequently, to a new vision in the understanding of ovarian cancer, which displaces classical clinical and morphological investigations (characterizing the late nineties) toward a genetic and proteomic trend (corresponding to the second decade of the new century).

In this context, we consider that molecular, genetic and proteic approaches are still in a pioneering stage, and that is why any assessment of the relationship between the clinical features and the complex biologic profile of ovarian cancer is valuable and deserves the full attention of the scientific community.

Obviously, ovarian carcinogenesis represents a topic of great interest. In the medium term, the research investment developed all around the world will be able to bring the expected progress, namely, robust connections between genetic and proteomic hallmarks, signaling pathways, tumoral environment and the clinical behavior of the disease.

*Acknowledgements.* The first author acknowledges the support of the POSDRU/88/1.5/S/58965 project, financed by the European Social Fund and the Romanian Government.

## References

Acloque H., Adams M.S., Fishwick K., Fraser M.B. and Nieto M.A. (2009). Epithelial-mesenchymal transitions:the importance of changing cell state in development and disease. J. Clin. Invest. 119, 1438-1449.

Aghajanian C., Dizon D.S., Sabbatini P., Raizer J.J., Dupont J. and Spriggs D.R. (2005). Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. J. Clin. Oncol. 23, 5943-5949.

Ahmed N., Maines-Bandiera S., Quinn M.A., Unger W.G., Dedhar S. and Auersperg N. (2006). Molecular pathways regulating EGF-induced epitheliomesenchymal transition in human ovarian surface epithelium. Am. J. Physiol. Cell. Physiol. 290, 1532-1542.

American Cancer Society. (2009). Cancer facts and figures http://www.cancer.org/downloads/STT/500809web.pdf.

Anderson G.L., McIntosh M., Wu L., Barnett M., Goodman G., Thorpe J.D., Bergan L., Thornquist M.D., Scholler N., Kim N., O'Briant K., Drescher C. and Urban N. (2010). Assessing lead time of selected ovarian cancer biomarkers: a nested case-control study. J. Natl. Cancer Inst. 102, 26-38.

Annunziata C.M., Azad N., Dhamoon A.S., Whiteley G. and Kohn E.C. (2008). Ovarian cancer in the proteomics era. Int. J. Gynecol. Cancer 18 (Suppl 1), 1-6.

Annunziata C.M., Stavnes H.T., Kleinberg L., Berner A., Hernandez L.F., Birrer M.J., Steinberg S.M., Davidson B. and Kohn E.C. (2010). Nuclear factor kappaB transcription factors are coexpressed and convey a poor outcome in ovarian cancer. Cancer 116, 3276-3284.

Auersperg N., Pan J., Grove B.D., Peterson T., Fisher J., Maines-Bandiera S., Somasiri A. and Roskelley C.D. (1999). E-cadherin induces mesenchymal-to-epithelial transition in human ovarian surface epithelium. Proc. Natl. Acad. Sci. USA 96, 6249-6254.

Bagnato A. and Rosanò L. (2007). Epithelial-mesenchymal transition in ovarian cancer progression: a crucial role for the endothelin axis. Cells Tissues Organs 185, 85-94.

Banz C., Ungethuem U., Kuban R.J., Diedrich K., Lengyel E. and Hornung D. (2010). The molecular signature of endometriosis-associated endometrioid ovarian cancer differs significantly from endometriosis-independent endometrioid ovarian cancer. Fertil. Steril. 94, 1212-1217.

Barnett J.C., Bean S.M., Whitaker R.S., Kondoh E., Baba T., Fujii S., Marks J.R., Dressman H.K., Murphy S.K. and Berchuck A. (2010). Ovarian cancer tumor infiltrating T- regulatory T(reg) cells are associated with a metastatic phenotype. Gynecol. Oncol. 116, 556-562.

Bast R.C., Hennessy B. and Mills G.B. (2009). The biology of ovarian cancer: new opportunities for translation. Nat. Rev. Cancer 9, 415-428.

Bast R.C. Jr, Feeney M., Lazarus H., Nadler L.M., Colvin R.B. and Knapp R.C. (1981). Reactivity of a monoclonal antibody with human ovarian carcinoma. J. Clin. Invest. 68, 1331-1337.

Bonome T., Lee J.Y., Park D.C., Radonovich M., Pise-Masison C., Brady J., Gardner G.J., Hao K., Wong W.H., Barrett J.C., Lu K.H., Sood A.K., Gershenson D.M., Mok S.C. and Birrer M.J. (2005). Expression profiling of serous low malignant potential, low-grade, and high-grade tumors of the ovary. Cancer Res. 65, 10602-10612.

Braem M.G., Schouten L.J., Peeters P.H., den Brandt P.A. and Onland-Moret N.C. (2011). Genetic susceptibility to sporadic ovarian cancer: A systematic review. Biochim. Biophys. Acta 1816, 1321-1346.

Cadron I., Van Gorp T., Timmerman D., Amant F., Waelkens E. and Vergote I. (2009). Application of proteomics in ovarian cancer: which sample should be used? Gynecol. Oncol. 115, 497-503.

Cancer Genome Atlas Research Network. (2011). Integrated genomic analyses of ovarian carcinoma. Nature 474, 609-615.

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

Cho K.R. and Shih I.M. (2009). Ovarian Cancer. Annu. Rev. Pathol. 4, 287-313.

Coward J., Kulbe H., Chakravarty P., Leader D., Vassileva V., Leinster D.A., Thompson R., Schioppa T., Nemeth J., Vermeulen J., Singh N., Avril N., Cummings J., Rexhepaj E., Jirström K., Gallagher W.M., Brennan D.J., McNeish I.A. and Balkwill F.R. (2011). Interleukin-6 as a therapeutic target in human ovarian cancer. Clin. Cancer Res. 17, 6083-6096.

Curiel T.J., Coukos G., Zou L., Alvarez X., Cheng P., Mottram P., Evdemon-Hogan M., Conejo-Garcia J.R., Zhang L., Burow M., Zhu Y., Wei S., Kryczek I., Daniel B., Gordon A., Myers L., Lackner A.,

Disis M.L., Knutson K.L., Chen L. and Zou W. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat. Med. 10, 942-949.

Despierre E., Lambrechts D., Neven P., Amant F., Lambrechts S. and Vergote I. (2010). The molecular genetic basis of ovarian cancer and its roadmap towards a better treatment. Gynecol. Oncol. 117, 358-365.

Ding J.X., Feng Y.J., Yao L.Q., Yu M., Jin H.Y. and Yin L.H. (2006). The reinforcement of invasion in epithelial ovarian cancer cells by 17,-Estradiol is associated with up-regulation of Snail. Gynecol. Oncol. 103, 623-630.

Dubeau L. (2008). The cell of origin of ovarian epithelial tumours. Lancet Oncol. 9, 1191-1197.

Gallo D., Ferlini C. and Scambia G. (2010). The epithelial-mesenchymal transition and the estrogen-signaling in ovarian cancer. Curr. Drug. Targets 11, 474-481.

Gates M.A., Rosner B.A., Hecht J.L. and Tworoger S.S. (2010). Risk factors for epithelial ovarian cancer by histologic subtype. Am. J. Epidemiol. 171, 45-53.

Geisler J.P., Hatterman-Zogg M.A., Rathe J.A. and Buller R.E. (2002). Frequency of BRCA1 dysfunction in ovarian cancer. J. Natl. Cancer Inst. 94, 61-67.

Gómez-Raposo C., Mendiola M., Barriuso J., Hardisson D. and Redondo A. (2010). Molecular characterization of ovarian cancer by gene-expression profiling. Gynecol. Oncol. 118, 88-92.

Grant K., Loizidou M. and Taylor I. (2003). Endothelin-1: a multifunctional molecule in cancer. Br. J. Cancer 88, 163-166.

Hays J.L., Kim G., Giuroiu I. and Kohn E.C. (2010). Proteomics and ovarian cancer: Integrating proteomics information into clinical care. J. Proteomics 73, 1864-1872.

Hegymegi-Barakonyi B., Eros D., Szántai-Kis C., Breza N., Bánhegyi P., Szabó G.V., Várkondi E., Peták I., Orfi L. and Kéri G. (2009). Tyrosine kinase inhibitors - small molecular weight compounds inhibiting EGFR. Curr. Opin. Mol. Ther. 11, 308-321.

Hennessy B.T., Coleman R.L. and Markman M. (2009). Ovarian cancer. Lancet 374, 1371-1382.

Hernandez L., Hsu S.C., Davidson B., Birrer M.J., Kohn E.C. and Annunziata C.M. (2010). Activation of NF-kappaB signaling by inhibitor of NF-kappaB kinase beta increases aggressiveness of ovarian cancer. Cancer Res. 70, 4005-4014.

Hilton J.L., Geisler J.P., Rathe J.A., Hattermann-Zogg M.A., DeYoung B. and Buller R.E. (2002). Inactivation of BRCA1 and BRCA2 in ovarian cancer. J. Natl. Cancer Inst. 94, 1396-1406.

Husseinzadeh N. (2011). Status of tumor markers in epithelial ovarian cancer: has there been any progress? A review. Gynecol. Oncol. 120, 152-157.

Inan S., Vatansever S., Celik-Ozenci C., Sanci M., Dicle N. and Demir R. (2006). Immunolocalizations of VEGF, its receptors flt-1, KDR and TGF-,'s in epithelial ovarian tumors. Histol. Histopathol. 21, 1055-1064.

Jazaeri A.A. (2009). Molecular profiles of hereditary epithelial ovarian cancers and their implications for the biology of this disease. Mol. Oncol. 3, 151-156.

Jelovac D. and Armstrong D.K. (2011). Recent progress in the diagnosis and treatment of ovarian cancer. CA Cancer J. Clin. 61, 183-203.

Kalluri R. and Weinberg R.A. (2009). The basics of epithelial-mesenchymal transition. J. Clin. Invest. 119, 1420-1428.

Kandalaft L.E., Motz G.T., Duraiswamy J. and Coukos G. (2011). Tumor immune surveillance and ovarian cancer. Lessons on immune mediated tumor rejection or tolerance. Cancer Metastasis Rev. 30, 141-151.

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.

Kindelberger D.W., Lee Y., Miron A., Hirsch M.S., Feltmate C., Medeiros F., Callahan M.J., Garner E.O., Gordon R.W., Birch C., Berkowitz R.S., Muto M.G. and Crum C.P. (2007). Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am. J. Surg. Pathol. 31, 161-169.

Kozak K.R., Su F., Whitelegge J.P., Faull K., Reddy S. and Farias-Eisner R. (2005). Characterization of serum biomarkers for detection of early stage ovarian cancer. Proteomics 5, 4589-4596.

Kuk C., Kulasingam V., Gunawardana C.G., Smith C.R., Batruch I. and Diamandis E.P. (2009). Mining the ovarian cancer ascites proteome for potential ovarian cancer biomarkers. Mol. Cell. Proteomics 8, 661-669.

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.

Kurman R.J. and Shih I.M. (2010). The origin and pathogenesis of epithelial ovarian cancer. A proposed unifying theory. Am. J. Surg. Pathol. 34, 433-443.

Kuwabara Y., Yamada T., Yamazaki K., Du W.L., Banno K., Aoki D. and Sakamoto M. (2008). Establishment of an ovarian metastasis model and possible involvement of E-cadherin down-regulation in the metastasis. Cancer Sci. 99, 1933-1939.

Lalwani N., Prasad S.R., Vikram R., Shanbhogue A.K., Huettner P.C. and Fasih N. (2011). Histologic, molecular, and cytogenetic features of ovarian cancers: implications for diagnosis and treatment. Radiographics 31, 625-646.

Lauchlan S.C. (1972). The secondary Müllerian system. Obstet. Gynecol. Surv. 27, 133-146.

Lee J.M., Han J.J., Altwerger G. and Kohn E.C. (2011). Proteomics and biomarkers in clinical trials for drug development. J. Proteomics 74, 2632-2641.

Lurie G., Wilkens L.R., Thompson P.J., Mcduffie K.E., Carney M.E., Terada K.Y. and Goodman M.T. (2009). Genetic polymorphisms in the estrogen receptor beta (ESR2) gene and the risk of epithelial ovarian carcinoma. Cancer Causes Control 20, 47-55.

Markman B., Atzori F., Pérez-García J., Tabernero J. and Baselga J. (2010). Status of PI3K inhibition and biomarker development in cancer therapeutics. Ann. Oncol. 21, 683-691.

Meinhold-Heerlein I., Bauerschlag D., Hilpert F., Dimitrov P., Sapinoso L.M., Orlowska-Volk M., Bauknecht T., Park T.W., Jonat W., Jacobsen A., Sehouli J., Luttges J., Krajewski M., Krajewski S., Reed J.C., Arnold N. and Hampton G.M. (2005). Molecular and prognostic distinction between serous ovarian carcinomas of varying grade and malignant potential. Oncogene 24, 1053-1065.

Milliken D., Scotton C., Raju S., Balkwill F. and Wilson J. (2002). Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. Clin. Cancer Res. 8, 1108-1114.

Moore R.G., MacLaughlan S. and Bast R.C. Jr (2010). Current state of biomarker development for clinical application in epithelial ovarian cancer. Gynecol. Oncol. 116, 240-245.

Nelson B.H. (2008). The impact of T-cell immunity on ovarian cancer outcomes. Immunol. Rev. 222, 101-116.

Nosov V., Su F., Amneus M., Birrer M., Robins T., Kotlerman J., Reddy S. and Farias-Eisner R. (2009). Validation of serum biomarkers for detection of early stage ovarian cancer. Am. J. Obstet. Gynecol. 200, 639. e1-5.

Oyesanya R.A., Lee Z.P., Wu J., Chen J., Song Y., Mukherjee A., Dent P., Kordula T., Zhou H. and Fang X. (2008). Transcriptional and post-transcriptional mechanisms for lysophosphatidic acid-induced cyclooxygenase-2 expression in ovarian cancer cells. FASEB J. 22, 2639-2651.

Park S.H., Cheung L.W., Wong A.S. and Leung P.C. (2008). Estrogen regulates snail and slug in the down-regulation of e-cadherin and induces metastatic potential of ovarian cancer cells through estrogen receptor alpha. Mol. Endocrinol. 22, 2085-2098.

Pejovic T., Anderson L. and Odunsi K. (2009). Molecular biology of ovarian cancer. In: Genomic and personalized medicine. Ginsburg W. (ed). Elsevier. Amsterdam. pp 913-920.

Peters D.G., Kudla D.M., Deloia J.A., Chu T.J., Fairfull L., Edwards R.P. and Ferrell R.E. (2005). Comparative gene expression analysis of ovarian carcinoma and normal ovarian epithelium by serial analysis of gene expression. Cancer Epidemiol. Biomarkers Prev. 14, 1717-1723.

Petri A.L., Simonsen A.H., Yip T.T., Hogdall E., Fung E.T., Lundvall L. and Hogdall C. (2009). Three new potential ovarian cancer biomarkers detected in human urine with equalizer bead technology. Acta. Obstet. Gynecol. Scand. 88, 18-26.

Petricoin E.F., Ardekani A.M., Hitt B.A., Levine P.J., Fusaro V.A., Steinberg S.M., Mills G.B., Simone C., Fishman D.A., Kohn E.C. and Liotta L.A. (2002). Use of proteomic patterns in serum to identify ovarian cancer. Lancet 359, 572-577.

Plebani M. (2005). Proteomics: the next revolution in laboratory medicine? Clin. Chim. Acta 357, 113-122.

Press J.Z., De Luca A., Boyd N., Young S., Troussard A., Ridge Y., Kaurah P., Kalloger S.E., Blood K.A., Smith M., Spellman P.T., Wang Y., Miller D.M., Horsman D., Faham M., Gilks C.B., Gray J. and Huntsman D.G. (2008). Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer 8, 17.

Rho S.B., Kim B.R. and Kang S. (2011). A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3?-kinase (PI3K)/AKT pathway in ovarian cancer cells. Gynecol. Oncol. 120, 121-127.

Ricciardelli C. and Oehler M.K. (2009). Diverse molecular pathways in ovarian cancer and their clinical significance. Maturitas 62, 270-275.

Roberts A.B., Anzano M.A., Lamb L.C., Smith J.M. and Sporn M.B. (1981). New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues. Proc. Natl. Acad. Sci. USA 78, 5339-5343.

Rosanò L., Cianfrocca R., Spinella F., Di Castro V., Nicotra M.R., Lucidi A., Ferrandina G., Natali P.G. and Bagnato A. (2011). Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cells. Clin. Cancer Res. 17, 2350-2360.

Rosanò L., Spinella F., Di Castro V., Nicotra M.R., Dedhar S., De Herreros A.G., Natali P.G. and Bagnato A. (2005). Endothelin-1 promotes epithelial- mesenchymal transition in human ovarian cancer cells. Cancer Res. 65, 11649-11657.

Sabatier R., Finetti P., Cervera N., Birnbaum D. and Bertucci F. (2009). Gene expression profiling and prediction of clinical outcome in ovarian cancer. Crit. Rev. Oncol. Hematol. 72, 98-109.

Sabbah M., Emami S., Redeuilh G., Julien S., Prévost G., Zimber A., Ouelaa R., Bracke M., De Wever O. and Gespach C. (2008). Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug. Resist. Updat. 11, 123-151.

Sato E., Olson S.H., Ahn J., Bundy B., Nishikawa H., Qian F., Jungbluth A.A., Frosina D., Gnjatic S., Ambrosone C., Kepner J., Odunsi T., Ritter G., Lele S., Chen Y.T., Ohtani H., Old L.J. and Odunsi K. (2005). Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc. Natl. Acad. Sci. USA 102, 18538-18543.

Saunders K.H., Nazareth S. and Pressman P.I. (2011). Case report: BRCA in the Ashkenazi population: are current testing guidelines too exclusive? Hered. Cancer Clin. Pract. 9, 3.

Schindl M., Schoppmann S.F., Ströbel T., Heinzl H., Leisser C., Horvat R. and Birner P. (2003). Level of Id-1 protein expression correlates with poor differentiation, enhanced malignant potential, and more aggressive clinical behavior of epithelial ovarian tumors. Clin. Cancer Res. 9, 779-785.

Shepherd T.G. and Nachtigal M.W. (2003). Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144, 3306-3314.

Sinclair J., Metodieva G., Dafou D., Gayther S.A. and Timms J.F. (2011). Profiling signatures of ovarian cancer tumour suppression using 2D-DIGE and 2D-LC-MS/MS with tandem mass tagging. J. Proteomics 74, 451-465.

Song Y., Wu J., Oyesanya R.A., Lee Z., Mukherjee A. and Fang X. (2009). Sp-1 and c-Myc mediate lysophosphatidic acid-induced expression of vascular endothelial growth factor in ovarian cancer cells via a hypoxia-inducible factor-1-independent mechanism. Clin. Cancer Res. 15, 492-501.

Strauss R., Li Z.Y., Liu Y., Beyer I., Persson J., Sova P., Möller T., Pesonen S., Hemminki A., Hamerlik P., Drescher C., Urban N., Bartek J. and Lieber A. (2011). Analysis of epithelial and mesenchymal markers in ovarian cancer reveals phenotypic heterogeneity and plasticity. PloS. One 6, 16186.

Sueblinvong T. and Carney M.E. (2009). Current understanding of risk factors for ovarian cancer. Curr. Treat. Options Oncol. 10, 67-81.

Sunde J.S., Donninger H., Wu K., Johnson M.E., Pestell R.G., Rose G.S., Mok S.C., Brady J., Bonome T. and Birrer M.J. (2006). Expression profiling identifies altered expression of genes that contribute to the inhibition of transforming growth factor-b signaling in ovarian cancer. Cancer Res. 66, 8404-8412.

Tainsky M.A. (2009). Genomic and proteomic biomarkers for cancer: a multitude of opportunities. Biochim. Biophys. Acta 1796, 176-193.

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.

Vergara D., Merlot B., Lucot J.P., Collinet P., Vinatier D., Fournier I. and Salzet M. (2010). Epithelial-mesenchymal transition in ovarian cancer. Cancer Lett. 291, 59-66.

Visintin I., Feng Z., Longton G., Ward D.C., Alvero A.B., Lai Y., Tenthorey J., Leiser A., Flores-Saaib R., Yu H., Azori M., Rutherford T., Schwartz P.E. and Mor G. (2008). Diagnostic markers for early detection of ovarian cancer. Clin. Cancer Res. 14, 1065-1072.

Voulgari A. and Pintzas A. (2009). Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to

overcome drug resistance in the clinic. Biochim. Biophys. Acta 1796, 75-90.

Woolas R.P., Xu F.J., Jacobs I.J., Yu Y.H., Daly L., Berchuck A., Soper J.T., Clarke-Pearson D.L., Oram D.H. and Bast R.C. Jr (1993). Elevation of multiple serum markers in patients with stage I ovarian cancer. J. Natl. Cancer Inst. 85, 1748-1751.

Wu C., Cipollone J., Maines-Bandiera S., Tan C., Karsan A., Auersperg N. and Roskelley C.D. (2008). The morphogenic function of E-cadherin-mediated adherens junctions in epithelial ovarian carcinoma formation and progression. Differentiation 76, 193-205.

Yamada S.D., Baldwin R.L. and Karlan B.Y. (1999). Ovarian carcinoma cell cultures are resistant to TGF-ß1-mediated growth inhibition despite expression of functional receptors. Gynecol. Oncol. 75, 72-77.

Yamaguchi K., Mandai M., Oura T., Matsumura N., Hamanishi J., Baba T., Matsui S., Murphy S.K. and Konishi I. (2010). Identification of an ovarian clear cell carcinoma gene signature that reflects inherent disease biology and the carcinogenic processes. Oncogene 29, 1741-1752.

Yang X., Fraser M., Moll U.M., Basak A. and Tsang B.K. (2006). Akt-mediated cisplatin resistance in ovarian cancer: modulation of p53 action on caspase-dependent mitochondrial death pathway. Cancer Res. 66, 3126-3136.

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.

Zhang B., Barekati Z., Kohler C., Radpour R., Asadollahi R., Holzgreve W. and Zhong X.Y. (2010). Proteomics and biomarkers for ovarian cancer diagnosis. Ann. Clin. Lab. Sci. 40, 218-225.

Zhang B., Cai F.F. and Zhong X.Y. (2011). An overview of biomarkers for the ovarian cancer diagnosis. Eur. J. Obstet. Gynecol. Reprod. Biol. 158, 119-123.

Zhang L., Conejo-Garcia J.R., Katsaros D., Gimotty P.A., Massobrio M., Regnani G., Makrigiannakis A., Gray H., Schlienger K., Liebman M.N., Rubin S.C. and Coukos G. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N. Engl. J. Med. 16, 203-213.

Zhang Z., Bast R.C. Jr, Yu Y., Li J., Sokoll L.J., Rai A.J., Rosenzweig J.M., Cameron B., Wang Y.Y., Meng X.Y., Berchuck A., Van Haaften-Day C., Hacker N.F., de Bruijn H.W., van der Zee A.G., Jacobs I.J., Fung E.T. and Chan D.W. (2004). Three biomarkers identified from serum proteomic analysis for detection of early stage ovarian cancer. Cancer Res. 64, 5882-5890.

Accepted January 18, 2012