

# Ovarian cancer: Origin and factors involved in carcinogenesis with potential use in diagnosis, treatment and prognosis of the disease

## Minireview

L. ZAVESKY<sup>1</sup>\*, N. JANCARKOVA<sup>1,2</sup>, M. KOHOUTOVA<sup>1</sup>

<sup>1</sup>Institute of Biology and Medical Genetics of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Albertov 4, 128 00 Prague 2, Czech Republic; <sup>2</sup>Department of Gynaecology and Obstetrics of the First Faculty of Medicine and General Teaching Hospital, Apolinarska 18, 128 51 Prague 2, Czech Republic

\*Correspondence: ludek.zavesky@vfn.cz

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Ovarian cancer representing the most lethal gynecologic malignancy escapes from the efforts to manage the disease. We reviewed the current state of the research considering three main concepts on origin of ovarian cancer including epithelial-mesenchymal transition, secondary origin from Müllerian system and cancer stem cell hypothesis. Cytogenetic and molecular characteristics of ovarian cancer are focused particularly on microRNA expression studies revealing huge potential in recent years, although other transcriptomic, proteomic, epigenetic, epidemiologic and immunological factors are touched upon, too. Routine and investigated diagnostic and treatment methods are outlined and several factors revealed to be associated with prognosis of the disease. Despite the huge progress on elucidating factors involved in ovarian cancer carcinogenesis, still remains urgent need to improve both the diagnostics as well as the treatment.

*Key words: ovarian cancer, genetics, expression, diagnosis, prognosis, microRNA*

**I. Introduction.** Among other gynecological cancers, ovarian cancer is specific due to its malignancy, high heterogeneity, tendency to recurrence and failure of medical efforts to manage diagnostic and therapeutic tools to get it under control. This most lethal malignancy of the female reproductive system and the fifth most fatal cancer for women at all [1] causes about 125.000 women deaths worldwide [2]. Ovarian cancer is generally presented as a complex disease and consists of several types of tumors, divided into two main groups: 1) epithelial and 2) non-epithelial, the latter one including germ cell and sex cord-stromal cell tumors [3]. Common epithelial tumors (EOC) account for about 90% of all ovarian cancers and are divided into four main histological subtypes: I. serous, comprising approximately 50% of all ovarian carcinomas [4], II. endometrioid (about 20–25% [5]), III. mucinous (about 10% [6]), and IV. clear cell tumors (about 4–12% [7]) occurring in four FIGO stages [8]. Three grades reflecting the differentiation extent of the

tumor are recognised: grade 1 (well), grade 2 (moderately) and grade 3 (poorly or undifferentiated).

Time of initial diagnosis is cardinal for the survival rate, so that detection during stage I or II results in a 60-90% 5-year survival, however diagnosis at stages III and IV leads to highly decreased survival rate previously reported less than 20-25% [9, 10], more recently about 33% [11]. Women with stage IV have the worst prognosis – less than 12% survived previously 5 years after diagnosis [12], recently about 19% [11]. However, most of cases (more than two thirds) are diagnosed in advanced stages with disseminated disease [11]. The relevant and early diagnosis associated with knowledge of exact causes, and therapeutic treatment without recurrence remain the major challenges in ovarian cancer research despite its huge progress in last years.

**II. Disputed origin of the epithelial ovarian cancer.** The six essential alterations in cell physiology have been suggested

to be associated with cancer: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [13].

However, the basic information on how ovarian cancer evolves remains still obscure and questionable. There are three main concepts which should be taken into consideration, 1) epithelial-mesenchymal transition (EMT) and its reversion, i.e. mesenchymal-epithelial transition [14, 15], 2) secondary origin of ovarian cancer from Müllerian system [16, 17], and 3) cancer stem cells hypothesis [18].

**1. Epithelial-mesenchymal transition (and its reverse) in ovarian cancer.** Ovarian epithelial cancers are supposed to originate from the ovarian surface epithelium (OSE) or inclusion cysts lined with OSE cells that were exposed to inflammatory stimuli, prolonged gonadotropin stimulation or incessant ovulation [19, 20, 21, 22, 23]. Ovarian surface epithelium (OSE) is considered to be a modified pelvic mesothelium originated from the mesoderm coelom, and has been found to have both mesenchymal and epithelial characteristics (in contrast to tubal epithelium which has epithelial only). Inclusion cysts have epithelial characteristics but lost mesenchymal characteristics indicating that mesenchymal-epithelial transition (MET) occurs during inclusion cyst formation [24].

Epithelial ovarian carcinogenesis thus may initially involve changes of surface mesothelial cells which acquire characteristics of epithelial cells (high miR-200, low ZEB1/2, high E-cadherin levels) due to MET [14, 25, 26, 27, 28]. Generally, epithelial cells have a highly baso-apical polarization suitable for processes as endocytosis, exocytosis and vesicle transport, and are closely associated with neighbouring cells. Mesenchymal cells, in contrast, lack the baso-apical polarization but have front-rear polarization, necessary for cell migration [29].

As ovarian tumor further progresses, epithelial-mesenchymal transition (EMT) may occur which is characterised by functional changes of cells, i.e. detachment from neighbouring cells (cells reduce intercellular adhesion and acquire fibroblastoid properties) and migration in the adjacent tissue [15]. Changes characteristic for EMT involve down-regulation of epithelial markers, e.g. E-cadherin and plakoglobin, up-regulation of mesenchymal markers, e.g. vimentin and N-cadherin, and translocation of  $\beta$ -catenin from membrane into the nuclear compartment [15, 30, 31]. Epithelial cells use the transmembrane glycoprotein of type I cadherin superfamily E-cadherin (encoded by CDH1) as the main molecule in the adherent junctions. Loss (or down-regulation) of E-cadherin correlates with susceptibility to EMT and acquisition of a metastatic phenotype in ovarian cancer [30]. Transcriptional repressors of E-cadherin include the zinc finger factors Snail (also known as Snail1, [32]) and Slug (also known as Snail2), the zinc finger E-box binding homeobox 2 known as ZEB2 (with its homolog ZEB1) and basic helix-loop-helix (bHLH) transcription factor Twist (see [14, 30, 33]).

There exist more genes involved in the regulation of E-cadherin expression in ovarian cancer, for example gene C4orf7, renamed FDC-SP (follicular dendritic cell secreted protein, FDC-SP) where its over-expression resulted in Akt ser473 phosphorylation and decreased E-cadherin expression [34]. Similarly, MUC4 mucin over-expression in invasiveness of ovarian cancer cells was associated with a decreased expression of epithelial markers (E-cadherin and cytokeratin (CK)-18) and an increased expression of mesenchymal markers (N-cadherin and vimentin) [35]. In addition to transcriptional repression, gene mutation, promoter hypermethylation and post-translational modification have been reported for inactivation of E-cadherin in malignant neoplasms (see [33]). Five main factors further identified to promote EMT in ovarian cancer cells are transforming growth factor  $\beta$  (TGF- $\beta$ ), epidermal growth factor (EGF), hepatocyte growth factor (HGF), endothelin-1 (ET-1) and bone morphogenetic protein 4 (BMP4) (reviewed in [15]). It has been shown recently that hepatocyte growth factor leads to down-regulation of E-cadherin, beta-catenin and caveolin-1, and is associated with invasion and metastasis enhancement in ovarian cancer cells [31].

**2. Secondary origin of ovarian cancer.** Particularly based on anatomical/morphological observations is a model of secondary Müllerian origin of ovarian cancer cells [16, 36]. This model assumed spreading of tumor cells from parts of mesonephric origin, i.e. of the Müllerian tract, to the ovary. Expression of the same set for HOX genes in serous, endometrioid and mucinous ovarian carcinomas as epithelial cells from normal fallopian tube, endometrium and endocervix [37] has been proposed as the further evidence for this theory, however, a compatibility with the above mentioned and the following model should be investigated. An association between endometrioid type of ovarian tumors and endometrioid tumors suggesting that they share their etiopathogenesis has been shown recently [38].

The evidence that high-grade serous ovarian carcinomas (most common and most lethal of all ovarian neoplasms) may have the origin in the fimbriae of the fallopian tubes [39] has underlined the controversy on origin of ovarian cancers either in ovarian surface epithelium, or in fimbrial epithelium [17]. On the contrary, mucinous, low-grade serous, some high-grade serous carcinomas and borderline tumors may originate in the OSE. Finally, the endometrioid and clear cell carcinoma are assumed to be derived from endometriosis, and remaining epithelial ovarian cancers may originate either in the OSE or in the fimbrial epithelium [17, 40].

The novel unifying hypothesis suggests that OSE and fimbrial epithelium are not separate and independent, either developmentally or anatomically. Here, the immunohistochemically-based study revealed further support and evidence that the OSE and fimbrial epithelium may not be fully determined, share differentiation markers, and represent junctional, or transitional epithelium extending from one form of fully differentiated epithelium (pelvic serosa) to another (tubal ampulla) [17].

**3. Cancer stem cells in ovarian cancer.** The existence of cancer stem cells (CSCs) described as multipotent cells capable of forming heterogeneous tumors in immunodeficient mice [41]) suggests an interesting point of view how cancer may evolve. CSCs are thought to be somatic stem cells that have undergone mutations and thus acquiring a cancerous phenotype [42]). Although the presence of ovarian somatic stem cells has been suggested previously [43, 44] there exists another theory dealing with CSCs as to be formed from lineage-restricted or differentiated cells with a cancer phenotype that dedifferentiate into CSCs [45]. CSCs were identified in ovarian carcinomas [46, 47] besides many other cancers (see [18]). Expression of stem cell markers CD133 and CD44 in ovarian cancers has been shown to be associated with ovarian cancer cells showing heightened aggressiveness and ability to form xenograft tumors in mice (CD44<sup>+</sup>CD117<sup>+</sup> [47] or having a higher proliferation potential at least (CD133<sup>+</sup>, [48]). As a prognostic marker, however, the role of CD133<sup>+</sup> is questionable [49]). Recently, a novel phenotype of ovarian cancer stem cell-like cells (CSC-LCs) CD44<sup>+</sup>CD24<sup>-</sup> has been identified with the characteristics of self-renewal, high tumorigenicity, multi-differentiation potential and marked resistance to conventional chemotherapeutic drugs in ovarian cancer [50]. Further, two types of ovarian tumor cells recently discovered differed in response to chemotherapy: Type I EOC cells were chemoresistant, while Type II EOC cells were chemosensitive; both the types differed also in expression of numerous genes. For example, Type I EOC cells (having stem-like properties and slower growth) express IL6, IL8, MCP-1, GRO $\alpha$ , Cytokeratin 18, the TLR adapter protein, MyD88 and stemness-associated genes as CD44, Oct-4, SSEA-4 (see [51]). Although CSCs have been implicated in tumor initiation, progression, metastasis, and drug resistance, there is lack of consensus about the general molecular characteristics of ovarian CSCs [52].

### III. Cytogenetic and molecular characteristics, and further factors in ovarian cancer.

#### 1. Germline mutations of genes involved in ovarian cancer.

Mutations in BRCA1 and BRCA2 genes have been shown to be in association with developing breast [53, 54], ovarian [55, 56] and fallopian tube [57] cancers. The inherited mutations in BRCA1 (3-6%), BRCA2 (1-3%) and HNPCC DNA mismatch repair genes (1-2%) associated with the invasive epithelial ovarian cancer represent only about 10% of the cancers to be attributed to that mutations which may significantly increase lifetime risk of ovarian cancer (BRCA1 carriers: 15-30%, BRCA2 carriers: 10-15%), but overall occurrence of these mutations is low (<0.5% of individuals, [58]). Although detailed cellular functions remain to be further elucidated, BRCA1 and BRCA2 are supposed to act as tumor suppressor genes and their protein products affect transcriptional regulation and DNA damage repair [59, 60].

**2. Chromosomal rearrangements, cytogenetic aspects and genetic polymorphisms in ovarian cancer.** Ovarian cancer is often characterized by genetic alterations including amplifi-

cations and deletions of large chromosomal regions. In our previous studies using Comparative Genomic Hybridization (CGH) method along with other cytogenetic methods in ovarian cancer [61, 62], gains were observed on 3q, 1q and 20q chromosomal regions similarly to Nowee et al. [63]. Similar results were observed also for losses on 4q, 4p and 18q; however, there were some different regions with observed amplifications and deletions. Frequent observation of deletion on 22q (36.2% cases) is congruent with other reports (e.g. [64]). We should mention that deletions in some regions may represent common deletion polymorphisms not necessarily associated with the disease as it was demonstrated for this region [65]. On the other hand, in another study, there was tumor suppressor gene, MYO18B on 22q suggested to be involved in ovarian cancer [66] and also in other cancers (e.g. [67]). Other important results based on conventional banding, FISH, CGH, chromosome microdissection, loss of heterozygosity, chromosome microcell-mediated chromosome transfer were reviewed in Wang [68]. Further, recurrent deletions have been identified on chromosomes 4, 6, 9, 12, 13, 15, 16, 17, 18, 22 and most prevalently on X and 8 [69]. Genetic alterations specific to chemoresistant (gains on chromosome 9) and chemosensitive disease (losses on chromosome 8) have been identified recently [70].

Research focused on mapping the genetic polymorphisms within genome-wide association studies (GWAS) has identified several millions common genetic polymorphisms in humans but for most diseases the discovered associations cover a small portion of the estimated total heritability [71, 72]. Particularly in ovarian cancer these studies have failed to find relevant associations previously [73], however a recent study confirmed susceptibility locus at 9p22, revealed several candidate loci and two susceptibility loci 8q24 and 2q31 have been confirmed [74]. Several further polymorphisms investigated previously (concerning sex hormone pathways or cell cycle genes) proved not to be convincingly usable for the identifying a strong association with the disease [75, 76].

#### 3. Transcriptomic/proteomic research in ovarian cancer.

**a. mRNA expression and proteomics.** Historically, the mRNA expression profiling has been often used for a prediction of expression of relevant proteins and for determining their up- or down-regulation. In ovarian cancer, studies of protein deregulations were aimed particularly at establishing early detection biomarkers. Promising biomarkers include family of serine proteases, i.e. kallikreins (KLKs), further interleukins (ILs), glycoproteins (osteopontin, CA-125), or antitumor antibodies [77, 78, 79]. Also roles of B7-H4 (expressed in activated T-cells), spondin 2 (extracellular matrix protein) and DcR3 (tumor necrosis factor receptor superfamily) have been investigated previously [80], and lamin A (nuclear membrane organisation), SSRP1 and IRF6 (regulation of transcription), RALBP1 (transport), FUSIP1 (RNA splicing), CBLB (signal transduction), TADA31 (regulation of cell cycle) have been shown to express different pattern in sera [81].

Genome-wide analyses based on microarray technology revealed (when reviewed) more than 150 genes highly

up-regulated or down-regulated in short-term survivors, involved in cell signaling, growth factors, transcription factors, proteinases, metabolism, cell adhesion, extracellular matrix component, cell proliferation and anti-apoptosis. Moreover, among 154 genes in aggressive ovarian cancers, 108 (70.1%) were associated with epithelial-mesenchymal transition (EMT)-related genes [82]. Further, detailed transcriptome analysis of chromosome 3 genes in serous epithelial ovarian cancers including EOC cell lines and malignant tumors revealed among 278 differentially expressed genes, three genes (RIS1, GBE1 and HEG1) that were similarly under-expressed in all the cancer samples studied [83], however still without further detailed evidence in other investigations.

Many other previous studies in ovarian cancer were aimed particularly at finding the expression signature associated with the disease stage, long-term survival of patients and resistance to chemotherapeutics [84, 85]. However, there is a poor association between the mRNA expression and abundance of mRNA encoded proteins and qualitative and quantitative analyses of proteomes are required [86]. Details on promising serum protein markers (e.g. [87]) are given in the later part Diagnosis of ovarian cancer.

**b, Non-coding RNA/miRNA expression studies and a role of microRNAs in ovarian cancer.** Non-coding RNA and progress in their investigation achieved in the last decade have dramatically changed the view of regulation of gene expression [88]. The most remarkable class represent microRNA (miRNAs), which are one-stranded, 19 to 25 nucleotides long molecules of RNA. They are implicated in regulation of gene expression at post-transcriptional level by block of translation and/or cleavage and degradation of target mRNA [89, 90]. Different levels of miRNAs expression in different cell types (including cancer cells) and developmental stages suggested their involvement in cell growth, differentiation and programmed cell death [91]. In cancer, over-expressed miRNAs may function as oncogenes (i.e. cancer promoters) by down-regulating tumor suppressor genes and/or genes involved in control of cell differentiation or apoptosis, for example miR-21 targets tumor suppressor PTEN. On the other hand, miRNAs may act as tumor suppressors (i.e. cancer inhibitors) by regulating above mentioned processes, for example by repression of oncogenes, as does let-7 by targeting the oncogenes K-Ras, Myc and HMGA-2 [92, 93]. There have been reports on up- or down-regulation of miRNAs involved in cancer cell proliferation, regulation of apoptosis, replicative potential or angiogenesis of cancer cells. Further, miRNAs are involved in regulation of immune responses, tumor invasion and metastasis and regulation of genomic instability of cancer cells where the changes in miRNA expression levels are correlated with copy number changes in regions of genomic instability or fragile sites [94]. MiRNAs may serve as predictors and modifiers of chemo- and radiotherapy in different tumor types in addition to a great potential for biomarker establishing and future treatment possibilities [95].

In ovarian cancer, miRNA signatures using microarray hybridization and quantification revealed 39 miRNAs, out of them 25 were down-modulated and 4 up-modulated; these 29 miRNAs were able to classify normal and tumor samples and also characterized different ovarian carcinoma histotypes [96]. Most significantly up-modulated were miR-200a and miR-141 (the same family), and miR-200b. Among the down-modulated genes there were the miR-125b1 and miR-145 altered also in breast cancer [97] and miR-199a was shown to be down-modulated in other tumors (e.g. hepatocellular carcinoma [98]).

An exceptional approach where integrated transcriptome and miRNA analyses were used, revealed miR-9 (down-regulated) and miR-223 (up-regulated) as potential biomarkers in recurrent ovarian cancer [99]. Detection of differentially expressed microRNAs in the serum (data from tumors not available) was obtained in another study [100] where eight miRNAs were found to be significantly differentially expressed between cancer and normal serum specimens. Here, miRNAs-21, 92, 93, 126 and 29a were over-expressed, and miRNAs-155, 127 and 99b were under-expressed in cancer specimens [100]. Using breakthrough massively parallel sequencing approach (454 Life Sciences platform), six novel miRNAs and 39 candidate miRNAs were discovered [101]. Moreover, a set of 124 miRNAs differentially expressed in normal versus cancer samples and 38 miRNAs differentially expressed across histological subtypes were identified [101]. Role of overexpressed miR-21 in targeting PTEN (tumor suppressor) was highlighted recently in ovarian cancer [102].

Impact of expression of components of miRNA processing machinery (enzymes Dicer and Drosha) has not been proved to be included in deregulation of miRNAs in ovarian cancer previously [99]. However, correlation of levels of mRNA and corresponding proteins of Dicer and Drosha with tumor stage has been found recently [103]. In this study, low Dicer expression was significantly associated with advanced tumor stage and low Drosha expression with suboptimal surgical cytoreduction. The low levels of Dicer expression in ovarian cancer tissues in comparison to normal tissues have been found recently [104]. Generally, it should be noted that miRNA expression may be dysregulated by various ways, e.g. mechanisms targeting miRNA genes (1. genomic alterations – deletions, amplifications, translocations, epigenetics changes (methylation, histone modification), polymorphisms or mutations and transcriptional alterations, and 2. mechanisms modulating the activity of the multistep processing enzymes (Drosha, DGCR8, Exportin-5, Dicer and TRBP)) [105].

Based on expression studies of miR-200 family along with ZEB transcription factors, over-expression of this miRNA family was observed in ovarian cancer [14] in contrast to another study where differential expression did not occur [106]. The controversial results are ascribed to using E6/E7 immortalized HOSE cells as the normal control in the latter study which may suggest activity of E6/E7 viral oncoproteins [14]. Expression

of ZEB1 and ZEB2 was inverse to miR-200 family. Data in this study supported the model of mesothelial-to-epithelial transition and *vice versa* during tumor progression and further dissemination [see 14].

When we compare some recent studies discrepancies can be found in miRNAs expression profiles of tumors versus controls in ovarian cancer. For example, miR-21 was shown to be up-modulated [96] or down-modulated [101], down-modulated expression of miR-126, miR-143, miR-195, miR-29c and miR-99a [96] was not followed in Wyman et al. [101] where these miRNAs were up-modulated; miR-214 and miR-199a found to be over-expressed in Yang et al. [107] were found to be under-expressed elsewhere [101]. In many instances, however, expression profiles of certain miRNAs were similar. The discrepancies in detected microRNA expression patterns in many studies may be for example attributed to different tissue types (heterogeneity, classification), processing and analyzing methods, normalization strategies (choice of endogenous controls), choice of normal controls (calibrators), sample numbers or geographical differences. For a comprehensive review of microRNA expression in ovarian cancer and therapy resistance see [108].

**4. Epigenetic factors involved in ovarian carcinogenesis.** DNA methylation profiling studies proceeding during last years revealed several potential target genes (being methylated) involved in ovarian carcinogenesis. For example, tumor specific hypermethylation status of BRCA1 and RASSF1A tumor suppressor genes with corresponding values in patient serum/plasma DNA have been reported previously in ovarian cancer [109]. Particularly, HOXA genes cluster methylation is the common feature in cancer [110] and these genes are essential for differentiation of the reproductive tract, e.g. HOXA9 is expressed at high levels in areas becoming fallopian tube [111]. The possibly relevant markers for screening may be for example HOXA9, HOXB5, SCGB3A1 and CRABP1 identified in ovarian tumor samples [112]. However, in another study genes HOXA10 and HOXA11 appeared to be highly methylated in comparison to normal ovary tissue and results for SCGB3A1 [112] were not supported [113]. Methylation of HOXA 9, 10 and 11 genes was further confirmed and possibility of detection of methylation status of the endometrial epithelium as the marker for ovarian cancer has been proposed recently [114]. DNA methylation profiles in a panel of 56 genes using sections of ovarian serous papillary adenocarcinomas and also in plasma samples revealed ten of the profiles as potentially informative in tissues and five genes were identified as informative in plasma [115]. Another study on methylation provides further profiling analyses, and most importantly, it challenges the usage of cell lines as tumor models [116].

**5. Epidemiological factors in ovarian cancer.** From an epidemiological point of view, there have been reported several risks known to influence a women's lifetime risk for ovarian cancer (see [117]). We can mention associations with age, duration of breastfeeding, age at natural menopause, and duration of estrogen use, all these factors have been shown to

differ significantly by histologic subtype, although duration of breastfeeding was inversely associated with all the subtypes but with strongest association found in mucinous tumors. Age among women under 50 was associated with serous invasive and endometrioid tumors, among older women (50 years and older) age may implicate modest increase in risk of serous invasive cancers and modest decrease in risk of endometrioid tumors. The increase in number of ovulatory years is associated with high risk of serous invasive and endometrioid tumors (each 1-year brings 8% increase of risk) and 3% risk of mucinous tumors. Parity has been shown most protective for endometrioid and clear-cell tumors, but has the protective effect among all the subtypes, similarly as use of oral contraceptive which exhibited similar protective effect for all the subtypes (see [117]).

In contrast to another gynecological malignancy, i.e. the cervical cancer (HPV associated one), no causal association of ovarian cancer and bacterial (*Mycoplasma genitalium*, *Neisseria gonorrhoeae*) or viral infection (HPV or polyomavirus) has been confirmed recently [118].

**6. Role of immune system in ovarian cancer.** Association of tumor infiltrating leukocytes with clinical outcome of ovarian cancer patients have been demonstrated previously (see later), however, the exact immunological basis for the tumor escape from host immunosurveillance remains elusive. Two basic general models deal with this issue (reviewed in [119]). The first one suggests that immune rejection of the tumor does not occur due to fact the tumor is recognized as immunologically normal tissue. Tumor cells express tumor-associated antigens (TAA), which can be normally present on host cells, so induction of an effective anti-tumor immune response is not achieved [120, 121]. In ovarian cancer, it has been demonstrated that loss of HLA class I antigen on tumor cells correlates with poor outcome, as the interaction of T cells and the HLA receptor is needed to elicit their function (see [122]).

The second general model suggests that a host mechanism of immunosurveillance exists and plays an active role in suppressing the initiation and further tumor growth. Mechanisms of immunologic escape of the tumor involve change of immunogenicity and production of various tumor-derived immunoregulatory molecules (see [119]). In ovarian cancer, inflammatory cells may be attracted by chemokines produced by tumor islets (e.g CXCL9, see [123]), or chemotoxic substances may be released by tumor to enhance apoptosis of the cytotoxic T cells (see [124]). Regulatory CD4+ T lymphocytes suppress immune responses by secreting transforming growth factor beta and interleukin 10, or by direct cell-cell contact, and concentrate in peri-tumoral areas (see [124]). The exact role and functions of these and other leukocytes subsets in ovarian carcinogenesis remain to be elucidated.

**IV. Current diagnosis, treatment and prognosis in ovarian cancer patients.** All above-mentioned issues, such as origin, cytogenetic alterations, and consequently changes in gene expression reflected in mRNA and protein levels in-

cluding regulations by non-coding RNAs may have potential applications in diagnosis, prognosis or treatment of disease. However, studies focused specifically on tumor tissues have limited potential for the diagnosis as it is not known how the changes are reflected in body fluids (e.g. blood, urine) which can be used for routine disease screening. Despite several clinical trials ongoing nowadays, most of the results of the current research need to be validated extensively before the use in the clinical practice.

**1. Diagnosis of ovarian cancer.** There exists no proven effective screening method which could be used for the early detection of ovarian cancer. Routine diagnostic methods for ovarian cancer involve pelvic examination, assessment of serum CA-125 antigen level (tumor marker) and transvaginal ultrasound (TVU), potentially associated with analysis of several tumor markers [125]. Although use of CA-125 marker along with a computerised algorithm (Risk of Ovarian Cancer Algorithm, ROCA) has improved this common marker relevance previously [126], for the early stage ovarian cancer detection this marker has poor sensitivity [127, 128].

Tumor markers being under investigation for the potential use in ovarian cancer screening include mucin related glycoproteins (include abovementioned CA-125, and OVX1, CA-125 II, CA-72-4, CA15-3, HE4 (human epididymis protein 4), mesothelin (MES)), hepatic and acute phase proteins (haptoglobin- $\alpha$ (HP- $\alpha$ ), bikunin, C-reactive protein) and several other markers with various specificities and sensitivities according to different combinations (reviewed in [128]). The usage of one marker alone usually has a worse sensitivity and specificity than their combination and some authors use the composite index, however, there remains the urgent need for sensitive tumor markers [128].

Moreover, when comparing different diagnostic strategies, i.e. histologic, cytologic and clinical in predicting final pathology, cytology-based approaches (paracentesis, thoracentesis, or fine needle aspirate; diagnostic accuracy 98%) and histology-based ones (core biopsy, surgery; accuracy 92%) revealed to be more accurate than clinical ones (radiology and CA-125, accuracy 87%) [129].

The abovementioned differential expression of miRNAs showing potential in ovarian cancer detection has to be validated extensively for the blood/serum/plasma samples, and is not currently used in diagnostic routine.

**2. Treatment of ovarian cancer.** Primary cytoreductive surgery followed by chemotherapy is used usually as the initial management of ovarian cancer. Despite the fact that more effective surgery and optimized combinational chemotherapy, i.e. platinum-based drugs combined with taxanes have improved the management of ovarian cancer over the last two decades, the overall cure rate is only 30% [108, 130, 131, 132]. Following primary treatment for ovarian cancer, clinical assessment and CA-125 are routinely used to monitor patients. For suspected recurrence, ultrasound, computed tomography (CT) and positron emission tomography (PET)/CT appeared suitable particularly in patients with negative CT or magnetic

resonance imaging (MRI) [133]. Second-line chemotherapy applied in recurrent disease has a palliative character, and is used along with the above-mentioned procedures.

**3. Prognosis of the ovarian cancer development.** Several alterations in gene expression have been revealed to be associated with prognostic outcome for patients. High expression levels of Dicer, Drosha and eIF6, proteins involved in miRNA maturation, were shown to be associated with a favourable prognosis of ovarian cancer patients [134, 135]. On the contrary, high expression levels of the miR-200 family have been associated with decreased progression-free survival and overall survival of ovarian cancer patients [136]. Low expression levels of let-7b in serous ovarian carcinomas have been associated with poor prognosis [136]. High expression levels of Lin28 and Lin28b (inhibitors of let-7 miRNA processing, [137]), correlated with shorter progression-free survival and overall survival in patients with ovarian cancer [138]. Several miRNAs altered expressions have been also reported to be associated with chemotherapy resistance in both directions (reviewed in [108]). Currently, down-regulation of miR-153 and up-regulation of miR-519a has been shown to be correlated significantly with advanced clinical stage, and higher expression of miR-519a in late stage serous carcinoma was significantly associated with poor progression-free survival [139].

It has been also found recently that high levels of Wnt5a expression were associated with FIGO stage and a poorer overall survival and progression-free survival compared with low Wnt5a expression [140]. Similarly, positive NAC1 expression significantly correlated with shorter disease-free and overall survival and revealed as an independent prognostic factor for these characteristics after standard platinum-taxane chemotherapy in another recent study [141]. Wnt7a expression has been found to be correlated with serous subtype, elder age, advanced stage and high grade, suggesting the association with poor prognosis [142]. Patients with p53-positive tumors (alone/or combined with p27 and/or C-MYC) had significantly worse survival (DFS) compared with patients with p53-negative tumors and continued to have recurrences after the 5-year follow-up [143].

Correlations have been also found between numbers of particular tumor infiltrating lymphocytes (e.g. CD3<sup>+</sup>, including CD4<sup>+</sup> and CD8<sup>+</sup> T cells) and disease prognosis previously. Elevated proportions of tumor-infiltrating CD3<sup>+</sup> T cells and CD8<sup>+</sup> T cells (cytotoxic T cells) have been often associated with favorable prognosis (see [144]). Contrary to results for CD3<sup>+</sup> T cell infiltration, also a correlation with brief (<12 months) disease-free interval has been reported previously both for CD3<sup>+</sup> and  $\gamma\delta$  T cells [145]. Further, an association of tumor-infiltrating CD25<sup>+</sup>FoxP3<sup>+</sup> T cells with decreased survival has been found previously (e.g. [146]). Significant correlation was found between higher numbers of CD8<sup>+</sup> cells and macrophages with malignancy of the tumors [147].

**V. Conclusions.** Epithelial ovarian cancer remains the controversial issue for further investigations. Heterogeneity in

cancer tissues (intratumoral and among the subtypes) resulting from possibly different origin and development, and reflected also in various clinical manifestations of histological subtypes providing different potential markers with disputed relevancy, and high tendency to recurrence/chemoresistance make this disease the great challenge of the current medicine. Huge number of diagnostic markers is being tested to obtain relevant diagnostic non-invasive tools. Many prognostic factors have been revealed. Novel therapeutic experimental approaches include use of RNAi (RNA interference), immunotherapy, or use of oncolytic viruses. Several novel drugs are investigated in clinical trials. As there exist many novel approaches differing in the methodology used, the resulting bias may lead to limited usefulness for the diagnostic purposes and for relevant comparisons. We can mention for example differences noted in the microRNA expression which may possibly be particularly attributed to use of various calibration and normalization strategies. Further, the investigations usually do not integrate DNA, RNA (coding and non-coding ones) and proteins analyses and expression studies, and the heterogeneity of tumor tissues may be underestimated. In comparison to tumor tissue profiling, the non-invasive methods and analyses (e.g. blood, leukocytes, plasma/serum, saliva, and urine) particularly considering non-coding RNA are much rarer or lacking, but may have higher potential for the diagnostic purposes. Moreover, the associations of such expression profiles with tumor tissue profiling (and *vice versa*) are not usually known. Therefore, despite all the efforts (many have not been mentioned) and recent research progress, diagnosis and treatment at the current state provide the issue with much potential to improve.

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