NON-THEMATIC REVIEW

Ovarian cancer molecular pathology

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Abstract Ovarian cancer (OVC) is the fourth leading cause of cancer mortality among women in Europe and the United States. Its early detection is difficult due to the lack of specificity of clinical symptoms. Unfortunately, late diagnosis is a major contributor to the poor survival rates for OVC, which can be attributed to the lack of specific sets of markers. Aside from patients sharing a strong family history of ovarian and breast cancer, including the BRCA1 and BRCA2 tumor suppressor genes mutations, the most used biomarker is the Cancer-antigen 125 (CA-125). CA-125 has a sensitivity of 80 % and a specificity of 97 % in epithelial cancer (stage III or IV). However, its sensitivity is 30 % in stage I cancer, as its increase is linked to several physiological phenomena and benign situations. CA-125 is particularly useful for at-risk population diagnosis and to assess response to treatment. It is clear that alone, CA-125 is inadequate as a biomarker for OVC diagnosis. There is an unmet need to identify additional biomarkers. Novel and more sensitive proteomic strategies such as MALDI mass spectrometry imaging studies are well suited to identify better markers for both diagnosis and prognosis. In the present review, we will focus on such proteomic strategies in regards to OVC signaling pathways, OVC development and escape from the immune response.

Keywords Ovarian cancer · Molecular pathology · Cancer antigen · MALDI imaging · Signaling pathway · Immuno-oncology

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1 Introduction

Age, genetic profile, hormonal profile (early onset of menses, high number of ovulatory cycles, late menopause, infertility, endometriosis), environmental factors (e.g., diet, obesity, smoking, virus), geographical areas, the racial and ethnic variation (correlated with the genetic inheritance) are all factors that have been implicated in OVC development ([1,2]); [3,4] .; [5] Only 5–10 % of OVC is hereditary [6]. In this context, CA-125 is insufficient as a single biomarker for OVC diagnosis [7]. OVC etiology is multiple, thus, like finding a needle in a haystack, identifying and validating a single specific biomarker appears to be a very low probability event. There is an unmet medical need to aid in screening and diagnosis, and thus alternative strategies need to be considered.

2 OVC signaling pathways

Major intracellular signaling pathways are involved in OVC cell development. The understanding of the malignant mechanisms and the transformation of ovary cells to epithelial OVC are seen as a path to identify specific OVC biomarkers. The genetic studies reflect that OVC polymorphisms are highly unstable [8]. In fact, genic amplification, mutations, hypermethylations, and numerous chromosomal deletions have been found in OVC pointing the identification of several main categories of genes involved in OVC development such as tumor suppressor genes and oncogenes (Table 1) [6,9–12]. Thus, these genes are directly linked to cell signaling pathways, which play a central role in cancer cell growth, survival, invasion, and metastasis [13]. Discovering the "circuit maps" of these signaling pathways in OVC seems a good challenge for detecting novel therapeutic strategies [14–16]. According to literature, the signaling pathways associated with OVC are the followings: the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, the activator of transcription 3 (Jak-STAT 3) pathway, the mitogen-activated protein kinase (MAPK) pathway, the proto-oncogene tyrosine-protein kinase Src pathway, the ErbB activation pathway, the lysophosphatidic acid (LPA) pathway, the phosphatidylinositol 3-kinases (PI3K) pathway, the Mullerian inhibitory substance receptor pathway, the EGF and VGEF pathways and the ER beta pathway [17–40].

2.1 Nuclear factor kappa-light-chain-enhancer of activated B cells pathway

A correlation has been demonstrated between nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and OVC clinical profile showing that the expression of NF-κB p65 in OVC tumors is mainly nuclear and that their levels correlate with poor differentiation and late FIGO stage [41]. Positive patients for NF-κB p65 subunit staining had lower cumulative survival rates and lower median survival (20 % and 24 months, respectively) than negative patients (46.2 % and 39 months, respectively). Recently, microRNA-9 (miR-9) has been shown to downregulate the levels of NF-κB1 [42]. Moreover, results from gene expression microarray, using the highly specific IKKβ small-molecule inhibitor ML120b or IKKβ siRNA to decrease IKKβ expression, showed that IKKβ-NF-κB pathway controls genes associated with OVC cell proliferation, adhesion, invasion, angiogenesis, and the creation of a proinflammatory microenvironment. The NF-κB family proteins are implicated in signaling pathways driving tumor development and progression by activating anti-apoptotic genes. It also activates genes involved in cell cycle progression and the secretion of tumor necrosis factor (TNF) α, interleukin (IL)-6, and growth hormones. Moreover, NF-κB

regulates genes promoting pro-angiogenic environment through enhanced production of IL-8 and vascular endothelial growth factor and creation of a microenvironment that may prevent immune surveillance [43–45].

2.2 The mitogen-activated protein kinase pathway

Previous studies identified that mitogen-activated protein kinase (MAPK) pathway is activated in OVC (ref). The downregulation of CL100, an endogenous dual-specificity phosphatase known to inhibit MAPK, plays a role in progression of human OVC by promoting MAPK pathway [46]. OVC epithelial cells display 10–25 times less activity of C100 compared to normal ovarian epithelial cells. The MEK inhibitor PD98059 sensitized OVC cell lines to Cisplatin. Upregulation of CL100 in ovarian cancer cells decreases adherent and non-adherent cells growth and induces phenotypic changes, including loss of filopodia and lamellipodia in association to decreased cell motility [46]. Thus, the development of specific MAPK pathway inhibitors is currently in process [47].

2.3 The ErbB activation pathway

Overexpression of c-erbB-2 protein in tumors has been reported from approximately 25 % of patients with epithelial OVC [48,49]. Multivariate analysis showed that c-erbB-2 overexpression and residual tumors, greater than 2 cm, decrease survival rates [48,49].

2.4 The Mullerian inhibitory substance receptor pathway

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor β (TGF-β) family In absence of AMH, Müllerian ducts of both sexes develop into uterus, Fallopian tubes, and the upper part of the vagina. Also, AMH exerts inhibitory effects on the differentiation and steroidogenesis of the immature ovaries, the follicle of adult ovaries and as well as fetal and postnatal Leydig cells. Other proposed targets of AMH actions include breast, prostate, ovarian, and uterine cancer cells [50].

2.5 Lysophosphatidic acid signaling pathway

Lysophosphatidic acid (LPA) is generated through hydrolysis of lysophosphatidyl choline by lysophospholipase D/autotaxin or via hydrolysis of phosphatidic acid by phospholipase A_2 or A_1. LPA is a ligand for at least four different heptahelical transmembrane G protein-coupled receptors (GPCR; LPA_1/endothelial differentiation gene (Edg)2, LPA_2/Edg4, LPA_3/Edg7, and LPA_4/GPR23/P_2Y_9) results in activation of at least three distinct G protein subfamilies (G_q, G_i, $G_{12/13}$) and initiation of multiple signaling pathways, including Ras/

Raf/mitogen-activated protein kinase, phosphoinositide-3-kinase/Akt, phospholipase C/protein kinase C, or RhoA small GTPase signaling [51]. Activation of these G-proteins stimulates release of cell surface metalloproteases, like the ADAM family, inducing subsequently cleavage of EGF-like ligand precursors and thus EGFR (HER/erbB) family of. This transactivation process seems to involve many signaling pathways, e.g., mitogen-activated protein kinase (p38 and p44/42), protein kinase C or c-Src. LPA induces cancer cell proliferation, survival, drug resistance, invasion, opening of intercellular tight junctions and gap junction closure, cell migration, or metastasis [51].

2.6 Phosphatidylinositol 3-kinases pathway

The phosphoinositide 3-kinase (PI3K)/AKT pathway is a key component of cell survival and is implicated in OVC cell motility, adhesion, and contributes to metastatic/invasive phenotypes of various cancer cells. PI3K was also reported to be involved in cell growth and transformation. Its effect is related to indirect or direct deregulation of its signaling pathway and causes aberrant cell-cycle progression and transformation of normal cells into tumor cells. Due to hyperactivation of the PI3K/Akt pathway in many cancers and its role in multiple aspects of cancer progression, inhibition of the PI3K/Akt signaling pathway has been investigated as a treatment for cancer [52]. A proof of concept is based on the loss of function of inositol polyphosphate 4-phosphatase type II (INPP4B) from the PI3K/Akt pathway acting as a tumor suppressor. Remarkably, loss of INPP4B in ovarian cancer correlates with poor patient outcomes [52].

2.7 Estrogen receptors pathway

The mitogenic action of estrogen seems to be critical to the etiology and progression of human gynecologic cancers [53,54]. Estrogens influence the growth, differentiation, and function of reproductive tissues by interacting with their receptors to mediate various signaling pathways associated with the risk of ovarian cancer [53,54]. Estrogen receptors exist in two forms, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) which is the predominant estrogen receptor in the ovaries [26]. Recent *in vivo* and *in vitro* studies suggest that ERβ is involved with the control of cellular proliferation, motility and apoptosis in ovarian cancer; and loss of ERβ expression is associated with tumor progression [55,56].

Taken together, the above studies reveal that all these signaling pathways are implicated in OVC cell differentiation, cell movement and apoptosis, and are directly linked to OVC tumor suppressor genes and oncogenes (Table 1). At this moment, it is relevant to establish a link between these signaling pathways and proteins specific to OVC cells previously identified by classical proteomic studies or mass spectrometry imaging (MSI).

3 Proteomic studies

Since the last decade, proteomic studies have been widely used to identify key proteins implicated in different ovarian cancer processes. Using these new technologies, many authors identified panels of biomarkers and tested these for their relevant utility to screen early stages of the disease. Candidate biomarkers have been detected in serum or tissues using SELDI chips analysis. From this method, a biomarker, the alpha chain of haptoglobulin, has been identified and found in higher levels in samples from OVC patient [57]. Then, a protein pattern has been determined by Yu et al. [58] with 96.7 % specificity, 96.7 % sensitivity, and a predictive positive value of 96.7 %. Another group reported the use of the following markers: transthyretin, beta-hemoglobin, apolipoprotein A1, and when used in combination with CA 125, has been found to have a ROC of 0,959 for the detection of ovarian cancer, when CA 125 used alone 0.613 [59]. Two separate classical proteomic studies in 2006 [60] and 2008 [61], using liquid chromatography (LC) separation followed by MS (LC-MS), have shown that early and late stage endometroid ovarian carcinoma MS profiles can be distinguished using a clustering analysis, which separates profiles based on feature similarity; in

Table 1 The genetic polymorphism of the OVC

Gene classification	Tumors suppressors		Oncogenes	
	ARHI, RASSF1A, DLEC1, SPARC, DAB2, PLAGL1, RPS6KA2, PTEN, OPCML, BRCA2, ARL11, WWOX, TP53, DPH1, BRCA1, PEG3		RAB25, EVI1, EIF5A2, PRKCI, PIK3CA, MYK, EGFR, NOTCH3, KRAS, ERBB2, PIK3R1, CCNE1, AKT2, AURKA	
Gene modulation	Amplification	Mutation	Hypermethylation	Deletion
Activation	RAB25, PRKCI, EVI1 and PIK3CA, FGF1, MYC, PIK3R1 and AKT2, AURKA	KRAS, BRAF, CITNNB1, CDKN2A, APC, PIK3CA, KIT, SMAD4	IGF2, SAT2	
Deletion		BRCA1, BRCA2, PTEN, TP53	MUC2, PEG3, MLH1, ICAM1, PLAGL1, ARH1	BRCA1, BRCA2, PTEN, TP53, PEG3, PLAGL1, ARHI

this case, similar protein masses. These studies demonstrate that "classical" proteomics can generate molecular fingerprints of a disease. However, the disadvantage of this method is the loss of molecular discrimination of tissues subtypes regarding the anatomical context. This effect can be seen in heterogeneous carcinomas where different structural elements express specific proteome. Laser capture micro-dissection (LCM) is one of the techniques used to solve this problem/inconvenient, but this method is time consuming and based on cells phenotypes rather than the molecular content of the cells. MALDI mass spectrometry imaging (MSI) keeps the spatial localization of the markers and discriminates cells to theirs molecular content. Using this technology, our group has been able to discriminate several novel biomarkers in ovarian cancer (Fig. 3 [62,63]) e.g., the C-terminal fragment of the immunoproteasome 11S, Reg alpha or mucin 9. In 2010, Hoffmann's team was able to identify 67 proteins, using this technology on formalin-fixed paraffin-embedded (FFPE) archived ovarian tissue [64,65]. In sum, proteomic studies performed at the level of the serum, ascites, and tissues have also clearly shown the presence of protein associated the intracellular signaling pathways activation, i.e., protein associated with cell proliferation, signaling to skeleton, invasion, resistance (Table 2).

3.1 Proteins associated with cell proliferation

The protein family S100 has been previously detected in aggressive ovarian tumors [66] and more specifically, S100 A11 and S100 A12 proteins have been identified by MALDI-MSI [67]. S100 A11 has been detected in ovarian ascites [68] and this protein (or calgizzarin) is known to regulate cell growth through the inhibition of DNA synthesis [69,70]. S100 A12 is known to promote leukocyte migration in chronic inflammatory responses [71]. The expression of oviduct-specific glycoprotein (OGP, Mucin-9), a marker of normal oviductal epithelium has also been reported in conjunction with S100 proteins and cytoskeleton modifying proteins [67]. Supportive data were provided by Woo and associates who found that OGP is a tubal differentiation marker and may indicate early events in ovarian carcinogenesis. These data also support the hypothesis, recently reported, of oviduct ascini as the origin of serous ovarian carcinoma [1]. From immune components, stromal cell-derived factor-1 (SDF-1), induces proliferation in OVC by increasing the phosphorylation and activation of extracellular signal-regulated kinases (ERK)1/2, which correlates to epidermal growth factor (EGF) receptor transactivation. Similarly, TGF-β produced by Treg cells stimulates tumor cell proliferation, increases matrix metalloproteinase's (MMP) production, and enhances invasiveness of OVC cells [72–76]. In OVC, IL7 has been found in ascites and plasma, and it's acting as a growth factor like in breast cancer [77–79].

Table 2 Biomarkers identified by genomic, classical proteomic, or MSI approaches

Marker name	Genomic	Proteomic	MALDI imaging
Mesothelin-MUC16	[192]		
STAT3	[192]		
LPAAT-β (lysophosphatidic acid acetyl transferase beta)	[193]		
Inhibin	[194]		
Kallikrein Family [9,11,13,14]	[195]		[67]
Tu M2-PK	[196]		
c-MET	[197–199]		
MMP-2, MMP-9, MT1-MPP: matrix metalloproteinase	[158,200,201]		[67]
EphA2	[202–204]		
PDEF (prostate-derived Ets factor)	[116,205]		
IL-13	[177]		
MIF (macrophage inhibiting factor)	[116,205]		
NGAL (neutrophil gelatinase-associated lipocalin)	[206]		[67]
CD46	[207–209]		
RCAS 1 (Receptor-binding cancer antigen expressed on SiSo cells)	[117,210]		
Annexin 3	[211]		
Destrin	[212,213]		
Cofilin-1		[214]	
GSTO1-1		[212,213]	
IDHc		[212,213]	
FK506 binding protein		[215]	
Leptin		[216,217]	
Osteopontin		[211]	
Insulin-like growth factor-II		[218]	
Prolactin		[219]	
78 kDa glucose-regulated Protein		[220]	
Calreticulin		[220]	
Endoplasmic reticulum protein ERp29		[220]	
Endoplasmin		[220]	
Protein disulfideisomerase A3		[220]	
Actin, cytoplasmic 1		[220]	
Actin, cytoplasmic 2		[220]	
Macrophage capping protein		[220]	
Tropomyosin alpha 3 chain, alpha-4 chain		[220]	
Vimentin		[220]	[67]
Collagen alpha		[220]	

Table 2 (continued)

	Genomic	Proteomic	MALDI imaging
1(VI) chain			
Dihydrolipoyllysineresidue		[220]	
succinyltransferase			
component of 2-oxoglutarate			
Dehydrogenase			
Pyruvate		[220]	
dehydrogenase E1			
component beta			
Superoxide		[220]	
dismutase [Cu-Zn]			
Chromobox protein		[220]	
homologue 5			
Lamin B1, B2		[220]	
14-3-3 protein		[220]	
Cathepsin B		[220]	
Heterogeneous		[220]	
nuclear			
ribonucleoprotein K			
Nucleophosmin		[220]	
Peroxiredoxin 2		[220]	
Prohibitin		[220]	
Receptor		[220]	
tyrosine-protein			
kinase erbB-3			
Fibrinogen gamma		[220]	
Chain			
Splicing factor, arginine/ serine-rich 5		[220]	
Elongation factor		[220]	
1-beta			
Lysosomal protective protein		[220]	
Hemoglobin beta		[220]	
subunit			
Transitional endoplasmic		[220]	
reticulum ATPase			
Serum albumin		[220]	
Protein KIAA0586		[220]	
Similar to testis expressed sequence 13A		[220]	
SNRPF protein		[220]	
Fibrinogen gamma chain		[220]	
Transitional endoplasmic reticulum ATPase		[220]	
Heat shock 70 kDa protein 1, 60 K protein		[220]	
Heterogeneous nuclear ribonucleoprotein K		[220]	
Keratin, type I cytoskeletal 7, 9, 18, 19 ?		[220]	[67]
Adenylosuccinate Lyase		[220]	

Table 2 (continued)

	Genomic	Proteomic	MALDI imaging
Peroxiredoxin 2		[220]	
Glutathione S-transferase P		[220]	
Ras-related protein		[220]	
Rab-7			
Prohibitin		[220]	
Cathepsin B		[220]	
Heterogeneous nuclear		[220]	
ribonucleoprotein K			
Tumor protein D54		[220]	
Rho GDPdissociation		[220]	
inhibitor 1			
Annexin A2		[220]	
ATP synthase beta Chain		[220]	
Heterogeneous nuclear ribonucleoprotein K		[220]	
Actin, cytoplasmic 1		[220]	
Heterogeneous nuclear ribonucleoprotein A/B		[220]	
Immunoprotease activator fragment 11 S			[62]
Mucin-9			[67]
Tetranectic			[67]
Urokinase plasminogen activator			[67]
Orosomucoid			[67]
S100-A2			[67]
S100-A11			[67]
Apolipoprotein A1			[67]
Transgelin			[67]
Prolargin			[67]
Lumican Precursor			[67]
Siderophilin			[67]
Alpha 1 antiprotease			[67]
Phosphatidyl Ethanolamine Binding Protein			[67]
Hemopexin			[67]
Profilin -1			[67]
HLA G			[1]
Chorionic Gonadotropin Hormone			[1]

pDcs are also present in tumor environment and stimulate tumor growth by releasing TNF-α and IL8. The sum of these data reflects that cytokines exert pleiotropic effects in OVC and exert a major role in tumor proliferation.

3.2 Signaling to the cytoskeleton

Several candidate proteins, including profilin-1, cofilin-1, vimentin, and cytokeratin 19 are involved in the intracellular

signaling to the cytoskeleton. Changes in cell phenotypes, such as the conversion of epithelial cells to mesenchymal cells, are integral, not only to embryonic development, but also to cancer invasion and metastasis. Cells undergoing epithelial–mesenchymal transition (EMT) lose their epithelial morphology, reorganize their cytoskeleton, and acquire a motile phenotype through the up- and downregulation of several molecules, including tight and adherent junction proteins and mesenchymal markers. TGF-β has been described to induce EMT in ovarian adenosarcoma cells [80]. In the human lung adenocarcinoma cell line A549, this differentiation is followed by modification in the expression of several cytoskeleton proteins including β-actin, cofilin 1, moesin, filamin A and B, heat-shock protein beta-1, transgelin-2, S100 A11, and calpactin. Most likely, these changes increase migratory and invasive abilities [81]. Treatment of the OVC cell line SKOV-3 with TGF-β increases the expression of cofilin and profilin-1 at mRNA and protein levels and modifies its cytoskeletal organization assessed by confocal microscopy analysis [76]. After binding to its receptor, TGF-β stimulates the reorganization of the actin cytoskeleton and triggers the formation of stress fibers and cellular protrusions [82].

3.3 Hormonal pathways

Several hormones have been detected during proteomic studies like leptin, prolactin, osteopontin, insulin-like growth factor-II, anti-mullerian hormone, and HCG [83]. Leptin is produced by adipocytes and known to stimulate the growth of BG-1 ovarian cancer cells via the extracellular signal-regulated kinase signaling pathway. Recent studies have demonstrated the involvement of the estrogen receptor (ER) pathway in the mechanism of leptin-induced OVC growth via STAT-3 [84]. Elevated seric levels of prolactin (PRL) in ovarian and endometrial cancers have been reported, indicating a potential role for PRL in carcinogenesis [85]. Binding of PRL to its receptor was followed by rapid phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, mitogen-activated protein kinase/ERK kinase 1, signal transducer and activator of transcription 3, CREB, ATF-2, and p53 and activation of 37 transcription factors in ovarian and endometrial carcinoma cells. Osteopontin (OPN) is known to increase the survival of OVC under stress conditions *in vitro* and promotes the late progression of ovarian cancer *in vivo*. The survival-promoting functions of OPN are mediated through Akt activation and the induction of HIF-1alpha expression [86]. Insulin-like growth factor binding protein 2 (IGFBP2) is also overexpressed in ovarian malignant tissues and in the serum and cystic fluid of ovarian cancer patients, suggesting its important role in the biology of ovarian cancer by increasing invasion capability of ovarian cancer cells [87]. HCG is a glycoprotein

consisting of subunits alpha and beta, which are non convalently linked. The hormone is normally produced by the syncytiotrophoblastic cells of the placenta and is elevated during pregnancy. Recently, beta-hCG and alpha-fetoprotein have been detected in OVC germ cell tumors [88,89]. These proteins have been detected at early stages of the cancer, as well as lysophosphatidic acid, mesothelin, HE4, osteopontin, VEGF, IL-8, M-CSF, different kallikreins [90–92], and the C-terminal fragment of the 11S immunoproteasome (Reg-alpha) [1,67].

Most of the above proteins are directly linked to signaling pathways for cytoskeleton remodeling, contractility, junction remodeling, adhesion, invasion, migration, cell cycle progression, proliferation, metastasis, apoptosis/necrosis, angiogenesis, endothelial permeability, vascular remodeling, metalloprotease activation, and EGFR transactivation (Fig. 1). The immune response modulation is another dysregulated cell signaling pathway found in OVC. In fact, cancer cells use various strategies to escape from the immune response. In this context, it is interesting to link activated intracellular signaling pathways to the proteins involved in immune response escape.

3.4 Proteins involved in immune response modulation

Several 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) have been detected in OVC. However, recent studies have shown that ovarian cancer-associated ascites may provide an immunosuppressive environment [77]. A high CD4/CD8 ratio, which may indicate the presence of regulatory T cells, is associated with poor outcomes. Recently, Clarke et al. [93] have validated in a cohort of 500 ovarian cancer patients that the presence of intraepithelial CD8+ T cells correlates with improved clinical outcomes for all stages of the diseases. Curiel et al. [94] demonstrated in 104 ovarian cancer patients that CD4+CD25+FoxP3+Tregs suppress tumor-specific T cell immunity and contributes to growth of the tumor *in vivo*. These data suggest a mechanism of immune suppression in ovarian cancer either through overexpression of Tregs or by the capability of tumors to escape from the immune response (using molecular mimicry) or immunosurveillance [95,96]. Additional evidences have reinforced the involvement of Tregs in ovarian cancer. CCL22, a protein secreted by dendritic cells and macrophages, highly expressed in tumor ascites is known to have a role in Treg cell migration in tumors [94]. Overexpression of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) has also been demonstrated in ovarian cancer [97–100]. IDO suppresses

Fig. 1 Ovarian cancer intracellular signaling pathways scheme. LPA/hormones bind to RPCG receptors. Cytokines/growth factors bind RTK receptors. Intracellular pathways and signaling out are detailed. Cellular output after signaling pathways activation are presented
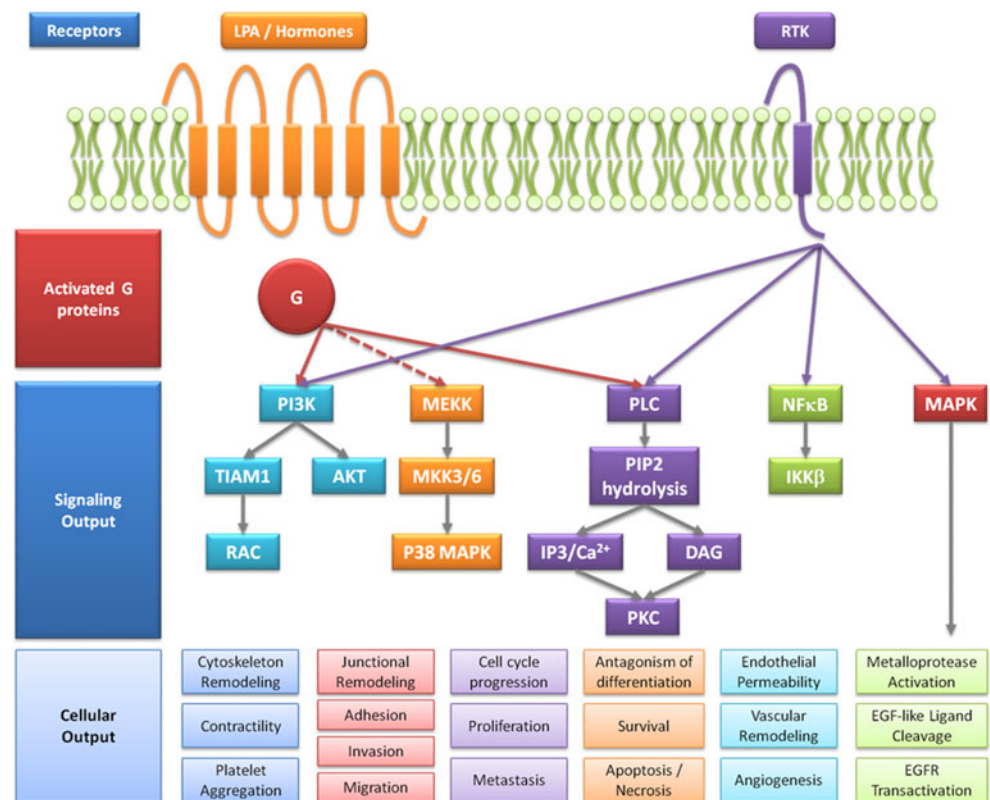
the proliferation of effector T cells or natural killer cells and their killer functions [98,101]. In ovarian cancer, high IDO expression in tumor cells was correlated with a reduced number of tumor-infiltrating lymphocytes [97]. Reduced IL-2 combined to elevated TGF-β and IL-10 levels favor induced Tregs [102]. On the other hand, tumor cells escape the immune response by inducing peripheral mature DCs to induce IL-10 CCR7+CD45RO+CD8+Tregs. Primary suppressive CCR7+CD45RO+CD8+ T cells are found in the tumor environment of patients with ovarian cancer [103]. Another way that tumor cells escape immunosurveillance is through the expression of human leukocyte antigen (HLA-G) ([104–106]{Sheu, 2007 #5782). Recent studies have shown that the expression of HLA-G was detected in 22/33 (66.7 %) primary tumor tissues but was absent in normal ovarian tissues ($P<0.01$). Cytotoxicity studies showed that HLA-G expression dramatically inhibits cell lyses by NK-92 cells ($P<0.01$), which could be restored by the anti-HLA-G conformational mAb 87 G ($P<0.01$). HLGA-G5 type has been detected in tumor while soluble form of HLA-G was found in ascites [107,108] and in the blood of patients [109]. HLA-G seems to be implicated in the immune response modulation through NKT cell inhibition [110]. In the tumor cells expressing a B7 co-stimulatory family molecule, B7H4 is known to inhibit antigen-dependent induction of T cell proliferation and activation. B7-H4 promotes the malignant transformation of epithelial cells by protecting them from apoptosis and seems to be expressed at an early stage of tumor

development [111–113]. In the same way, tumor cells highly express the mesothelin-Mucin 16 (MUC16), which inhibits the formation of immune synapses between NK cells and ovarian tumor targets [114] (Fig. 2).

Transcriptomic and proteomic studies performed at the tumor level confirmed the active role of the tumor cells to escape from the immune response. For example, the over-expression of the macrophage migration inhibitory factor (MIF) [115,116] and the receptor-binding cancer antigen expressed on SiSo cells [117] (known to be implicated in lymphocytes apoptosis), were shown using transcriptomic approaches MIF contributes to the inhibition of antitumoral CD8+ T and NK cells by downregulating NKG2D levels (NK cell receptor NK group 2D). [118]. From MALDI-MSI studies, five factors involved in immune response modulation in mucinous tumors have been identified, namely a C-terminal fragment of the 11S immunoproteasome (Reg-alpha), orosomucoid, apolipoprotein A1, hemopexin, and lumican, which have also been detected in ascites [62,63,67,119,120]. Cleavage of PSME1 (proteasome activator complex subunit 1, 11S regulator complex [syn: PA28 alpha]) into the Reg-alpha fragment could lead to default self-antigen presentation [62] (Fig. 3). PA28 is a regulatory complex associated with 20S proteasome that consists of three subunits: alpha, beta, and gamma [121]. Binding of the 11S regulator complex to the 20S proteasome does not depend on ATP hydrolysis and, unlike the 19S regulatory subunit, the 11S regulator complex does not catalyze degradation of large proteins. Rather, it is
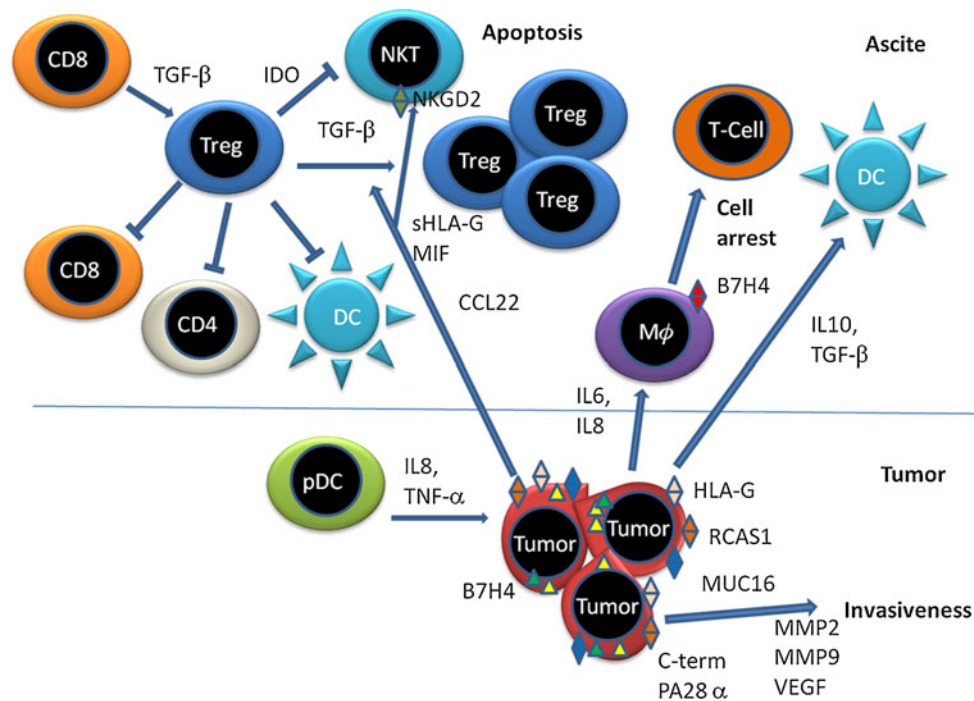
Fig. 2 Tumor cell factors production for escaping immune response. Apolipoprotein A1 has been detected in conjunction with transthyretin and transferrin in early-stage mucinous tumors. Lumican, which is a small LRR proteoglycan in the extracellular matrix is known to be overexpressed in breast cancer and to play a role in tumor progression. ApoA-I is known to decrease expression of surface molecules such as CD1a, CD80, CD86, and HLA-DR in dendritic cells, and it stimulates the production of IL-10. Hemopexin has recently been demonstrated to reduce TNF and IL-6 from macrophages during inflammation, and it limits TLR4 and TLR2 agonist-induced macrophage cytokine production. Orosomucoid have immunosuppressive properties in ovarian carcinoma ascites through inhibition of IL-2 secretion by lymphocytes. The tumor environment expresses molecules that can convert functional APCs into dysfunctional ones. These dysfunctional APCs in turn stimulate Treg differentiation and expansion.The tumor produces IL6, IL8, MUC18, MIF, RCAS1, sHLA-G exerting negative effects on the T cells.PA28 activator belongs to the antigen processing machinery (APM). Its alteration by cleavage by (furin, PACE4) in ovarian carcinomas participates in a mechanism to evade immune recognition

responsible for MHC-class 1 antigen processing [122–124], which is greatly improved by interferon gamma-induced expression of the alpha and beta subunits [125]. Several viral proteins that interact with these proteasome subunits have been reported and may interfere with host anti-viral defenses, thereby contributing to cell transformation [126]. The manner in which they bind to the core particle (via its subunits C-terminal tails) and induce a α-ring conformational change (to open the 20S gate), suggests a mechanism similar to the 19S particle [121]. No role in ovarian cancer has been demonstrated for the 11S regulator complex. Our data demonstrate a high expression level of PA28 in carcinomas, especially in epithelial cells at stage III/IV (Fig. 3a,b), also at early stages Ia (Fig. 3c) [1] and as a marker of tumor relapse after chemotherapy (Fig. 3d). The PA28 activator belongs to the antigen processing machinery (APM). Its alteration by cleavage in ovarian carcinomas may be a mechanism to evade immune recognition. Similar hypothesis has already been proposed in the case of APM chaperones such as TAP, LMP2, LMP10, and tapasin in colon carcinoma, small cell lung carcinoma, and pancreatic carcinoma cell lines. In fact, IFN-γ treatment of these carcinoma cell lines corrects the TAP, LMP, and tapasin deficiencies and enhances PA28α,

LMP7, calnexin and calreticulin expression, which is accompanied by increased levels of MHC class 1 antigens [127]. PSEM2 (proteasome activator complex subunit 2, PA28 beta) has also been detected in ascites fluid, implicating an immune cell tolerance toward carcinoma cells and confirms the dysregulation of self-antigen processing in ovarian tumors [68]. Additionally, PA28 alpha seems to be a target for Epstein-Barr virus (EBV) and herpes virus (HV), as our proteomic and qPCR data indicates [128,129]. Pudney et al. [130] have also shown that as EBV-infected cells move through the lytic cycle, their susceptibility to EBV-specific CD8+ T cell recognition falls dramatically, concomitant with a reduction in transporter associated with antigen processing (TAP) function and surface human histocompatibility leukocyte antigen (HLA) class 1 expression. The implication of virus in the etiology of ovarian cancer is also sustained by the over-expression of furin enzyme [1,67], which is known to be implicated in glycoprotein B cleavage through a motif R-X-K/R-R in both EBV and HV [128,129].

Among the other four factors that might participate in the tolerance phenomenon by inhibiting immune activation, the acute phase protein orosomucoid (ORM, also known as
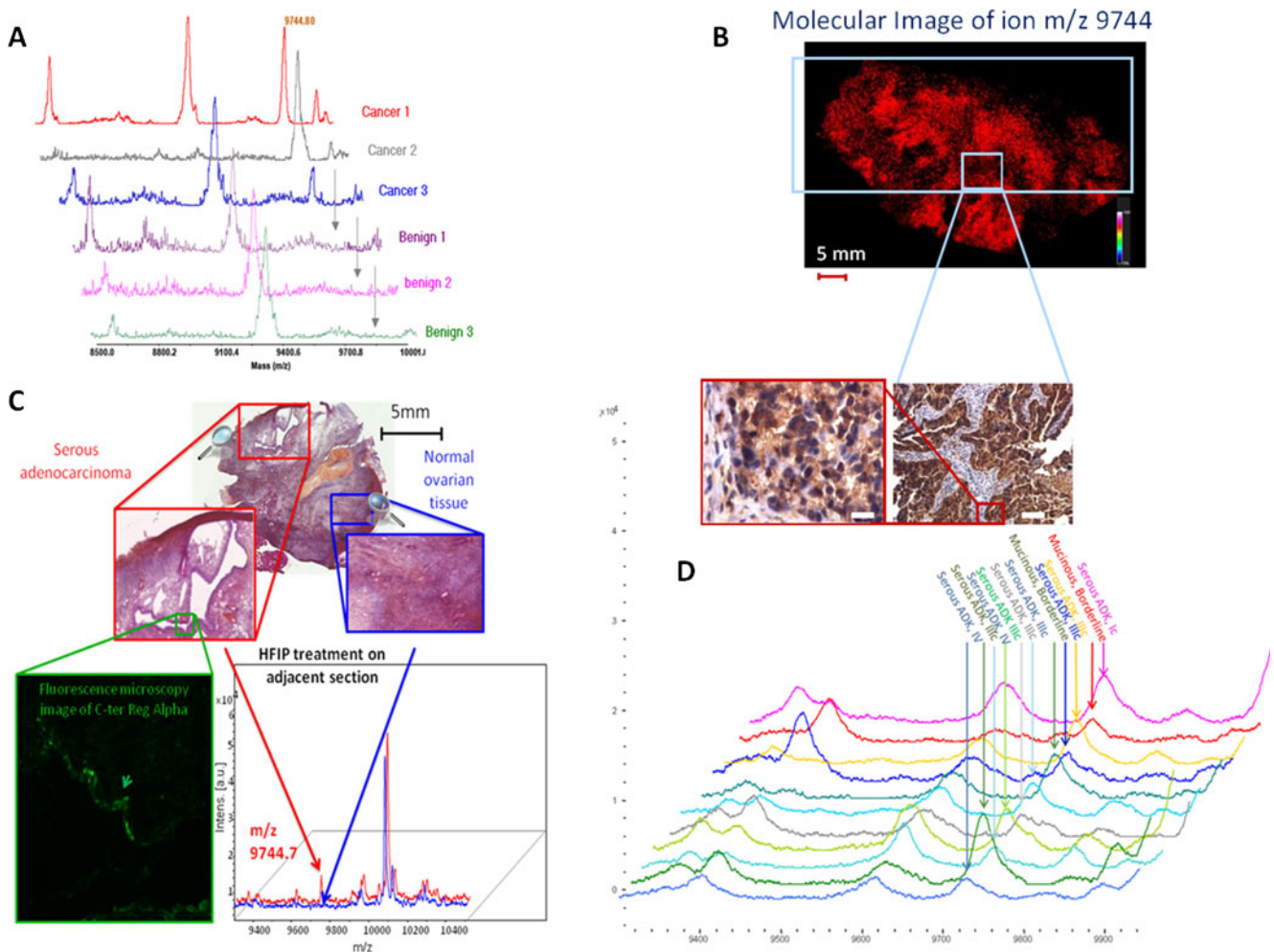
Fig. 3 Example of MALDI MSI and profiling workflow for ovarian cancer biomarker discovery and validation: **a** MALDI mass spectrometry profiling spectra of six patients, i.e., three suffering of adenocarcinoma and three benign tumors. The *m/z* at 9744 corresponds to the Cter fragment of RegAlpha. **b** Epithelial ovarian cancer tissue section with a benign and a cancerous parts submitted to an automatic matrix deposition using a micro spotting machine (CHIP 1000, Shimadzu). Molecular image corresponding of the *m/z* 9744 Cter fragment of RegAlpha in the tumoral area of the ovarian biopsy with *inset* pictures of immunohistocytochemical validation of the presence of the biomarker using an antibody designed against the Cter part of RegAlpha. **c** The MALDI MS spectra of tissue extract from stage 1a serous OVC biopsies. The C-terminal Reg-alpha fragment (*m/z* 9,744) is detected in the acini

cells. The *inset* shows fluorescent immunocytochemistry data using the anti-C terminal Reg alpha fragment and hematoxylin and eosin (H&E) staining of the tissue subjected to the MALDI MS profiling analyses (scale bar 5 mm) [191]. **d** The MALDI MS spectra of the tissue extract from the carcinoma regions of ten patients at different pathologic stages after neoadjuvant chemotherapy. Mass spectra analyses of extracts from stages I, IIIc, and IV serous tumors after neoadjuvant chemotherapy. The ion with an *m/z* ratio of 9774 corresponds to the C-terminal Reg-alpha fragment, is found in each patient and is indicated by an *arrow*. These data confirm that the C-terminal fragment of Reg alpha can be a good marker for the early diagnosis of tumor relapse (all tissues came from patients collected after neoadjuvant chemotherapy with 6 cycles of carboplatin/Taxol followed by carboplatin with Caelyx before complete surgery) [191]

alpha1-acid glycoprotein or AGP), which is normally increased in infection, inflammation and cancer, seems to have immunosuppressive properties in ovarian carcinoma ascites through inhibition of IL-2 secretion by lymphocytes [131]. Similarly, apolipoprotein A1 has been detected in conjunction with transthyretin and transferrin in early-stage mucinous tumors [132]. ApoA-I is known to decrease expression of surface molecules such as CD1a, CD80, CD86, and HLA-DR in dendritic cells and stimulates the production of IL-10 [133] (Fig. 2). Interestingly, hemopexin has recently been demonstrated to reduce TNF α and IL-6

from macrophages during inflammation and limits TLR4 and TLR2 agonist-induced macrophage cytokine production [134]. In SKOV-3 epithelial ovarian carcinoma cells, all TLRs are overexpressed with the exception of TLR9 and TLR10 [1]. This is in line with the overexpression of lumican, which is a small LRR proteoglycan in the extracellular matrix. Along with other proteoglycans, such as decorin, biglycan, and prolargin, lumican is known to be overexpressed in breast cancer and playing a role in tumor progression [135,136]. However, as demonstrated for biglycan, which interacts with TLR2/4 on macrophages [137,138], we

speculate that lumican is also involved in the activation of the inflammasome through TLR2/4 interaction. The activation of all danger-sensing receptors in carcinoma cells can be explained through the regulation of inflammation by carcinoma cells to facilitate tumor progression. In a sense, this implies that ovarian cancer cells use molecular mimicry as "parasites" to escape the immune response, as they produce immunosuppressors to achieve tolerance [139].

4 Specific mechanisms underlying ovarian cancer metastasis

The clinical and biological behavior of epithelial ovarian cancers differs from any other type of cancers. While the dissemination of cancerous cells in other carcinomatous afflictions requires the implication of vascular mechanisms of intra- and extravasation for the migration from the primary site to distant organs, ovarian carcinoma metastasis is a more passive event. Indeed, cells coming from the ovarian tumor often migrate to peritoneal organs with the physiological movement of peritoneal fluid but rarely create metastasis outside the peritoneum. This passive clinical mechanism is supported by some molecular changes in the cell elements for its anchoring to the remote metastatic sites. The surrounding cellular and tissular environment also promotes hosting of the cells outside the ovarian tumor. The first biological mechanism for cell migration is the epithelial–mesenchymal transition (EMT) which facilitates the attachment of neoplastic cells to new tissue sites [82] (Fig. 4). Due to the lack of an anatomical barrier, ovarian carcinoma can spread directly throughout the peritoneal cavity, mainly by intra-abdominal dissemination and by lymphatic dissemination, enabling in this way the attachment to peritoneum and omentum. This event is characterized by proteins' and genes' modifications like epithelial intermediate filaments, whose expression is typically reduced, and in the overexpression of vimentin and matrix metalloproteases (MMPs). It has characterized by alterations in the expression of cell-to-cell adherents junctions and cell-matrix adhesion molecules including integrins and E-N-cadherin E-cadherin [140,141] (Fig. 4). It has recently also be validated that this event is related to an elevation of the expression of the glycoprotein MUC 4 [142]. E-cadherins allow the junction between the filaments of actin inside the cell and its cellular environment. This deficiency allows the cells to initiate its detachment from the tumor site. MUC4 is also associated to an elevation of other cadherins [143] such as N-cadherins, but also other mesenchymal markers such as Vimentin [142], which are also expressed and confer the cells an invasive phenotype. EMT confers as well the ability of cells to evolve in unfavorable conditions such as hypoxia [144]. Once the cell detached from the tumor site, the peritoneal environment of ascites starts the

promotion of ovarian carcinoma cells proliferation and implantation in peritoneal sites. Growth factors play a critical role in motility and invasiveness of these cells where VEGF is one of the principal factors [145] (Fig. 4). It acts through EGFR, which has been proven to be overexpressed in 70 % of carcinomas [146]. This process activates the MAPK pathway, which in turn contributes to the lack of cell–cell junctions. VEGF also activates the PI3K pathways, which contributes to the localization of the matrix metalloproteinase 9 at the membrane surface for the cleavage of E cadherins [147,148]. Moreover, VEGF stimulates ascite accumulation by increasing diaphragmatic and tumor associated vasculature [145,149]. Then, cancer cells can migrate through the peritoneum as single cells or groups of multiple cells called spheroid composed of multiple cells group. These spheroids present at their surface $\alpha5\beta1$ integrin, which play a role in these spheroid formation [150] and its ligand fibronectin, important for spheroid growth. Other couples integrin/ligand exist such as $\alpha6\beta1$integrin/laminin and $\alpha2\beta1$-integrin/type IV collagen, which intervenes in the attachment of the spheroids with the mesothelium of the peritoneum and the omentum [151]. In the process of cellular invasion, proteolytic enzymes are mandatory in order to release the spheroids to the peritoneal environment. Matrix metalloproteinase type I and II play this role in the primary cancerization steps [152,153]. After the transit in the peritoneum, cancerous cells acceed and attach to the peritoneum, which is constituted by mesothelial cells with collagen types I and IV, fibronectin and laminin. Coupled integrins then act for the attachment. At this step, VCAM is also a membrane protein at the surface of mesothelial cells that binds $\alpha4\beta1$ integrin from the cancerous cells surface [154]. With the integrins, CD44 can also be an element of binding, which attachs to hyaluronic acid [155]. Additionally, adhesion molecules such as NCAM can promote ovarian cancer metastasis via the interaction with receptors such as FGFR [156]. Proteolytic activity is a requested mechanism for cells attachment since MMP-2, at the surface of cancer cells, cleaves fibronectin and vitronectin (extracellular matrix proteins) into smaller fragments to increase adhesion, respectively, with $\alpha5\beta1$ $\alpha v\beta3$ integrins [157,158]. Furthermore, host cells produce MMP9 to improve adhesion of cancerous cells [159]. Transglutaminases are another family of enzymes for cellular adhesion, in which transglutaminase 2 plays a major role in the relaxation of extracellular matrix and the regulation of MMP2 [160]. Enzymes of the kallikrein family have also been found to play a role in extracellular matrix degradation [161] (Fig. 4).

The last phases of metastasis progression consist in the final hosting of the cells groups in the peritoneal tissues. This mainly consists in the recruitment of new blood vessels within the host tissue in order to provide a complete nutritional autonomy to the new tumor site. VEGFs are then needed to stimulate vascular lymphatic endothelium in order
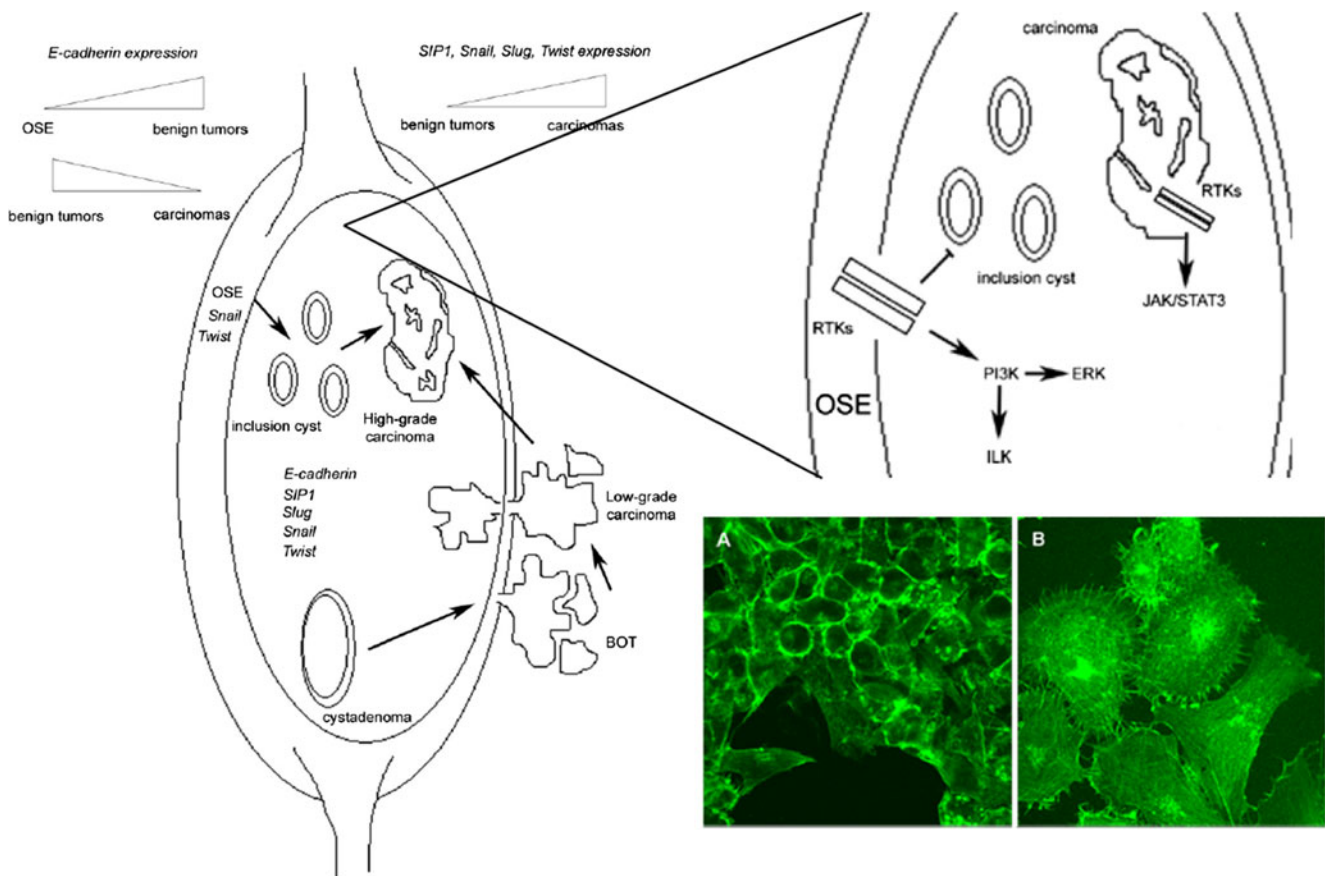
Fig. 4 Schematic illustration of E-cadherin, SIP1, Snail, Slug, and Twist during ovarian progression. A model has been proposed by Shih and Kurman in 2004 to explain ovarian cancer progression [83]. In this model, epithelial ovarian tumors have been classified into two broad categories: type I tumors including low-grade serous carcinomas, mucinous, endometrioid, and clear cells carcinomas seem to develop from their precursors, namely borderline ovarian tumors (BOTs), in a stepwise manner; type II including high-grade serous malignancies develop from the OSE or inclusion cysts without a common precursor.OSE cells covering the ovarian surface do not express E-cadherin but are positive for Snail and Twist expression. As depicted, E-cadherin expression changes during ovarian cancer progression showing an inverse correlation compared to SIP1, Snail, Slug and Twist expression. The zoom corresponds to A simplified overview of signalling network regulating EGF-induced EMT. In OSE cells, activation of the EGF receptor tyrosine kinases (RTKs) by EGF results in activation of the phosphatidylinositol 3-kinase (PI3K), which activates ILK and ERK pathways. EGF treated OSE cells display a molecular signature characteristic of EMT and are less likely to undergo a conversion in inclusion cysts. JAK/STAT3 pathway is required to induce EMT in ovarian cancer cells. Ovarian cancer cells that undergo EMT lose the expression of E-cadherin and NGAL and show an increased motility [82]). Pictures illustrate human epithelial ovarian cancer cell line SKOV3 treated with (**b**) or without (**a**) TGF-β (10 ng ml^{-1}). **a** Control cells have a typical epithelial-like morphology in culture flask/Petri with the tendency to form dense colonies. The filamentous actin cytoskeleton shows circumferential organization. **b** Protusive structures and actin stress fibers are clearly visible only in treated cells

to create new blood and lymphatic vessels. This process is the very last step of metastasis, that's why the expression of VEGF in ovarian carcinoma context is generally associated to poor prognosis [162]. Periostin has also been explored for its ability to require angiogenes, in addition to known effects on EMT process [163].

As mentioned above, the environmental context provided by the ascites is of great influence on properties of ovarian cancer cells. It has been established that chemokines such as CXCL12 induces migration, integrin expression, proliferation, and invasion [164]. CCL25–CCR9 interaction also contributes to ovarian cancer migration, metalloprotease expression, and invasion [165]. The hosting tissue is particularly important for metastasis efficiency since adipocytes of the omentum, which is a common site for ovarian tumor dissemination, can interact with the cells groups by adipokines such as IL8. IL8 can mediate homing, migration, and invasion of ovarian cancer cells. Besides, adipocytes can induce β-oxidation in cancer cells. Finally, the fatty-acid-binding protein 4 has been found to be overexpressed and plays a key role in ovarian cancer cells [166]. The cellular context in the peritoneum is also of high relevance. For example, cancer-associated fibroblasts (CAF) promote angiogenesis and lymphangiogenesis, tumor progression and metastasis by secreting different growth factors in the peritoneal environment [167].

Recent "omics" studies aimed to explore the complete molecular aspects of ovarian cancer metastasis.

In 2010, Xu team established an *in vivo* model of ovarian cancer metastasis by injecting the well-known SKOV-3 cell line in nude mice peritoneum [168]. Then, serum proteins of the mice were analyzed by LC-MS/MS. Thirteen human proteins were identified, including three proteins that were highly relevant biomarkers for ovarian cancer metastasis. These candidates were tested for their relevance in blood screening of biomarkers by ELISA assays for patients with different stages of cancer progression. 14-3-3 zeta was found to have 90 %, 82.5 %, 72 %, and 94.3 % sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), respectively, for stage I and stage II patients. 14-3-3 zeta is an adaptor protein that binds to other upstream or downstream signaling molecules containing tandem repeats of phosphoserine motifs [169,170] and has been found to form a ternary complex with integrin alpha-4 to accelerate cell migration [171]. This study highlights that metastasis of ovarian cancer is an early event since a protein involved in this behavior can be detected as a biomarker for early ovarian cancer. In another study, another member of the 14-3-3 family, 14-3-3 sigma, has been found to protect the cancer against oxidative stress by inducing an insensitiveness of the cells to high O_2 concentrations [172].

In 2011, Kakar team aimed for insights into the "metabolome" of ovarian cancer using GC/MS-TOF [173]. By this approach, they have been able to deduce the metabolic pathways involved in ovarian cancer metastasis behavior. This study revealed that ovarian cancer metastasis have altered carbohydrate metabolism. The ovarian cancer metastatic cells prefer to use glucose for anaerobic glycolysis instead of oxidative phosphorylation for the generation of ATP. This was revealed by an increased level of lactate. Fucose was also increased, coinciding with the elevated glycoproteins in ovarian cancer cells. Also, stimulation of proliferation has been related to the overconcentration of phenylactates. Antioxidants have also been found such as tocopherols and glutathione, concording with previous studies of protective behavior of ovarian metastatic cells against oxidative stress.

Recently, using MALDI mass spectrometry imaging coupled to on tissue microproteomics, we discovered in ovarian carcinoma proteins involved in metabolic processes particularly over-expressed like the pyruvate kinase isoenzyme M1/M2 (PKM2), the Vitamin D binding protein isoform 2 (DBP), the vitamin K epoxide reductase complex subunit 1 (VKORC1), the fatty acid-binding protein (FABP), the coactivator of PPAR-gamma-like proteins (Longuespée unpublished data). These results reinforced the close correlation between proteome and metabolome expression during ovarian cancer metastasis.

Clearly, the results prove that novel tools, combined with appropriate models could be used to understand the specific mechanisms required for ovarian cancer metastasis.

5 Relevant biomarkers for clinical diagnosis of ovarian cancer

Amongst the molecules discovered and integrated in the understanding of OVC process mechanisms, some have been evaluated for their clinical relevance. In this part of the review, we have summarized the most promising markers that could be used for OVC screening in women populations. To date, CA 125 is the most widely used marker for the disease screening. In normal physiological conditions, ovary surface epithelium does not express CA 125 [174]. The level of seric CA 125 is considered as normal when it is not higher than 35 U/mL [175]. Levels higher than 35 U/mL are found in 90 % of patients with advanced stage disease and in 50 % of stage I cancer patients [176]. CA 125 is exclusively used to monitor the effect of chemotherapies or targeted therapies on the disease. Thus, it is useless for the screening of large scale supervision of the healthiness of postmenopausal women.

The detection of molecular factors for ovarian cancer process was then undertaken in large cohorts of patients and found to provide relatively good results for ovarian cancer screening. Interleukin 13 (IL13) is a cytokine with an inflammatory activity that plays important roles in many biological activities. The level of this cytokine has been measured and found more elevated in cancerous tissues [177]. IL13 receptor is composed by two strands (ILA3Ra1 and IL13Ra2) and the second one has been found in high levels in 44 of 53 of ovarian cancer samples [178]. A cytotoxic therapy mediated by IL-13 have been designed and tested in phase I/II clinical trial. This therapy showed an antitumor activity and was very efficient when administered intraperitoneally, since it blocks the spread of ovarian cancer cells through the peritoneum in late stages of ovarian cancer [178]. The serum macrophage inhibitory factor MIF have also been tested for its presence in the blood of ovarian cancer patients and a sensitivity of 77.8 % and a specificity of 53.3 % were measured for this marker [116]. HE4 has also been identified as a potential discriminator of ovarian cancers [179]. This marker, tested in blinded studies on post menopausal patients, was found to have similar discriminating characteristics to CA 125. Many markers were found to be much more efficient for ovarian cancer discrimination, when used in conjunction of CA 125. Macrophage-colony stimulating factor (M-CSF) [180] has been used alone and detected ovarian cancer with a specificity of around 61–68 % and 93 % specificity [181], but the results for the detection were better when used in conjunction with CA 125 [182]. Indeed, the use of either CA 125 or M-CSF enabled the identification of 96–98 % of ovarian cancers and 81 % of early stages [180]. Another marker, the lysophosphatidic acid, has been reported to have a sensitivity of 100 % for high stages cancers and 90 % for stage I cancers[183]. Some

panels of biomarkers have also been proposed for high sensitivity/specificity tests of ovarian cancer screening. The use of the combination of 5 markers (CA 125, OVX1, LASA, CA 15-3, CA 72-4) showed sensitivity of 90.6 % and specificity of 93.2 %, when included in CART analysis (classification and regression tree analysis), which is a marker-based classification algorithm of the disease [184]. Four other markers (CA 125, CA72-4, CA 15-3, and PLA) were found to have a sensitivity from 68 % to 87 % and the same specificity as for CA 125 [185]. Thus, the use of panels of biomarkers seems to be mandatory for biomarker screening. This is particularly well illustrated by the study of Leiser and coll. who proposed the use of six markers (leptin, prolactin, osteopontin, IGFII, MIF, and CA 125) to discriminate ovarian cancer and benign tissues with an accuracy of 89 % for early stages cancers and 100 % for late stage disease. However, none of these markers used alone was able to discriminate properly diseased and unaffected samples [186]. Recently, association of some of the markers presented here, namely mesothelin, osteopontin, and HE4 have been selected by the Specialized Program of Research Excellence (SPORE) committee for their good sensitivity and specificity values [187].

6 Conclusion

This review attempts to link OVC genes polymorphisms to cell signaling pathways which play a central role in cancer cell growth, survival, invasion metastasis, and immune escape.

The integration of these OVC data, as a function of the grade and tumor type, with specific sets of markers is important in order to link the pathology with improved diagnosis and even for therapeutic benefits. The clinical relevance of these OVC molecular factors then need to be validated by large scale screening studies of patient samples, with an emphasis on attempting to screen in the early stages of the diseases. The development of proteomic approaches in the field of biomarkers research has greatly enhanced the discovery of relevant molecular markers for multiple diseases. In the case of OVC, these new markers could provide an alternative strategy to the actual CA 125 monitoring test.

In this review, we conclude that MALDI-MSI is one of the most promising proteomic methods for biomarker discovery, as it allows the direct analysis of tissue sections and large scale screening of markers in their anatomical context. The growing interest for MALDI-MSI in the proteomics community is due to the increasing ease of use, great accuracy, and statistical power, as it is now combined with adapted statistical tools. MALDI-MSI may soon become the primary approach for the biomarkers discovery. In this review, we also conclude as to the difficulty in correlating between predicting important genes in pathologies and biomarker detection in tissue and in serum. Nevertheless, the association multiplex panel of biomarkers has also been proposed to increase the sensitivity/specificity testing of ovarian cancer screening associated with CA125. Novel biomarkers identified by MALDI-MSI, i.e., the C-terminal fragment of Reg-alpha and Mucin 9 as well as specific viral signatures
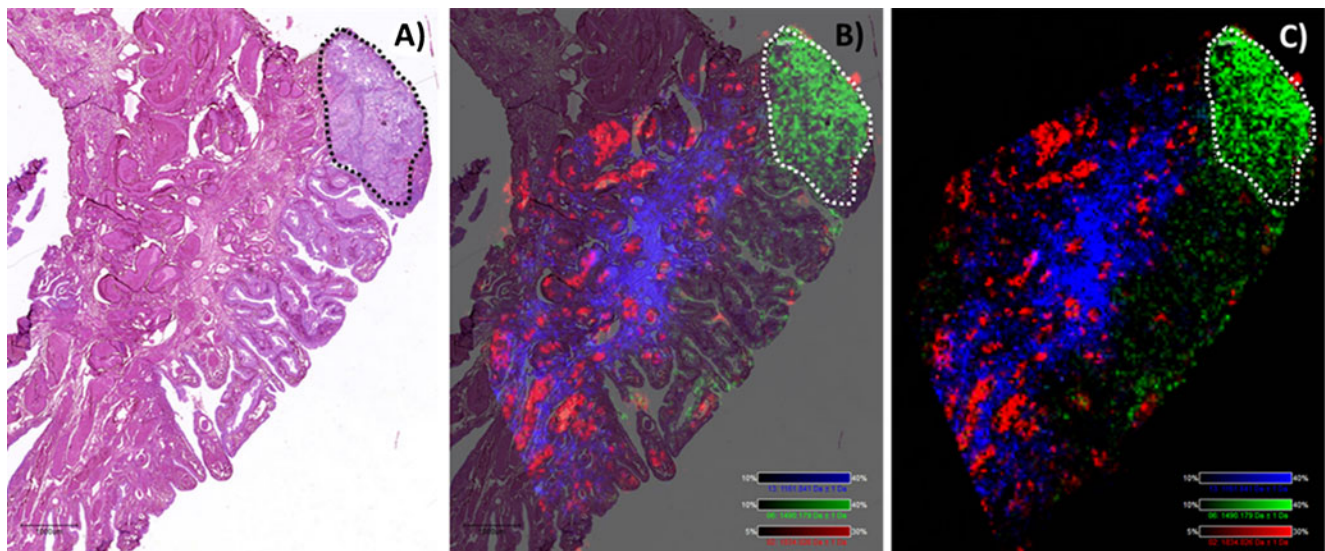
Fig. 5 Molecular localization of three different ions on a FFPE tissue section in the region Fimbria of the uterine tube. Histological section after hematoxylin eosin-safran (HES) coloration (**a**). Ions images at a spatial resolution of 50 μm with (**b**) or without (**c**) a superposition of the histological image. Three specific molecules were localized in the blood vessel (in *red*), in the mesothelium (in *blue*) and specific to the cancer region (in *green*)

(EBV, HHV6) may allow the assembly of more complete biomarker panels for early ovarian cancer diagnosis. In order to increase specificity, it will be necessary to take into account not only protein biomarkers linked to cell modifications but also the presence of specific viral proteins, the etiology of the pathology (e.g., the Mullerian cell origin of ovarian serous cancer) and their specific markers. Most ovarian carcinomas associated with deleterious mutations BRCA1/2 appear to derive from the tube, especially its fimbria. These cancers are almost always high-grade serous ovarian, tubal, or peritoneal. Given the ineffectiveness of current screening, bilateral salpingo-oophorectomy is the preferred prophylactic procedure. This procedure is not without consequences and in view of pathogenesis data reviewed, it may be possible to perform a temporary prophylaxis in the form of a bilateral radical fimbriectomie. Recent histopathological studies support the possibility of tumors forming at epithelial–mesothelial junctions (peritoneal mesothelioma between epithelium and Mullerian or between tubal epithelium and tubal and ovarian mesothelium lining). This hypothesis is in line with tumors cervix or the cardia which are also "pathologies junction" between coatings of different natures. The hypothesis of an abnormal spread of cells on the cortical tubal or ovarian inclusion cysts, and their development into carcinoma, is corroborated by very recent biomolecular studies. They confirm the expression by cancer cells of high-grade serous mullerian markers (like the duct) but not mesothelium (such as ovarian cortex) [188–190]. Hypothesis that can be drawn is the fact that high-grade serous carcinoma would be a secondary malignance of the ovary and not a primary ovarian tumor Type 1 lesions will find their origin in the carcinogenesis of inclusion cysts post-ovulation, which, during the healing of the breach ovarian tissue would then reflect a Mullerian origin [188–190]. The type of carcinoma depends on the origin of these Mullerian cells. It is therefore necessary to integrate these notions in ovarian cancer diagnosis and identify specific markers in relation with these Mullerian cells. MALDI-MSI (Fig. 5) can offer versatile and powerful methods to investigate these junctions, on specimens of prophylactic oophorectomy or in fimbriectomies with mutated BRCA 1/2, in order to detect early abnormalities and help explain tumor development while identifying early disease biomarkers.

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