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Novel biomarker candidates for the diagnosis of ovarian clear cell carcinoma (Review)

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Abstract. Ovarian clear cell carcinoma can arise from endometriosis; however, it is distinct from other types of epithelial ovarian carcinoma in terms of its clinicopathological and molecular features. Cancer antigen 125 lacks the sensitivity and specificity required for accurate clinical diagnosis of clear cell carcinoma. Therefore, the aim of the current review was to identify novel biomarker candidates for the immunohistochemical and serological diagnosis of clear cell carcinoma. A search of the relevant English language literature published between 1966 and 2014 was conducted using the PubMed MEDLINE online database. High-throughput tissue microarray technology and proteomic screening combined with mass spectrometry may provide additional information regarding diagnostic biomarker candidates for ovarian clear cell carcinoma. The present review summarizes the characteristics of potential genomic alterations that activate cancer signaling pathways and, thus, contribute to carcinogenesis. The major signaling pathways activated in clear cell carcinoma are associated with cell cycle regulation (hepatitis A virus cellular receptor 1 and tumor protein D52), growth factor signaling (insulin-like growth factor binding protein 1; KiSS-1 metastasis-suppressor; erb-b2 receptor tyrosine kinase 2; and fibroblast growth factor receptor 2), anti-apoptosis and survival pathways [sialidase 3 (membrane sialidase)], metabolism (γ-glutamyltransferase 1), chemoresistance (napsin A aspartic peptidase, glutathione peroxidase 3; and aldehyde dehydrogenase 1 family, member A1), coagulation [coagulation factor III (thromboplastin, tissue factor); and tissue factor pathway inhibitor 2], signaling (lectin, galactoside-binding and soluble, 3), and adhesion and the extracellular matrix [cadherin 1, type 1, E-cadherin (epithelial); versican; and laminin, α 5]. The present review of the relevant literature may provide a basis for additional clinical investigation of the ovarian clear cell carcinoma serum biomarker candidate proteins identified herein.

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Contents

1. Introduction

Epithelial ovarian carcinoma is commonly diagnosed at an advanced stage due to a lack of effective serum markers. For example, the conventional serum cancer antigen 125 (CA125) is a poor marker for early diagnosis (1). CA125 is elevated in the serum of >80% of patients with high-grade serous adenocarcinoma, but false-negative results for CA125 frequently occur in cases of ovarian clear cell carcinoma (2). However, CA125 is the only currently used diagnostic marker for ovarian clear cell carcinoma. Ovarian clear cell carcinoma occurs more frequently in Japan than in Western countries (3). It is considered to arise from endometriosis (4). Compared with other histological types, clear cell carcinoma has been recognized to display a chemoresistant phenotype, which leads to poorer prognosis (3); its response rate to paclitaxel plus carboplatin therapy is lower compared with other histological types, ranging from 22-56%. Therefore, the identification and development of more accurate clear cell carcinoma diagnostic markers is required.

Epithelial ovarian carcinoma is a heterogeneous disease with various histological subtypes, including serous, mucinous, endometrioid and clear cell. Each histological subtype is a classified as a separate disease with characteristic cytogenetic features, molecular signatures, oncogenic signaling pathways and clinicobiological behaviors (5). Epithelial ovarian carcinomas are classified into two major subtypes, types I and II, based on their distinctive epigenetic and gene expression profiles, in addition to their functional genomic mutations. Type I carcinomas include low-grade serous, mucinous, endometrioid and clear cell subtypes. They evolve from precursor lesions (typically borderline tumors) in a slow, step-wise process, with prolonged early-stage disease and a more favorable prognosis than type II high-grade tumors (5).

Type I carcinomas frequently exhibit mutations in the following genes: AT-rich interactive domain 1A (SWI-like) (ARID1A); phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA); protein phosphatase 2 regulatory subunit A-α; B-Raf proto-oncogene, serine/threonine kinase; Kirsten rat sarcoma viral oncogene homolog; phosphatase and tensin homolog; and catenin (cadherin-associated protein), β1, 88 kDa. In addition, type I epithelial ovarian carcinomas lack mutations in tumor protein p53 (TP53). By contrast, type II carcinomas originate *de novo* from the adnexal epithelia with no identifiable precursor lesion, develop rapidly and are of an aggressive nature. Type II tumors include high-grade serous, endometrioid, mixed and undifferentiated carcinomas (5). Mutations in TP53 are common in type II carcinomas and this distinction may serve as a marker of malignant growth (6).

There is a requirement for the development of novel serum markers for clear cell carcinoma to improve its early diagnosis, therapeutic stratification and prognosis, as well as for monitoring patients that have undergone treatment. The proteomic and genome-wide search techniques available for exploring sensitive and specific biomarkers may facilitate the early diagnosis of clear cell carcinoma in the future. The current review presents novel biomarker candidates obtained from a diverse cohort of patients diagnosed with ovarian clear cell carcinoma, and proposes that specific, overexpressed tumor self-antigens may be used as diagnostic serum marker candidates.

2. Search strategy and selection criteria

The current study aimed to identify markers for distinguishing clear cell carcinoma from high-grade serous adenocarcinoma. A literature review was conducted to investigate the molecular phenotype of ovarian clear cell carcinoma-specific genes. A PubMed MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed) search of the relevant literature published between 2005 and 2014 was performed using the following key words: 'Epithelial ovarian cancer', 'clear cell carcinoma', 'gene expression profiling', 'proteome' and 'marker'. English-language publication search results from PubMed and references within the relevant articles were analyzed. Furthermore, references within the references were searched to identify additional relevant studies.

A list of genes and their transcripts significantly differentially expressed in patients with clear cell carcinoma versus high-grade serous adenocarcinoma was created. Genes and their encoded proteins identified in two or more of the primary studies are listed on Table I. To minimize selection bias, screening of the studies was independently performed by two of the co-authors (HS and SO) after agreeing on the selection criteria.

A total of 2087 articles were identified by the search; around 350 articles were potentially relevant. Only 91 publications available for distinguishing clear cell carcinoma from high-grade serous adenocarcinoma were chosen based on the final selection, taking into account the title and the summary analysis. Others were excluded due to various reasons, including selection bias, detection bias, reporting bias and other possible sources of bias.

3. Overexpression of genes and their transcripts in ovarian clear cell carcinoma

Gene expression profiling and proteomic analysis have been used in previous studies to evaluate levels of gene and protein expression, respectively, in the following two subtypes of epithelial ovarian carcinoma: High-grade serous carcinoma and clear cell carcinoma. These have been characterized by gene and protein expression patterns of specific tissue markers, allowing objective detection of each disease. Furthermore, it was identified that atypical endometriosis and clear cell carcinoma share molecular alterations, such as inactivating mutations for ARID1A, activating mutations for PIK3CA and the hypomethylation of HNF1 homeobox B (HNF1B) (4). The HNF1B protein binds to DNA as a member of the homeodomain-containing superfamily of transcription factors. This transcription factor plays a role in endometrial regeneration, differentiation, decidualization, glycogen synthesis, detoxification, cell cycle regulation, implantation, uterine receptivity and a successful pregnancy (7). Furthermore, HNF1B regulates a subset of progesterone target genes and may act as a modulator of female reproduction (7). The immunohistochemical study revealed that the positive HNF1B staining was a frequent finding in clear cell carcinoma. Ovarian clear cell carcinomas exhibit increased activity of signaling pathways involved in cell cycle regulation, survival, anti-apoptosis, chemoresistance, metabolism, coagulation and angiogenesis (7). Table I includes possible clear cell carcinoma-specific biomarkers identified using the aforementioned search strategy (Table I).

Cell cycle regulation. Clear cell carcinoma exhibits a unique pattern of expression of cell cycle regulatory molecules among epithelial ovarian carcinomas (8). HNF1B induces cell cycle arrest and stimulates an anti-apoptotic response to oxidative stress-induced DNA damage (9). Therefore, elucidating the detailed association between HNF1B upregulation and the acquisition of cell cycle regulation under conditions of oxidative stress may improve understanding of the mechanisms used to repair DNA damage (7). A previous study identified the role of the HNF1B-mediated signaling pathway in a fail-safe mechanism for the oxidative stress-induced DNA damage response (10). The present study identified six genes, F-Box protein 5 (11,12), Cdk5 and Abl enzyme substrate 1 (13), hepatitis A virus cellular receptor 1 (HAVCR1) (14), cyclin E1 (15), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, θ (16) and TPD52 (17,18), associated with regulation of the cell cycle regulation that are abnormally expressed in ovarian clear cell carcinoma. Among them, HAVCR1 (14) and TPD52 (17,18) have been proposed as serum marker candidates in ovarian clear cell carcinoma.

Cell proliferation and survival. The growth factor receptor axis is frequently dysregulated in a wide variety of human cancer types via gene amplification, mutation, protein overexpression and aberrant activation. The overexpression of growth factor receptors has been associated with potentially aggressive tumors and correlated with poor prognosis. Microarray analysis and proteome studies identified a number of candidate marker genes for cell proliferation and survival in ovarian clear cell carcinoma, including PIK3CA (6,19,20),

Table I. Genes recognized as ovarian clear cell carcinoma-specific biomarkers.

Symbol	Full gene name	Proposed as a serum marker candidate	References
Cell cycle regulation			
FBXO5	F-box protein 5		10-12
CABLES1	Cdk5 and Abl enzyme substrate 1		8,13
HAVCR1	Hepatitis A virus cellular receptor 1	Yes	14
CCNE1	Cyclin E1		15
YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, θ		16
TPD5	Tumor protein D52	Yes	17,18
Cell proliferation and survival			
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α		4,19,20
MET	MET proto-oncogene, receptor tyrosine kinase		21
IGFBP1	Insulin-like growth factor binding protein 1	Yes	22,23
IGF2BP3	Insulin-like growth factor 2 mRNA binding protein 3		24
PDGFRA	Platelet-derived growth factor receptor, α polypeptide		25,26
KISS1	KiSS-1 metastasis-suppressor	Yes	27,28
KISS1R	KISS1 receptor		27,28
ERBB2	Erb-b2 receptor tyrosine kinase 2	Yes	29
FGFR2	Fibroblast growth factor receptor 2	Yes	30,31
ARHGDIA	Rho GDP dissociation inhibitor (GDI) α		32,33
PAX8	Paired box 8		34
Anti-apoptosis			
HNF1B	HNF 1 homeobox B		4,35,36
PPM1D	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1D		37
APPBP2	Amyloid β precursor protein (cytoplasmic tail) binding protein 2		38
BCL2	B-cell CLL/lymphoma 2		39
NEU3	Sialidase 3 (membrane sialidase)	Yes	40,41
Metabolism			
HNF1B	HNF 1 homeobox B		43,44
GGT1	γ-glutamyltransferase 1	Yes	45
VHL	von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase		40,46
MUC1	Mucin 1, cell surface associated		47
MAP1LC3A	Microtubule-associated protein 1 light chain 3 α		42
HOXA10	Homeobox A10		48
Chemoresistance			
XIAP	X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase		49
NAPSA	Napsin A aspartic peptidase	Yes	50
PPP1R13L	Protein phosphatase 1, regulatory subunit 13 like		51
GPC3	Glypican 3		52,53
ABCF2	ATP-binding cassette, sub-family F (GCN20), member 2		54
GPX3	Glutathione peroxidase 3	Yes	55
ANXA4	Annexin A4		56
ALDH1A1	Aldehyde dehydrogenase 1 family, member A1	Yes	57
Coagulation			
TFPI2	Tissue factor pathway inhibitor 2	Yes	59,60
F3	Coagulation factor III (thromboplastin, tissue factor)	Yes	60
Angiogenesis			
VEGFA	Vascular endothelial growth factor A		9,61

Table I. Continued.

Symbol	Full gene name	Proposed as a serum marker candidate	References
Angiogenesis			
HYAL1	Hyaluronoglucosaminidase 1		62
CXCR4	Chemokine (C-X-C motif) receptor 4		63
CXCL12	Chemokine (C-X-C motif) ligand 12		63
THBS2	Thrombospondin 2		64
Chromatin remodeling			
ARID1A	AT rich interactive domain 1A (SWI-like)		4,66
Signaling			
HILPDA	Hypoxia inducible lipid droplet-associated		67
LGALS3	Lectin, galactoside-binding, soluble, 3	Yes	12,68
SFRP5	Secreted frizzled-related protein 5		69
Adhesion and extracellular matrix			
MSLN	Mesothelin		70
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	Yes	71
VCAN	Versican	Yes	72,73
LAMA5	Laminin, α 5	Yes	74
Hormone			
ER	Estrogen receptor		4,75
PR	Progesterone receptor		4,75
Mucin			
PDPN	Podoplanin		76

MET proto-oncogene, receptor tyrosine kinase (21), insulin-like growth factor binding protein 1 (IGFBP1) (22,23), IGF2 mRNA BP3 (24), platelet-derived growth factor receptor, α polypeptide (25,26), KiSS-1 metastasis-suppressor (KISS1) (27,28), KISS1 receptor (27,28), Erb-b2 receptor tyrosine kinase 2 (ERBB2) (29), Erb-b2 receptor tyrosine kinase 2 (FGFR2) (30,31), Rho GDP dissociation inhibitor (GDI) α (32,33) and paired box 8 (34). In particular, additional clinical studies are required to assess the efficacy of circulating IGFBP1, KISS1, ERBB2 and FGFR2 protein expression for the diagnosis of ovarian clear cell carcinoma.

Anti-apoptosis. Genes involved in anti-apoptosis have previously been demonstrated to undergo epigenomic and genomic changes in clear cell carcinoma. HNF1B is involved in carcinogenesis through the upregulation of genes involved in anti-apoptosis. The present study identified key genes involved in anti-apoptosis in ovarian clear cell carcinoma, including HNF1B (6,35,36), protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1D (37), amyloid β precursor protein (cytoplasmic tail) binding protein 2 (38), B-cell CLL/lymphoma 2 (39) and sialidase 3 (membrane sialidase) (NEU3) (40,41). As NEU3 is a cell surface protein, it may be a good serum marker candidate for the detection of ovarian clear cell carcinoma (40,41).

Metabolism. Glucose metabolism is reprogrammed to generate energy by glycolysis in cancer cells and is one of the key clinical features of carcinoma used to facilitate cancer cell survival. The ovarian clear cell carcinoma histotype exhibits specific metabolic features in which tumor growth is dependent on anaerobic glycolysis as opposed to oxidative phosphorylation for rapid energy generation, a process termed the Warburg effect. This metabolic characteristic also confers resistance to apoptosis (42). Numerous genes are involved in metabolism in ovarian clear cell carcinoma, including HNF1B (43,44), γ-glutamyltransferase 1 (GGT1) (45), von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (44,46), mucin 1, cell surface associated (47), microtubule-associated protein 1 light chain 3 α (42) and homeobox A10 (48). In particular, GGT1 may be a candidate serum marker for diagnosing ovarian clear cell carcinoma.

Chemoresistance. Biologically diverse mechanisms are involved in the development of a chemoresistant phenotype in ovarian clear cell carcinoma. These include the activation of anti-apoptosis, detoxification and antioxidation systems in the cell. The resistance of ovarian clear cell carcinoma to platinum-based chemotherapy may be caused by low levels of cell proliferation and increased cell cycle arrest during the response to DNA damage. A number of up- and downregulated chemoresistance genes have been categorized into differentially-expressed functional groups. The expression of eight genes, X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase (49), Napsin A aspartic peptidase (NAPSA) (50), protein phosphatase 2 regulatory subunit 13 like (51), glypican 3 (52,53), ATP-binding cassette, sub-family F (GCN20), member 2 (54), glutathione peroxidase 3 (55), annexin A4 (56) and aldehyde dehydrogenase 1 family, member A1 (ALDH1A1) (57), were

demonstrated to be upregulated in ovarian clear cell carcinoma specimens. Among these eight genes, NAPSA, GPX3 and ALDH1A1 were markedly overexpressed and may be possible novel biomarker candidates.

Coagulation. HNF1B is a broad marker of the ovarian clear cell carcinoma phenotype (58). Microarray analysis conducted in a previous study revealed that HNF1B may be associated with the blood clotting signaling cascade, which includes the overexpression of fibrinogen, prothrombin, coagulation factor III (thromboplastin, tissue factor) (F3) and factor XIII. Thus, HNF1B acts as a molecular link between the ovarian clear cell carcinoma histotype and an increase in the risk of developing clinically significant venous thromboembolism (VTE) (43). In particular, the coagulation-associated biomarkers F3 and tissue factor pathway inhibitor 2 (TFPI2) may be useful for the detection of clear cell carcinoma (59,60). For example, tumor-derived F3 may initiate an extrinsic coagulation signaling cascade, triggering VTE formation in clear cell carcinoma.

Angiogenesis. Angiogenesis-associated signaling pathways are potentially the biological activity most associated with an ovarian clear cell carcinoma signature. Furthermore, analysis of gene expression data in previous studies demonstrated that vascular endothelial growth factor A (61), hyaluronoglucosaminidase 1 (62), chemokine (C-X-C motif) (CXC) receptor 4 (63), CXC ligand 12 (63) and thrombospondin 2(64) constitute the angiogenesis-associated signaling most significantly associated with upregulated gene expression in ovarian clear cell carcinoma.

4. Additional biomarker candidates in ovarian clear cell carcinoma

Chromatin remodeling. Chromatin remodeling may be important for the repair of DNA damage in oxidative lesions, in order to maintain genome stability (65). Components of the chromatin remodeling multiprotein complexes may harbor develop gene sequence mutations that lead to protein inactivation or loss of expression in various types of human cancer, including ovarian clear cell carcinoma (66).

Cancer-associated signaling pathways. Previous studies have identified a number of up- and downregulated genes involved in signaling pathways associated with clear cell carcinoma (12,67-69). One of these genes, lectin, galactoside-binding, soluble, 3 (LGALS3), is a potential serum biomarker for the diagnosis of ovarian clear cell carcinoma.

Adhesion and the extracellular matrix. Previous studies have revealed the clinical importance of certain cellular adhesion molecules for the diagnosis and prognosis of patients with ovarian clear cell carcinoma. These include mesothelin (70), cadherin 1, type 1, E-cadherin (epithelial) (CDH1) (71), versican (VCAN) (72,73) and laminin, α 5 (LAMA5) (74), the last three of which are serum biomarker candidates.

Hormones. Ovarian clear cell carcinoma is a subtype of epithelial ovarian carcinoma characterized by an estrogen- and progesterone receptor-negative phenotype (75). Thus, this phenotype may be used as an immunohistochemical diagnostic marker for patients with ovarian clear cell carcinoma.

Mucin. In addition, mucin is key in the process of malignant transformation via promotion of cell invasion, metastasis and increased aggressiveness of cancer cells (76). Among the mucin-type characteristics, podoplanin expression was significantly stronger in clear cell carcinoma than in other histological types (76). Although elevated mucin expression was considered to be a prognostic marker for ovarian cancer, no significant correlation was observed between podoplanin expression and overall survival. Further investigation is needed to clarify the relationship between podoplanin expression and the biological characteristics of clear cell carcinoma.

5. Discussion

To date, the early detection of ovarian clear cell carcinoma has been hindered by the absence of effective serum biomarkers. Previous studies have failed to identify a biomarker panel with significantly improved performance over CA125. Technological advances have permitted the development of novel methods for identifying genes and proteins responsible for disease predisposition. In particular, high-throughput techniques using microarray technology and proteomic screening have generated a collection of marker candidates for use in the diagnosis of ovarian clear cell carcinoma. Ovarian clear cell carcinoma exhibits increased activity of the following signaling pathways known to potentially drive carcinogenesis: Cell cycle regulation, survival, anti-apoptosis, chemoresistance, metabolism, coagulation and angiogenesis (7). The current review summarizes characteristics of each genomic alteration that results in activation of the aforementioned cancer signaling pathways, as well as other potential sources of diagnostic and prognostic biomarkers for ovarian clear cell carcinoma.

The present review highlights specific genes and their products that are aberrantly expressed in ovarian clear cell carcinoma tissues, thus indicating their role as potential serum markers for the diagnosis of the disease. Biomarkers identified in the current review are involved in cell cycle regulation (HAVCR1 and TPD52), growth factor signaling (IGFBP1, KISS1, ERBB2 and FGFR2), anti-apoptosis and survival pathways (NEU3), metabolism (GGT1), chemoresistance (NAPSA, GPX3 and ALDH1A1), coagulation (F3 and TFPI2), signaling (LGALS3), and adhesion and the extracellular matrix (CDH1, VCAN and LAMA5; Table I).

HAVCR1 functions as part of the regulatory apparatus for tight junctions and may serve as a marker for renal and ovarian clear cell carcinoma susceptibility, as well as tumor invasion and metastasis (14). Furthermore, the physiological function of TPD52 may be associated with cell proliferation and the generation of a metastatic phenotype (17). IGFBP1, KISS1, ERBB2 and FGFR2 regulate the functions of growth factors that may be molecular targets in patients with ovarian clear cell carcinoma, while NEU3 protects cancer cells from apoptosis and is a marker of favorable prognosis (40). In addition, GGT1 has been implicated in glucose and lipid metabolism (45). NAPSA and ALDH1A1 are correlated with resistance to certain anti-cancer agents (50); GPX3 acts as a potent tumor suppressor by decreasing the number of

reactive oxygen species and limiting DNA damage (55); F3 and TFPI2 have roles in the regulation of the extrinsic coagulation cascade and thrombosis (59,60); and LGALS3 is associated with the Wnt/β-catenin signaling pathway, which is important oncogenesis (12,68). Furthermore, CDH1 predicts a favorable prognosis (71). Thus, the current review may provide a basis for further clinical investigation of the aforementioned serum marker candidates. So far, there has been no data concerning the relationship between the expression of the identified biomarkers and the biological characteristics of clear cell carcinoma. Therefore, further investigation is required to clarify which of the identified biomarkers is the most interesting or important.

In conclusion, the present literature review selected a diagnostic panel of potentially useful markers for distinguishing clear cell carcinoma from high-grade serous adenocarcinoma. Additional investigation is required to determine whether these gene products are elevated in blood samples, and whether they will ultimately be useful in the diagnosis and monitoring of patients with ovarian clear cell carcinoma.

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