OncoTargets and Therapy

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REVIEW

Circulating Cell-Free DNA or Circulating Tumor DNA in the Management of Ovarian and Endometrial Cancer

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Ovarian cancer (OC) is the most lethal cancer of all gynecological malignancies, while endometrial cancer (EC) is the most common one. Current strategies for OC/EC diagnosis consist of the extraction of a solid tissue from the affected area. This sample enables the study of specific biomarkers and the genetic nature of the tumor. However, the tissue extraction is risky and painful for the patient and in some cases is unavailable in inaccessible tumors. Moreover, a tissue biopsy is expensive and requires a highly skilled gynecological surgery to pinpoint accurately which cannot be applied repeatedly. New alternatives that overcome these drawbacks are rising up nowadays, such as liquid biopsy. A liquid biopsy is the analysis of biomarkers in a non-solid biological tissue, mainly blood, which has remarkable advantages over the traditional method. The most studied cancer non-invasive biomarkers are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and circulating free DNA (cfDNA). These circulating biomarkers play a key role in the understanding of metastasis and tumorigenesis, which could provide a better insight into the evolution of the tumor dynamics during treatment and disease progression. Liquid biopsy is an emerging non-invasive, safe and effective method with considerable potential for clinical diagnosis and treatment management in patients with OC and EC. Analysis of cfDNA and ctDNA will provide a better characterization of biomarkers and give rise to a wide range of clinical applications, such as early detection of OC/EC, the prediction of treatment responses due to the discovery of personalized tumor-related biomarkers, and therapeutic response monitoring.

Keywords: ovarian cancer, endometrial cancer, liquid biopsy, circulating cell-free DNA, circulating tumor DNA

Introduction

In recent years, the continuous development in genomic detection and analysis technology has revealed a more complicated molecular picture of cancers. Such complexity may be caused by the inter/intra-tumoral heterogeneity and clonal evolution, which brings forward a higher demand on a precise and real-time monitoring system.[1]

Ovarian cancer (OC), endometrial cancer (EC) and cervical cancer (CC) are the three major cancers in gynecology. Established screening projects and the prevalence of vaccination have remarkably reduced the incidence and mortality of invasive CC,[2] but the situation of OC and EC is still not optimistic.

Ovarian cancer is an aggressive disease with a high mortality rate, largely due to a lack of effective screening methods or biomarkers with high sensitivity and specificity.[3]

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submit your manuscript | www.dovepress.com
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http://doi.org/10.2147/OTT.S227156

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A recent review on the benefits and harms of OC screening among average-risk women included 4 randomized clinical trials involving transvaginal ultrasound (TVS), cancer antigen 125 (CA125) testing, or their combination. The review illustrated no significant difference on mortality between screened women and non-screened or irregular-screened ones.[4] Furthermore, although the response rate of initial treatment is satisfactory, recurrence rate in advanced-stage OC patients remain high, partly because of insufficient understanding of drug resistance mechanisms.[5] As a widely used biomarker during the treatment and the follow-up, CA125 does not perform well in clinical work.[6]

Endometrial cancer is the most common cancer occurring in female reproductive tract.[7] It can be diagnosed and treated at an early stage, due to its typical symptom of abnormal genital bleeding and the subsequent detailed examination. However, some patients present nonspecific symptoms and those with intermediate to high-risk factors are still very vulnerable to recurrence, resulting in a higher mortality rate. The sensitivity and specificity of TVS is too limited to be a screening method for EC, which can only provide a rough evaluation.[8] Diagnostic dilatation and curettage is effective with high sensitivity and specificity for postmenopausal women,[9] while the invasive process and possible complications may reduce the compliance of patients. More importantly, it could not be used in the treatment surveillance once the uterus is removed.

Histopathology examination has long been regarded as the gold standard for the final diagnosis and genotypic analysis of OC and EC. However, tissue biopsy is incompatible for clinical longitudinal monitoring due to its invasive nature.

Based on the above, a new strategy for these two types of gynecological malignancies is urgently needed. As a non-invasive and cost-effective method, liquid biopsy is expected to overcome the above shortcomings and has become a research hotspot.[10] Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating free RNAs, tumor-derived extracellular vesicles (TEVs) and tumor-educated platelets (TEPs) are the main components released by tumors from either primary or metastatic sites that can be used in a variety of analysis.[11] In 2017, Cheng et al briefly reviewed the role of circulating cell-free DNA (cfDNA) and CTC in OC,[12] providing us with important information. In our review, we will focus on the potential value of cfDNA/ctDNA in OC and EC patients.

Origin and Detection of cfDNA/ctDNA

It took about 30 years from the first discovery of cfDNA in the blood of healthy people[13] to the report of its relationship with cancer in 1977.[14] Researchers found that higher levels of cfDNA appeared in cancer patients and those with metastatic disease compared to the healthy and non-metastatic ones, respectively. It was not until 1994 that somatic point mutations were identified in cfDNA.[15] Since then, more studies have focused on this circulating molecular, especially its potential role in cancer management.

A great proportion of cfDNA comes from normal cells of the body, a small part of which is related to tumors, coming from primary tumors, metastatic sites or CTCs, and is called ctDNA. The mechanism of cfDNA being released by cells into circulation was still not fully understood. Mouliere et al[16] investigated the sources of circulating DNA of three distinctive categories (normal extratumoral cells, tumor microenvironment cells and neoplastic tumor cells) in colorectal cancer patients, elucidating three sources of cfDNA: necrosis, apoptosis and active secretion. Apoptosis has been reported to be one of the sources of cfDNA, as cfDNA fragments are found to be similar in size to apoptotic DNA. Necrosis might be an important contributor to long cfDNA fragments (over 1000bp).[17] Another hypothesis of cfDNA release was active secretion, with DNA included in the exosomes or DNA-lipoprotein complexes.[16]

Analysis of ctDNA paves us the way for a comprehensive view of the tumor genomic landscape. The ctDNA fragments (~134-144 bp) were reported to be shorter than that of cfDNA (~166bp).[18,19] The proportion of ctDNA in the total cfDNA may vary greatly in accordance with clinical-pathologic features of tumor as well as the tumor microenvironment.[20,21] Relatively low ctDNA levels in standard blood samples and background cfDNA unrelated to tumor cells make detection a challenge. Therefore, the standard procedure for blood collection and DNA isolation should be finalized prior to analysis.[22,23]

Methodologies in Exploring cfDNA/ctDNA

For blood collection, the ratio of plasma germline cfDNA is lower than that of serum; thus, it is more suitable for ctDNA isolation.[24] Plasma separation should be performed within a short time since the half-time of ctDNA is only about 2 hrs in standard EDTA tubes.[25] Some other special blood

collection tubes with special preservatives, such as Streck, allow for delayed plasma separation.[26] After centrifugation, the separated plasma should be stored at −80°C for later use.

The extraction of cfDNA/ctDNA is the key to the subsequent test. Available methods include phase isolation, silicon membrane-based spin column, and magnetic bead-based isolation.[27] The extraction efficiency varies depending on the methods and the plasma volumes.[28]

New techniques have emerged with high sensitivity and high specificity for the analysis of cfDNA or ctDNA from a quantitative and qualitative perspective,[29–31] such as polymerase chain reaction (PCR) strategies and Next-Generation Sequencing (NGS) strategies.

There is a variety of PCR-based methods, each with its characteristics, including real-time PCR, co-amplification at lower denaturation temperature-PCR, methylation-specific PCR, digital PCR or droplet digital PCR (ddPCR), "Beads, Emulsion, Amplification, Magnetics digital PCR" (BEAMing) and so on.[24] Designed to detect hotspot mutations, these PCR-based methods are relatively time-saving and economical.

Next-Generation Sequencing (NGS)-based methods such as tagged-amplicon deep sequencing (TamSeq), the Safe Sequencing System (SafeSeqS), CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) and targeted error correction sequencing (TEC-Seq) are available to assess genetic alterations with different detection capacities.[24] Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) technologies allow for the identification of novel alterations with no pre-existing knowledge.[32,33] The ability of NGS-based methods to detect copy number variations (CNVs) is an advantage over PCR.[24] Though efficient, these methods are relatively time-consuming and require professional bioinformatics analysis.

Application of cfDNA/ctDNA in Ovarian and Endometrial Cancer

As a promising effective tool in oncology, cfDNA/ctDNA is of essential value in clinical management of ovarian and endometrial cancer patients from the diagnostic, predictive and prognostic aspects (Table 1; Figure 1).

Early Detection and Differential Diagnosis

As previously mentioned, the diagnostic value of blood markers and imaging examinations for early detection and differential diagnosis of ovarian and endometrial tumors is limited. Plenty of studies have been carried out to evaluate the competency of cfDNA/ctDNA in cancer screening.

Quantification of cfDNA/ctDNA

Ovarian Cancer

Changes in levels of cfDNA/ctDNA can be a hint for the existence of malignance, which could be more accurate than CA125 or HE4 in some OC studies. Shao et al reported a significant increment of cfDNA levels in the OC group compared with the control group ($P<0.01$). They also noticed a higher sensitivity and specificity (88.9% and 89.5%) of cfDNA than CA125 (75% and 52.6%) and HE4 (80.6% and 68.4%) in OC detection. Sensitivity and specificity were promoted (91.67% and 84.21%) when the above biomarkers were combined.[34] Similarly, a cohort study of OC achieved a sensitivity and specificity of 87–91.5% and 85-87%, respectively. By comparison, they found a lower false-positive rate of cfDNA level than CA125.[35] Another small sample study focusing on three endogenous loci (*GAPDH*, β-actin and β-globin) identified significantly higher cfDNA levels among OC patients compared to controls, with the greatest difference occurring at β-actin locus.[36] Quantitative alterations of circulating cell-free nuclear DNA and circulating cell-free mitochondrial DNA were evaluated to confirm their values in diagnosing OC with moderate sensitivity and specificity.[37] Dobrzycka et al found that the detection rate of ovarian serous carcinoma was higher than other types, suggesting the role of cfDNA in differential diagnosis.[38]

Endometrial Cancer

The role of cfDNA levels for EC screening and classification appears to be controversial. Significantly elevated total cfDNA levels were detected in EC group compared with the controls, with higher levels in high-grade groups (G2 and G3).[39] Another study reported a dramatic difference between the mean level of cfDNA in patients with type I and type II EC.[40] Vizza et al observed a remarkably increased total cfDNA content in high-grade EC patients. They adopted a special calculation method applying Alu-quantitative real-time PCR (qPCR) technology to investigate the role of cfDNA. The serum DNA integrity index, defined as qPCR-Alu247 value/qPCR-Alu115 value of each sample, was significantly reduced in high-grade EC patients with hypertension and obesity. Unfortunately, this index presented a low predictive accuracy to differentiate high grade from low-grade EC patients.[41] The study carried out by Tanaka et al showed no significant difference of cfDNA levels between EC and the controls, partly due to the small sample size or differences in methods.[42]

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Table 1 Studies with Analysis of cfDNA/ctDNA in OC or EC Patients

References	Country	Cancer Type	Sources	Cancer/ Control	Abnormalities	Methodology	Clinical Relevance
Shao et al[34]	China	OC	Serum	36/41	Level	bDNA technique	Diagnosis
Kamat et al[35]	USA	OC	Plasma	164/124	Level	RT-PCR	Diagnosis
Kamat et al[36]	USA	OC	Plasma	19/12	Level	RT-PCR	Diagnosis
Zachariah et al[37]	Switzerland	OC	Serum/plasma	21/83	Level	RT-PCR	Diagnosis
Dobrzycka et al[38]	Poland	OC	Plasma	126/NA	Level/*KRAS* mutation	PCR-RFLP	Diagnosis/ prognosis
Cicchillitti et al[39]	Italy	EC	seRum	59/NA	Level	RT-PCR	Diagnosis
Dobrzycka et al[40]	Poland	EC	Plasma	109/NA	Level	PCR-RFLP	Diagnosis
Vizza et al[41]	Italy	EC	Serum	60/NA	Level	RT-PCR	Diagnosis
Tanaka et al[42]	Japan	EC	Plasma	53/24	Level	RT-PCR	Diagnosis/ prognosis
Hickey et al[43]	United Kingdom	OC	Serum	20/NA	Mutations, LOH and MI	PCR	Diagnosis
Otsuka et al[46]	Japan	OC	Plasma	27/NA	*p53* mutation	PCR	Diagnosis/ prognosis
Park et al[47]	Korea	OC	Plasma	4/NA	*TP53* mutation	Digital PCR	Diagnosis
Cohen et al[49]	Australia	OC	Plasma	32/32	Chromosomal instability	Whole genome NIPT platform	Diagnosis
Vanderstichele et al[50]	Belgium	OC	Plasma	57/11	Chromosomal instability	NGS	Diagnosis
Phallen et al[51]	USA	OC	Plasma	42/NA	Somatic mutations	TEC-Seq	Diagnosis
Farkkila et al[52]	Finland	OC	Plasma	35/NA	*FOXL2* mutation	ddPCR	Diagnosis/ prognosis
Sun et al[53]	China	EC	PBLs	139/139	mtDNA copy number value	RT-PCR	Diagnosis
Giannopoulou et al[56]	Greece	OC	Plasma	59/NA	*RASSF1A* methylation	MSP technique	Diagnosis
Zhang et al[57]	China	OC	Serum	87/115	(*APC, RASSF1A, CDH1, RUNX3, TFPI2, SFRP5, OPCML*) methylation	MSP technique	Diagnosis
Liggett et al[58]	USA	OC	Plasma	30/60	(*RASSF1A, CALCA, EP300, BRCA1, CDKN1C, PGR-PROX*) methylation	MethDet 56	Diagnosis
Melnikov et al[59]	USA	OC	Plasma	33/33	(*BRCA1, HIC1, PAX5, PGR, THBS1*) methylation	MethDet 56	Diagnosis
Widschwendter et al[60]	UK	OC	Serum	25/598	(*COL23A1, C2CD4D, WNT6*) methylation	NGS	Diagnosis/ prognosis

(*Continued*)

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Table 1 (Continued).

References	Country	Cancer Type	Sources	Cancer/ Control	Abnormalities	Methodology	Clinical Relevance
Li et al[61]	China	OC	Peripheral blood	206/205	Methylation at multiple sites	NGS	Diagnosis
Wu et al[62]	China	OC	Plasma	47/24	*RASSF2A* methylation	MSP technique	Diagnosis
Ibanez et al[63]	USA	OC	Serum/plasma	50/NA	(*BRCA1, RASSF1A*) methylation	MSP technique	Diagnosis
Margolin et al[67]	USA	EC	A computational simulation	42/8	*ZNF154* methylation	NGS	Diagnosis
Wimberger et al[71]	Germany	OC	plasma	62/28	Level	RT-PCR	prognosis
Perkins et al[72]	United Kingdom	OC	Plasma	105/20	Level/mutations in 19 genes	NGS	Prognosis
Steffensen et al[73]	Denmark	OC	Plasma	144/NA	Level	RT-PCR	Prognosis
No et al[74]	Korea	OC	Plasma	36/16	(*B2M, RAB25, CLDN4, ABCF2*) mutation	RT-PCR	Prognosis
Kuhlmann et al[75]	Germany	OC	Serum	63/20	Level/LOH	Fluorescence-labeled PCR	Prognosis
Swisher et al[48]	USA	OC	Plasma/serum	137/NA	*p53* mutation	DNA sequencing	Prognosis
Giannopoulou et al[76]	Greece	OC	Plasma	129/NA	*ESR1* methylation	MSP technique	Prognosis
Bolivar et al[70]	USA	EC	Plasma	48/NA	(*CTNNB1, KRAS, PTEN, PIK3CA*) mutation	NGS	Prognosis
Harris et al[77]	USA	OC	Plasma	8/NA	Chromosomal junctions	Quantitative PCR	Prognosis
Pereira et al[78]	USA	OC and EC	Serum	22OC,17EC/ NA	Level	Digital PCR	Prognosis
Du et al[79]	China	OC	Plasma	21/NA	Mutations/CNV	NGS	Prognosis
Martignetti et al[80]	USA	OC	Plasma/serum	1/NA	*FGFR* fusions	NGS/RT-PCR	Prognosis
Choudhuri et al[82]	India	OC	Plasma	100/NA	Level	RT-PCR	Treatment response
Capizzi et al[83]	Italy	OC	Plasma	22/NA	Level	RT-PCR	Treatment response
Arend et al[84]	USA	OC	plasma	14/NA	level	NGS	Treatment response
Kamat et al[69]	USA	OC in mice	Plasma	–	Level	RT-PCR	Treatment response
Parkinson et al[68]	United Kingdom	OC	Plasma	40/NA	*TP53* mutation	Digital PCR	Treatment response

(*Continued*)

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Table 1 (Continued).

References	Country	Cancer Type	Sources	Cancer/ Control	Abnormalities	Methodology	Clinical Relevance
Gifford et al[85]	United Kingdom	OC	Plasma	138/NA	*hMLH1* methylation	Microsatellite PCR	Treatment response
Flanagan et al[86]	United Kingdom	OC	Peripheral blood	247/NA	Methylation at CpG sites	NGS	Treatment response
Matulonis et al[90]	USA	OC	Plasma	67/NA	Mutations in components/ modulators of the *PI3K* pathway	NGS/Sanger sequencing	Treatment response
Weigelt et al[87]	USA	OC	Plasma	19/NA	*BRCA* reversion mutation	NGS	Treatment response
Christie et al[88]	Australia	OC	Plasma	30/NA	*BRCA* reversion mutation	NGS	Treatment response
Lin et al[89]	USA	OC	Plasma	209/NA	*BRCA* reversion mutation	NGS	Treatment response

Abbreviations: OC, ovarian cancer; EC, endometrial cancer; bDNA, branched DNA; PCR-RFLP, PCR-restriction fragment length polymorphism; LOH, loss of heterozygosity; MI, microsatellite instability; NGS, next-generation sequencing; mtDNA, mitochondrial DNA; ddPCR, digital droplet PCR; PBLs, peripheral blood leukocytes; MSP, methylation-specific PCR; CNV, copy number variation; NK, do not know.

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Genomic Alterations of cfDNA/ctDNA

Ovarian Cancer

Detection of molecular alterations in cfDNA/ctDNA dates back to 1999, when Hickey et al used PCR to detect loss of heterozygosity (LOH) and microsatellite instability in 20 OC patients.[43] *TP53* mutations were reported as the most common in OC, accounting for approximately 96% of the somatic mutations.[44,45] Articles targeting *TP53* mutations in cfDNA/ctDNA with different detection techniques have been published, implying their roles in OC management.[46–48]

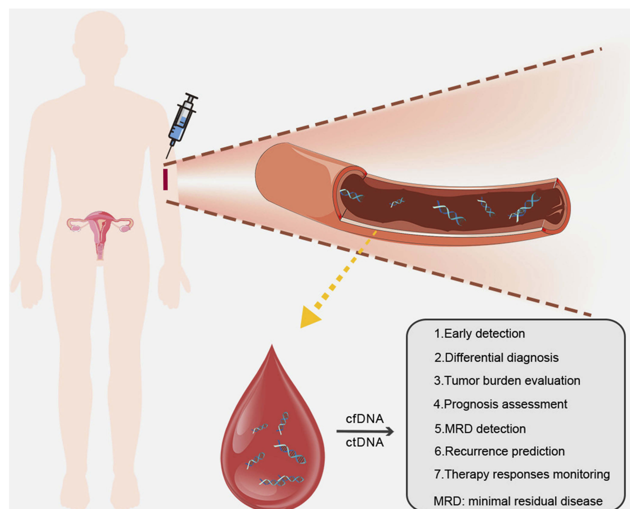

Figure 1 Applications of cfDNA/ctDNA in ovarian or endometrial cancer patients.

Researches centered on other genetic alterations or gene panels with several selected genes of cfDNA/ctDNA were also carried out. Cohen et al conducted a proof-of-concept study for subchromosomal with a low coverage sequencing approach called non-invasive prenatal testing platform, observing a relatively low sensitivity of 40.6%, but a high specificity of 93.8%.[49] A recent study focused on the chromosomal instability of cfDNA included 68 patients presenting adnexal masses. In terms of the area under the curve (AUC), a much higher sensitivity over CA125 appeared, when the specificity was set to 99.6%.[50] An ultrasensitive approach called TEC-Seq was applied to detect ctDNA alterations in different kinds of tumors based on a well-designed combination of genes. Of the 42 enrolled OC patients, 71% were found with ctDNA alterations, and the fraction turned into 68% when researchers focused on early-stage patients.[51] Farkkila et al developed a study to detect the *FOXL2* mutation in ctDNA of adult granulosa cell tumor (AGCT) patients with ddPCR assay, revealing a sensitivity and specificity of 23% and 90%, respectively.[52]

Endometrial Cancer

With regard to EC patients, Sun et al conducted a special research on mitochondrial DNA (mtDNA) copy number detection in peripheral blood leukocytes (PBLs) rather than serum or plasma.[53] Alterations in mtDNA may lead to mitochondrial dysfunction, thus contributing to tumorigenesis.[54]

They reported that low mtDNA copy number indicated a more than five-fold increase in the risk of EC.[53]

Epigenetic Changes of cfDNA/ctDNA

Alteration of DNA methylation has been proven to be an early event of tumorigenesis, making the analysis of circulating DNA methylation patterns a potential method to detect OC and EC.[55]

Ovarian Cancer

Published studies applied similar research methods for OC, while focused on different genes.[56–62] Ibanez et al developed a promoter hypermethylation analysis of 6-gene panel in the serum of OC patients (ranging from early to advanced-stage patients), observing a sensitivity of 82% and a specificity of 100%.[63] A 7-gene panel study showed higher sensitivity and specificity (85.3% and 90.5%, respectively) of cfDNA than CA125 (56.1% and 64.15%, respectively) in stage I OC.[57] There are similar studies published with different results of sensitivities and specificities, which may attribute to different markers they adopted.[58,59] Although Widschwendter's group observed sensitivities of 41.4% and 82.8%, and specificities of 90.7% and 87.1%, respectively, for a three-DNA-methylation-serum-marker panel and CA125, they noticed that cfDNA can detect OC much earlier than the exact diagnosis.[60] In addition, Li et al obtained a prediction accuracy of 77.3% in the included OC population for the six validated CPG sites.[61] It is worth noticing that four of the studies mentioned above selected *RASSF1A* gene as one of the biomarkers.[56,57,59,63] Wu et al evaluated the methylation status of *RASSF2A*, observing an aberrant rate of 51.1% in tissues and 36.2% in corresponding plasma samples.[62] The *RASSF* gene family has been reported to play vital roles in tumorigenesis of various malignancies.[64–66] Epigenetic alterations of *RASSF* gene family, especially *RASSF1A* and *RASSF2A* may be promising as a marker that warrants further investigations.

Endometrial Cancer

Margolin et al developed a study to detect the hypermethylation of the *ZNF154* CpG island in five types of tumor, including 42 EC patients. Apart from the tissue samples, a computational simulation of ctDNA (1% tumor DNA in 99% normal DNA) was also applied in their study, and an AUC of 0.79 was proved to show the best performance in endometrial tumors.[67]

Tumor Burden Evaluation and Prognosis Assessment

Analysis of cfDNA/ctDNA allows doctors to gain insight into tumor burden, thus providing references for subsequent treatment. Information on survival outcomes predicted by cfDNA/ctDNA could assist us in developing therapeutic projects and personal follow-up plans for cancer patients.

Tumor Burden Evaluation

Ovarian Cancer

A significant discrepancy of cfDNA levels between stage I-II and stage III-IV ($P<0.01$) was reported based on an analysis of 36 OC cases,[34] and a strong relationship between ctDNA level and tumor volume was also documented in a group of high-grade serous ovarian carcinoma patients,[68] suggesting the potential value of cfDNA/ctDNA in evaluating tumor burden. A study in mice confirmed that elevated ctDNA levels indicated elevated tumor burden.[69] In addition to quantification, specific genetic mutations can also reflect tumor burden. Larger median tumor size was observed in AGCT patients with *FOXL2* ctDNA mutations by a research group.[52]

Endometrial Cancer

Relative researches about EC are rarely seen, but one has found that the appearance of plasma DNA mutations was remarkably correlated with primary tumor size.[70]

Prognosis Assessment

Ovarian Cancer

Several studies drew similar conclusions on the prognostic role of cfDNA levels, that is, an increased cfDNA levels indicate a significant reduction in overall survival (OS) in OC patients.[35,38,71–73] From the perspective of genetic changes, certain genes were verified to be useful to assess prognosis. No et al reported that *RAB25* levels in serum cfDNA were remarkably related to disease-free survival (HR=18.2, 95% CI=2.0–170.0) and OS (HR=33.6, 95% CI=1.8–634.8) of advanced-stage OC patients.[74] LOH proximal to *M6P/IGF2R* locus (D6S1581) was found to be associated with OS ($P=0.030$) in another study.[75] The level of cfDNA[72] or presence of specific ctDNA mutation[48] was even identified as an independent predictor of OS for OC patients. In addition, the presence of *ESR1* methylation in primary tumor samples was significantly correlated with better survival outcomes, though the correlation was not significant in plasma samples.[76]

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Endometrial Cancer

Plasma DNA mutations in EC patients were found to be significantly related to deep myometrial invasion and lymphatic/vascular invasion, suggesting a possibly poorer survival outcome than those with only wildtype DNA.[70]

Minimal Residual Disease (MRD) Detection and Recurrence Prediction

Postoperative residual disease may occur in advanced OC and EC patients even under standard surgical procedures. As a potential source of relapse, it poses a serious threat to the patient's survival outcome. As aforementioned, despite emerging new treatment strategies, recurrences of OC and EC remain as urgent problems to be resolved. The evaluation of residual tumor burden and recurrence is thus important, and cfDNA/ctDNA may offer assistance.

Ovarian Cancer

Wimberger et al demonstrated that serum DNA levels of OC patients were significantly correlated with postoperative residual tumor load of >1 cm (p=0.0001) and a higher risk of relapse (p=0.002).[71] Similar relationship between preoperative cfDNA levels and residual tumor load (p=0.017) was mentioned in another study.[75] A research focusing on selected chromosomal junctions reported presence of ctDNA after surgery in patients with detectable disease, while no ctDNA in those without the disease.[77]

ctDNA may provide opportunities of timely treatment of recurrent lesions that were not detectable through imaging. One research revealed that ctDNA could make a diagnosis for relapsed OC cases 7 months earlier than CT scanning.[78] In patients with recurrent OC, the coincidence rates of *TP53* and *BRCA1* in cfDNA and tumor tissue DNA were 76.2% and 95.24%, respectively, which means that cfDNA/ctDNA could assist the monitoring of disease progression.[79] Otsuka et al noticed in a follow-up survey that one patient with re-emerging *p53* mutation after surgery died shortly after, while the other one with no *p53* mutation survived.[46] An American study revealed a more sensitive and specific biomarker, the fibroblast growth factor receptor 2 (*FGFR2*) fusion ctDNA biomarker, to detect OC. In a series of 28 measurements during a 4-year follow-up of a specific patient, *FGFR2* fusion better reflected the evolvement of the disease than CA125, especially the tumor recurrences.[80]

Endometrial Cancer

Levels of cfDNA were evaluated in a cohort of 25 EC patients before and after operation, and 3 cases whose postoperative cfDNA levels did not decrease as expected relapsed during the follow up.[41]

Therapy Responses Monitoring

As the molecular mechanisms of tumors continue to unlock, different types of targeted drugs have been validated in clinical trials. Treatment resistance occurs commonly, possibly because of the genomic heterogeneity or tumor subclonal evolution under selection pressure.[81] Real-time monitoring of therapeutic response is pivotal as it helps to understand the dynamic development of the disease. Detection of cfDNA/ctDNA may achieve the goal of screening out potential drug-resistant patients and instituting candidate-tailored therapeutic project to reduce the recurrence rate and improving survival rate.

Ovarian Cancer

Several studies, including a study of the OC mouse model, reported a decrease in cfDNA levels after ovarian cancer treatment, and some studies have shown that it is associated with post-therapy survival.[69,82,83] On the other hand, significant changes in genetic variants of cfDNA were noticed after chemotherapy.[84] In relapsed patients, a >60% reduction in *TP53* mutant allele fraction was reported as an independent predictor of time to progression after one cycle of chemotherapy.[68] In one study, methylation changes of the *hMLH1* gene in plasma DNA were detected (before chemotherapy and at relapse) in 138 patients with stage IC–IV OC who experienced a relapse. A significant (P<0.001) increase of the positive rate (from 12% to 33%) in *hMLH1* methylation was observed, together with a poor OS. This study provided evidence to support the idea that the loss of DNA mismatch repair (MMR) might involve in the process of acquired drug resistance.[85] Another study also documented changes in plasma DNA methylation in patients with OC who relapsed after platinum-based chemotherapy.[86] The identification of chemotherapy responders and non-responders was essential for the subsequent treatment plan, and the analysis of DNA-methylation-serum-marker panel will be useful.[58]

Recently, a couple of studies have been developed to explore the acquisition of *BRCA* reversion mutations and their roles in drug resistance by analyzing cfDNA/ctDNA in OC patients who received platinum-based chemotherapies or PARP inhibitors.[87–89] Detection of *BRCA* reversion mutations in cfDNA/ctDNA has been proven to be useful to predict possible drug resistance and guide treatment strategies.

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Endometrial Cancer

Matulonis et al conducted a study to assess treatment response of PI3K inhibitor pilaralisib in advanced or recurrent EC cases. Different degrees of consistency were noticed between ctDNA and paired tumor tissue on the status of *PIK3CA, KRAS*, and *BRAF* genes.[90]

Possible Applications of cfDNA/ctDNA in Specific Situations

Utility of cfDNA/ctDNA in Fertility-Sparing Treatment

Advanced techniques, including detection techniques of cfDNA/ctDNA, have improved the detection rate of early-stage disease. Choices of fertility-sparing approaches for gynecological cancer patients are receiving greater attention, since an increasing number of patients are diagnosed at productive age and desire to have children. Gynecological cancers have a negative impact on the sexuality and fertility of patients, increasing their stress level and reducing personal identity, which may pose a threat to the quality of their lives.[91]

Most OC cases are diagnosed at an advanced stage, for whom cytoreduction remains optimal. However, fertility-sparing surgery is still recommended for selected patients by several society recommendations, with the premise of a comprehensive surgical staging.[92,93]

As for EC patients, total hysterectomy and bilateral salpingo-oophorectomy with or without surgical staging are regarded as the standard treatment. Fertility-sparing surgery is a choice for reproductive-aged patients with stage IA type I and G2 EC.[94] Furthermore, previous studies have evaluated the accuracy of preoperative methods, such as magnetic resonance imaging (MRI), TVS and hysteroscopically directed biopsies, for predicting the nodal-spread risk of EC.[95,96] They concluded that these preoperative mapping methods worked well with high accuracy, avoiding over- or under-treatment of EC patients, while more evidence in this field is still needed. Conservative treatment such as progestational agents has attracted widespread attention among premenopausal women who are eager to give birth and have achieved favorable outcomes in early-stage patients.[97–100]

Given the risk of recurrences, monitoring must be strengthened for patients receiving conservative treatment. Non-invasive detection of cfDNA/ctDNA provides a more convenient tool for tracking disease progression, which helps to assess treatment response. Nevertheless, no relative data have been published by now, and the application of cfDNA/ctDNA analysis in this field needs further investigations.

Utility of cfDNA/ctDNA in the Management of Elderly Patients

The incidence of cancer in elderly population is much higher than the young group. However, the management of elderly patients remains a challenge. In the clinical setting, treatment patterns of elderly patients with OC and EC often differ from younger patients, and are less aggressive, due to their seemingly fragile bodies.[101,102] Studies supported that age itself was not a prognostic factor for survival outcome, and elderly patients could also receive standard treatments.[102] The management of elderly OC or EC patients should be personalized according to the performance status of the patients, the extent of the disease and their life goals.[102] The detection of cfDNA/ctDNA may act as an assistant examination during the whole course of the management, given its non-invasive nature.

Detection of cfDNA/ctDNA in Non-Blood Fluids

In addition to blood, cfDNA/ctDNA is also detectable in other body fluids, among which urine, peritoneal fluid and uterine lavage fluid were reported to be utilized in the management of OC or EC patients (Table 2). Compared to bloodstream, the concentration of ctDNA shed in non-blood fluids can be higher in some certain cancer types.[103] However, a lack of relative researches and protocols, as well as rare experience in the exploration of these tests remain problems for non-blood-based liquid biopsy.

Several studies focused on tumor-specific genetic alterations of DNA extracted from peritoneal fluid samples of OC patients, receiving relatively high sensitivities in detection.[43,48,104–106] In 2004, methylation of peritoneal DNA was first reported as an independent factor in OC survival.[107] Shortly after, hypermethylation was detected in peritoneal fluid DNA with relatively high sensitivity and specificity, including 3 cytologically negative patients.[63] Du and his colleagues included urine samples into their study for genetic mutation analysis, identifying a detection rate of 86%.[79] Nair et al conducted a study to detect endometrial driver mutations in uterine lavage in seven EC patients, of whom six were at early stage.[108] In another similar study, specific mutations were identified in 80% (24/30) OC and 100% (5/5) EC patients.[109] It has been confirmed that tumor

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Table 2 Studies with Analysis of cfDNA/ctDNA in Other Body Fluids of OC or EC Patients

References	Country	Cancer Type	Fluid Type	Number of Samples	Abnormalities	Methodology	Clinical Relevance
Hickey et al[43]	United Kingdom	OC	Peritoneal fluid	20	Mutations, LOH and MI	PCR	Diagnosis
Swisher et al[48]	USA	OC	Peritoneal fluid	30	*p53* mutation	DNA sequencing	Prognosis
Krimmel et al[104]	USA	OC	Peritoneal fluid	37	*TP53* mutation	Duplex sequencing	Diagnosis
Parrella et al[105]	Italy	OC	Peritoneal fluid	15	(*p53,KRAS*) mutation/ LOH	DNA sequencing	Diagnosis
Barquin et al[106]	Spain	OC	Peritoneal fluid	10	*BRCA* mutation	DNA sequencing	Prognosis
Muller et al[107]	Austria	OC	Peritoneal fluid	61	methylation of 15 selected genes	MethyLight	Prognosis
Ibanez et al[63]	USA	OC	Peritoneal fluid	42	(*BRCA1, RASSF1A*) methylation	MSP technique	Diagnosis
Du et al[79]	China	OC	Urine and ascites	21 and 13	Mutations/CNV	NGS	Prognosis
Nair et al[108]	USA	EC	Uterine lavage fluid	107	Mutations of selected genes	NGS	Diagnosis
Maritschnegg et al[109]	Austria	OC and EC	Uterine lavage fluid	30 and 5	Somatic mutations	NGS	Diagnosis
Kinde et al[110]	USA	OC and EC	Liquid-based Pap smear	22and 24	Somatic mutations	NGS	Diagnosis
Wang et al[111]	USA	OC and EC	Pap brush samples	245 and 382	Somatic mutation	Safe-sequencing system	Diagnosis

Abbreviations: OC, ovarian cancer; EC, endometrial cancer; LOH, loss of heterozygosity; MI, microsatellite instability; MSP, methylation-specific PCR; CNV, copy number variation; NGS, next-generation sequencing; Pap, Papanicolaou.

DNA is detectable in detached cells from ovarian and endometrial malignancies collected at the cervix.[110] Recently, genetic analysis of Papanicolaou (Pap) test using liquid-based methods rather than traditional Pap smear revealed potential advantages in early detection of OC and EC patients.[110,111]

Conclusion

On June 1, 2016, cobas EGFR Mutation Test v2 was approved by the U. S. Food and Drug Administration (FDA) to identify patients with metastatic non-small cell lung cancer (NSCLC) appropriate for treatment with Tarceva® (erlotinib).[112] This test, focusing on the mutation of the epidermal growth factor receptor (*EGFR*) gene in the cfDNA isolated from the blood samples of patients, is the first cfDNA/ctDNA test officially applied to clinical work.[112] Currently, no cfDNA/ctDNA-related test has been approved by FDA in the field of OC and EC. Encouragingly, as mentioned above, published studies supported the feasibility of cfDNA/ctDNA's application in the interrogation of tumor genome profiles and real-time tracing of the tumor conditions in OC and EC patients. The analysis of levels, genetic changes and epigenetic alterations of cfDNA/ctDNA hold tremendous promise in a wide range of applications: cancer screening, tumor burden evaluation, prognosis assessment, MRD detection, recurrence surveillance and treatment monitoring. In addition, a large number of clinical trials exploring the role of cfDNA/ctDNA in the management of OC and EC are underway (for example, NCT03691012), which may provide us with strong evidence.

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It should be noted that well-defined preanalytical, analytical and postanalytical protocols are the prerequisites to obtain convincing results from large-scale clinical trials. Efforts are still needed to overcome challenges in detection techniques such as low amounts of cfDNA/ctDNA and high background signals. Combination of this new liquid biopsy method with other traditional methods may yield more satisfactory results. Accumulated evidence for survival benefits is required to integrate this novel diagnostic approach into the clinical scenario in the foreseeable future.

Acknowledgments

This study was funded by CAMS Youth Talent Award Project (No. 2018RC320006) and CAMS Innovation Fund for Medical Sciences (CIFMS; No. 2016-I2M-1-002).

Disclosure

The authors report no conflicts of interest in this work.

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