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REVIEW

Circulating tumor cells as trigger to hematogenous spreads and potential biomarkers to predict the prognosis in ovarian cancer

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Abstract Despite several improvements in the surgical field and in the systemic treatment, ovarian cancer (OC) is still characterized by high recurrence rates and consequently poor survival. In OC, there is still a great lack of knowledge with regard to cancer behavior and mechanisms of recurrence, progression, and drug resistance. The OC metastatization process mostly occurs via intracoelomatic spread. Recent evidences show that tumor cells generate a favorable microenvironment consisting in T regulatory cells, T infiltrating lymphocytes, and cytokines which are able to establish an "immuno-tolerance mileau" in which a tumor cell can become a resistant clone. When the disease responds to treatment, immunoediting processes and cancer progression have been stopped. A similar inhibition of the immunosuppressive microenvironment has been observed after optimal cytoreductive surgery as well. In this scenario, the early identification of circulating tumor cells could represent a precocious signal of loss of the immune balance that precedes cancer immunoediting and relapse. Supporting this hypothesis, circulating tumor cells have been demonstrated to be a prognostic factor in several solid tumors such as colorectal, pancreatic, gastric, breast, and genitourinary cancer. In OC, the role of circulating tumor cells is still to be defined. However, as opposed to healthy women, circulating tumor cells have been demonstrated in peripheral blood of OC patients, opening a new research field in OC diagnosis, treatment monitoring, and follow-up.

Keywords Circulating tumor cells · Immunoediting · Ovarian cancer · Prognostic markers · Tumor immunology

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Introduction

In developed countries, ovarian cancer (OC) is the second most common malignant tumor of the female reproductive system, reaching the eight world position for morbidity and mortality rates [1]. Optimal cytoreduction followed by platinum- and taxane-based chemotherapy represents the cornerstone treatment of OC (http://www.cancer.gov/types/ovarian/hp/ovarian-epithelial-treatment-pdq). Approximately 75 % of all OC patients are diagnosed at stage III–IV when transperitoneal, hematogenous, and lymphatic dissemination have already occurred; in this scenario, surgery is often multivisceral and highly complex and survival chances limited. Despite surgical improvement [2], recurrences remain the most challenging obstacle to overcome. In the past decades, only the intraperitoneal administration of chemotherapy in optimally debulked OC patients and the adoption of bevacizumab as maintenance therapy have been associated with an improved survival [3, 4]. At present, we are still unable to identify patients who will have different oncologic outcomes at the time of diagnosis. In other words, pathologic and molecular prognostic factors are still lacking. As a matter of fact, OC patients are subdivided into platinum refractory, resistant, and sensitive based on their progression-free survival.

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In an attempt to identify molecular markers that may serve as prognostic indicator and as target for new molecular chemotherapeutic agents, a number of studies have investigated the prognostic value of oncogenes and tumor suppressor genes presumed to be involved in the development and progression of OC, such as overexpression of protein p185 and amplification of the encoding oncogene HER-2/neu [5]. Unfortunately, data regarding overexpression and clinical significance of molecular targets are still conflicting and far from clinical applicability.

Recently, it has been found that host's cellular adaptive immune system mounts a response to many solid tumors, including OC, mediated by tumor-infiltrating T lymphocytes (TILs) [6]. In this view, biomarkers to capture the TIL immunosurveillance for cancer prognosis and prediction of therapeutic response could be developed [7]. Similarly, the microRNA-200 family and circulating tumor DNA (ctDNA) were found to be useful OC biomarkers [8–10]. Finally, circulating tumor cells (CTCs) recruited in peripheral blood were identified as a marker of hematogenous spread in various solid tumors [11]. So far, their predictive and prognostic value has been proven in breast [12], colorectal [13], lung [14], esophageal [15], liver [16], pancreatic [17], and prostate cancers [18]. Recently, the presence of positive CTCs was associated with deep myometrial invasion and lymph node positivity in endometrial cancer [19].

The role of CTCs in OC is still to be defined. However, conversely to healthy women, CTCs have been demonstrated in peripheral blood of OC patients [20]. This finding could open new scenarios in OC diagnosis and treatment monitoring.

Disseminated tumor cells and circulating tumor cells

Disseminated tumor cells (DTCs) and CTCs have first been identified and considered the potential precursors of metastatic disease in the 1990s [21]. Fidler et al. highlighted that CTCs reach distant organs developing metastases by three processes: endosmosis (invasion of surrounding tissue, blood, and lymph circulation), exosmosis (exudation from microvascular architecture), and oecesis (germination into remote organs where visible tumor lesions are developed) [22].

It is unclear whether DTCs and CTCs represent identical cell populations observed at a different anatomic location (bone marrow and bloodstream, respectively) and distinct stage of tumor progression. Overall, the existence of CTCs in the bloodstream and the settlement of these cells in secondary organs such as liver, lungs, and mostly bone as DTCs is generally accepted. In this scenario, bone marrow sampling (such as sampling from other organs) is a rather invasive procedure, which is not widely accepted in the clinical management. Surely, detection of CTCs seems more practical than DTCs due to a systematically feasible evaluation of CTCs in peripheral bloodstream, with respect to bone biopsy. Furthermore, CTCs seems to be more sensitive than DTCs in evaluating tumor progression [23]. For these reasons, focus on DTCs has been shifted to the detection of tumor cells in peripheral blood.

Identification of circulating tumors cells in ovarian cancer

CTCs are tumor cells that spread into the bloodstream from the primary tumors, recurrences, or metastases and possess antigenic and genetic tumor-specific characteristics. CTCs have been identified in epithelial cancer patients, while they were absent in healthy subjects [19]. Particularly, six genes appeared very highly expressed in the cancer cell lines and absent in healthy women; this identification of tumor cells may demonstrate the potential utility for early detection, clinical monitoring, and treatment control of gynecological malignancies [20, 24].

Detection methods consist in immunocytochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR). Compared with IHC, RT-PCR seems to be more sensitive (HR 3.49 vs 1.70) [25], suggesting RT-PCR as the promising methods in identifying CTCs in OC patients. However, so far, the US Food and Drug Administration (FDA) has approved only IHC as the method of choice for detecting CTCs of epithelial origin in clinical practice.

Unfortunately, CTCs in OC are present in low concentration (1/109 blood cells or 1/106 nucleated blood cell); hence, pre-enrichment methods to highlight their presence are not only needed but mandatory. Methods to enrich and detect these clusters of cells are size-based, density-based, immunomagnetic separation, microfluidic-based [26].

Association of CTCs and clinical outcome

In accordance with other studies, Obermayr et al. [20] has detected CTCs at baseline in 24 % of the patients with primary [27–29]. Studies of CTCs in OC patients demonstrated that CTCs are associated with poor clinical outcome [30–34].

Pearl et al. showed significant differences in CTCs' detection rates in OC patients with regard to International Federation of Gynecology and Obstetrics (FIGO) tumor stage (90.7 % in stage III–IV patients vs 46.4 % in stage I–II patients, $p<0.00001$), PFS (4 vs 30 months $p=0.024$), and OS (5 vs 41 months, $p=0.0219$) [30]. CTC detection rates did not differ based on age, tumor grade, histology, amount of residual disease, and platinum sensitivity [30]. Considering that in OC patients, progression from intraperitoneal tumor residues generally occurs much earlier than the development

of distant metastasis (median lead time of 23 to 56 months) [35], CTC detection in OC could be associated with adverse clinicopathological features and a worse clinical outcome.

A significant decrease in OS was found in OC patients with detectable CTCs (35 vs 15 months of median survival, respectively $p=0.042$) [36]. Another study showed that patients with complete resection of the primary tumor had a significantly lower CTC detection rate than patients with macroscopic residual disease after surgery, thus suggesting a correlation with prognosis [29]. A similar correlation had been previously shown with the Treg cell population [37, 38]. These data have been later confirmed assuming that a persistence of CTCs after chemotherapy could be a strong indicator of poor therapy response as well [39].

On the contrary, some evidences have shown a negative association in terms of progression-free survival and overall survival and CTCs [29, 36]. Thus, the prognostic value of CTCs in OC remains controversial. In order to solve this unanswered question, three meta-analyses interrogating on the prognostic value of CTCs in OC have been performed in 2015 [23, 40, 41]. These meta-analyses have found a strong relationship of CTCs with advanced FIGO stage and treatment response in patients with OC. No significant association was observed between CTCs and histological subtypes, macroscopic residual disease, lymph nodes metastasis [23], and oncologic outcome [40]. Furthermore, the presence of CTCs was closely associated with elevated CA-125 blood values [40].

Interestingly, the meta-analytic data did not support a significant association with residual disease after surgery [23, 40]. Such an association has only been demonstrated only in few studies [20, 29].

A limitation of these meta-analyses is represented by the heterogeneity of the methods adopted in the different studies such as method used to identify CTCs and the cutoff used to predict clinical outcome [39, 42, 43].

Globally, despite the conflicting results reported in the literature (Table 1), all data arising from the recent meta-analyses [23, 40, 41] support a strong correspondence between CTCs detection and oncologic outcome.

Future directions

The identification of new targets in OC has recently permitted to test new drugs that are now trying to change the biological history of this disease [44–48]. Unfortunately, there is a lack of knowledge in selecting patients and in monitoring the response to these treatments. The immune system could offer a potential environment in which target drugs and immunotherapy could be better monitorized. However, this kind of monitoring represents an indirect method that could be affected by many variables (e.g., immunosoppressive medications with steroids, autoimmune disease, or immune-response exhaustion). A treatment response

Table 1 Oncologic significance of circulating tumor cells in ovarian cancer

Author	Year	Study type	Population	Pts n	Methods	FIGO/Grade	OS	DFS	Follow-up, months (range)
Marth et al.	2002	Case series	POC	90	Immunomagnetic beads (Dynabeads®)	I–IV/G1-3	NS	NS	25 (16–39)
Judson et al.	2003	Case series	POC/ROC PC	53 6	Veridex CellSearch System	I–IV/G1-3	NS	NS	18.7 (12–25)
Fan et al.	2009	Retrospective cohort	POC ROC	25 33	CAM cell invasion assay	I–IV/G1-4	NS	$p=0.042$	18 (1–80)
Aktas et al.	2011	Prospective	POC	122	Immunomagnetic selection+multiplex RT-PCR	Ia–IV/G1-4	$p=0.054$–0.047	NS	28.4 (2–169)
Behbacht et al.	2011	Prospective	ROC	54	CellSearch System	Recurrent/G1-3	NA	NA	>6
Poveda et al.	2011	Prospective	ROC	216	CellSearch System (EpCAM cell isolation)	NA	$p=0.0017$	$p=0.0024$	10 (1–25)
Kuhlman et al.	2012	Prospective	POC Healthy	63 20	PCRbased fluoresc microsatellite analisys (QIAamp DNA minikit)	I–IV/G1-4	$p=0.030$	NS	36 (1–70)
Liu et al.	2013	Prospective	POC ROC	30 48	CellSearch System (EpCAM cell isolation)	III–IV/G1-3	NS	NS	30 (2–60)
Obermayr et al.	2013	Multicenter	POC	216	CellSearch System (EpCAM cell isolation)	II–IV/G1-3	$p=0.001$	$p=0.001$	52 (1–69)
Pearl et al.	2014	Retrospective	Benign POC PC Fallopian	41 76 9 3	CAM cell invasion assay	I–IV/G1-3	$p<0.0001$	$p<0.0001$	21.4 (0–140.9)
Pearl et al.	2015	Prospective	POC Benign Healthy	123 49 64	CAM cell invasion assay	I–IV/NA	NA	$p<0.00001$	NA

POC, primary OC, *ROC* recurrent OC, *CAM* cell adhesion matrix, *ICC* immunocytochemistry, *PC* peritoneal cancer, *NA* not available, *NS* not significant

surveillance model based on clinical response and the effective quantification of tumor cells detected in the bloodstream could be hypothesized in the future. However, an international agreement of the definition of 'positive' CTCs in future trial is necessary. Furthermore, more reproducible test to detect and amplify CTCs populations is required. If these goals will be achieved, the detection of CTCs may become a valuable tool to integrate in clinical management in future years.

Conclusion

Treatment of OC is fortunately evolving into a more individualized approach, with a better understanding of the molecular composition of each patient's tumor. Data from the literature support a correlation of CTCs not only with advanced stage and poor prognosis in patients with OC but also with treatment response, suggesting that CTCs could be used as an early predictive marker of tumor response in OC patients undergoing conventional or targeted therapy.

Compliance with ethical standards

Conflicts of interest None

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics. CA Cancer J Clin. 2013;63(1):11–30.
2. Papadia A, Morotti M. Diaphragmatic surgery during cytoreduction for primary or recurrent epithelial ovarian cancer: a review of the literature. Arch Gynecol Obstet. 2013;287(4):733–41.
3. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med. 2006;354(1):34–43.
4. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26):2473–83.
5. Verri E, Guglielmini P, Puntoni M, Perdelli L, Papadia A, Lorenzi P, et al. HER2/neu oncoprotein overexpression in epithelial ovarian cancer: evaluation of its prevalence and prognostic significance. Clinical study. Oncology. 2005;68(2–3):154–61.
6. Gasparri ML, Attar R, Palaia I, Perniola G, Marchetti C, Di Donato V, et al. Tumor infiltrating lymphocytes in ovarian cancer. Asian Pac J Cancer Prev. 2015;16(9):3635–8.
7. Emerson RO, Sherwood AM, Rieder MJ, Guenthoer J, Williamson DW, Carlson CS, et al. High-throughput sequencing of T-cell receptors reveals a homogeneous repertoire of tumour-infiltrating lymphocytes in ovarian cancer. J Pathol. 2013;231(4):433–40.
8. Kan CW, Hahn MA, Gard GB, Maidens J, Huh JY, Marsh DJ, et al. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. BMC Cancer. 2012;12:627.
9. Martignetti JA, Camacho-Vanegas O, Priedigkeit N, Camacho C, Pereira E, Lin L, et al. Personalized ovarian cancer disease surveillance and detection of candidate therapeutic drug target in circulating tumor DNA. Neoplasia. 2014;16(1):97–103.
10. Attar R, Gasparri ML, Donato VD, Yaylim I, Halim TA, Zaman F, et al. Ovarian cancer: interplay of vitamin D signaling and miRNA action. Asian Pac J Cancer Prev. 2014;15(8):3359–62.
11. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004;10(20):6897–904.
12. Pukazhendhi G, Glück S. Circulating tumor cells in breast cancer. J Carcinog. 2014;13:8.
13. Romiti A, Raffa S, Di Rocco R, Roberto M, Milano A, Zullo A, et al. Circulating tumor cells count predicts survival in colorectal cancer patients. J Gastrointestin Liver Dis. 2014;23(3):279–84.
14. Fiorelli A, Accardo M, Carelli E, Angioletti D, Santini M, Di Domenico M. Circulating tumor cells in diagnosing lung cancer: clinical and morphologic analysis. Ann Thorac Surg. 2015;99(6):1899–905.
15. Reeh M, Effenberger KE, Koenig AM, Riethdorf S, Eichstädt D, Vettorazzi E, Uzunoglu FG, Vashist YK, Izbicki JR, Pantel K, Bockhorn M. Circulating tumor cells as a biomarker for preoperative prognostic staging in patients with esophageal cancer. Ann Surg 2015.
16. Liu Y, Wang YR, Wang L, Song RM, Zhou B, Song ZS. Significance of detecting circulating hepatocellular carcinoma cells in peripheral blood of hepatocellular carcinoma patients by nested reverse transcription-polymerase chain reaction and its clinical value: a retrospective study. Tumori. 2014;100(5):536–40.
17. Tjensvoll K, Nordgård O, Smaaland R. Circulating tumor cells in pancreatic cancer patients: methods of detection and clinical implications. Int J Cancer. 2014;134(1):1–8.
18. Goodman Jr OB, Symanowski JT, Loudyi A, Fink LM, Ward DC, Vogelzang NJ. Circulating tumor cells as a predictive biomarker in patients with hormone-sensitive prostate cancer. Clin Genitourin Cancer. 2011;9(1):31–8.
19. Bogani G, Liu MC, Dowdy SC, Cliby WA, Kerr SE, Kalli KR, et al. Detection of circulating tumor cells in high-risk endometrial cancer. Anticancer Res. 2015;35(2):683–7.
20. Obermayr E, Castillo-Tong DC, Pils D, Speiser P, Braicu I, Van Gorp T, et al. Molecular characterization of circulating tumor cells in patients with ovarian cancer improves their prognostic significance—a study of the OVCAD consortium. Gynecol Oncol. 2013;128(1):15–21.
21. Cain JM, Ellis GK, Collins C, Greer BE, Tamimi HK, Figge DC, et al. Bone marrow involvement in epithelial ovarian cancer by immunocytochemical assessment. Gynecol Oncol. 1990;38(3):442–5.
22. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003;3(6):453–8.
23. Cui L, Kwong J, Wang CC. Prognostic value of circulating tumor cells and disseminated tumor cells in patients with ovarian cancer: a systematic review and meta-analysis. J Ovarian Res. 2015;8(1):38.
24. Obermayr E, Sanchez-Cabo F, Tea MK, Singer CF, Krainer M, Fischer MB, et al. Assessment of a six gene panel for the molecular detection of circulating tumor cells in the blood of female cancer patients. BMC Cancer. 2010;10:666.
25. Ring AE, Zabaglo L, Ormerod MG, Smith IE, Dowsett M. Detection of circulating epithelial cells in the blood of patients with breast cancer: comparison of three techniques. Br J Cancer. 2005;92(5):906–12.
26. Lowes LE, Allan AL. Recent advances in the molecular characterization of circulating tumor cells. Cancers (Basel). 2014;6(1):595–624.
27. Wimberger P, Heubner M, Otterbach F, Fehm T, Kimmig R, Kasimir-Bauer S. Influence of platinum-based chemotherapy on

disseminated tumor cells in blood and bone marrow of patients with ovarian cancer. Gynecol Oncol. 2007;107(2):331–8.
28. Judson PL, Geller MA, Bliss RL, Boente MP, Downs Jr LS, Argenta PA, et al. Preoperative detection of peripherally circulating cancer cells and its prognostic significance in ovarian cancer. Gynecol Oncol. 2003;91(2):389–94.
29. Aktas B, Kasimir-Bauer S, Heubner M, Kimmig R, Wimberger P. Molecular profiling and prognostic relevance of circulating tumor cells in the blood of ovarian cancer patients at primary diagnosis and after platinum-based chemotherapy. Int J Gynecol Cancer. 2011;21(5):822–30.
30. Sang M, Wu X, Fan X, Sang M, Zhou X, Zhou N. Multiple MAGE-A genes as surveillance marker for the detection of circulating tumor cells in patients with ovarian cancer. Biomarkers. 2014;19(1): 34–42.
31. Pearl ML, Zhao Q, Yang J, Dong H, Tulley S, Zhang Q, et al. Prognostic analysis of invasive circulating tumor cells (iCTCs) in epithelial ovarian cancer. Gynecol Oncol. 2014;134(3):581–90.
32. Kuhlmann JD, Wimberger P, Bankfalvi A, Keller T, Schöler S, Aktas B, et al. ERCC1-positive circulating tumor cells in the blood of ovarian cancer patients as a predictive biomarker for platinum resistance. Clin Chem. 2014;60(10):1282–9.
33. Fehm T, Banys M, Rack B, Janni W, Marth C, Blassl C, et al. Pooled analysis of the prognostic relevance of disseminated tumor cells in the bone marrow of patients with ovarian cancer. Int J Gynecol Cancer. 2013;23(5):839–45.
34. Schindlbeck C, Hantschmann P, Zerzer M, Jahns B, Rjosk D, Janni W, et al. Prognostic impact of KI67, p53, human epithelial growth factor receptor 2, topoisomerase IIalpha, epidermal growth factor receptor, and nm23 expression of ovarian carcinomas and disseminated tumor cells in the bone marrow. Int J Gynecol Cancer. 2007;17(5):1047–55.
35. Dauplat J, Hacker NF, Nieberg RK, Berek JS, Rose TP, Sagae S. Distant metastases in epithelial ovarian carcinoma. Cancer. 1987;60(7):1561–6.
36. Fan T, Zhao Q, Chen JJ, Chen WT, Pearl ML. Clinical significance of circulating tumor cells detected by an invasion assay in peripheral blood of patients with ovarian cancer. Gynecol Oncol. 2009;112(1):185–91.
37. Bellati F, Visconti V, Napoletano C, Antonilli M, Frati L, Panici PB, et al. Immunology of gynecologic neoplasms: analysis of the prognostic significance of the immune status. Curr Cancer Drug Targets. 2009;9(4):541–65.
38. Gasparri ML, Bellati F, Napoletano C, Panici PB, Nuti M. Interaction between Treg cells and angiogenesis: a dark double track. Int J Cancer. 2013;132(10):2469.
39. Poveda A, Kaye SB, McCormack R, Wang S, Parekh T, Ricci D, et al. Circulating tumor cells predict progression free survival and overall survival in patients with relapsed/recurrent advanced ovarian cancer. Gynecol Oncol. 2011;122(3):567–72.
40. Zhou Y, Bian B, Yuan X, Xie G, Ma Y, Shen L. Prognostic value of circulating tumor cells in ovarian cancer: a meta-analysis. PLoS One. 2015;10(6):e0130873. **eCollection 2015**.
41. Zeng L, Liang X, Liu Q, Yang Z. The predictive value of circulating tumor cells in ovarian cancer: a meta analysis. Int J Gynecol Cancer 2015.
42. Behbakht K, Sill MW, Darcy KM, Rubin SC, Mannel RS, Waggoner S, et al. Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a Gynecologic Oncology Group study. Gynecol Oncol. 2011;123(1):19–26.
43. Liu JF, Hirsch MS, Lee H, Matulonis UA. Prognosis and hormone receptor status in older and younger patients with advanced-stage papillary serous ovarian carcinoma. Gynecol Oncol. 2009;115(3): 401–6.
44. Marchetti C, Imperiale L, Gasparri ML, Palaia I, Pignata S, Boni T, et al. Olaparib, PARP1 inhibitor in ovarian cancer. Expert Opin Invest Drugs. 2012;21(10):1575–84.
45. Leone Roberti Maggiore U, Bellati F, Ruscito I, Gasparri ML, Alessandri F, Venturini PL, et al. Monoclonal antibodies therapies for ovarian cancer. Expert Opin Biol Ther. 2013;13(5):739–64.
46. Bellati F, Napoletano C, Gasparri ML, Visconti V, Zizzari IG, Ruscito I, Caccetta J, Rughetti A, Benedetti-Panici P, Nuti M. Monoclonal antibodies in gynecological cancer: a critical point of view. Clin Dev Immunol 2011; 890758. Epub 2011 Dec 26. Review.
47. Bellati F, Napoletano C, Gasparri ML, Ruscito I, Marchetti C, Pignata S, et al. Current knowledge and open issues regarding bevacizumab in gynaecological neoplasms. Crit Rev Oncol Hematol. 2012;83(1):35–46.
48. Bellati F, Napoletano C, Ruscito I, Visconti V, Antonilli M, Gasparri ML, et al. Past, present and future strategies of immunotherapy in gynecological malignancies. Curr Mol Med. 2013;13(4): 648–69.