Chapter 17

Disseminated Tumor Cells and Cancer Stem Cells in Ovarian Cancer

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1. Introduction

Ovarian cancer is currently the fifth most lethal malignancy of women in Europe and the United States [1, 2]. The prognosis of ovarian cancer patients is limited due to lack of specific early symptoms and a high rate of relapse; more than half of all patients will suffer from disease recurrence, resulting in a poor overall survival [3]. Most cases are diagnosed in advanced stages, and although the initial response to chemotherapy is generally good, a significant proportion of patients will suffer from a relapse despite optimal cytoreductive surgery [4]. Since treatment strategies are mainly developed to control locoregional cancer growth, it may be anticipated that more women will die of distant metastatic disease. The identification of novel molecular markers, reflecting current tumor activity, may improve prediction and therapy monitoring and provide valuable insights into process of carcinogenesis. In this regard, oncologic research have increasingly focused on disseminated and circulating tumor cells.

The presence of disseminated tumor cell (DTC) in bone marrow (BM) is a phenomenon observed in almost all solid tumors of epithelial origin. For breast cancer, DTC presence has been demonstrated as a strong independent prognostic factor (level I evidence) [5]. Available data support the notion that hematogenous tumor cell dissemination may be clinically relevant in ovarian cancer as well [6-10]. Detection rates of DTC, as a surrogate parameter for occult hematogenous spread, vary between 30-50% primary ovarian cancer patients.

A provocative hypothesis has been introduced recently with respect to natural history and progression of ovarian cancer. While the 'classical' stochastic model of cancer development holds that any cell may become source of malignant transformation, emerging evidence sup-

ports the view that only a minor subpopulation of cancer cells has the potential to initiate cancer growth. These cells, called cancer stem cells (CSC), have the ability to self-renew, propagate tumorigenesis and are usually drug-resistant [11]. Experimental studies on stem cell biology have given new impetus to the cancer stem cell theory. CSC are assumed to play important role in development of various tumor entities, such as breast and gastrointestinal cancer, retinoblastoma and ovarian cancer [12], [13]. Interestingly, ovarian cancer cell lines feature "side population" cells with potential to differentiate into cancers with different histologies, suggesting the pluripotent character of stem cells [14]. Whether DTC in extraperitoneal sites, such as bone marrow, reflect a stem cell-like sub population of tumor cells, remains yet to be cleared.

Of all prognostic factors, monitoring of minimal residual disease is the only one available after the tumor has been removed. Beside monitoring of tumor markers, there is currently a major effort to identify other biological markers which can be assessed with minimally invasive methods and persist beyond surgery. We previously reported on a significant correlation of positive BM status with shortened relapse-free survival in ovarian cancer patients [6]. DTC persistence after completion of platinum-based chemotherapy was also found to be prognostically relevant [15]. Recently, attempts have been made to target DTC by using antibody-based therapy with the trifunctional antibody catumaxomab. Wimberger et al. reported a marked decrease in tumor cells in peripheral blood following intraperitoneal catumaxomab treatment for malignant ascites, indicating a systemic effect of the therapy [16]. However, in comparison with breast cancer, data on DTC detection in gynecological malignancies are so far limited [7], [17], [18], [19], [9].

In this chapter we discuss recent advances in ovarian cancer research with respect to disseminated tumor cells and cancer stem cell hypothesis. Data on prognostic and clinical relevance are presented.

2. Disseminated and circulating tumor cells in ovarian cancer

Detection and characterization of disseminated tumor cells in bone marrow and blood of patients with epithelial carcinomas can be accomplished by various techniques. For the detection of isolated tumor cells, both antibody-based assays and molecular assays have been established [20, 21]. Despite advances in this field, no specific antigen or marker gene has been described for ovarian cancer so far. Therefore, immunocytochemical identification of these cells based on expression of epithelial markers remains the gold standard (Figure 1). Commonly targeted antigens are cytokeratin and EpCAM due to relatively constant and universal expression pattern in cells of epithelial origin [9, 15, 22]. A major difficulty in detecting and characterizing tumor cells is their relatively low frequency. Most protocols include therefore a cell enrichment step (e.g. density gradient centrifugation, immunomagnetic enrichment). These obstacles highlight the need for optimization of the assay (e.g. by minimizing cell loss, preserving cell morphology and producing reliable immunophenotypic and genotypic data) as it is essential for detecting, enumerating and characterizing single tumor cells [21].

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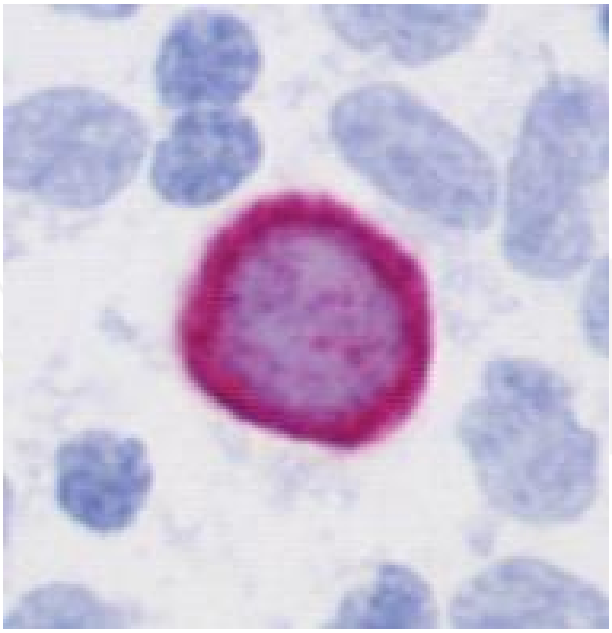

Figure 1. Disseminated tumor cell from ovarian cancer patient with typical cytomorphology and immunophenotype (positive cytokeratin-staining, large nucleus, high nuclear to cytoplasmic ratio, nucleus partially covered by CK-staining, nucleus granular [23].

Detection rates of disseminated tumor cells in ovarian cancer patients stage FIGO I-III reach 20-60% [7, 9, 15, 22]. These results suggest hematogenous spread to be a comparatively frequent phenomenon in ovarian malignancies and indicate the ability of single tumor cells to disseminate to bone marrow in a very early stage of disease. DTC are routinely detected in 13-18% FIGO I ovarian cancer patients [6, 19, 22]. Since bone metastases are relatively rare in ovarian cancer patients, BM seems to serve as a temporary `homing site´ for single tumor cells, from where they are able to migrate and subsequently cause distant metastasis or local recurrence [24]. Assuming that DTC may spread by means of blood stream we cannot exclude that those may also be able to repopulate the peritoneal cavity, an environment which easily supports ovarian cancer growth [11, 25].

Based on numerous studies, no significant relationship has been reported between clinicopathological characteristics of primary tumor and DTC detection. In our latest trial with 414 ovarian cancer patients, DTC status did not correlate with FIGO stage, tumor size, lymph node status, histopathologic grading or resection status [26, 27]. Braun et al., in a cohort of 108 primary ovarian cancer patients, showed no concordance between classical prognostic factors and DTC positivity. The only factor associated with positive DTC status was tumor grading ($p = 0.02$) [7]. These results could be also confirmed in our earlier study with 112 ovarian cancer patients [6]. Similar findings were obtained by other investigators [8, 9, 15, 19].

2.1. Prognostic relevance of DTC/CTC in ovarian cancer

Detection of disseminated tumor cells in patients with primary ovarian cancer was shown to be of prognostic value (Table 1). However, the currently available data are sparse. In our latest trial bone marrow status of 414 ovarian cancer patients was correlated with clinical follow-up [26]. The presence of DTC predicted a shorter OS ($p < 0.001$) and DFS ($p = 0.035$) compared with BM negative patients [27]; this association was highly significant and confirmed in a multivariable Cox regression analysis. Similar results were found in several smaller studies. Braun et al. demonstrated unfavorable prognosis with regard to distant DFS in BM positive patients at the time of diagnosis [7]. DTC presence remained a strong prognostic factor also in a subset of 64 optimally debulked patients ($p = 0.002$), which highlights the role of DTC detection especially in patients who received successful surgical cytoreduction. We previously reported a significant correlation of positive BM status with reduced DFS in a group of 112 stage FIGO I-III ovarian cancer patients [6]. Interestingly, in some studies, the presence of isolated tumor cells in secondary sites, such as BM and blood, was also associated with higher risk for recurrence [10], [6]. Therefore, it might be speculated that hematogenous tumor cell dissemination may serve as an indicator of a more aggressive phenotype of the primary disease that is likely to cause local relapse. In contrast, other authors reported no significant correlation between DTC detection and clinical outcome in ovarian cancer [19, 28]. This discrepancy might be due to differences in study protocols, e.g. time point of BM sample collection (pre- vs. postoperative aspiration). Hypothetically, a transient increase in cancer cell dissemination from the primary tumor due to intraoperative manipulation could contribute to false-positive results and therefore affect further analysis [29].

2.2. Circulating tumor cells

Bone marrow biopsy represents an invasive procedure not well tolerated by many patients. Therefore, detection of circulating tumor cells (CTC) by simple blood drawing has increasingly become a focus of translational research. Prognostic significance of CTC in peripheral blood has been evaluated in breast cancer both in primary and metastatic disease [33, 34]. Two commercially available kits are currently in use for CTC detection in blood of breast cancer patients: antibody-based CellSearch and RT-PCR-based AdnaTest. Both assays were modified and validated in ovarian cancer patients (Table 1). Recently published trial by Poveda et al. based on a cohort of 216 patients with recurrent ovarian cancer, represents the largest study so far on the impact of CTC presence on survival [10]. Using CellSearch test increased CTC numbers (> 1 cell / 7.5 ml blood) were found in 14% of these patients before the beginning of treatment. Detection of CTC in peripheral blood was associated with significantly impaired prognosis. In the study by Aktas et al., including 86 ovarian cancer patients, a modified AdnaTest kit was used to detect cells expressing EpCAM, MUC-1, HER-2 or CA 125-transcripts [8]. CTC positivity rate of 19% observed in this cohort was associated with significantly shorter survival independent of the time of blood sampling (before surgery or after chemotherapy). Similar results were obtained by Fan et al. in a trial of 66 primary ovarian cancer patients [32]. In contrast, Marth et al. reported a 12% positivity rate

irrespective of tumor stage but observed no correlation with clinical outcome [19]. Interestingly, positive finding in the blood was highly associated with DTC detection in bone marrow. Smaller studies showed varying CTC incidence, depending on methodology [35, 36].

Author	N	Method	Median follow-up [months]	Positivity rate	Prognostic significance
Fehm [26]	414	DTC (ICC)	34	27%	OS, DFS [1]
Banys [6]	112	DTC (ICC)	12	25%	DFS
Braun [7]	108	DTC (ICC)	45	30%	DFS
Aktas [8]	95	DTC (ICC)	28	35%	n.s.
Schindlbeck [9]	90	DTC (ICC)	28	23%	DDFS
Marth [19]	73	DTC (immunobeads)	25	21%	n.s.
Wimberger [30]	62	DTC (ICC)	18	54%	DFS [2]
Poveda [10]	216	CTC (ICC: CellSearch) [3]		14% [4]	PFS, OS
Sehouli [17]	167	CTC (ICC)	46		n.s.
Marth [19]	90	CTC (immunomagnetic beads)	25	12%	n.s.
Aktas [8]	86	CTC (Multiplex-RT-PCR: AdnaTest)	28	19%	OS [5]
Heubner [31]	68	Circulating 20S-proteasomes	19	-	OS
Fan [32]	66	CTC (immunofluorescence, cell invasion assay)	18	61%	DFS
Wimberger [30]	62	Circulating nucleosomes, DNA, protease and caspase activity	18	-	DFS, OS

Abbreviations: DFS – disease-free survival, DDFS – distant disease-free survival, DTC – disseminated tumor cells in bone marrow, ICC – immunocytochemistry, n.s. – not significant, PFS – progression-free survival

[1] Determined by multivariate Cox regression analysis

[2] DTC detected after chemotherapy

[3] Relapsed ovarian cancer

[4] Two or more CTC

[5] Both before and after chemotherapy

Table 1. Prognostic relevance of disseminated and circulating tumor cells in ovarian cancer.

2.3. Therapy monitoring

Beyond the prognostic value of DTC/CTC detection, monitoring of minimal residual disease (MRD) during and after treatment offers the opportunity to assess response to therapy and evaluate the residual risk of recurrence. Changes in MRD represent the only clinical parame-

ter available after surgical removal of the primary tumor. While tumor markers are established tools for the evaluation of treatment efficacy in patients with advanced ovarian cancer, CA 125 levels fall during adjuvant chemotherapy and remain often below cut-off values after completion of first line systemic treatment even though significant number of patients will suffer from relapse within five years. Furthermore, the clinical relevance of serial CA125 measurements for early detection and treatment of disease recurrence is currently being controversially discussed [37]. In this regard, the detection of isolated tumor cells in bone marrow or peripheral blood might serve as a parameter for occult tumor load after completion of first line therapy. DTC persistence despite adjuvant treatment is so far an independent prognostic factor in patients with primary breast cancer [38]. Whether persistent DTC influence prognosis in ovarian cancer patients, is currently being investigated. In the study by Wimberger et al. DTC counts before and after the first line systemic treatment were correlated to clinical course of disease in 30 ovarian cancer patients; 54% of these patients presented with DTC after first-line chemotherapy. Marked increase in DTC counts was associated with shortened progression free survival [15].

Evaluation of treatment efficacy in ovarian cancer patients after optimal surgical cytoreduction and completion of first line systemic therapy is based on clinical, radiological and biological (tumor marker CA125) criteria. However, a reliable tool to assess long-term prognosis has so far not been established. Therefore, a solid therapy monitoring tool could help to identify a group of high-risk patients who potentially benefit from additional treatment. New studies are required to evaluate whether persistent DTC indeed predict a worse prognosis and if these cells may be targeted by secondary adjuvant therapy.

3. Cancer stem cell model

An important hypothesis on tumor initiation and progression has attracted much attention in the ovarian cancer research in the past decade. In contrast to stochastic model that postulates every cell as a potential source of malignant transformation, the cancer stem cell model, a theory introduced over a century ago, proposes that tumors are organized in a cellular hierarchy, in which cancer stem cell (CSC) are the only cells with tumorigenic potential. Accordingly, tumors are initiated in cancer stem cells or their immediate progeny through imbalance of self-renewal and apoptosis; these tumors contain a cellular subpopulation that retains key stem cell feature [39]. This small cell group with unlimited proliferative potential is assumed to play a marked role in initiation and development of several tumor entities like retinoblastoma, gastrointestinal cancer as well as breast and ovarian cancer [12]. The cancer stem cell concept has important implications for understanding the process of carcinogenesis as well as for designing new treatment strategies. Due to their long life, CSC are more likely to acquire transforming mutations; further, they seem more resistant to apoptosis and DNA damage and are therefore able to persist beyond therapy. New evidence in support of the cancer stem cell model has arisen due to advances in stem cell biology and the introduction of novel animal models to assess self-renewal and challenge the validity of this concept.

The cancer stem cell hypothesis holds that CSC are responsible for phenomena like drug resistance, tumor dormancy or minimal residual disease and may persist beyond chemotherapy and repopulate the tumor leading to relapse [11]. The stem cell subpopulation, but not the remaining differentiated cancer cells in the tumor, can sustain tumor formation and growth due to their high tumor-initiating potential. So far, such cells have been found in several solid tumors, such as colon [40], breast [41] and ovarian cancer [13, 42, 43]. Indeed, in accordance with recent studies, ovarian cancer cell lines feature ´side population´ cells (SP) with potential to differentiate into other morphological entities. Thus, this pluripotent subclone with stem cell-like features is considered a marker of CSC presence [14]. The detection of CSC is based on the presence of extracellular markers assumed to be stem cell-specific; commonly identified markers are CD44, CD133, and CD24, which are found in prostate, breast, pancreas, and ovarian cancer. It remains under discussion whether these parameters are universal markers relevant for CSC derived from all tumor types; for ovarian cancer, multiple markers have been described for the stem cell-like tumor initiating cells.

Owing to aggressive natural course of disease and emergence of multidrug resistance, an essential role of cancer stem cells has been postulated in ovarian cancer [13, 44]. In the study by Szotek et al. SP cells have been encountered in ovarian cancer cell lines as well as in primary ascites cancer cells [13]. In the trial by Hosonuma et al. in 18 of 28 ovarian cancer patients samples side population cells could be detected. SP cells occurred more often in relapsed and metastatic patients and SP positivity predicted significantly reduced overall survival [15]. A high proportion of CD44+ stem cells in ovarian cancer was reported to be an independent predictor of poor progression-free survival [45].

As previously mentioned, CSC are considered responsible for high emergence of drug resistance in the natural history of ovarian cancer, since standard therapies fail to target pluripotent tumor-initiating cells [13]. Latifi et al. could show in their recently published trial, the ability of cisplatin chemotherapy to generate residual tumor cells with mesenchymal stem cell-like characteristics *in vitro* [46]. Accordingly, new therapeutic strategies have to be developed to target these cells by identifying their specific antigens. However, very few tumor characteristics have been described to target the subset of CSC.

Based on an animal model, Bapat et al. reported the isolation and identification of ovarian cancer stem cells [42]. In an *in vitro* model comprised of 19 spontaneously immortalized clones derived from an advanced-stage patient, the authors demonstrated the ability of two clones with stem cell-like characteristics to differentiate to grow as spheroids and form xenografts in an animal model (nude mice). These cells were shown to express CD44, E-cadherin, and the stem cell factors Nestin, Nanong, and Oct-4.

3.1. DTC and cancer stem cell model

A currently discussed theory postulate DTC and CTC, the presumed precursor cells of systemic metastatic disease, to be in fact cancer stem cells. These observations have been so far reported in breast cancer studies. Balic et al. analyzed bone marrow specimens from 50 primary breast cancer patients; 33-100% of DTC of every patient exhibited stem cell-like phenotype: CD44+ / CD24 low/- [47]. This prevalence is estimated for less than 10% in primary

tumor suggesting much higher, stem cell like self-renewal and tumorigenic potential in DTC. Aktas et al. detected stem cells markers on CTC in peripheral blood of metastatic breast cancer patients [41]. Moreover, Abraham et al. reported that high proportion of stem cell-like subpopulation in primary breast cancer correlate with a higher prevalence of distant metastases [48]. As breast cancer stem cells have been shown to be generally triple-negative, basal-like CTC, independent of the phenotype of the primary tumor, support the cancer stem cell theory [20, 49, 50]. However, this aspect has not been researched in ovarian cancer so far. Therefore, futher studies have to be performed to evaluate whether isolated tumor cells in extraperitoneal sites, such as blood and bone marrow, may reflect ovarian cancer stem cell population.

4. Conclusions

Despite advances in surgical and systemic therapy, ovarian cancer leads to relapse in 60% of patients within 5 years, resulting in impaired clinical outcome. In the last decades, novel biomarkers have been introduced for better prediction and prognostication [51]. Early hematogenous dissemination of single tumor cells is a general phenomenon observed in most solid tumors of epithelial origin; recent data supports the clinical relevance of disseminated and circulating tumor cells (DTC/CTC) in ovarian cancer. A currently discussed hypothesis postulates isolated tumor cells in secondary sites to be not only the presumed precursors of systemic metastatic disease but in fact pluripotent 'cancer stem cells'. Future research will clarify the implications of these findings for clinical management of ovarian cancer patients. While the published data do not support the use of DTC/CTC detection for early detection or screening purposes, its role as an important prognostic factor has been confirmed in several studies. One of the most promising applications of DTC detection is their use as a therapy monitoring tool. DTC persistence beyond surgery and adjuvant chemotherapy may help to identify patients at risk of developing a relapse.

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References

[1] Ries LAG, Harkins D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, Clegg L, Eisner MP, Horner MJ, Howlader N et al: SEER Cancer Statistics Review, 1975-2003. Bethesda, MD 2006.

[2] Black RJ, Bray F, Ferlay J, Parkin DM: Cancer incidence and mortality in the European Union: cancer registry data and estimates of national incidence for 1990. Eur J Cancer 1997, 33(7):1075-1107.

[3] Tingulstad S, Skjeldestad FE, Halvorsen TB, Hagen B: Survival and prognostic factors in patients with ovarian cancer. Obstet Gynecol 2003, 101(5 Pt 1):885-891.

[4] Cannistra SA: Cancer of the ovary. N Engl J Med 2004, 351(24):2519-2529.

[5] Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, Schlimok G, Diel IJ, Gerber B, Gebauer G et al: A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 2005, 353(8):793-802.

[6] Banys M, Solomayer EF, Becker S, Krawczyk N, Gardanis K, Staebler A, Neubauer H, Wallwiener D, Fehm T: Disseminated tumor cells in bone marrow may affect prognosis of patients with gynecologic malignancies. Int J Gynecol Cancer 2009, 19(5): 948-952.

[7] Braun S, Schindlbeck C, Hepp F, Janni W, Kentenich C, Riethmuller G, Pantel K: Occult tumor cells in bone marrow of patients with locoregionally restricted ovarian cancer predict early distant metastatic relapse. J Clin Oncol 2001, 19(2):368-375.

[8] 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):822-830.

[9] Schindlbeck C, Hantschmann P, Zerzer M, Jahns B, Rjosk D, Janni W, Rack B, Sommer H, Friese K: 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):1047-1055.

[10] Poveda A, Kaye SB, McCormack R, Wang S, Parekh T, Ricci D, Lebedinsky CA, Tercero JC, Zintl P, Monk BJ: Circulating tumor cells predict progression free survival and overall survival in patients with relapsed/recurrent advanced ovarian cancer. Gynecol Oncol 2011, 122(3):567-572.

[11] Dyall S, Gayther SA, Dafou D: Cancer stem cells and epithelial ovarian cancer. J Oncol 2010, 2010:105269.

[12] Setoguchi T, Taga T, Kondo T: Cancer stem cells persist in many cancer cell lines. Cell Cycle 2004, 3(4):414-415.

[13] Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT, Donahoe PK: Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci U S A 2006, 103(30):11154-11159.

[14] Hosonuma S, Kobayashi Y, Kojo S, Wada H, Seino K, Kiguchi K, Ishizuka B: Clinical significance of side population in ovarian cancer cells. Hum Cell 2011, 24(1):9-12.

[15] 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):331-338.

[16] Wimberger P, Heubner M, Lindhofer H, Jager M, Kimmig R, Kasimir-Bauer S: Influence of catumaxomab on tumor cells in bone marrow and blood in ovarian cancer. Anticancer Res 2009, 29(5):1787-1791.

[17] Sehouli J, Konsgen D, Nimpsch R, Stengel D, Oskay G, Mustea A, Oertel J, Lichtenegger W: Prognostic significance of epithelial cells in the blood of patients with gynaecological malignancies. Anticancer Res 2003, 23(5b):4133-4140.

[18] Janni W, Hepp F, Strobl B, Rack B, Rjosk D, Kentenich C, Schindlbeck C, Hantschmann P, Pantel K, Sommer H et al: Patterns of disease recurrence influenced by hematogenous tumor cell dissemination in patients with cervical carcinoma of the uterus. Cancer 2003, 97(2):405-411.

[19] Marth C, Kisic J, Kaern J, Trope C, Fodstad O: Circulating tumor cells in the peripheral blood and bone marrow of patients with ovarian carcinoma do not predict prognosis. Cancer 2002, 94(3):707-712.

[20] Fehm T, Hoffmann O, Aktas B, Becker S, Solomayer EF, Wallwiener D, Kimmig R, Kasimir-Bauer S: Detection and characterization of circulating tumor cells in blood of primary breast cancer patients by RT-PCR and comparison to status of bone marrow disseminated cells. Breast Cancer Res 2009, 11(4):R59.

[21] Fehm T, Solomayer EF, Meng S, Tucker T, Lane N, Wang J, Gebauer G: Methods for isolating circulating epithelial cells and criteria for their classification as carcinoma cells. Cytotherapy 2005, 7(2):171-185.

[22] Fehm T, Becker S, Bachmann C, Beck V, Gebauer G, Banys M, Wallwiener D, Solomayer EF: Detection of disseminated tumor cells in patients with gynecological cancers. Gynecol Oncol 2006, 103(3):942-947.

[23] Fehm T, Braun S, Muller V, Janni W, Gebauer G, Marth C, Schindlbeck C, Wallwiener D, Borgen E, Naume B et al: A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation. Cancer 2006, 107(5):885-892.

[24] Pantel K, Brakenhoff RH: Dissecting the metastatic cascade. Nat Rev Cancer 2004, 4(6):448-456.

[25] Lengyel E: Ovarian cancer development and metastasis. Am J Pathol 2010, 177(3): 1053-1064.

[26] Fehm T, Banys M, Marth C, Neubauer H, Hartkopf A, Trope C, Kimmig R, Krawczyk N, Wallwiener D, Wimberger P et al: Detection of disseminated tumor cells in the bone marrow is an independent prognostic factor in primary ovarian cancer patients. in press 2012.

[27] Wimberger P, Banys M, Kasimir-Bauer S, Hartkopf AD, Krawczyk N, Marth C, Kimmig R, Neubauer H, Wallwiener D, Fehm TN: Detection of disseminated tumor cells in bone marrow as an independent prognostic factor in primary ovarian cancer patients (ASCO 2012). J Clin Oncol 2012, 30 (suppl; abstr 5042).

[28] Cain JM, Ellis GK, Collins C, Greer BE, Tamimi HK, Figge DC, Gown AM, Livingston RB: Bone marrow involvement in epithelial ovarian cancer by immunocytochemical assessment. Gynecol Oncol 1990, 38(3):442-445.

[29] Camara O, Kavallaris A, Noschel H, Rengsberger M, Jorke C, Pachmann K: Seeding of epithelial cells into circulation during surgery for breast cancer: the fate of malignant and benign mobilized cells. World J Surg Oncol 2006, 4:67.

[30] Wimberger P, Roth C, Pantel K, Kasimir-Bauer S, Kimmig R, Schwarzenbach H: Impact of platinum-based chemotherapy on circulating nucleic acid levels, protease activities in blood and disseminated tumor cells in bone marrow of ovarian cancer patients. Int J Cancer 2010.

[31] Heubner M, Wimberger P, Dahlmann B, Kasimir-Bauer S, Kimmig R, Peters J, Wohlschlaeger J, Sixt SU: The prognostic impact of circulating proteasome concentrations in patients with epithelial ovarian cancer. Gynecol Oncol 2011, 120(2):233-238.

[32] 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):185-191.

[33] Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW et al: Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004, 351(8):781-791.

[34] Rack BK, Schindlbeck C, Andergassen U, Schneeweiss A, Zwingers T, Lichtenegger W, Beckmann M, Sommer HL, Pantel K, Janni W: Use of circulating tumor cells (CTC) in peripheral blood of breast cancer patients before and after adjuvant chemotherapy to predict risk for relapse: The SUCCESS trial. ASCO Annual Meeting 2010. J Clin Oncol 2010, 28:15s (suppl; abstr 1003).

[35] Ntouroupi TG, Ashraf SQ, McGregor SB, Turney BW, Seppo A, Kim Y, Wang X, Kilpatrick MW, Tsipouras P, Tafas T et al: Detection of circulating tumour cells in peripheral blood with an automated scanning fluorescence microscope. Br J Cancer 2008, 99(5):789-795.

[36] Engel H, Kleespies C, Friedrich J, Breidenbach M, Kallenborn A, Schondorf T, Kolhagen H, Mallmann P: Detection of circulating tumour cells in patients with breast or ovarian cancer by molecular cytogenetics. Br J Cancer 1999, 81(7):1165-1173.

[37] Rustin GJ, van der Burg ME, Griffin CL, Guthrie D, Lamont A, Jayson GC, Kristensen G, Mediola C, Coens C, Qian W et al: Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. Lancet 2010, 376(9747):1155-1163.

[38] Janni W, Rack B, Schindlbeck C, Strobl B, Rjosk D, Braun S, Sommer H, Pantel K, Gerber B, Friese K: The persistence of isolated tumor cells in bone marrow from patients with breast carcinoma predicts an increased risk for recurrence. Cancer 2005, 103(5):884-891.

[39] Wicha MS, Liu S, Dontu G: Cancer stem cells: an old idea--a paradigm shift. Cancer Res 2006, 66(4):1883-1890; discussion 1895-1886.

[40] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R: Identification and expansion of human colon-cancer-initiating cells. Nature 2007, 445(7123):111-115.

[41] Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S: Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res 2009, 11(4):R46.

[42] Bapat SA, Mali AM, Koppikar CB, Kurrey NK: Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 2005, 65(8):3025-3029.

[43] Challen GA, Little MH: A side order of stem cells: the SP phenotype. Stem Cells 2006, 24(1):3-12.

[44] Ponnusamy MP, Batra SK: Ovarian cancer: emerging concept on cancer stem cells. J Ovarian Res 2008, 1(1):4.

[45] Steffensen KD, Alvero AB, Yang Y, Waldstrom M, Hui P, Holmberg JC, Silasi DA, Jakobsen A, Rutherford T, Mor G: Prevalence of epithelial ovarian cancer stem cells correlates with recurrence in early-stage ovarian cancer. J Oncol 2011, 2011:620523.

[46] Latifi A, Abubaker K, Castrechini N, Ward AC, Liongue C, Dobill F, Kumar J, Thompson EW, Quinn MA, Findlay JK et al: Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. J Cell Biochem 2011.

[47] Balic M, Lin H, Young L, Hawes D, Giuliano A, McNamara G, Datar RH, Cote RJ: Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res 2006, 12(19):5615-5621.

[48] Abraham BK, Fritz P, McClellan M, Hauptvogel P, Athelogou M, Brauch H: Prevalence of CD44+/CD24-/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. Clin Cancer Res 2005, 11(3):1154-1159.

[49] Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS: Stem cells in normal breast development and breast cancer. Cell Prolif 2003, 36 Suppl 1:59-72.

[50] Asselin-Labat ML, Shackleton M, Stingl J, Vaillant F, Forrest NC, Eaves CJ, Visvader JE, Lindeman GJ: Steroid hormone receptor status of mouse mammary stem cells. J Natl Cancer Inst 2006, 98(14):1011-1014.

[51] Banys M, Krawczyk N, Fehm T: New Tumor Biomarkers in Ovarian Cancer and Its Prognostic and Clinical Relevance. In: Ovarian Cancer - Basic Science Perspective. Edited by Farghaly SA: InTech; 2012.

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