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The direct molecular analysis of metastatic precursor cells in breast cancer: A chance for a better understanding of metastasis and for personalised medicine

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ABSTRACT

The search for disseminated cancer cells has become a routine procedure in many clinical centres since the pioneering work of Riethmüller and Schlimok was published in the mid 1980s. Until today, clinical studies have mostly focused on the prognostic role of disseminated cancer cells that can be detected in bone marrow samples before manifestation of metastasis. As a more recent development, the field is increasingly concentrating on the prognostic information provided by tumour cells circulating in the peripheral blood instead of analysing the nature of disseminated tumour cells that have successfully homed to a new microenvironment and may eventually grow into metastases. This review critically questions that direction and proposes exploiting the unique opportunities provided by the direct molecular analysis of metastatic precursor cells for a better understanding of metastasis, tumour dormancy, therapy target identification, and personalised medicine in an adjuvant therapy setting.

1. Brief historical and critical introduction

Soon after the Halsted-paradigm of a continuous lymphatic cancer spread from the local mammary cancer to distant metastatic sites had been falsified in large clinical studies,[1] the idea was born to search systematically for disseminated tumour cells (DTCs) in bone marrow samples of breast cancer patients. The concept was equally simple and convincing: since increasing numbers of patients with initially non-metastatic disease presented to the hospital, it became obvious that metastases emerging months to years after surgery are derived from tumour cells that had disseminated before resection of the primary tumour. Therefore, metastatic precursor cells should be detectable at distant sites and their eradication should enable prevention of later arising metastases. Both facets of the concept were promoted and actively pursued from the beginning by G. Riethmüller[2,3] who influenced many scientists working in the field today. To detect DTCs, bone marrow was chosen as the distant organ since specific histogenetic markers, epithelial cytokeratins, were identified as not being expressed in bone marrow but abundantly in epithelial cancer cells, and therefore enabled specific detection of DTCs. Moreover, by demonstrating *in vivo* binding of the antibody 17-1A, directed against the epithelial cell adhesion molecule (CD326 or EpCAM),[4] to single, early-disseminated tumour cells in patients,[3] and by testing the antibody in a randomised, prospective clinical trial,[5,6] Riethmüller promoted what could retrospectively be defined as the first cancer stem cell-directed therapy in an adjuvant setting. The story has taken this interesting turn since today EpCAM is commonly used to identify cancer stem cells, also termed tumour initiating cells, of various epithelial malignancies.[7–10] Together, these and other early studies paved the way for the currently emerging synthesis of research on early

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metastasis, single cell genomics, cancer stem cell biology, and personalised medicine.

During the first years after successful establishment of a detection assay, clinical and experimental studies concentrated on proving that cytokeratin-positive cells in bone marrow are indeed tumour cells, thereby also addressing fundamental questions of early metastasis and minimal residual disease (MRD). In contrast, recent efforts rather aim to facilitate implementation of MRD detection into clinical practice – mostly by substituting bone marrow for blood as the source of tumour cells. Therefore, far too few groups are actively engaged in exploring the opportunities that the characterisation of DTCs provides for cancer research and too little progress has been made in the molecular characterisation of DTCs. Thus, the reader may be referred to relatively old reviews[11,12] to catch up on most available data. Basic research on micrometastases - as DTCs were originally called - has got stuck in a premature stage with more reviews being published than original work as a typical symptom. This review hopes to raise interest in studying DTCs by summarising some recent and potentially thought-provoking findings.

2. A sceptical view on the role of DTCs or CTCs as prognostic marker

In 1987 the first systematic and relatively large series of 212 patients was published comprising patients with breast and colorectal cancer as well as an additional 75 noncarcinoma patients that were tested for the presence of cytokeratin-positive cells in bone marrow.[3] Cytokeratin-positive cells were only found in carcinoma patients. Subsequently, follow-up studies demonstrated prognostic relevance for positive samples, first for colorectal cancer[13,14] and then for breast cancer.[15] Since then, at least in breast cancer, the prognostic impact of DTCs could be firmly established by combining data from several studies.[16] However, 20 years after the first systematic search for DTCs, it becomes increasingly questionable whether the prognostic factor 'DTC' will be included in clinical routine outside of academic centres. While the enormous diversity of methodological approaches for DTC detection have largely been reduced by a consensus protocol,[17] recent technical developments[18,19] will further position the detection of circulating tumour cells in peripheral blood (CTCs) into the centre of clinical attention. Easier access to blood compared to bone marrow clearly supports this rationale; however, one should be aware that the prognostic role of CTCs has yet to be established (here, I do not consider PCR-based studies as they cannot differentiate between CTCs and circulating nucleic acids). While the prognostic significance of CTCs in metastatic patients could be confirmed for breast cancer[18,20] after a first report for melanoma had been published,[21] currently available data point to a lack of prognostic impact of CTCs in early stage patients.[22] Thus, the field is more or less starting *de novo* and it has to be awaited whether the numerous studies under way will eventually identify early stage patients with CTCs in their blood sample at risk of relapse or amenable for monitoring studies.[23] In the most optimistic scenario, CTC detection may eventually substitute DTC detection in bone marrow as prognostic marker.

But even if CTC or DTC detection will enable monitoring of systemic disease, it has to be demonstrated that it will provide prognostic information as precise as that derived by one of the many 'poor prognosis' signatures derived from microarray studies of the primary tumour.[24] Some of these signatures have been reduced to a number of genes that can be handled in routine laboratories either by small microarrays or a limited number of PCR reactions and are currently under prospective evaluation in large studies.[24] Eventually, we will need to compare the prognostic impact of DTC or CTC with these or other molecular assays. It can be foreseen that counting DTCs and CTCs, even during follow-up, is unlikely to change clinical decision-making unless these representatives of smouldering MRD will provide predictive information, i.e. clues for the adequate selection of systemic therapies.

3. A less sceptical view on the role of DTCs for the understanding of metastasis

For the reasons mentioned above, simple counting of DTCs or CTCs in bone marrow or blood, respectively, or limited phenotypic or genotypic characterisation by double staining or FISH analysis, may fall short in the attempt to significantly alter clinical routine to the benefit of the patients. However, comprehensive molecular characterisation of DTCs and possibly also of CTCs may provide important insights into the biology of metastasis. This, in turn, may then be translated into a novel diagnostic pathology of systemic disease that indeed may improve patient care. First examples are already emerging.

3.1. *The time point of tumour cell dissemination*

Traditionally, metastasis is viewed as the last step of breast cancer progression for several, partially obvious reasons. Most importantly, distant growths are usually detected after diagnosis of a primary tumour and breast cancer patients die on average within 2½ years after diagnosis of clinically manifest metastases.[25] The fact that metastasis is associated with increased tumour diameter (i.e. T-stage) was also commonly interpreted as evidence for late dissemination of tumour cells. The prevailing concept holds that tumour cells evolve mostly within the primary tumour, acquire the capability to metastasis by additional mutations, disseminate and grow out.[26] The theoretical flaws of this concept within an evolutionary paradigm of cancer progression have been pointed out before.[27] More importantly, we recently demonstrated that the central predictions from the concept could neither be verified in mouse models nor in patients.[28]

Firstly, if the association of large tumour size and metastasis is caused by increasing numbers of disseminating cells able to home to and survive in an ectopic environment, one would expect to detect higher numbers of DTCs in patients with large tumours when compared to patients with small tumours. However, this is not the case. Patients with tumours larger than 5 cm (pT3) do not have more DTCs in bone marrow than patients with small tumours and conflicting data exist whether or not the prevalence of DTCs in pT3-staged patients is marginally higher than in pT1-stage (tumour diameter < 2 cm) patients or not.[16,28] Nevertheless, even the

increased detection of a single DTC in 10^6 bone marrow cells in 10% of patients in the study by Braun and coworkers[16] is strikingly out of proportion to the 90–350 times higher number of tumour cells in pT3/pT4 tumours compared to pT1 tumours. In addition, it is not clear whether the marginally higher detection rate of DTCs in late stage tumours reflects an increased rate of dissemination or the onset of active proliferation of DTCs in bone marrow as consequence of the longer duration of a pT3-disease compared to a pT1-disease (i.e. as consequence of the lead time effect).[29]

Secondly, if repeated rounds of selection and clonal expansion precede dissemination, characteristic genetic changes that accumulate late in primary tumours should be detectable in DTCs. Analysis of primary tumours demonstrated that many mutations such as *TP53* mutations are relatively rare in pT1 tumours and significantly more frequent in pT4 tumours (11% versus 35%, $p<0.001$),[30] and therefore represent events that accumulate during tumour growth in advanced tumours. The linear progression model described above predicts that DTCs in bone marrow display most of the genetic aberrations present in the predominant cellular genotype in the primary tumour plus additional changes. However, so far we have detected only one case with *TP53* mutations in M0 stage breast cancer patients[31] and also rarely found the most prevalent chromosomal aberrations of the primary tumours, such as 8q gain, 13q loss, 16q, 17p loss.[32]

Thirdly, a higher number of aberrations in the DTCs than in the primary tumour would be consistent with a model predicting linear accumulation of mutations. However, in breast cancer, DTCs display significantly fewer aberrations than the cells in the primary tumour. Moreover, about 50% of cytokeratin-positive cells displayed normal karyograms.[32,33] This observation is puzzling as chromosomal aberrations are acquired before the invasive stage of local progression.[34] However, we could confirm by identification of small allelic losses that most of these genomically normal-appearing cytokeratin-positive cells are of cancerous origin.[33] From this data it was clear that dissemination is not commonly a late event on the genomic trajectory of progression and the question arose as to when it takes place during morphologically defined stages of tumour growth.

Therefore, we attempted to pinpoint the onset of tumour cell dissemination in spontaneous mouse models. In two mouse models, the MMTV-driven HER2-transgenic mouse and the MMTV-driven PyMT transgenic mouse, we noted that breast cancer cells leave the primary site before invasive cancers became morphologically detectable and can be found in bone marrow and lung tissue.[28] Transmission electron microscopy revealed that individual cells break through the basement membrane at the stage of atypical hyperplasia and molecular analysis revealed both upregulation of proteolytic enzymes at this stage as well as of Twist, a master regulator of invasion.[35] Notably, in the HER2 model, Twist expression was higher at the stage of atypical hyperplasia than in the invasion front of the primary tumour. When breast cancer patients with DCIS, a non-invasive lesion, were subsequently investigated, cytokeratin-positive cells were detected in bone marrow at rates significantly higher than published for patients without malignancies.[28]

Together these results suggest that dissemination of tumour cells is neither dependent on tumour size nor on the presence of chromosomal aberrations. In many patients, tumour cells may have disseminated in an early stage of tumour development.

3.2. *Is a dissemination-phenotype only transiently expressed?*

During exponential tumour growth the number of DTCs in bone marrow neither increases substantially in mouse models nor in breast cancer patients. The underlying mechanism for this observation is neither understood nor is it clear whether tumour subgroups exist that behave differently. As mentioned above, expression of Twist, a transcription factor regulating several key steps of genetic programs for invasion and migration, was higher in earlier, rather than later, lesions of HER2 transgenic mice. One possible explanation is that cancer cells are selected for survival and proliferation within the primary tumour. Such cells, when disseminating, may fail to successfully colonise distant sites although they may be circulating in the peripheral blood stream. Alternatively, but not mutually exclusive, a genetic or epigenetic activation of a migratory and invasive behaviour may be more frequently followed by cellular proliferation than vice versa. After disseminating from the primary site, homing to the distant site, initiating colonisation, and differentiating during tumour mass formation, cancer cells may thus lose the ability to spread further. It is interesting in this context that it is still not clear whether the first diagnosed metastases seed secondary metastases. Data from large epidemiological databases suggest that in breast cancer such a metastatic cascade is not frequent[29] because the time courses cannot be fit into a sequential model. However, in very late stages of systemic disease, general dissemination from various tumour colonies may also contribute to shortened survival as suggested by the prognostic impact of CTCs on survival in metastatic breast cancer.[18,20]

3.3. *The divergence of local and systemic cancer and personalised medicine*

Apparently, primary tumours and disseminated cancer cells diverge early and acquire different genotypes during systemic progression. Cancer progression may also involve continuous genomic remodelling. It is currently unclear whether cells fit for dissemination preserve their genotype during metastatic outgrowth or whether colonisation and outgrowth requires ongoing selection of chromosomal aberrations. We first gathered evidence for the latter scenario, which would explain why primary tumours and manifest metastasis share more chromosomal aberrations than primary tumours and DTCs (Hodak and Klein, unpublished data). Outgrowth seems to require different genotypes than dissemination and cells at the primary site and at ectopic sites may recruit similar mechanisms for tumour expansion, such as the loss of tumour suppressor genes.

Therefore, early dissemination and clonal divergence of local and systemic disease will have significant impact on therapeutic decision-making. If response to therapies depends on

the genetic activation of the targeted pathway, as has been mostly observed so far,[36,37] it will be mandatory to assess the genetics of systemically spread cancer cells and not of primary tumours. From this perspective, personalised medicine should be based on molecular characteristics of the target cells of adjuvant therapy, i.e. the DTCs, and not on the local disease that has been resected by the surgeon. One striking example is the recent finding that HER2 gains in oesophageal DTC are highly predictive for poor outcome while HER2 amplifications in primary tumours were not informative. Therefore, patient selection for HER2-targeting therapies based on HER-status of DTCs should be explored.[38]

3.4. *Migrating cancer stem cells (MCSC)*

The molecular analysis of DTCs will further allow the testing of some of the predictions derived from the cancer stem cell concept. Here, only a subpopulation of a hierarchically ordered tumour is able to initiate tumour growth, the so-called cancer stem cells (CSCs). Currently, CSCs are identified by transplantation of sorted tumour cell populations into immunodeficient mice and growth within weeks must be observed for defining CSCs,[7] a rationale that is not unequivocally accepted.[39] These subpopulations are characterised by expression of a specific marker-phenotype. Subsequently, the existence of migrating cancer stem cells was proposed[40] which eventually gives rise to metastasis. However, so far, a direct link between CSCs, MCSCs, and their phenotype has not been firmly established. In one study based on two selected cell lines, co-expression of the CSC marker CD133 with CXCR4 was proposed to define the MCSC population that can give rise to pancreatic cancer metastases.[41]

The analysis of DTCs may help to identify MCSCs directly at the sites of metachronous metastasis. The currently discussed markers for CSCs should be tested for being expressed on DTCs. In a first study, the putative stem-like phenotype of mammary CSCs ($CD24^{low}$, $CD44^{+}$, ref.(7)) was found in all bone marrow samples that harboured cytokeratin-postive cells.[42] However, due to the double-staining and triple-staining method applied, which cannot exclude cross-reaction of the secondary antibodies, these first data must be viewed with caution. Thus, there is ample opportunity for DTC research to clarify the role of CSCs in metastasis and to identify putative therapy targets on metastasis-initiating cells.

4. Tasks ahead

The proposed dynamic model of cancer progression leads to different consequences than the linear progression model[26] or the static model deduced from transcriptional profiling of primary tumours and metastases, which rejects evolutionary selection processes.[43,44] The two latter concepts suggest that primary tumours are adequate models of the systemic disease, which may be true to a certain degree since all cancer cells, i.e. from local and from systemic tumours, are selected within the genetic background of the individual patient. The individual genetic background certainly has a major impact on the power of microarray studies to predict outcome.[45] Primary tumours may therefore be useful to estimate prognosis and susceptibility to systemic therapies; however, due to genetic divergence of locally growing and systemically spreading cancer cells, the predictive power for selection of targeted therapies will inevitably be imperfect. What is needed is an in-depth analysis of the individual disease, in particular of the systemically spread cancer cells instead of focussing on the local disease. We need to gather as much information as possible on the target cells of systemically administered therapies. This includes an understanding of the cellular interactions between DTCs and the ectopic environment, the mechanisms inducing, maintaining and breaking cellular dormancy, and, of immediate importance, the presence or absence of molecular therapy targets. This information at hand, minimal residual cancer may eventually turn out to be the Achilles' heel of the disease and successful targeting may enable the prevention of lethal metastasis.[46]

Conflict of interest statement

None declared.

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REFERENCES

1. Fisher B. The evolution of paradigms for the management of breast cancer: a personal perspective. *Cancer Res* 1992;**52**(9):2371–783.
2. Riethmuller G, Johnson JP. Monoclonal antibodies in the detection and therapy of micrometastatic epithelial cancers. *Curr Opin Immunol* 1992;**4**(5):647–55.
3. Schlimok G, Funke I, Holzmann B, et al. Micrometastatic cancer cells in bone marrow: in vitro detection with anti-cytokeratin and in vivo labeling with anti-17–1A monoclonal antibodies. *Proc Natl Acad Sci U S A* 1987;**84**(23):8672–6.
4. Gottlinger HG, Funke I, Johnson JP, Gokel JM, Riethmuller G. The epithelial cell surface antigen 17–1A, a target for antibody-mediated tumor therapy: its biochemical nature, tissue distribution and recognition by different monoclonal antibodies. *Int J Cancer* 1986;**38**(1):47–53.
5. Riethmuller G, Holz E, Schlimok G, et al. Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial. *J Clin Oncol* 1998;**16**(5):1788–94.
6. Riethmuller G, Schneider-Gadicke E, Schlimok G, et al. Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17-1A Study Group. *Lancet* 1994;**343**(8907):1177–83.
7. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;**100**(7):3983–8.
8. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;**67**(3):1030–7.
9. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;**445**(7123):106–10.

10. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;**445**(7123):111–5.
11. Klein CA. The biology and analysis of single disseminated tumour cells. *Trends Cell Biol* 2000;**10**(11):489–93.
12. Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. *Nat Rev Cancer* 2004;**4**(6):448–56.
13. Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmuller G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. *Lancet* 1992;**340**(8821):685–9.
14. Schlimok G, Funke I, Bock B, Schweiberer B, Witte J, Riethmuller G. Epithelial tumor cells in bone marrow of patients with colorectal cancer: immunocytochemical detection, phenotypic characterization, and prognostic significance. *J Clin Oncol* 1990;**8**(5):831–7.
15. Braun S, Pantel K, Muller P, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med* 2000;**342**(8):525–33.
16. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med* 2005;**353**(8):793–802.
17. Fehm T, Braun S, Muller V, 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):885–92.
18. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004;**351**(8):781–91.
19. Nagrath S, Sequist LV, Maheswaran S, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 2007;**450**(7173):1235–9.
20. Bidard FC, Vincent-Salomon A, Sigal-Zafrani B, et al. Prognosis of women with stage IV breast cancer depends on detection of circulating tumor cells rather than disseminated tumor cells. *Ann Oncol* 2008.
21. Ulmer A, Schmidt-Kittler O, Fischer J, et al. Immunomagnetic enrichment, genomic characterization, and prognostic impact of circulating melanoma cells. *Clin Cancer Res* 2004;**10**(2):531–7.
22. Wong NS, Kahn HJ, Zhang L, et al. Prognostic significance of circulating tumour cells enumerated after filtration enrichment in early and metastatic breast cancer patients. *Breast Cancer Res Treat* 2006;**99**(1):63–9.
23. Rack BK, Schindlbeck C, Hofmann S, et al. Circulating tumor cells (CTCs) in peripheral blood of primary breast cancer patients. *ASCO Meeting Abstracts* 2007:10595.
24. Sotiriou C, Piccart MJ. Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? *Nat Rev Cancer* 2007;**7**(7):545–53.
25. Engel J, Eckel R, Kerr J, et al. The process of metastasisation for breast cancer. *Eur J Cancer* 2003;**39**(12):1794–806.
26. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;**61**(5):759–67.
27. Bernards R, Weinberg RA. A progression puzzle. *Nature* 2002;**418**(6900):823.
28. Husemann Y, Geigl JB, Schubert F, et al. Systemic spread is an early step in breast cancer. *Cancer Cell* 2008;**13**(1):58–68.
29. Klein CA, Holzel D. Systemic cancer progression and tumor dormancy: mathematical models meet single cell genomics. *Cell Cycle* 2006;**5**(16):1788–98.
30. Olivier M, Langerod A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1, 794 patients with breast cancer. *Clin Cancer Res* 2006;**12**(4):1157–67.
31. Klein CA, Blankenstein TJ, Schmidt-Kittler O, et al. Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. *Lancet* 2002;**360**(9334):683–9.
32. Schmidt-Kittler O, Ragg T, Daskalakis A, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci U S A* 2003;**100**(13):7737–42.
33. Schardt JA, Meyer M, Hartmann CH, et al. Genomic analysis of single cytokeratin-positive cells from bone marrow reveals early mutational events in breast cancer. *Cancer Cell* 2005;**8**(3):227–39.
34. Chin K, de Solorzano CO, Knowles D, et al. In situ analyses of genome instability in breast cancer. *Nat Genet* 2004;**36**(9):984–8.
35. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;**117**(7):927–39.
36. Arteaga CL, Baselga J. Tyrosine kinase inhibitors: why does the current process of clinical development not apply to them? *Cancer Cell* 2004;**5**(6):525–31.
37. Weinstein IB, Joe AK. Mechanisms of disease: Oncogene addiction–a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 2006;**3**(8):448–57.
38. Stoecklein NH, Hosch SB, Bezler M, Stern F, Hartmann CH, Vay C, et al. Direct genetic analysis of single disseminated cancer cells for prediction of outcome and therapy selection in esophageal cancer. *Cancer Cell* 2008;**13**(5):441–53.
39. Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science* 2007;**317**(5836):337.
40. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005;**5**(9):744–9.
41. Hermann PC, Huber SL, Herrler T, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;**1**(September):313–23.
42. Balic M, Lin H, Young L, et al. 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):5615–21.
43. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nat Genet* 2003;**33**(1):49–54.
44. Weigelt B, Glas AM, Wessels LF, Witteveen AT, Peterse JL, van't Veer LJ. Gene expression profiles of primary breast tumors maintained in distant metastases. *Proc Natl Acad Sci U S A* 2003;**100**(26):15901–5.
45. Hunter K. Host genetics influence tumour metastasis. *Nat Rev Cancer* 2006;**6**(2):141–6.
46. Riethmüller G, Pantel K, Funke I, Schlimok G. Micrometastasis of epithelial tumors: Signum mortis for the patient or the Achilles' heel of cancer? In: Messmer K, Stein M, editors. *Pathways in Applied Immunology.* Berlin: Springer-Verlag; 1991. p. 103–7.