

# Hematogenous and lymphatic tumor cell dissemination may be detected in patients diagnosed with ductal carcinoma in situ of the breast

Malgorzata Banys · Ines Gruber · Natalia Krawczyk · Sven Becker ·  
Ralph Kurth · Diethelm Wallwiener · Jolanta Jakubowska · Jürgen Hoffmann ·  
Ralf Rothmund · Annette Staebler · Tanja Fehm

Received: 17 December 2010 / Accepted: 19 March 2011 / Published online: 1 April 2011  
© Springer Science+Business Media, LLC. 2011

**Abstract** Tumor cell dissemination in bone marrow (BM) and lymph nodes is considered an important step in systemic disease progression and is associated with poor prognosis. Only invasive cancers are assumed to shed isolated tumor cells (ITC) into the bloodstream and infiltrate lymph nodes. However, latest studies indicate that tumor cell dissemination may occur before stroma invasion, i.e., in ductal carcinoma in situ (DCIS). Therefore, the purpose of this study was to examine the incidence of ITC in bone marrow and sentinel lymph nodes (SN) in patients diagnosed with DCIS and its correlation with clinicopathological factors. 266 patients who were treated at the Department of Gynecology and Obstetrics (University Hospital Tuebingen, Germany) between 2003 and 2009 with DCIS were included into this study. BM aspirates were analyzed by immunocytochemistry (pancytokeratin antibody A45-B/B3) using ACIS system (Chromavision) according to the ISHAGE evaluation criteria. SN were analyzed in 221 of these patients by extensive step sectioning and hematoxylin–eosin staining. In 34 of 266 patients (13%), ITC in BM could be detected. There was no correlation found between tumor size, grading, histology, or Van Nuys Prognostic Index and tumor cell dissemination. In two cases, metastatic spread into lymph nodes was observed

(pN1mi), whereas in one case, ITC in lymph nodes were detected; however, additional sectioning and immunohistochemical staining of the primary lesion in the cases with positive SN did not reveal invasive cancer. Interestingly, all the three patients were BM negative. Tumor cell dissemination may be detected in patients diagnosed with DCIS. Either these cells have started already to disseminate from preinvasive mammary lesions or from occult invasive tumors or represent the earliest step of microinvasion in a preinvasive lesion. The clinical relevance of these cells has to be further evaluated.

**Keywords** Ductal carcinoma in situ · Breast cancer · Disseminated tumor cell

## Abbreviations

BM	Bone marrow
CK	Cytokeratin
DIN	Ductal intraepithelial neoplasia
DCIS	Ductal carcinoma in situ
DTC	Disseminated tumor cell(s)
HE	Hematoxylin-eosin
ITC	Isolated tumor cell
n.s.	Not significant
SN	Sentinel node
SLNB	Sentinel lymph node biopsy
VNPI	Van Nuys Prognostic Index

Malgorzata Banys and Ines Gruber contributed equally to this work.

M. Banys · I. Gruber · N. Krawczyk · S. Becker ·  
D. Wallwiener · J. Jakubowska · J. Hoffmann · R. Rothmund ·  
T. Fehm (✉)

Department of Obstetrics and Gynecology, University  
of Tuebingen, Calwerstrasse 7, 72076 Tuebingen, Germany  
e-mail: tanja.fehm@t-online.de

R. Kurth · A. Staebler  
Department of Pathology, University of Tuebingen,  
Calwerstrasse 7, 72076 Tuebingen, Germany

## Introduction

Ductal carcinoma in situ (DCIS), the most common kind of non-invasive breast cancer, is defined as a proliferation of malignant cells at their site of origin, without invasion

across the ductal basement membrane [1]. The incidence of DCIS seems to have increased dramatically in the past two decades as a result of widespread application of screening mammography, accounting for 10–15% of all newly diagnosed breast cancer cases [2, 3]. It is widely assumed that preinvasive lesions are unable to produce metastasis since the tumor is limited to epithelial layer and does not reach blood or lymphatic vessels. However, several epidemiology studies with long follow-up estimated breast cancer specific mortality in DCIS patients at ca. 1–2% despite surgical *in sano* removal of the tumor [4]. This might be due to the axillary micrometastasis observed in up to 3% DCIS patients and/or the presence of occult (micro-)metastases at the time of surgery, implying occult microinvasion or an invasive lesion that was not detected by standard pathological workup of the specimen [5, 6].

Based on animal models, Hüseemann et al. [7] showed recently that tumor cells can disseminate even from earliest epithelial alterations, such as carcinoma in situ, and may subsequently cause metastasis. These findings add to our understanding of breast cancer progression and challenge the prevailing view of tumor cell dissemination as a relatively late event in the metastatic cascade [8]. Unclear natural history of DCIS, its biological heterogeneity, and inability to predict potential invasive course of the lesions have made an optimal management of DCIS challenging. The pathology of ductal carcinoma in situ requires thus further investigation.

Further, it is very likely that microinvasive foci in the primary tumor during conventional histopathological analysis are not detected. The currently available techniques can only perform a representative sampling of a ductal carcinoma in situ. This is especially true for large lesions involving one or more segments of the breast. Therefore, additional assays, such as sentinel node biopsy and detection of disseminated tumor cells (DTC), may help to identify patients with occult or missed microinvasion and thus contribute to adequate staging.

The aim of the present study was to evaluate tumor cell dissemination to bone marrow (BM) in a large cohort of 266 patients treated for DCIS in Women's University Hospital in Tuebingen, Germany.

## Materials and methods

After written informed consent bone marrow samples were intraoperatively obtained from 266 primary DCIS patients who were treated at the Department of Gynaecology and Obstetrics (University Hospital of Tuebingen, Germany, a certified and multidisciplinary breast center) during March 2003–December 2009. Patients with DCIS larger than 2 cm (based on mammogram) received a sentinel lymph

node biopsy (SLNB) and BM aspiration as a routine procedure since in case of invasive breast cancer a second surgery can be avoided. None of the patients had history of cancer. 10–20 ml BM was aspirated from posterior iliac crest into syringes containing heparin anticoagulant under local or general anesthesia using Jamshidi's technique [9]. This analysis was approved by the ethics committee of the University of Tuebingen (502/2010A).

## Immunocytochemistry

Tumor cell isolation and detection was performed based on the recommendations for standardized tumor cell detection [10]. Bone marrow samples were separated by density centrifugation using Bicolll (density 1,077 g/ml, Biochrom, Germany). Mononuclear cells were collected from the interphase layer and were spun down onto a glass slide (Hettich cytocentrifuge, Germany) ( $10^6$  MNC/spot). For detection of cytokeratin (CK)-positive tumor cells, slides were fixed in 4% neutral buffered formalin for 10 min and rinsed in PBS. Automatic immunostaining was performed on the DAKO Autostainer using the monoclonal mouse A45-B/B3 antibody (Micromet, Germany) and the DAKO-APAAP detection kit (DakoCytomation, Denmark) according to the manufacturer's instructions. The A45-B/B3 antibody is directed against common cytokeratin epitopes including the CK heterodimers 8/18 and 8/19. The malignant breast cell line MCF-7 was used as a positive control. Leukocytes of a healthy volunteer served as negative control. In addition, isotype-matched myeloma protein conjugated to FITC was included as negative staining controls (Sigma, Deisenhofen). For each patient,  $2 \times 10^6$  cells were analyzed on two slides. Analysis was performed on the Automated Cellular Imaging System (ACIS, ChromaVision Medical Systems, San Juan, Capistrano, CA). Details of this system have been described in detail elsewhere [11]. Criteria for detection of disseminated tumor cells were based on the recommendations of the European ISHAGE Working group for standardization of tumor cell detection and the consensus statements [10], [12].

## Histopathological evaluation of sentinel lymph nodes and primary tumors

Sentinel lymph nodes were sliced in 2-mm intervals, fixed in 10% buffered formalin for at least 24 h and embedded in paraffin. At least three step sections in 500 micron intervals were cut, stained with routine hematoxylin-eosin (HE) stain, and evaluated for metastasis by light microscopy by a pathologist. In equivocal cases, suspicious areas were immunostained with anti-cytokeratin-antibody (AE1/AE3). Primary tumors were sampled for histology in at least 0.5-cm intervals. Sections were evaluated by HE-staining. Cases with areas suspicious for invasion were submitted for

immunohistochemical staining with an antibody against smooth muscle myosin heavy chain. This antibody specifically stains the myoepithelial cell layer surrounding non-invasive intraductal carcinomas. Therefore, lack of this staining would indicate invasive growth. All primary tumors were examined by an experienced pathologist; cases with lymph node metastasis were evaluated by at least two independent pathologists.

#### Statistical analysis

Chi-squared test was used for examining the association between positive bone marrow status and clinicopathological factors. Statistical analysis was performed using SPSS (Version 15). *P*-values less than 0.05 were considered statistically significant.

## Results

#### Patients' characteristics

A total of 266 patients diagnosed with DCIS were included into the study. 66 of 266 tumors (25%) were  $\leq 15$  mm, 99 (37%) between 16 and 40 mm, and 101 (40%)  $\geq 41$  mm. With regard to grading, 51 of these 266 patients (19%) presented with ductal intraepithelial neoplasia (DIN) grade 1c, 110 (41%) with DIN 2, and 105 (40%) with DIN 3. 200 of 266 (75%) primary tumors were ER-positive, and 202 of 266 (76%) showed PR-positivity. 44 tumors were both estrogen and progesterone receptor negative. Clinicopathological data are summarized in Table 1. The distribution of patients is summarized in recommendations for tumor marker prognostic studies (REMARK) diagram (Fig. 1) [13].

#### Incidence of DTC

Bone marrow aspirates were obtained intraoperatively from 266 patients with newly diagnosed ductal carcinoma in situ of the breast (Fig. 2). Disseminated tumor cells were detected in 34 of 266 (13%) of all BM aspirates. The number of CK-positive cells ranged from 1 to 3 per  $2 \times 10^6$  mononuclear cells (Fig. 3).

#### Correlation between presence of DTC and clinicopathological factors

17% of the patients at Van Nuys Prognostic Index (VNPI) Group I were BM positive compared to 12 and 19% at VNPI Group II and III, respectively. There was no statistical correlation between bone marrow involvement and

**Table 1** Clinical characteristics of the included patients

	n (%) <i>N</i> = 266	BM positive (%)	<i>P</i> -value
Total	266	34 (13)	
Menopausal status			n.s.
Premenopausal	77 (29)	10 (13)	
Postmenopausal	189 (71)	24 (13)	
Histology			n.s.
DIN 1c	51 (19)	10 (20)	
DIN 2	110 (41)	13 (12)	
DIN 3	105 (40)	11 (11)	
Tumor size			n.s.
$\leq 15$ mm	66 (25)	6 (9)	
16–40 mm	99 (37)	14 (14)	
$\geq 41$ mm	101 (38)	14 (14)	
ER status			n.s.
Negative	66 (25)	5 (8)	
Positive	200 (75)	29 (15)	
PR status			n.s.
Negative	64 (24)	6 (9)	
Positive	202 (76)	28 (14)	

BM bone marrow, DIN ductal intraepithelial neoplasia, n.s. not significant

established clinicopathological factors including VNPI (*P* = 0.312) (Tables 1, 2).

#### Metastatic spread into the lymph node

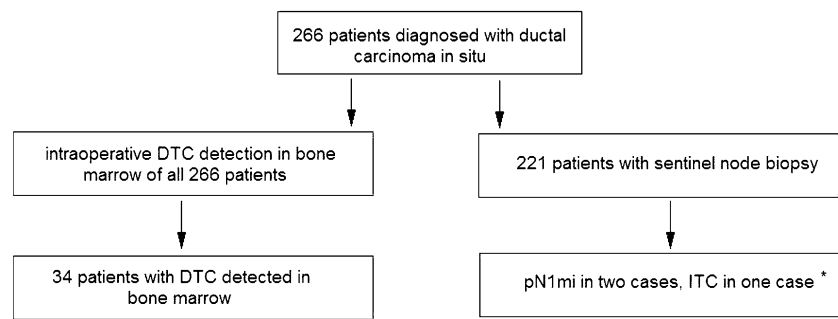
221 patients underwent SLNB. In two out of 221 (1%), patients metastatic spread into lymph nodes was observed (pN1mi; histology of the primary tumor in both cases: DIN 2, tumor size: 6.0 and 4.5 cm, respectively). In addition, in one patient with a large (10 cm) DIN 1c-lesion isolated tumor cells in the lymph node were detected (Fig. 4). In these three cases additional sectioning of the primary lesion was performed, but it did not reveal invasive cancer. Interestingly, all the three patients were BM negative.

## Discussion

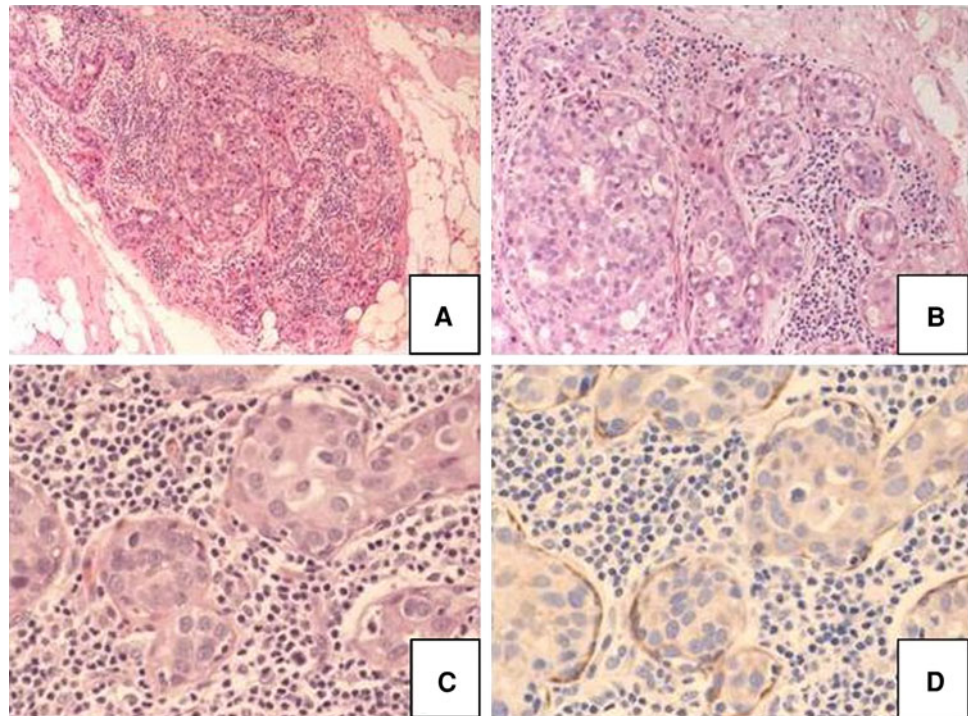
#### Incidence of disseminated tumor cells in DCIS

This is the largest study so far concerning both hematogenous and lymphatic tumor cell dissemination in primary DCIS patients. A total of 266 patients were included in the study. Tumor cells in BM were detected by morphologic criteria in addition to typical immunocytologic staining. Overall incidence of positive BM status in patients with pure DCIS was 13%, independent of prognostic factors

**Fig. 1** Patient distribution diagram according to the recommendations for tumor marker prognostic studies (REMARK) \*None of the three patients had disseminated tumor cells in bone marrow



**Fig. 2** Ductal carcinoma in situ with cancerisation of lobules without evidence of invasion. **a–c** HE-staining: **a** 50×, **b** 100×, **c** 400× magnification. **d** Lack of invasive growth is demonstrated by positive brown staining of myoepithelial cells surrounding the DCIS cells (anti-smooth muscle myosin heavy chain; magnification 400×)



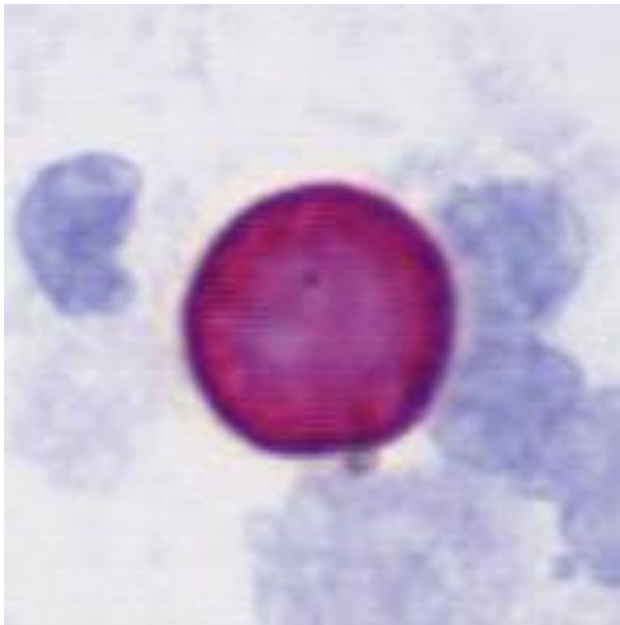
(Tables 1, 2). Similar findings were reported by Hüsemann et al. [7]. High incidence of DTC detection in our study raises several questions to be addressed by further investigation: (a) Do in situ carcinomas have the ability to shed single tumor cells into the bloodstream or lymphatic system? (b) Do cells derived from preinvasive lesions have metastatic potential and is their potential similar to cells shed by invasive tumors? and (c) Are in situ lesions with tumor cells in secondary sites in fact “in-situ”?

#### Early systemic spread of tumor cells

The concept of an early stage in tumor progression which is not yet able to produce metastasis developed in the first half of the twentieth century with the introduction of the term *in situ carcinoma* [14], [15], [16]. It is widely accepted that in situ lesions may progress to invasive

disease and thus require therapeutic intervention. Moreover, it has been shown that chromosomal instability occurs in breast cancer before histologic cancer invasion [17]. As preinvasive lesions do not shed isolated tumor cells into the lymph system or bloodstream, optimal surgical removal of the tumor should by definition result in a survival comparable to patients without cancer history. However, breast cancer-specific mortality in women diagnosed and treated for DCIS, though low, is higher than in general population [4]. 1–2% of DCIS patients will eventually die from breast cancer-associated death. In a study of Cutuli et al. [18], distant metastasis in DCIS patients has been observed, despite complete resection of their mammary tissue. This might support the hypothesis that a part of this group might already have had occult (micro-)metastasis or foci of undetected invasive carcinoma at the time of surgery. Nevertheless, in some cases, subsequent invasive lesion may have evolved independently.



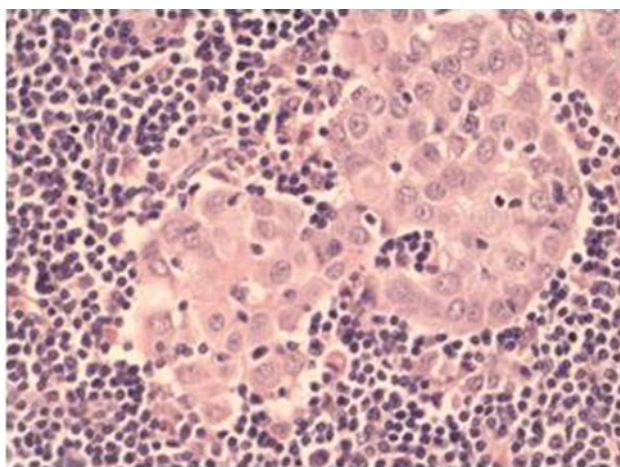


**Fig. 3** Immunocytochemistry. Disseminated tumor cell with typical cytomorphology and immunophenotype (positive cyokeratin-staining, large nucleus, high nuclear to cytoplasmic ratio, nucleus partially covered by cyokeratin staining, and nucleus granular)

**Table 2** DTC and Van Nuys Prognostic Index in DCIS patients

	N (%)	BM positive (%)	P-value
Total	254	35 (14)	n.s.
VNPI			
Group 1 (1–4 points)	30 (12)	5 (17)	n.s.
Group 2 (5–7 points)	172 (68)	20 (12)	n.s.
Group 3 (8–9 points)	52 (20)	10 (19)	n.s.

BM bone marrow, DCIS ductal carcinoma in situ, DTC disseminated tumor cells, n.s. not significant, VNPI Van Nuys Prognostic Index



**Fig. 4** Micrometastasis in a lymph node of the same patient as in Fig. 1 (HE-staining; magnification 400×)

### Sentinel node biopsy in DCIS patients

In accordance with our findings, evidence has recently accumulated that a significant proportion of DCIS patients present with isolated tumor cells either in the blood system or in the lymphatic nodes [6, 19, 20]. The value of axillary staging in DCIS patients remains controversial. Numerous studies reported that lymph node metastases were routinely found in 1–7% patients diagnosed with DCIS without definite evidence of invasion (Table 3). In our series, 1% of DCIS patients showed metastatic spread into the lymph node but additional sectioning of the primary tumor did not reveal invasive growth. This seems low enough to abandon complete axillary dissection due to its significant morbidity [21]. The introduction of SLNB, however, has enabled adequate axillary staging with minimal complication rate. Several advantages of SLNB in DCIS patients are currently under debate. (a) In a part of patients with DCIS diagnosed using core biopsy, an invasive focus will be found at the time of definite surgery, thus leading to a re-operation with secondary SLNB [22, 23]. Since some concern has been raised as to whether the disruption of lymphatic drainage patterns during primary surgery affects the reliability of secondary SLNB [24], one-step approach including excisional biopsy combined with SLNB in selected patients (e.g., high-risk DCIS, palpable and/or large lesions) is currently recommended [25] (b) We cannot exclude the possibility that microinvasion was missed in the primary tumor. However, the rate of positive sentinel lymph nodes in our patients was 1%. In comparison to other published data this rate is one of the lowest (Table 3). Therefore, we may assume that, in our collective, the histopathologic evaluation of the primary specimen was at least adequate, if not superior, in sensitivity. Nevertheless, considering that even the most diligent histopathological analysis involves representative stochastic assessment of a very low percentage (generally  $\leq 0.1\%$ ) of the specimen, it may lead to understaging of the disease [21]. In case of larger lesions, it is practically impossible under diagnostic conditions to evaluate every case of DCIS completely in step sections. The calculated number of slides for a 30-mm DCIS would be at least 600 and for a 50-mm DCIS close to 1000. Therefore, additional diagnostic tools, such as SLNB or DTC detection, have the potential to identify patients whose (micro-)invasive cancer was misdiagnosed as pure DCIS.

### Models of cancer progression

Hypothetical models of cancer progression have been developed over the years. Two fundamental models of tumor growth were discussed recently by Klein [8]. According to the linear progression model, local progression within the

**Table 3** Sentinel node involvement in patients with ductal carcinoma in situ

Reference	No. of patients with SN biopsy	SN positive (%)
Intra et al. [6]	854	12 (1)
Intra et al. [32]	223	7 (3)
Cox et al. [22]	195	26 (13)
Kelly et al. [5]	134	3 (2)
Mabry et al. [33]	171	10 (6)
Katz et al. [34]	110	8 (7)
Zavagno et al. [35]	102	1 (1)
Pendas et al. [36]	86	4 (5)
Klauber-DeMore et al. [37]	72	5 (7)
Leidenius et al. [38]	74	5 (7)
Sakr et al. [39]	39	4 (10)
Di Saverio et al. [40]	32	4 (13)

SN sentinel node

primary tumor microenvironment leads to stepwise accumulation of genetic and morphological abnormalities, after which fully malignant cells leave the primary tumor and form distant metastasis. However, epidemiological and genetic data do not support the concept of linear cancer progression [26, 27]. Engel et al. [28] indicated in a large epidemiological study of more than 12,000 breast cancer patients that metastasis might be initiated at a very early, asymptomatic stage of the primary disease, suggesting that cancer cells capable of metastasis are independent of primary tumor size. The parallel progression model addresses these important issues. In this model, tumor cell dissemination occurs at the earliest stages of the disease, years before diagnosis of the primary tumor. Consequently, persistent tumor cells in secondary sites are evolving independently of primary tumor. This phenomenon may lead to development of cancer cell clones adapted to specific microenvironments. Previously, we have reported a discrepancy in phenotype between tumor cells from primary tumor and those detected in blood and bone marrow of early breast cancer patients [29, 30].

#### Fate of early-disseminated tumor cells

The key steps in the metastatic cascade are intravasation at the primary site and subsequent extravasation at secondary sites. As regards the first step, Hüsemann et al. [7] reported that preinvasive lesions as early as ADH may show microinvasion, which could be detected by electron microscopy, even though no evidence of invasion was observed after careful light microscopic inspection by an experienced pathologist. These cells had the ability to disseminate to bone marrow and other organs and displayed genomic aberrations. Hypothetically, such cells

may form metastasis independently and even before the transition of primary lesion from non-invasive to invasive which could be an explanation for lymph node involvement in ductal carcinoma in situ. Experiments in animal models suggest that such a phenomenon is indeed possible. Podsypanina et al. [31] injected mammary cells engineered to express inducible oncogenic transgenes into the blood circulation of a mouse. Interestingly, these cells were able to bypass transformation at the primary site and independently develop into metastasis at distant sites upon oncogene induction.

#### Conclusions

In this article, we have shown that a significant proportion of patients diagnosed with preinvasive mammary lesions by current standards present with isolated tumor cells either in bone marrow or in lymph nodes. This hypothesis suggests that pathological diagnosis of preinvasive lesion in the breast may nevertheless be accompanied by hematogenous and/or lymphatic tumor cell dissemination. One has to consider that, in these cases, tumor cells disseminate from occult invasive tumors or represent the earliest step of microinvasion. SLNB and DTC detection may hypothetically add to adequate staging in DCIS patients with occult or missed microinvasion. However, follow-up data are not yet available. The issue of whether these patients should be treated as patients with (micro)invasive disease remains unclear. A deeper understanding of early metastatic outgrowth is essential for effective systemic cancer therapies in the future.

**Acknowledgments** The authors thank Professor Ludwig Spätling (Department of Gynecology and Obstetrics, Klinikum Fulda) and Dr. Mustafa Aydogdu (Department of Gynecology and Obstetrics, Klinikum Bremen-Mitte) for their respective supports.

**Conflict of interest** None.

#### References

1. Sakorafas GH, Farley DR, Peros G (2008) Recent advances and current controversies in the management of DCIS of the breast. *Cancer Treat Rev* 34(6):483–497
2. Ernster VL, Ballard-Barbash R, Barlow WE, Zheng Y, Weaver DL, Cutter G, Yankaskas BC, Rosenberg R, Carney PA, Kerlikowske K, Taplin SH, Urban N, Geller BM (2002) Detection of ductal carcinoma in situ in women undergoing screening mammography. *J Natl Cancer Inst* 94(20):1546–1554
3. Ernster VL, Barclay J, Kerlikowske K, Grady D, Henderson C (1996) Incidence of and treatment for ductal carcinoma in situ of the breast. *JAMA* 275(12):913–918
4. Ernster VL, Barclay J, Kerlikowske K, Wilkie H, Ballard-Barbash R (2000) Mortality among women with ductal carcinoma in

- situ of the breast in the population-based surveillance, epidemiology and end results program. *Arch Intern Med* 160(7):953–958
5. Kelly TA, Kim JA, Patrick R, Grundfest S, Crowe JP (2003) Axillary lymph node metastases in patients with a final diagnosis of ductal carcinoma in situ. *Am J Surg* 186(4):368–370
  6. Intra M, Rotmensz N, Veronesi P, Colleoni M, Iodice S, Paganelli G, Viale G, Veronesi U (2008) Sentinel node biopsy is not a standard procedure in ductal carcinoma in situ of the breast: the experience of the European institute of oncology on 854 patients in 10 years. *Ann Surg* 247(2):315–319
  7. Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, Forni G, Eils R, Fehm T, Riethmuller G, Klein CA (2008) Systemic spread is an early step in breast cancer. *Cancer Cell* 13(1):58–68
  8. Klein CA (2009) Parallel progression of primary tumours and metastases. *Nat Rev Cancer* 9(4):302–312
  9. Jamshidi K, Swaim WR (1971) Bone marrow biopsy with unaltered architecture: a new biopsy device. *J Lab Clin Med* 77(2):335–342
  10. Fehm T, Braun S, Muller V, Janni W, Gebauer G, Marth C, Schindlbeck C, Wallwiener D, Borgen E, Naume B, Pantel K, Solomayer E (2006) A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation. *Cancer* 107(5):885–892
  11. Bauer KD, de la Torre-Bueno J, Diel IJ, Hawes D, Decker WJ, Priddy C, Bossy B, Ludmann S, Yamamoto K, Masih AS, Espinoza FP, Harrington DS (2000) Reliable and sensitive analysis of occult bone marrow metastases using automated cellular imaging. *Clin Cancer Res* 6(9):3552–3559
  12. Borgen E, Naume B, Nesland JM, Kvalheim G, Beiske K, Fodstad O, Diel IJ, Solomayer EF, Theoharous P, Coombes RC, Smith BM, Wunder E, Marolleau J-P, Garci J, Pantel K (1999) Standardization of the immunocytochemical detection of cancer cells in BM and blood I establishment of objective criteria for the evaluation of immunostained cells. *Cytotherapy* 1(5):377–388
  13. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2006) Reporting recommendations for tumor marker prognostic studies (REMARK). *Breast Cancer Res Treat* 100(2):229–235
  14. Wellings SR, Jensen HM (1973) On the origin and progression of ductal carcinoma in the human breast. *J Natl Cancer Inst* 50(5):1111–1118
  15. Broders AC (1932) Carcinoma in situ contrasted with benign penetrating epithelium. *JAMA* 99:1670–1674
  16. Bloodgood JC (1931) Border-line breast tumors. *Ann Surg* 93:235–249
  17. Chin K, de Solorzano CO, Knowles D, Jones A, Chou W, Rodriguez EG, Kuo WL, Ljung BM, Chew K, Myambo K, Miranda M, Krig S, Garbe J, Stampfer M, Yaswen P, Gray JW, Lockett SJ (2004) In situ analyses of genome instability in breast cancer. *Nat Genet* 36(9):984–988
  18. Cutuli B, Cohen-Solal-Le Nir C, De Lafontan B, Mignotte H, Fichet V, Fay R, Servent V, Giard S, Charra-Brunaud C, Auvray H, Penault-Llorca F, Charpentier JC (2001) Ductal carcinoma in situ of the breast results of conservative and radical treatments in 716 patients. *Eur J Cancer* 37(18):2365–2372
  19. Lara JF, Young SM, Velilla RE, Santoro EJ, Templeton SF (2003) The relevance of occult axillary micrometastasis in ductal carcinoma in situ: a clinicopathologic study with long-term follow-up. *Cancer* 98(10):2105–2113
  20. Weaver DL (2003) Occult “micrometastases” in ductal carcinoma in situ: investigative implications for sentinel lymph node biopsy. *Cancer* 98(10):2083–2087
  21. van Deurzen CH, Hobbelenk MG, van Hillegersberg R, van Diest PJ (2007) Is there an indication for sentinel node biopsy in patients with ductal carcinoma in situ of the breast? A review. *Eur J Cancer* 43(6):993–1001
  22. Cox CE, Nguyen K, Gray RJ, Salud C, Ku NN, Dupont E, Hutson L, Peltz E, Whitehead G, Reintgen D, Cantor A (2001) Importance of lymphatic mapping in ductal carcinoma in situ (DCIS): why map DCIS? *Am Surg* 67(6):513–519 discussion 519–521
  23. Yen TW, Hunt KK, Ross MI, Mirza NQ, Babiera GV, Meric-Bernstam F, Singletary SE, Symmans WF, Giordano SH, Feig BW, Ames FC, Kuerer HM (2005) Predictors of invasive breast cancer in patients with an initial diagnosis of ductal carcinoma in situ: a guide to selective use of sentinel lymph node biopsy in management of ductal carcinoma in situ. *J Am Coll Surg* 200(4):516–526
  24. Feldman SM, Krag DN, McNally RK, Moor BB, Weaver DL, Klein P (1999) Limitation in gamma probe localization of the sentinel node in breast cancer patients with large excisional biopsy. *J Am Coll Surg* 188(3):248–254
  25. Lyman GH, Giuliano AE, Somerfield MR, Benson AB 3rd, Bodurka DC, Burstein HJ, Cochran AJ, Cody HS 3rd, Edge SB, Galper S, Hayman JA, Kim TY, Perkins CL, Podoloff DA, Sivasubramanian VH, Turner RR, Wahl R, Weaver DL, Wolff AC, Winer EP (2005) American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. *J Clin Oncol* 23(30):7703–7720
  26. Friberg S, Mattson S (1997) On the growth rates of human malignant tumors: implications for medical decision making. *J Surg Oncol* 65(4):284–297
  27. Schmidt-Kittler O, Ragg T, Daskalakis A, Granzow M, Ahr A, Blankenstein TJ, Kaufmann M, Diebold J, Arnholdt H, Muller P, Bischoff J, Harich D, Schlimok G, Riethmuller G, Eils R, Klein CA (2003) From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci USA* 100(13):7737–7742
  28. Engel J, Eckel R, Kerr J, Schmidt M, Furstemberger G, Richter R, Sauer H, Senn HJ, Holzel D (2003) The process of metastasisation for breast cancer. *Eur J Cancer* 39(12):1794–1806
  29. Fehm T, Krawczyk N, Solomayer EF, Becker-Pergola G, Durr-Storzer S, Neubauer H, Seeger H, Staebler A, Wallwiener D, Becker S (2008) ER alpha-status of disseminated tumour cells in bone marrow of primary breast cancer patients. *Breast Cancer Res* 10(5):R76
  30. Krawczyk N, Banys M, Neubauer H, Solomayer EF, Gall C, Hahn M, Becker S, Bachmann R, Wallwiener D, Fehm T (2009) HER2 status on persistent disseminated tumor cells after adjuvant therapy may differ from initial HER2 status on primary tumor. *Anticancer Res* 29(10):4019–4024
  31. Podsypnina K, Du YC, Jechlinger M, Beverly LJ, Hambarzumyan D, Varmus H (2008) Seeding and propagation of untransformed mouse mammary cells in the lung. *Science* 321(5897):1841–1844
  32. Intra M, Veronesi P, Mazzarol G, Galimberti V, Luini A, Sacchini V, Trifiro G, Gentilini O, Pruneri G, Naninato P, Torres F, Paganelli G, Viale G, Veronesi U (2003) Axillary sentinel lymph node biopsy in patients with pure ductal carcinoma in situ of the breast. *Arch Surg* 138(3):309–313
  33. Mabry H, Giuliano AE, Silverstein MJ (2006) What is the value of axillary dissection or sentinel node biopsy in patients with ductal carcinoma in situ? *Am J Surg* 192(4):455–457
  34. Katz A, Gage I, Evans S, Shaffer M, Fleury T, Smith FP, Flax R, Drogula C, Petrucci P, Magnant C (2006) Sentinel lymph node positivity of patients with ductal carcinoma in situ or microinvasive breast cancer. *Am J Surg* 191(6):761–766
  35. Zavagno G, Carcoforo P, Marconato R, Franchini Z, Scalco G, Burelli P, Pietrarota P, Lise M, Mencarelli R, Capitano G, Ballarin A, Pierobon ME, Marconato G, Nitti D (2005) Role of

- axillary sentinel lymph node biopsy in patients with pure ductal carcinoma in situ of the breast. *BMC Cancer* 5:28
36. Pendas S, Dauway E, Giuliano R, Ku N, Cox CE, Reintgen DS (2000) Sentinel node biopsy in ductal carcinoma in situ patients. *Ann Surg Oncol* 7(1):15–20
  37. Klauber-DeMore N, Tan LK, Liberman L, Kaptain S, Fey J, Borgen P, Heerdt A, Montgomery L, Paglia M, Petrek JA, Cody HS, Van Zee KJ (2000) Sentinel lymph node biopsy: is it indicated in patients with high-risk ductal carcinoma-in situ and ductal carcinoma-in situ with microinvasion? *Ann Surg Oncol* 7(9):636–642
  38. Leidenius M, Salmenkivi K, von Smitten K, Heikkila P (2006) Tumour-positive sentinel node findings in patients with ductal carcinoma in situ. *J Surg Oncol* 94(5):380–384
  39. Sakr R, Barranger E, Antoine M, Prugnotte H, Darai E, Uzan S (2006) Ductal carcinoma in situ: value of sentinel lymph node biopsy. *J Surg Oncol* 94(5):426–430
  40. Di Saverio S, Catena F, Santini D, Ansaloni L, Fogacci T, Mignani S, Leone A, Gazzotti F, Gagliardi S, De Cataldis A, Taffurelli M (2008) 259 Patients with DCIS of the breast applying USC/Van Nuys Prognostic Index: a retrospective review with long term follow up. *Breast Cancer Res Treat* 109(3):405–416