

# Strong correlation between N-cadherin and CD133 in breast cancer: role of both markers in metastatic events

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## Abstract

**Purpose** Epithelial mesenchymal transition is a major mechanism to explain metastatic events in breast cancer. Another important aspect is that cells with stem cell properties are able to become resistant against chemotherapeutics. Our main goal was to investigate the role of the EMT marker, N-cadherin, and of the stem cell marker, CD133, in breast cancer.

**Methods** The expressions of N-cadherin and CD133 were assessed by immunohistochemistry in 307 primary tumors

from breast cancer patients and for 30 patients, in the related recurrences and/or metastases. We studied the correlation between both markers, their associations with known clinico-pathological parameters and their role as predictive markers for survival time. Different expressions of both markers in primary tumor and recurrences or metastases were examined. **Results** N-cadherin and CD133 expressions correlated positively in the 261 primary tumor samples ( $p = 0.000$ ) and in the 45 primary tumor, recurrence or metastasis samples ( $p = 0.010$ ). In patients without lymph node metastases, the 10-year survival time was significantly lower when the tumor was N-cadherin-positive ( $p = 0.042$ ). Expression of N-cadherin was also significantly higher in metastases than in the related primary tumors ( $p = 0.039$ ).

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**Conclusion** N-cadherin and CD133 expressions are strongly correlated and N-cadherin appears as a potential metastases marker in a specific patient subpopulation.

**Keywords** N-cadherin · CD133 · Breast cancer · EMT · Stem cell

### Abbreviations

CSC	Cancer stem cell
DAB	3, 3'-Diaminobenzidine
EMT	Epithelial to mesenchymal transition
ER $\alpha$	Estrogen receptor $\alpha$
FGFR	Fibroblast growth factor receptor
HER2	Human epidermal growth factor receptor 2
IRS	Immunoreactive score
MET	Mesenchymal to epithelial transition
MMP	Matrix metalloproteinase
PBS	Phosphate buffered saline
PI(3)K	Phosphatidylinositol 3-kinase
PR	Progesterone receptor
TNM classification	Tumor-node-metastasis classification

### Introduction

Breast cancer is the most frequent cancer and cause of death for women worldwide (Jemal et al. 2008; Levi et al. 2005). In order to characterize the primary tumor, estrogen receptor  $\alpha$  (ER $\alpha$ ), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expressions are evaluated routinely at time of diagnosis or surgery. These biomarkers have prognostic and predictive relevance for individualized therapy regimes (Patani et al. 2013). But still, patients suffering from recurrences or distant metastases have a worse prognosis and a lower benefit from the known therapies (Comen 2012).

Mechanisms leading to metastases are still poorly understood. One of the events, which appears to play an important role in the development of metastases is the epithelial to mesenchymal transition (EMT), initially described for its physiological function in embryogenesis (Grogger et al. 2012; Hay 1995). EMT also allows cancer cells to lose their epithelial properties and acquire mesenchymal features. They can then escape the initial cell association in the primary tumor and invade into blood vessels or into the lymphatic system, to reach distant sites in the body. There, cells are able to reactivate their epithelial properties (mesenchymal to epithelial transition or MET) to form metastases (Kalluri and Weinberg 2009).

Another major theory to explain the metastatic potential of tumor cells is that some cancer cells may have intrinsic stem cell properties, meaning that they have the ability

for self-renewal, differentiation, tumor initiation and driving tumor progression. Resistance to chemotherapy may also have its origin in stem cell features (Dave et al. 2012; Schott et al. 2013).

The interplay between the two mechanisms of EMT and stem cell features has to be further evaluated (Mani et al. 2008; May et al. 2011). Many new markers may be relevant for the outcome of patients, combining the evaluation of these two mechanisms. Today, major EMT markers are N-cadherin, vimentin or fibronectin, among others (Armstrong et al. 2011; ElMoneim and Zaghloul 2011; Jeong et al. 2012; Park and Schwarzbauer 2013) and regarding cancer stem cells (CSCs), relevant markers are CD44<sup>+</sup>/CD24<sup>low</sup>, ALDH-1 or CD133 as a more recent marker (Takebe et al. 2011). In colon cancer, a connection has been demonstrated between mesenchymal and stem cell properties, which results in a highly malignant and even drug resistant phenotype of the tumor (Singh et al. 2013). In pancreatic cancer, significant correlations were observed between EMT markers and stem cell markers (Zhang et al. 2012). Also in circulating tumor cells of primary breast cancer patients, simultaneous expression of EMT and stem cell markers was described (Kasimir-Bauer et al. 2012). Comparatively, circulating tumors cells from castration-resistant prostate cancer and metastatic breast cancer patients more frequently over-expressed both groups of markers, especially the EMT marker, N-cadherin, and the stem cell marker CD133 [(Aktas et al. 2009; Armstrong et al. 2011) and unpublished data in breast cancer patients].

Cadherins are calcium-dependent glycoproteins, responsible for calcium-dependent cell–cell-adhesions, with an intracellular domain that allows the association with catenins and thereby with the cytoskeleton (Peinado et al. 2004; Saito et al. 2012). N-cadherin (neuronal Cadherin or Cadherin-2), is a subtype that plays an important role in gastrulation, neurulation and cardiogenesis, while in adults N-cadherin is almost only found in neural tissue and cells of mesenchymal origin (Radice et al. 1997; Stemmler 2008). Today, N-cadherin is a well-known marker for EMT as the “Cadherin switch” describes the reciprocal downregulation of E-Cadherin (epithelial) and upregulation of N-cadherin (mesenchymal) that characterizes EMT. Literature shows that N-cadherin-positive cancer cells are able to establish cell–cell-adhesion with N-cadherin-expressing cells of the stroma, promoting invasion (Christofori 2006). N-cadherin also activates Fibroblast Growth Factor Receptors (FGFRs) with downstream activation of other signaling pathways (e.g., PI(3)K), supporting cell survival, migration and invasion. Moreover, matrix metalloproteinases (MMPs) and  $\gamma$ -secretase-like proteases can trigger activation signals by cleaving the extracellular domain or the carboxy-terminal fragment of N-cadherin, respectively. All these features support the involvement of N-cadherin expression in tumor progression and N-cadherin

is recognized as both a marker for mesenchymal cell properties (Armstrong et al. 2011; Jeong et al. 2012; Stoyianni et al. 2012) and a prognostic marker for breast cancer patients (ElMoneim and Zaghloul 2011).

CD133 (AC133 or Prominin-1) is also a transmembraneous glycoprotein, mainly localized in cellular protrusions. Its physiological functions are still unknown except that it is involved in embryogenesis by activation of the Wnt-signaling pathway resulting in an enhanced transcription activity and consequently in intensified cell proliferation (Mizrak et al. 2008). CD133 is a potential upcoming marker for stem cells, and CD133-positive cancer cells are called CSCs. Their role in cancer evolution has still to be elucidated further. Some studies already investigated the role of CD133 in breast cancer as a potential predictive marker for aggressiveness and treatment optimization (Crocker et al. 2009; Ieni et al. 2011; Liu et al. 2009) and its correlation with prognosis in triple-negative breast cancer (Zhao et al. 2011). Moreover, CD133-positive tumor cells in breast cancer patients exhibit resistance to chemotherapy (Aomatsu et al. 2012; Nadal et al. 2013). This is why new targeting for anticancer therapy is required, potentially against CD133 as a marker for CSCs (May et al. 2011; Swaminathan et al. 2013; Takebe et al. 2011).

In this work, we investigated the expression of N-cadherin and/or of CD133 in 307 primary breast cancer tissues, comparing their expression in primary tumors, related recurrences and/or metastases (30 cases), and their correlation to survival and metastasis data.

## Materials and methods

### Characteristics of the patients

A total of 307 paraffin-embedded primary tumor tissue samples from breast cancer patients were randomly included in this study. For 30 patients, 30 primary tumors, 25 recurrences and 9 metastatic tissue samples were available for comparison of the expressions. The study has been approved by the Ethical Committee of the Ludwig Maximilians University of Munich, Germany (LMU, ethical number 048-08) and has been carried out in concordance with the guidelines of the Helsinki Declaration. Written informed consent was obtained from the patients.

The tissues we used for our investigations were sampled between 2000 and 2002 from patients of the Department of Obstetrics and Gynaecology of the LMU. The women's average age was 68.66 years (range from 38 to 98). All the tumors were classified by the tumor-node-metastasis classification (TNM classification). Histology was determined by an experienced pathologist (Doris Mayr) of the Department of Pathology of the LMU, according to a modification of Elston and Ellis grading proposed by Bloom and

**Table 1** Patient characteristics

	Case number	
	<i>n</i>	%
Patients	307	100 %
Samples		
Primary tumor	307	100 %
Recurrence	25	n.a.
Metastasis	9	n.a.
Tumor size <sup>a</sup>		
pT1	197	64.2 %
pT2–4	110	35.8 %
Lymph node metastases		
No	166	54.1 %
Yes	134	43.6 %
Unknown	7	2.3 %
Local recurrences		
No	264	86.0 %
Yes	43	14.0 %
Metastases		
No	240	78.2 %
Yes	67	21.8 %
ER status <sup>a</sup>		
Negative	45	14.7 %
Positive	202	65.8 %
Unknown	60	19.5 %
PR status <sup>a</sup>		
Negative	92	30.0 %
Positive	155	50.5 %
Unknown	60	19.5 %
HER2 status <sup>a</sup>		
Negative	95	30.9 %
Positive	95	30.9 %
Unknown	117	37.1 %
Triple negative <sup>a</sup>	20	6.5 %

*n.a.* not applicable

<sup>a</sup> All information given refers to the primary tumor

Richardson (Elston and Ellis 1993). Hormone receptor status (ER $\alpha$  and PR) was evaluated by immunohistochemistry, where a nucleic staining of more than 10 % of the tumor cells was considered as hormone-receptor-positive tumor. HER2 expression was analyzed and evaluated also in the Department of Pathology with an automated staining system (Ventana; Roche, Mannheim, Germany). The detailed characteristics of the patients are shown in Table 1.

### Immunohistochemistry

Paraffin-embedded tissue was used for preparation of 3  $\mu$ m sections. For staining procedure, tissue sections were

dewaxed in xylol (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for 15 min at room temperature. For reducing endogenous peroxidase reaction, sections were immersed in a solution of 3 % hydrogen peroxide (VWR International S.A.S., Fontenay-sous-Bois, France) in methanol (Sigma-Aldrich, Steinheim, Germany) for 20 min. After rehydrating in decreasing concentrations of ethanol (100–0 %) in distilled water, slides were boiled for 5 min in a pressure cooker with sodium citrate buffer (pH 6) for epitope retrieval. Then, the sections were washed with distilled water and phosphate buffered saline (PBS), before blocking with Powerblock (Biogenex, San Ramon, CA, USA) in distilled water (1:10) for 3 min. Tissue sections were then incubated with either a polyclonal rabbit antibody against N-cadherin (Abcam, Cambridge, UK) or with a monoclonal mouse antibody against CD133 (clone 3F10; Thermo Scientific, Waltham, MA, USA) for 1 h. After incubation with a biotinylated secondary anti-rabbit or anti-mouse IgG antibody, respectively, and with the associated avidin–biotin–peroxidase–complex (both Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA), visualization was performed with substrate and chromogen 3,3'-diamino-benzidine (DAB; Dako, Glostrup, Denmark). Sections were counterstained with acidic hematoxylin and afterward dehydrated in increasing concentrations of ethanol (70–100 %). The slides were immediately mounted with Eukitt (Merck, Darmstadt, Germany) before manual analysis with a Diaplan light microscope (Leitz, Wetzlar, Germany) with 2.5×, 10× or 40× magnifications. Pictures were obtained with a digital CCD camera system (JVC, Tokio, Japan). Positive controls were carried out with heart muscle tissue for N-cadherin and kidney tissue for CD133. Negative controls were performed by replacement of the primary antibodies by species-specific isotype control antibodies (Dako, Glostrup, Denmark).

#### Data analysis

N-cadherin or CD133 status was classified by evaluation of the percentage of brown stained tumor cell membranes and staining intensity, allowing assessment of an immunoreactive score (IRS = percentage score × intensity score). For the percentage score of stained tumor cells, we considered no staining (score = 0), <10 % stained (score = 1), 10–50 % stained (score = 2), 51–80 % stained (score = 3) and 81–100 % stained (score = 4). Staining intensity was evaluated as no staining (score = 0), weak staining (score = 1), moderate staining (score = 2) and strong staining (score = 3). For both markers, IRS of 0 or 1 was considered as negative, 2–4 as low expression, 6–8 as moderate expression and 9–12 as high expression. In doubtful cases, slides were evaluated by two or three independent examiners (CB, UJ, CK) with consent at the end.

#### Statistical analysis

Statistical analyses were performed using SPSS 21 (IBM SPSS Statistics, IBM Corp., Armonk, NY, USA). Correlation analysis was performed by calculating the Spearman's–Rho correlation coefficient or a chi-square test. To visualize the correlation, a scatter blot was performed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). For the comparison of survival times, Kaplan–Meier curves were generated. The chi-square statistic of the log-rank test was calculated to test differences between survival curves for significance. The Wilcoxon test was used to compare marker expressions in different tissues, depicted in a box plot. Multivariate analysis for prognostic value was performed using the Cox regression model. P values below 0.05 were considered statistically significant.

## Results

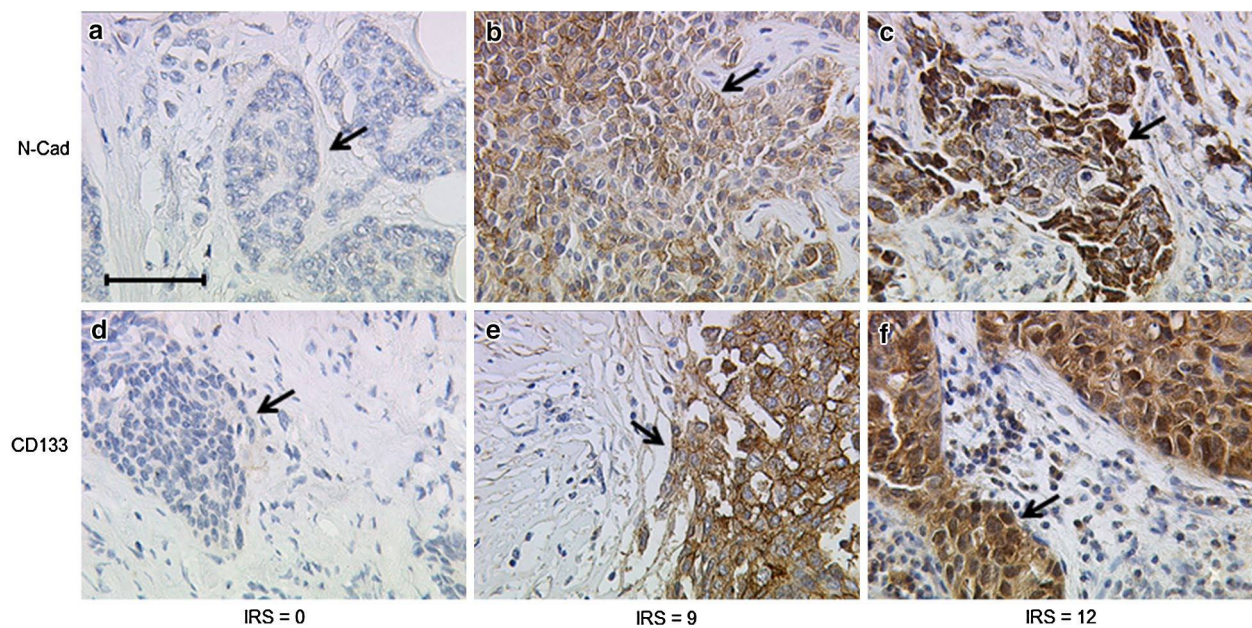
#### Immunohistochemical staining

We first stained 307 primary breast cancer tissue samples. In Fig. 1, staining results are shown exemplary for N-cadherin (a–c) and CD133 (d–f). Positive staining for both markers, with an IRS higher than 1, resulted in a membranous and partially cytoplasmatic brown coloration, observed specifically in the tumor tissue. Pictures a and d show patients classified as not expressing N-cadherin or CD133, respectively, (IRS = 0). Patients with a high expression of N-cadherin or CD133 (IRS = 9 and 12) are also shown (b, c and e, f, respectively).

Table 2 presents the immunohistochemical analysis that resulted in 275 evaluable cases for N-cadherin, 278 for CD133 and 261 for both markers (in some cases, the quality and/or quantity of material did not allow a correct evaluation). 71.3 and 42.1 % of the evaluable tumors were positive for N-cadherin and CD133, respectively, with an IRS higher than 1. The two markers were co-expressed in 37.5 % of the tumors, with 50.0 % (98/196) of the N-cadherin-positive tumors were also positive for CD133, and 83.8 % (98/117) of the CD133-positive tumors were also positive for N-cadherin.

Additionally, 25 recurrences and 9 metastatic tissue samples were stained in parallel with the related primary tumors from 30 patients (Table 3). For these 30 primary tumors, 23 cases were evaluable for N-cadherin, 27 for CD133 and 21 for both. While again the majority of the primary tumor (69.6 %) were N-cadherin-positive, only 3.7 % were CD133-positive and 4.8 % were positive for both markers. For the recurrences and metastases, 47.4 and 100 % were, respectively, positive for N-cadherin, 5.6 and 33.3 % for CD133 and 6.3 and 37.5 % for both markers. It





**Fig. 1** Immunohistochemical stainings of N-cadherin and CD133 expressions. Determination of the expression of N-cadherin (a–c) and CD133 (d–f) in primary tumors from breast cancer patients, with no expression (a, d) or high expression (b, c, e, f). Scale bar: 50  $\mu$ m

**Table 2** Results of the N-cadherin and CD133 stainings for the primary tumors from 307 patients

	Total	N-cadherin	CD133	Both markers
Primary tumors (n)	307			
Evaluable (n)	275	278	261	
IRS > 1 (n, % <sup>a</sup> )	196, 71.3 % <sup>a</sup>	117, 42.1 % <sup>a</sup>	98, 37.5 % <sup>a</sup>	

<sup>a</sup> Percentages refer to evaluable cases for either N-cadherin, CD133 or both markers

**Table 3** Results of the N-cadherin and CD133 stainings for the primary tumors, recurrences and metastases from 30 patients

	Total	N-cadherin	CD133	Both markers
Primary tumors (n)	30			
Evaluable (n)	23	27	21	
IRS > 1 (n, % <sup>a</sup> )	16, 69.6 % <sup>a</sup>	1, 3.7 % <sup>a</sup>	1, 4.8 % <sup>a</sup>	
Recurrences (n)	25			
Evaluable (n)	19	18	16	
IRS > 1 (n, % <sup>a</sup> )	9, 47.4 % <sup>a</sup>	1, 5.6 % <sup>a</sup>	1, 6.3 % <sup>a</sup>	
Metastases (n)	9			
Evaluable (n)	8	9	8	
IRS > 1 (n, % <sup>a</sup> )	8, 100 % <sup>a</sup>	3, 33.3 % <sup>a</sup>	3, 37.5 % <sup>a</sup>	

<sup>a</sup> Percentages refer to evaluable cases for either N-cadherin, CD133 or both markers

is noteworthy that all the CD133-positive primary tumors, recurrences and metastases ( $n = 1$ , 1 and 3) were N-cadherin-positive, whereas only 6.3, 11.1 and 37.5 %,

respectively, of the N-cadherin-positive one ( $n = 16$ , 9 and 8) were CD133-positive, too.

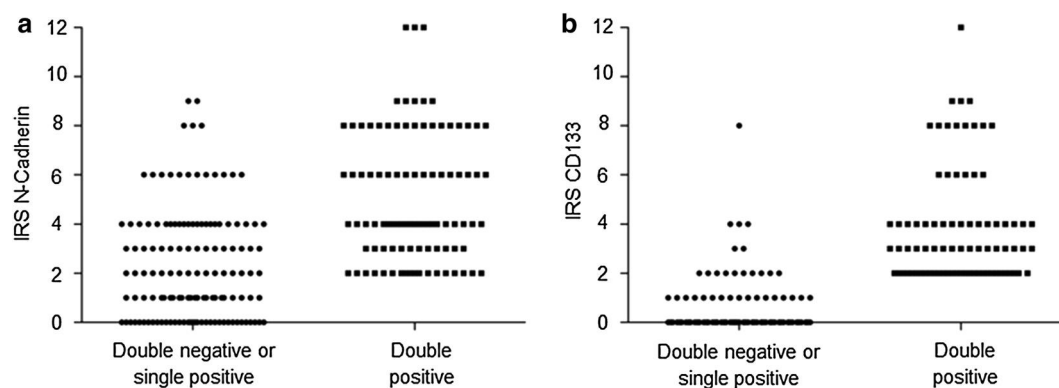
#### Correlation between N-cadherin and CD133 expressions

We first analyzed any positive or negative correlation between N-cadherin and CD133 expressions for the 261 primary tumors that were stained for both markers. We performed a Spearman's correlation test and could demonstrate a very high statistical significance for a positive correlation coefficient ( $p = 0.000$ ), significant at the 0.01 level (2-tailed). Scatter blots showing the IRS values for N-cadherin or CD133 of each individual sample are presented in Fig. 2a, b, respectively. In the two Figures, samples expressing none or only one marker ( $n = 163$ , on the left) are compared to samples expressing both markers ( $n = 98$ , on the right).

We performed the same test with the 30 patients, from whom the tissue samples of the primary tumor ( $n = 21$ ), recurrence ( $n = 16$ ) and/or metastasis ( $n = 8$ ) were analyzed, with a total of 45 evaluable cases for both markers. N-cadherin and CD133 again correlated positively ( $p = 0.010$ ), confirming the result obtained above on an almost 10 times smaller scale and with different tissue origins indicating the metastatic process.

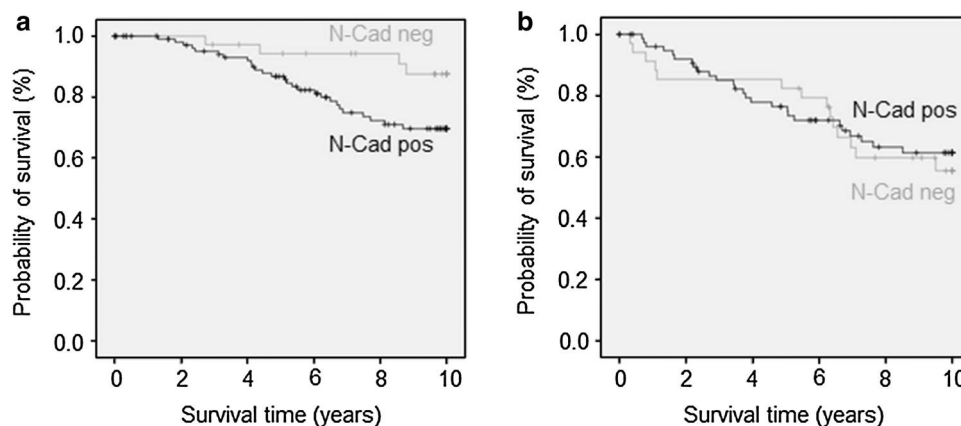
#### Correlation of N-cadherin and/or CD133 with patient characteristics

In addition, we calculated correlations between N-cadherin and/or CD133 expressions and the clinicopathological



**Fig. 2** **a** Scatter blot showing the IRS values for N-cadherin of each individual sample. Samples negative for both markers or positive for only one marker are shown on the left ( $n = 163$ ). On the right, IRS values of N-cadherin are shown for all the samples that are positive for N-cadherin and CD133 ( $n = 98$ ). **b** Scatter blot showing the IRS

values for CD133 of each individual sample. Samples negative for both markers or positive for only one marker are shown on the left ( $n = 163$ ). On the right, IRS values of CD133 are shown for all the samples that are positive for CD133 and N-cadherin ( $n = 98$ )



**Fig. 3** **a** Kaplan–Meier survival analysis of patients without lymph node metastases. For patients without lymph node metastases ( $n = 150$ ), N-cadherin negatively correlates with the survival time,  $p = 0.042$ ; N-cadherin-positive in black and N-cadherin-negative in

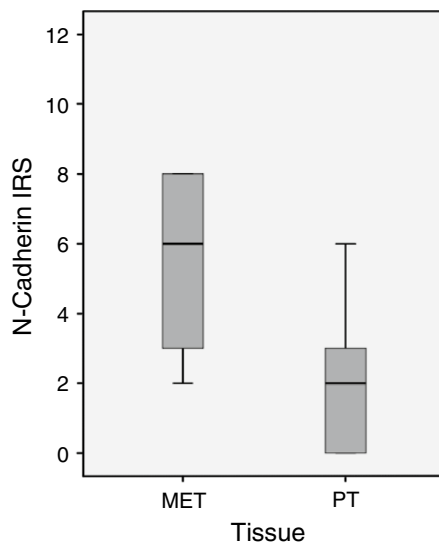
gray. **b** Kaplan–Meier survival analysis of patients with lymph node metastases. For patients with lymph node metastases ( $n = 118$ ), N-cadherin does not correlate with the survival time,  $p = 0.741$ ; N-cadherin-positive in black and N-cadherin-negative in gray

characteristics of the patients (described in Table 1), such as hormone receptor status, HER2 status, grading or age. No chi-square correlation coefficient was found significant (Additional data 1).

#### Survival analysis

To determine the relevance of both markers' expression for prognosis and survival time, we correlated the 10-year survival with the expression of N-cadherin and/or CD133. Considering the whole cohort, we found no statistically significant difference. Consequently, we performed various stratifications of the cohort, based on their clinicopathological characteristics. The stratification in patients having or not axillary lymph node metastases allowed to show that

within the lymph node-negative patients strata, N-cadherin expressing patients had a significantly lower survival time than patients not expressing the EMT marker ( $p = 0.042$ ). This result is presented in a Kaplan–Meier curve in Fig. 3a. Multivariate analysis for the lymph node-negative patients using the Cox regression model showed tumor staging to be the only independent prognostic marker for survival but not N-cadherin or CD133 (Additional data 2). For the lymph node-positive patients, N-cadherin expressions showed no statistical difference in the Kaplan–Meier curve (Fig. 3b). In total, we analyzed data of 150 lymph node-negative patients and 118 lymph node-positive patients, in addition we had 7 patients with unknown lymph node status. Consequently, our results strongly suggest that N-cadherin is a negative predictor for 10-year survival time in patients



**Fig. 4** Box plot of N-cadherin expression in primary tumors versus metastases. N-cadherin is significantly higher expressed in the metastases (MET, left box) than in the primary tumors (PT, right box),  $n = 5$  patients,  $p = 0.039$

without lymph node metastases. Investigation of simultaneous N-cadherin and CD133 expression gave no indication for a better possibility to predict survival times or patient's outcome (data not shown).

Different expressions of N-cadherin in the primary tumors and the related metastases

Focusing on the potential role of N-cadherin on the metastatic evolution, we compared the expression of N-cadherin in the primary tumors and related metastases, available for 5 patients (Wilcoxon test). The data we generated demonstrate a statistically significant higher expression of N-cadherin in the metastases in contrast to the related primary tumors ( $p = 0.039$ , Fig. 4). For CD133 expression, no significant difference was found between the primary tumors and the metastases (9 evaluable patients).

## Discussion

In this study, we evaluated the expression of N-cadherin and CD133 in primary breast cancer tissue of 307 breast cancer patients randomly included. For 30 patients, we also investigated related recurrences and/or metastases for both markers.

Although the majority of breast cancer patients show no evidence of metastasis at time of diagnosis, nearly 30 % of these women will recur metastatic lesions sometimes years later (O'Shaughnessy 2005; Redig and McAllister 2013).

Both EMT and stem cell properties are strongly suggested to be part of the metastatic events (May et al. 2011), while in parts being regulated via the same embryonic signaling pathways (Takebe et al. 2011). The existence of these pathways, e.g. Wnt, can support the connection between EMT and stem cell features and explain their deregulation in some cancers, also in breast cancer (Liu et al. 2010). Consequently, along such deregulations, the association of EMT and stem cell properties may gain importance in breast cancer, strongly suggesting further evaluations of EMT and CSCs markers. In our study, we found a very strong correlation between the EMT marker N-cadherin and the stem cell marker CD133 in breast cancer specimens. As already shown for pancreatic cancer, EMT markers (E-Cadherin and vimentin) correlated significantly with stem cell markers (CD24, CD44 and CD133) (Zhang et al. 2012). It is reported that EMT may induce stem cell properties and vice versa in human mammary epithelial cells (Mani et al. 2008). Here, we clearly demonstrated that a strong connection exists between EMT and stem cell features not only in cell models but also in breast tissue. We were able to show this association not only in a consequent primary tumor tissue collection ( $p = 0.000$ ,  $n = 261$ ), but also in their related recurrences and/or metastases, and this in a much smaller group ( $p = 0.010$ ,  $n = 45$ ). This connection between both markers, with 83.8–100 % of the CD133-positive samples being N-cadherin-positive too, can display that EMT is essential to trigger and/or accompany stem cell properties, reflecting the aggressive behavior of such cells. Our results obtained with CD133, a recent upcoming stem cell marker in breast cancer, have to be extended now to established stem cell markers in breast cancer, mainly CD24 and CD44.

Authors showed correlations between CD133 or N-cadherin expression and few clinicopathological parameters, but this was investigated either in small cohorts or with definite breast cancer subgroups, e.g., ER $\alpha$ -positive patients or mainly triple-negative patients [for N-Cad (Carvalho et al. 2011; ElMoneim and Zaghloul 2011; Jeong et al. 2012; King et al. 2008; Nagi et al. 2005; Nakagawa et al. 2011; Sarrio et al. 2008; van Nes et al. 2012), for CD133 (Ieni et al. 2011; Liu et al. 2009; Zhao et al. 2011)]. Although we found no correlation between any clinicopathological parameter and N-cadherin/CD133 expressions, N-cadherin or CD133 analysis can be of interest as marker for a definite subpopulation of patients, to study on a larger scale. The risk of recurrences, lymph node metastases or distant metastases may be decreased by targeting N-cadherin and/or CD133-related pathways. Swaminathan et al. (2013) recently showed that CD133-targeted nanoparticles loaded with paclitaxel obtained a significant decrease in tumor initiating cell populations compared to free paclitaxel treatment (Swaminathan et al. 2013). Knowing this, N-cadherin



and CD133 may be further used as novel targets for new targeted and individualized therapies in breast cancer to limit tumor spread and aggressiveness (Creighton et al. 2010).

Focusing on the survival analyses, N-cadherin and CD133 do not appear as predictors for survival time for every breast cancer patient. Splitting the cohort in lymph node metastasis-negative and -positive patients, we demonstrated a statistically significant difference for N-cadherin expression. In the subgroup of our cohort having no lymph node metastasis, a significant lower 10-year survival rate characterized the patients expressing N-cadherin compared to the patients expressing not the EMT marker. This suggests that N-cadherin could be a potential marker for distant metastases. Even among patients having no afflicted lymph nodes, the majority dies because of distant metastases. Consequently, tumor cells must have taken their way to distant organs not via the lymphatic system, but via the blood circulation and N-cadherin could be a predictor for this scenario. Indeed, preliminary results of our group show that circulating tumor cells in the blood circulation of breast cancer patients express different amounts of N-cadherin (unpublished data). By investigating the primary tumors and the related metastatic tissue samples available for 5 patients, a significant higher expression of N-cadherin was observed in metastases than in the primary tumors. Previously published data of our group show that 80 % of the metastases also express E-Cadherin (Jeschke et al. 2007), meaning that a co-expression of mesenchymal and epithelial markers exists in metastases. Due to the very limited number of samples in our study, this promising result has to be validated in larger scale studies.

## Conclusions

Altogether, we could demonstrate that N-cadherin and CD133 expressions are highly correlated in breast cancer tissue, meaning that EMT and stem cell properties may be intricate. Further studies have to be conducted on larger cohorts to investigate correlations between both markers and relevant clinicopathological parameters in definite subpopulations of patients. By examining specifically N-cadherin expression, we clearly elucidated that it can be a negative predictor for 10-year survival time in patients without lymph node metastases. Moreover, we demonstrated a significantly higher expression of N-cadherin in metastatic tissues than in the primary tumors. All these observations highlight the aggressive behavior and the metastatic potential of tumor cells expressing especially N-cadherin, and potentially co-expressing CD133.

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**Conflict of interest** The authors declare that they have no competing interests. We confirm that the authors have full control of all primary data and agree that the journal is allowed to review these data if requested.

**Ethical standards** The study has been approved by the Ethical Committee of the Ludwig Maximilians University of Munich, Germany (LMU, ethical number 048-08) and has been carried out in concordance with the guidelines of the Helsinki Declaration. Written informed consent was obtained from the patients.

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