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Immunophenotypic profile of tumor buds in breast cancer

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ABSTRACT

Background: Tumor buds are associated with lympho-vascular invasion and lymph node metastases leading to the assumption that they are involved in the early metastatic process. Hence, it would be important to know if tumor buds can be targeted with the most widely used targeted therapies in breast cancer (BC) and if changes in hormone and Her2 status occur. The aim of this study was to answer these questions by determining whether hormone receptor (HR) and Her2 status are expressed in the tumor buds of a large cohort of BCs.

Design: We constructed a tumor bud next-generation tissue microarray (ngTMA) consisting of n = 199 BCs of non-special type. Generally, two 1 mm punches were taken from the tumor bud areas in the periphery (PTB) and within the tumor center (ITB). HR and Her2 status was assessed using immunohistochemistry and fluorescence in situ hybridization, respectively. HR status was positive if ≥1% of tumor bud cells were positive. Her2 status was considered positive if bud cells showed strong complete membranous Her2 over-expression or *Her2* amplification.

Results: Most tumor buds were positive for estrogen (ER) (PTB: 86%; ITB: 88.3) and progesterone receptor (PgR) (PTB: 72%; ITB: 72.8%) and Her2 was positive in: PTB 11.5% and ITB 11%. A difference between the main tumor mass and tumor buds (PTB and ITB) was seen for PgR in 3.5% of cases (n = 7). No differences were seen for ER and Her2 between tumor buds and main tumor mass.

Conclusion: Most tumor buds (96.5%) share the same HR and Her2 expression profile of the main tumor mass, implying that tumor buds relay on the same pathways as the main tumor mass and might be equally responsive to targeted therapies.

1. Introduction

Tumor buds are small cell clusters or single tumors cells, detaching from the main tumor mass. This phenomenon can be seen within the tumor (intra-tumoral budding), or at the tumor periphery (peripheral tumor budding) [22,27,19]. Tumor budding is best characterized in colon cancer [6,19,31] but it is increasingly recognized and described in other tumor types such as e.g. breast, pancreatic-, esophagus-, larynx- and other cancers [16–18,22–25,27]. In breast cancer (BC) and other tumor types, high numbers of tumor buds are associated with lympho-vascular invasion (LVI) and/or lymph node metastasis [16,18,22,27]. Additionally, high numbers of tumor buds are associated with shorter overall and cancer-specific survival in BC [8,18] and this has been described in other tumor types as well [6,15]. The association of vascular invasion and tumor budding led to the assumption that tumor buds are involved in the early metastatic process by undergoing epithelial-mesenchymal transition (EMT) [8,19]. It is well-known that tumor cells undergoing EMT are more invasive and prone to metastasize that can lead to worse overall survival in cancer patients [1,10,23].

Inhibiting tumor cells involved in the early metastatic process would be of great clinical value since metastatic disease remains the major cause of cancer deaths with around 30% of BC patients developing metastasis [4,20]. This indicates that a better understanding of the metastatic process is needed in order to develop novel targeted approaches for highly aggressive and invasive cancer to improve patient outcomes. If tumor buds are involved in the early metastatic process, then it would be advantageous to determine whether and how they can be uniquely targeted. Tumor buds are known to have

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characteristics of EMT and in breast cancer, estrogen and Her2 overexpression was shown to be involved in EMT [11,13]. However, little is known regarding the expression of markers commonly targeted in breast cancer such as the estrogen and progesterone receptors and Her2 status in tumor buds.

Hence, the aim of this study was to examine the immunophenotypic profile of tumor buds to determine if they are targetable with the most widely used targeted therapies in BC, such as anti-hormonal and anti-Her2 therapy. In the current study, we report the estrogen, progesterone and Her2 receptor status of a large number of tumor buds in BC of non-special type (NST) using a next-generation tissue microarray (ngTMA).

2. Material and methods

Patients: We selected 199 NST BCs out of our previously described cohort of 356 therapy naïve, unilateral BCs diagnosed in female patients that underwent surgery between 2005 and 2011 at the Inselspital Bern, Switzerland [7]. T category was available for all BCs and N category was available for n = 181 (91%). Tumor grading, estrogen-, progesterone-, and Her2 receptor status (ER, PgR and Her2) and the molecular subtypes according to the St. Gallen 2013 criteria from the main tumor mass was available from our previous studies [7,22]. The median age at diagnosis was 64 years (range: 33–98 years) and clinical information regarding chemotherapy was available for 100 (50.2%) cases; for anti-hormone therapy for n = 102 (51.3%), for anti-her2 therapy in n = 95 (47.7%); and for radiation therapy for n = 140 (70.9%) cases. The clinic-pathological characteristics are shown in Table 1. The study was approved by the ethical commission of the University of Bern (Registration 200/2014).

Next-generation tissue microarray (ngTMA) of tumor buds: The ngTMA was constructed as previously described (3DHistech, Budapest, Hungary) [33]. In brief, pathologists reviewed breast cancer cases using H&E slides [7]. The decision, what block should be use for the peripheral and intra-tumoral bud ngTMA was made by one pathologist (CT). The H&E slides where then scanned and uploaded to the digital platform to perform annotation on the computer screen. Whenever feasible, two areas from peripheral and central tumor buds were punched. We successfully made two 1 mm punches in 199 and 193 of PTB and ITB cases, respectively.

Definition and assessment of tumor buds: We used our previous definition of tumor buds: One isolated tumor cell or a small tumor cell clusters of up to 5 tumor cells [22]. The slides of the tumor bud ngTMA were stained with ER, PgR and Her2 using the same antibodies and conditions as in our previous study [22]. Briefly, any nuclear ER and PgR staining, regardless of intensity, was considered as positive. The cases were then dichotomized into negative and positive cases according to the cut-off of ≥1% [9]. Her2 status was evaluated according to ASCO/CAP guidelines 2013 [30]. ER and PgR positive tumor cells were estimated in the tumor buds and a positive rate of ≥1% positive tumor cells was regarded as a positive hormone (HR) status. For HR status any intensity of nuclear staining was regarded as positive and the cases were dichotomized into negative and positive cases according to the cut-off of ≥1%. A strong, complete membranous staining for Her2, or a *Her2* amplification was considered Her2 positive. Difference in ER and PgR status was defined according to the dichotomized result obtained for the main tumor and the tumor buds or according to the defined positive expression of Her2 status.

Statistics: We used the Pearson Chi-Square test to calculate significant correlations between categorical variables. A p-value of < 0.05 was considered statistically significant. Analyses were carried out using the IBM SPSS Statistics 22.0 (IBM, Armonk, NY, USA).

Table 1
Patient characteristics of the whole cohort (n = 199).

Feature		Frequency n (%)
Age at diagnosis (years)	Median (min, max)	64 (33, 98)
Tumor size (centimeter)	Mean (min, max)	2.4 (0.6, 8.4)
ER	positive	165 (82.9)
	negative	34 (17.1)
PgR	positive	142 (71.4)
	negative	57 (28.6)
Her2	negative	177 (88.9)
	positive	21 (10.6)
	No data	1 (0.5)
Nottingham Grad	G1	21 (10.6)
	G2	97 (48.7)
	G3	81 (40.7)
LVI	Yes	82 (41.2)
	No	104 (52.3)
	No data	13 (6.5)
V1	Yes	21 (10.6)
	No	161 (80.9)
	No data	17 (8.5)
Pn1	Yes	26 (13.1)
	No	148 (74.3)
	No data	25 (12.6)
pT	T1	92 (46.2)
	T2	93 (46.8)
	T3	7 (3.5)
	T4	7 (3.5)
pN	N0	84 (42.3)
	N1mi	11 (5.5)
	N1	60 (30.2)
	N2	17 (8.5)
	N3	9 (4.5)
	No data	18 (9.0)
Recurrence	Yes	12 (6.0)
	No data	187 (94)
Molecular subtypes (St. Gallen 2013)	Luminal A	106 (53.3)
	Luminal B (her2 negative)	44 (22.1)
	Luminal B (her2 positive)	14 (7.0)
	Her2 non-luminal	7 (3.5)
	Triple negative	26 (13.1)
	no data	2 (1.0)
Anti-hormonal therapy	Yes	42 (21.1)
	No	60 (30.2)
	No data	97 (48.7)
Chemotherapy	Yes	37 (18.6)
	No	63 (31.7)
	No data	99 (49.7)
Anti-her2 therapy	Yes	5 (2.5)
	No	90 (45.2)
	No data	104 (52.3)
Radiation therapy	Yes	100 (50.3)
	No	40 (20.1)
	no data	59 (29.6)

3. Results

3.1. Peripheral tumor buds (PTB)

Informative ER, PgR and Her2 results were available for 172 (86.9%), 168 (84.8%), and 156 (78.8%) cases. ER status was positive in 148 (86%) cases. Her2 status was positive in 18 (11.5%). PgR was positive in 121 (72%). No difference in ER or Her2 status was seen between main tumor mass and tumor buds. However there was a difference in receptor status between main tumor mass and buds for PgR in 6 (3.6%; 6/168) cases. All cases showed a positive PgR status in the main tumor mass but were negative in PTBs. Comparing the differences of PgR status with the molecular subtypes of the main tumor mass differences in 2 (2%) of luminal A, and 4 luminal B (Her2-negative) (10%) were observed. No differences in other molecular subtypes were seen.