Discordance of the ER- and HER2-Status on Disseminated Tumor Cells Compared to the Primary Tumor in Patients With Early Breast Cancer

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Background

Differences in ER- and HER2-expression on metastases compared to the primary tumor (PT) are a known phenomenon and may have clinical implications in respect of targeted systemic treatment approaches. The aim of this study was to evaluate both ER- and HER2-status on disseminated tumor cells (DTCs) in the bone marrow (BM) of patients (pts) with early breast cancer (EBC; see table 1) and to compare these with the corresponding PT.

Methods

BM aspirates were obtained at the time of first surgery. After Ficoll enrichment for mononuclear cells two cytospins with 10^6 BM cells were evaluated for ER-, HER2- and cytokeratin (CK) -expressions simultaneously by immunocytochemistry using a triple fluorescence staining method with antibodies directed against human ER (secondly labeled with Cy3, red), HER2 (Coumarin-AMCA, blue) and CK (DyLight488, green). The manual analysis was conducted using a computerized fluorescence microscope (Axioskop, Zeiss, Germany). Criteria for CK- and HER2-positivity were the ring-like appearance of the respective membrane stainings and for ER-expression a nuclear staining (see figure 1). Only pts with the detection of CK positive cells (DTC+) and known ER- and HER2-status of the PT (n= 54) were selected for this analysis.

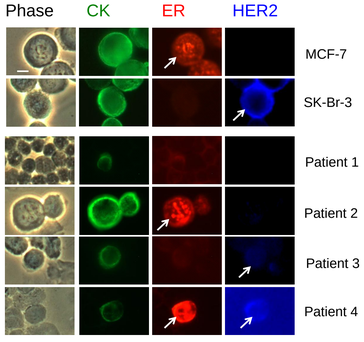

Figure 1: Phase, CK, ER and HER2 staining for ER-positive cell line (MCF-7), HER2-positive cell line (SK-Br-3) and 4 patients with 4 different subtypes

Menopausal status	pre	15 (27.8%)
	post	39 (72.2%)
pT	pT1b	6 (11.1%)
	pT1c	23 (42.6%)
	pT2	18 (33.3%)
	pT3	7 (13.0%)
pN	pN0	34 (63.0%)
	pN1	10 (18.5%)
	pN2	3 (5.6%)
	pN3	5 (9.3%)
	n/a	2 (3.7%)
Histological grading	G1	5 (9.3%)
	G2	31 (57.4%)
	G3	18 (33.3%)
Histological type	ductal	40 (74.1%)
	lobular	10 (18.5%)
	other	4 (7.4%)
Estrogen receptor status	negative	12 (22.2%)
	positive	42 (77.8%)
Progesteron receptor status	negative	20 (37.0%)
	positive	34 (63.0%)
HER2 status	negative	48 (88.9%)
	positive	6 (11.1%)

Table 1: Patient characteristics.

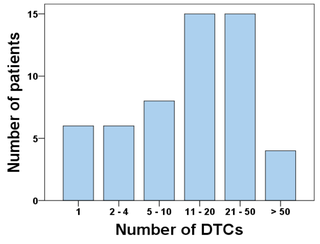

Figure 2: Frequency distribution of the number of DTCs detected per patient (n = 54)

A	ER status	DTC: only ER-negative DTCs (%)	DTC: at least one ER-positive DTC (%)	Total (%)
Tumor	ER negative (%)	6 (11)	6 (11)	12 (22)
	ER positive (%)	8 (15)	34 (63)	42 (78)
	Total (%)	14 (26)	40 (74)	54 (100)*

B	HER2 status	DTC: only HER2-negative DTCs (%)	DTC: at least one HER2-positive DTC (%)	Total (%)
Tumor	HER2 negative (%)	26 (48)	22 (41)	48 (89)
	HER2 positive (%)	4 (7)	2 (4)	6 (11)
	Total (%)	30 (55)	24 (45)	54 (100)**

Table 2: Association between ER-(A) and HER2-(B) status of PT and DTC. * p = 0.031 (Chi-Square-test). ** p = 0.56 (Chi-Square-test).

Primary tumor	DTC status	1 DTC profile				2 DTC profiles			3 DTC profiles		4 DTC profiles
	ER+/HER2-	+	-	-	-	+	-	-	+	+	+
	ER+/HER2+	-	+	-	-	-	+	-	+	-	+
	ER-/HER2-	-	-	+	-	+	+	+	+	+	+
	ER-/HER2+	-	-	-	+	-	-	+	-	+	+
ER+/HER2-	n	2	1	5		13	1	2	2	4	8
(n = 38)	%	5.3	2.6	13.1		34.2	2.6	5.3	5.3	10.5	21.1
ER+/HER2+	n			1		2					1
(n = 4)	%			25.0		50.0					25.0
ER-/HER2-	n			3	1	3		1		1	1
(n = 10)	%			30.0	10.0	30.0		10.0		10.0	10.0
ER-/HER2+	n			1						1	
(n = 2)	%			50.0						50.0	
Total	n	2	1	10	1	18	1	3	2	6	10
(n = 54)	%	3.7	1.9	18.5	1.9	33.3	1.9	5.5	3.7	11.1	18.5

Table 3: Combined ER/HER2-status of DTCs and association with the ER/HER2-status of the PT.

Results

The median number of DTCs was 13 (range 1-95; total number of DTCs detected: 1082; see figure 2). 40 (74%) of the pts had at least one ER-positive (pos) DTC, 24 (44%) at least one HER2-pos DTC, 14 (26%) at least one ER-pos/HER2-pos DTC, and 50 (93%) at least one ER-negative/HER2-negative (neg) DTC, while 10 (19%) pts had only ER-neg/HER2-neg DTCs.

The concordance rate between ER-status on DTCs and PT was 74%. Pts with an ER-pos PT were significantly more likely to have at least one ER-pos DTC (34 out of 42) than pts with an ER-neg PT (6 out of 12; Chi-square test, $\chi 2 = 4.66$, $p = 0.031$). 39 (93%) of the 42 pts with ER-pos PT had at least 1 ER-neg DTC (see table 2A).

The concordance rate between HER2-status on DTCs and PT was 52%. The probability of having at least one HER2-pos DTC was not related to the HER2-status of the PT (Chi-square test, $\chi 2 = 0.34$, $p = 0.56$). 22 (46%) of the 48 pts with a HER2-neg PT had at least one HER2-pos DTC. All of the 6 pts with a HER2-pos PT had at least one HER2-neg DTC (see table 2B).

7 out of 10 pts with a triple-neg PT had at least one DTC pos for ER, HER2 or both. Further the heterogeneity of the ER- and HER2-expression on DTCs compared to the PT for different DTC counts was evaluated. We detected all possible combinations of ER- and HER2-experssion on DTCs regardless of the respective status of the PT (for details refer to table 3).

Conclusions

Our study confirms that the ER- and/or HER2-status on DTCs may differ compared to the PT. This discordance could be especially important for pts with a triple-neg PT and ER-pos or HER2-pos DTCs, since they might respond favorably to an endocrine or HER2-targeted therapy. On the other hand, the presence of ER-neg or HER2-neg DTCs in pts with ER-pos or HER2-pos PT might explain some of the failures of adjuvant endocrine or HER2 targeted therapy.

Acknowledgment

We would like to thank all patients for participating at this study and donating their BM samples for research purposes.