Correlation between breast cancer associated mutations in BRCA1/2 genes and circulating tumor cells in primary breast cancer patients

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Background

- Breast cancer is the most commonly diagnosed malignancy among women with metastatic disease that is typically an incurable condition.
- The metastatic cascade is characterized as a multi-step process that is triggered by circulating tumor cells (CTCs).
- The prognostic and predictive value of CTCs was consistently reported by numerous studies, not only in metastatic, but also in primary breast cancer (PBC).
- Experimental and translational research data indicate that the generation of CTCs is a continuous process spanning from one end of the spectrum (epithelial phenotype) to the other end (mesenchymal phenotype), and involves those with a partial EMT phenotype.
- CTCs may be considered a heterogeneous population of cells and these subpopulations have different clinical and biological properties.
- The most common somatic mutations in breast cancer are TP53, PIK3CA and GATA3, which are present in >10% of all breast cancers (16).
- Several published studies have investigated the mutational status of CTCs (18-21); however, data on the association between gene mutations in primary tumor tissue and the presence of CTCs in the peripheral blood are lacking.

Study aim

- **The aim of the study was to examine mutational status of primary breast cancer (PBC) tumor tissues and correlate findings with clinical parameters as well as CTCs status.**

Results

- Six of the 78 samples (7.7%) were excluded due to poor quality of DNA for analysis. Mutations were detected in 68.1% (49/72) of tumor samples, 36.1% (26/72) were classified as likely pathogenic and 61.1% (44/72) as pathogenic while in 27.8% (20/72) of tumors no mutation was detected. In 43.1% (31/72) of tumor samples the patient's single pathogenic or likely pathogenic mutation was detected, in 18.1% (13/72) patient mutations in two genes were found, while in 4.2% (3/72) of tumor samples patient mutations in 3 genes were detected.
- The most commonly affected genes were *TP53*, mutated in 25.0% (18/72) tumors, followed by *PIK3CA* mutated in 22.2% (16/72) tumors, *BRCA1/2* in 9.7% (7/72) tumors (2 for *BRCA1* and 5 for *BRCA2*), *CDH1* and *GATA3* in 6.9% (5/72) tumors. *RUNX1* and *PTEN* were mutated in 4.2% (3/72) tumors, *NF1*, *BRIP1* and *ATM* in 2.8% (2/72) tumors while *BARD1*, *CDKN1B*, *GNPTAB*, *KRAS*, *PIK3R1* and *PMS2* were mutated in 1.4% (1/72) tumor.
- There were no differences in the number of tumors with pathogenic or likely pathogenic mutations between participants with detectable CTCs in the peripheral blood compared with patients with non-detectable CTCs (67.9 vs. 72.7%, respectively; P=0.79). This difference remained unchanged when likely pathogenic mutations were excluded and no mutations vs. pathogenic mutations were compared (46.4 vs. 52.3%, respectively; P=0.78). Similarly, there were no differences in CTC status regarding mutations of TP53 and PIK3CA and/or between tumors with single mutations vs. those with double/triple mutations. However, no *BRCA1/2* mutations were detected in CTC-negative tumors compared with 9.7% of *BRCA1/2* mutations (P=0.08) in CTCs-positive tumors. Moreover, 4 (23.5%) patients with epithelial CTCs in peripheral blood had *BRCA1/2* mutations compared to 0 (0%) patients without *BRCA1/2* mutations (P=0.02), while there was no significant correlation between mutation in the specific gene and presence of CTC_EMT-positive cells. Similarly, there was a trend for an increased mutation rate of genes other than *TP53*, *PIK3CA* and *BRCA1/2* in CTC-positive compared with CTC-negative patients (61.4 vs. 39.3%, respectively; P=0.09) (see Table).
- Examination of specific mutations revealed a significant association between *TP53* mutation and HER2-positive status, hormone receptor negativity, high grade and increased tumor cell proliferation, as determined by the expression of Ki67. *PIK3CA* mutations were associated with lower grade and low proliferation rate, as determined by Ki67 (see Table).

Table: Association between mutation status and clinicopathological characteristics (bold - statistically significant).

		Any mutation vs. no mutation		*TP53*		*PIK3CA*		*BRCA1/2*		Other than *TP53, PIK3CA, BRCA*		Double/triple hit vs. zero/single	
Variables	**No.**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Grade													
1 and 2	42	29	69.0	9	21.4	13	31.0	3	7.1	23	54.8	9	21.4
3	25	18	72.0	11	44.0	2	8.0	3	12.0	13	52.0	6	24.0
P-value			1.00		0.06		**0.04**		0.66		1.00		1.00
Hormone receptor status													
Negative for both	12	11	91.7	9	75.0	1	8.3	2	16.7	11	91.7	4	33.3
Positive for either	60	40	66.7	12	20.0	15	25.0	4	6.7	27	45.0	14	23.3
P-value			0.16		**0.0004**		0.28		0.26		**0.004**		1.00
HER2 status													
Positive	16	13	81.3	9	56.3	1	6.3	1	6.3	10	62.5	4	25.0
Negative	56	38	67.9	12	21.4	15	26.8	5	8.9	28	50.0	14	25.0
P-value			0.37		**0.01**		0.10		1.00		0.41		1.00
Ki 67 (cut-off 20%)													
Low	42	29	69.0	5	11.9	13	31.0	2	4.8	18	42.9	10	23.8
High	30	22	73.3	16	53.3	3	10.0	4	13.3	20	66.7	8	26.7
P-value			0.80		**0.0002**		**0.05**		0.23		0.06		0.79
CTC epithelial													
Negative	**28**	19	67.9	8	28.6	4	14.3	0	0.0	11	39.3	7	25.0
Positive	**17**	13	76.5	3	17.6	4	23.5	4	23.5	11	64.7	3	17.6
P-value			0.74		0.49		0.45		**0.02**		0.13		0.72

Conclusions

- **To the best of our knowledge, the present study was the first that revealed the association between the presence of epithelial CTCs in the peripheral blood and mutations of the *BRCA1/2* genes in primary tumor tissue. We observed a numerically higher mutation rate in genes other than *TP53* and *PIK3CA* and *BRCA1/2* in patients with CTC-positive compared with CTC-negative breast tumors; however, the differences did not reach statistical significance. The most commonly mutated genes in our patient cohort included *TP53*, *PIK3CA*, *BRCA1/2*, *CDH1* and *GATA3*, corresponding to the observed incidence in published datasets**
- **In conclusion, a correlation between the presence of epithelial CTCs in the peripheral blood and mutations of *BRCA1/2* genes in primary tumor tissue was identified, while there was no mutation in specific genes associated with CTC_EMT. The number of mutated breast cancer-associated genes was not associated with the presence of CTCs or the mutation of genes other than *BRCA1/2*, suggesting that different factors may be involved in the generation and migration of CTCs. These data support the concept that CTCs are of high biological and clinical value in breast cancer.**

Patients and Methods

Patients

- Patient cohort included PBC patients with stages I-III after definitive surgery. Patients enrolled in this study were selected from a cohort of 427 PBC patients analyzed in a previous study detecting CTCs with EMT phenotype in 77 (18.0%) patients with early breast cancer. Fresh frozen tumor tissue and status of CTCs in peripheral blood were available for all enrolled participants. Patients' data were also recorded and statistically analyzed. Healthy donors (n=60) were age-matched women without breast cancer. The present study was approved by the Institutional Review Board (IRB) of the National Cancer Institute of Slovakia. Written informed consent was obtained from each participant prior to study enrollment..

Methods

- CD45+ depleted blood taken at day of surgery were used for RNA extraction using Trizol LS (Sigma Aldrich). CTCs were detected by quantitative RT-PCR assay for expression of epithelial (EP; *CK19*) or epithelial-mesenchymal transition (EMT) genes *(TWIST1, SNAIL1, SLUG, ZEB1*).
- DNA was extracted from fresh frozen primary tumor tissue and used for targeted resequencing using SureSelect XT (Agilent Technologies) probes and enrichment protocol and sequenced on MiSeq platform (Illumina). Following BaseSpace-based sequencing data analysis, read mapping and variant calling sequencing data were annotated and filtered using Ingenuity Variant Analysis (Qiagen).
- The patients' characteristics were recorded and summarized as the median (range) for continuous variables and frequency (percentage) for categorical variables. Categorical data were tested by Fisher's exact test or Chi-squared test. All P-values were two-sided, and P≤0.05 was considered to indicate statistically significant differences. Statistical analyses were performed using NCSS 2007 statistical software (NCSS LLC).

Reference

Mego M, Karaba M, Sedlackova T, et al. Mol Clin Oncol. 2020 Jun;12(6):565-573. doi: 10.3892/mco.2020.2026.

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