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Short Communication

$(CA)_n$ Microsatellite polymorphism of *ERBB-1* in breast cancer

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ABSTRACT

The aim of this study was to determine polymorphism of repeated sequences $(CA)_n$ in the *ERBB-1* gene. The study group included 197 breast cancer patients and 180 healthy women. DNA was isolated from fresh-frozen tumour tissue and from peripheral blood. *ERBB-1* $(CA)_n$ microsatellite polymorphism was examined by polymerase chain reaction (PCR). A polymorphic simple sequence repeat region of 9–23 CA repeats was detected in both groups. Homozygotes comprised 22% and 34% of breast cancer patients and controls, respectively ($P = 0.009$). An allelic imbalance (AI), mostly in the shorter allele, was found in 27% of breast cancer patients. AI occurrence was associated with the lack of oestrogen receptors in tumour cells ($P = 0.05$); otherwise, there were no correlations between histoclinical features and $(CA)_n$ microsatellite polymorphism of *ERBB-1*. It was concluded that an allelic imbalance is a common feature in breast cancer patients and may coincide with the lack of oestrogen receptors in tumour cells. The clinical relevance of *ERBB-1* microsatellite polymorphism in breast cancer remains to be established.

1. Introduction

Overexpression of ERBB-1 is a common feature in breast cancer and is considered an adverse prognostic factor.[1–3] ERBB-1 overexpression seems to be caused by upregulated transcriptional activity, which is modulated by the number of CA short sequence repeats (SSRs) in the polymorphic region of *ERBB-1* intron 1.[4–8] Indeed, in breast cancer a low number of CA repeats was found to be associated with increased ERBB-1 expression.[4–7] Additionally, it was postulated that allelic imbalance (AI) in breast cancer tissue at the *ERBB-1* locus may carry an adverse prognosis.[9] Microsatellite polymorphism of *ERBB-1* seems to be related to ethnicity.[10] In this study we determined polymorphism of repeated sequences $(CA)_n$ and allelic imbalance (AI) in the *ERBB-1* gene in relation to histoclinical features of breast cancer in Polish women.

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2. Patients and methods

2.1. Patients

The study group included 197 consecutive breast cancer patients who underwent multidisciplinary treatment between 1998 and 2002 (Table 1). The control group consisted of 180 healthy women. Tumour specimens collected during surgery (before systemic treatment) and blood samples from breast cancer patients and controls were frozen immediately for further analysis. All patients signed informed consent forms.

2.2. DNA isolation

DNA was isolated from fresh-frozen tumour tissue and from peripheral blood with the QIAamp DNA Mini Kit (Qiagen, Germany) and Whole Blood Specimen Preparation Kit (Roche Diagnostics System, Poland), respectively. The control for polymerase chain reaction (PCR) fragment length was DNA from the tumour cell line DNA MDA-MB-468.

2.3. Microsatellite PCR

PCR amplification was performed in a 50-μl reaction volume containing 50 pmol of previously described primers,[8] one unit of *Taq* DNA polymerase, 200 nmol of each deoxynucleotide triphosphate, 1 × reaction buffer, 1.5 mM $MgCl_2$ (all from Promega, United States of America (USA)) and 200 ng DNA. The PCR products were diluted 10 times, mixed with formamide containing 0.5 μl of GeneScan 500 (Tamra) fluorescent size standard (Applera) and separated with capillary electrophoresis system (ABI PRISM 310 DNA Analyzer). The collected data was evaluated with GeneScan Analysis Software (Applera). All analyses were performed in duplicates of independent PCR reactions.

To standardise the analysis, the allelic imbalance (AI) score was calculated using the following equation:[11] $AI = T2 \times N1/T1 \times N2$, where *N*1 is the first (shorter) allele area in non-tumour tissue, *N*2 is the second allele area in non-tumour tissue, *T*1 is the first allele area in tumour tissue, and *T*2 is the second allele area in tumour tissue. AI cut-off values of < 0.6 and > 1.67 were used for the loss of longer and shorter allele, respectively.

2.4. Statistical methods

Statistical analysis was performed with Statistica for Windows software (Statsoft Co, USA, version 6.0.). The level of significance was set at $P < 0.05$. Shapiro-Wilk test was applied to test the normality of distribution. A set of non-parametric tests, including χ^2 test, Yates corrected χ^2 test and Fisher's exact test, was used to analyse correlations between the

Table 1 – Patient characteristics

Variable	No. of patients (%)[a]
pT stage	
TX	2 (1)
$T1_1$	60 (31)
T2	80 (41)
T3	26 (13)
T4	26 (13)
pN stage	
N0	108 (55)
N1	52 (26)
N2	34 (17)
Histology	
Ductal	136 (69)
Lobular	32 (16)
Other types	23 (12)
Grade (Richardson-Bloom)	
G1	11 (6)
G2	52 (26)
G3	60 (31)
Steroid receptor status	
ER+	107 (54)
ER-	89 (45)
PgR+	106 (54)
PgR–	90 (46)

a Numbers do not add to 197 (100%) due to a few missing data.

Table 2 – Distribution of allele combinations of the CA short sequence repeats (CA-SSR) in the ERBB-1 gene in breast cancer patients and in control group

Allele 1	Allele 2	Breast cancer patients		Control group	
		No. of cases	%	No. of cases	%
9	16	1	0.5	0	0
14	18	0	0	1	0.55
14	20	2	1.0	0	0
14	21	0	0	1	0.55
15	15	0	0	1	0.55
15	16	2	1.0	0	0
15	18	0	0	1	0.55
15	19	0	0	1	0.55
15	20	2	1.0	0	0
16	16	29	14.8	34	18.9
16	17	10	5.1	1	0.55
16	18	38	19.4	39	21.6
16	19	3	1.5	1	0.55
16	20	32	16.3	27	15.0
16	21	13	6.6	16	8.9
16	22	2	1.0	1	0.55
16	23	1	0.5	0	0
17	17	0	0	10	5.6
17	18	10	5.1	1	0.55
17	19	1	0.5	1	0.55
17	20	4	2.0	1	0.55
17	21	2	1.0	4	2.2
17	23	1	0.5	0	0
18	18	6	3.0	7	3.9
18	19	1	0.5	1	0.55
18	20	15	7.6	14	7.8
18	21	6	3.0	5	2.8
19	19	1	0.5	0	0
19	20	3	1.5	2	1.1
20	20	8	4.1	10	5.6
20	21	3	1.5	0	0
21	22	1	0.5	0	0
Total		197	100	180	100

molecular markers' status and clinicopathological patient data.

3. Results

The most common allele in both groups was 16 CA repeats (42% and 41% in controls and breast cancer patients, respectively). Alleles containing 16 CA, 18 CA and 20 CA repeats comprised more than 80% in both controls and breast cancer patients. In total, 32 different diploid genotypes were found (Table 2). Of those, the most prevalent in both groups was 16/18 CA repeat combination (22% and 19% in controls and patients, respectively). The 16/16 CA combination was found in 20% of controls and 15% of breast cancer patients, and 16/20 CA in 15% and 16%, respectively. The 16 CA containing combinations: 16/16 CA, 16/18 CA and 16/20 CA, accounted for 56% and 50% of the controls and breast cancer patients, respectively. Homozygotes comprised 34% and 22% of controls and breast cancer patients, respectively (P = 0.009).

Short (less than 19 CA repeats) and long (19 or more CA repeats) alleles were distributed equally in both groups. The most common short alleles occurred in 73% of controls and 70% of breast cancer patients. The combinations of two short alleles occurred in 53% and 49% of controls and breast cancer patients, respectively, and the combinations of two long alleles in 7% and 8%, respectively. The allelic imbalance (AI) in heterozygotes occurred in 27% of cases, 22% of which was the loss of the shorter allele.

AI was more common among oestrogen receptor negative compared with oestrogen receptor positive breast cancer cases (34% and 19%, respectively; P = 0.05). Otherwise, there were no significant correlations between AI and major clinicopathological characteristic including pT, pN, histological tumour grade and progesterone receptor status.

4. Discussion

The *ERBB* gene family is critically involved in the mammary gland carcinogenesis.[12,13] The increased activity of *ERBB* genes in breast cancer, expressed by gene amplification, protein overexpression or abnormal transcriptional regulation, carries an adverse prognosis.[14,15] Additionally, this feature serves as a predictive factor and target for therapy.[16]

Animal studies showed that increased expression of *ERBB* oncogenes, particularly *ERBB-1*, is involved in early breast cancer carcinogenesis.[17] A polymorphic SSR region with 14 to 21 CA dinucleotides was found in a Caucasian reference pedigree close to the downstream enhancer element.[8] Most common alleles in that population were 16 CA, 18 CA and 20 CA repeats (42%, 20% and 26% of cases, respectively). Similar frequencies of these alleles were found in our series. Additionally, we demonstrated the occurrence of previously not reported alleles containing 22 CA and 23 CA repeats, and a very short allele containing 9 CA repeats. It was noteworthy that the distribution of alleles in the group of breast cancer patients and healthy controls was similar, except for the proportion of homozygotes, which was higher in the control group (P = 0.009). The frequency of homozygotes in the control group was similar to that in the Caucasian CEPH reference pedigrees.[8]

The distribution of allele combinations in our series is similar to that found in German women.[9] Remarkably, in both populations the three most frequent allele combinations were 16/16, 16/18 and 16/20 CA repeats.[18] Neither study found any correlation between the frequency of allele combinations and histoclinical features. Our population seems to be more homogenous than that from Germany, which included 44 different diploid genotypes.[18] Otherwise there were no apparent differences in the distribution of *ERBB-1* genotypes in both populations.

The allelic frequencies of the *ERBB-1* intron 1 polymorphisms seem to vary considerably in geographically distant populations, with short alleles prevailing in Europe and long alleles in Asia.[10,19] Interestingly, preliminary results in non-small cell lung cancer patients receiving ERBB-1-specific tyrosine kinase inhibitor gefitinib (ZD1839) showed the presence of ethnic differences in drug response, with consistently higher response rates in Japanese than in Caucasian patients.[10,20] Additionally, the susceptibility to gefitinib was found to be strongly related to *ERBB-1* somatic mutations,[21] which are more frequent in the Asian population.[22]

The rate of AI in the present study (27%) was similar to that in the group of German breast cancer cases (34%),[7,9] and lower than in informative breast cancer cases from Japan (55%).[20] The high rate of AI in the Japanese series may be explained by a large proportion of long alleles, which are associated with the higher likelihood of AI compared with the short alleles.[7,9]

In our series AI occurrence was associated with oestrogen receptor status (P = 0.05). To the best of our knowledge this correlation has not been reported previously and requires confirmation. Similarly to other studies,[9] we have not found any other correlations between histoclinical variables and $(CA)_n$ microsatellite polymorphism of *ERBB-1*. Due to relatively short follow-up and small number of events we have not analysed here the clinical relevance of this feature. This issue, however, certainly warrants further research.

Conflict of interest statement

None declared.

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REFERENCES

1. Gullick WJ, Marsden JJ, Ward B, et al. Expression of epidermal growth factor receptors on human cervical, ovarian, and vulval carcinomas. *Cancer Res* 1997;**46**:285–92.
2. Gasparini G, Boracci P, Bevilacqua P, et al. Multiparametric study on the prognostic value of epidermal growth factor receptor in operable breast carcinoma. *Breast Cancer Res* 1994;**29**:59–71.

3. Benz CC, Brandt BH, Zanker KS. Gene diagnostics provide insights into breast cancer prognosis and therapy. *Gene* 1995;**159**:3–7.
4. Gebhardt F, Burger H, Brandt B. Modulation of EGFR gene transcription by secondary structures, a polymorphic repetitive sequence and mutations – links between genetics and epigenetics. *Histol Histopathol* 2000;**15**:929–36.
5. Gebhardt F, Zanker KS, Brandt B. Modulation of epidermal growth factor receptor gene transcription by polymorphic dinucleotide repeat in intron 1. *J Biol Chem* 1999;**274**:13176–80.
6. Gebhardt F, Rotger A, Zanker KS, Brandt B. Regulation of epidermal growth factor receptor in human carcinoma cell lines by structurally different 5'-transcripts. *Proc Amer Assoc Cancer Res* 1997;**38**:184.
7. Tidow N, Boecker A, Schmidt H, et al. Distinct amplification of an untranslated regulatory sequence in the egfr gene contributes to early steps in breast cancer development. *Cancer Res* 2003;**63**:1172–8.
8. Chi DD, Hing AV, Helms C, Steinbrueck T, Mishra SK, Donis-Keller H. Two chromosome 7 dinucleotide repeat polymorphisms at gene loci epidermal growth factor receptor (EGFR) and pro alpha 2 (I) collagen (COL1A2). *Hum Mol Genet* 1992;**1**:135.
9. Buerger H, Gebhardt F, Schmidt H, et al. Length and loss of heterozygosity of an intron 1 polymorphic sequence of EGFR is related to cytogenetic alterations and epithelial growth factor receptor expression. *Cancer Res* 2000;**60**:854–7.
10. Liu W, Innocenti F, Chen P, et al. Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. *Clin Cancer Res* 2003;**9**:1009–12.
11. Canzian F, Salovaara R, Hemmiki AI. Semiautomated assesment of loss of heterozygosity and replication error in tumours. *Cancer Res* 1996;**56**:3331–7.
12. Holbro T, Civenni G, Hynes NE. The ErbB receptors and their role in cancer progression. *Exp Cell Res* 2003;**284**:99–110.
13. Gullick WJ, Srinivasan R. The type 1 growth factor receptor family: new ligands and receptors and their role in breast cancer. *Breast Cancer Res Treat* 1998;**52**:43–53.
14. van de Vijver MJ. Assessment of the need and appropriate method for testing for the human epidermal growth factor receptor-2 (HER2). *Eur J Cancer* 2001;**37**(suppl 1):11–7.
15. Zaczek A, Brandt B, Bielawski KP. The diverse signaling network of EGFR, HER2, HER3 and HER4 tyrosine kinase receptors and the consequences for therapeutic approaches. *Histol Histopathol* 2005;**20**:1005–15.
16. Ross JS, Fletcher JA, Bloom, et al. Targeted therapy in breast cancer: the HER-2/neu gene and protein. *Mol Cell Proteomics* 2004;**3**:379–98.
17. Brown NM, Wang J, Cotreno MS, et al. Prepubertal genistein treatment modulates TGF-α, EGF and EGF-receptor mRNAs and proteins in the rat mammary gland. *Mol Cell Endocrinol* 1998;**144**:149–65.
18. Brandt B, Hermann S, Straif K, Tidow N, Buerger H, Chang-Claude J. Modification of breast cancer risk in young women by a polymorphic sequence in the egfr gene. *Cancer Res* 2004;**64**:7–12.
19. Bielawski KP, Klos P, Welnicka-Jaskiewicz M, et al. An epidermal growth factor receptor intron 1 polymorphism in healthy women in Poland. *Int J Biol Markers* 2005;**20**:184–8.
20. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small cell lung cancer. *J Clin Oncol* 2003;**21**:2237–46.
21. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;**350**:2129–39.
22. Paez G, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlations with clinical response to gefitinib therapy. *Science* 2004;**304**:1497–500.