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TITLE: Microsatellite and Chromosomal Instability in Breast Cancer

PRINCIPAL INVESTIGATOR: Svetlana Baranovskaya, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute
La Jolla, California 92037

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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

During the reporting period, we analyzed a set of breast tumors for chromosomal and microsatellite instabilities, two fundamental pathways of genomic instability that play a critical role in the pathogenesis of several types of human cancers.

We found that 22% of all breast cancer cases have allelic imbalances in one or more of 14 microsatellite markers, which covered a region of 21MB of chromosome 7 around the EGFR gene. We found both amplifications (8%) and losses of heterozygosity (14%) of the EGFR-containing region of chromosome 7.

Microsatellite instability (MSI) was assessed with the use of 3 mononucleotide and 4 dinucleotide markers. None of the 202 samples analyzed showed any frameshift mutations in the mononucleotide repeats including polyA sequences in cancer susceptibility genes BRCA1 and BRCA2. One breast cancer tumor showed MSI at all four dinucleotide markers used for MSI status evaluation, but not at the mononucleotide markers. These data indicate that microsatellite instability is very uncommon (less than 0.5%) in breast cancer tumors. Our data show that MSI is not important in the pathogenesis or progression of breast cancer in contrast to other genetic mechanisms, notably recurrent chromosomal imbalances that dysregulate function of genes controlling cell growth, differentiation and apoptosis.

14. SUBJECT TERMS
breast cancer, gene amplification, LOH, EGFR, MSI

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Introduction.

Two major types of genetic aberrations have been found to play a critical role in tumor development: chromosomal segment deletions/amplifications (chromosomal instability) and point mutations, which often involve microsatellite sequences due to inactivation of the DNA mismatch repair machinery (microsatellite instability).

Chromosomal instability is found frequently in cancers and is believed to contribute to their development and progression through amplification of oncogenes or inactivation of tumor suppressor genes (reviewed in Gollin SM, 2004). The overexpression of the well known oncogene epidermal growth factor receptor (EGFR) is frequently detected in breast cancer (reviewed in Klijn JG, 1992). Chromosomal instability involving the EGFR containing locus of the chromosome 7p12 could be responsible for the EGFR regulation in breast cancer.

Microsatellite instability (MSI) is a distinct tumor phenotype that is being increasingly reported in a number of tumors including gastric, colon, endometrium and ovarian cancers (reviewed in Yamamoto H., 2002; Perucho M., 2003). The spectrum of the cancer genes that are targets for microsatellite instability may be cancer site specific. For example, BRCA1 and BRCA2 genes, which are found to be involved in breast cancer susceptibility, contain microsatellite repeats in their coding regions and, thus, may be targets for MSI in breast cancer. The prevalence of MSI in breast cancer, however, was not assessed.

During the reporting period, we estimated the relative involvement of the chromosomal and microsatellite instabilities in development of breast cancer.

Body.

Evaluation of the chromosomal instability in breast cancer (tasks#1 and 3).

We performed microallelotyping analysis of the 7p12 chromosomal region with 14 microsatellite markers, which covered a region of 21 Mbp in length. We also used distal telomeric markers D7S2477, D7S531, D7S2423 and D7S550 to determine if the whole chromosomal arm was gained or deleted in tumors. We employed multiplexed PCR to unambiguously distinguish chromosomal gains versus chromosomal losses (Fig. 1). The results of this analysis are summarized in Fig. 2.

This allelotyping analysis revealed two amplicons, 1,318 kbp and 2,202 kbp in length. The overlapping part of the two amplicons spanned 846 kbp and contained the EGFR gene. Two more cases (120, 108) showed allelic gain at several consecutive markers (including telomeric), which indicated a copy number gain of 7p for the first case and the entire chromosome 7 for the second one.

In addition to the gains of the 7p12 region, 14% of breast tumors demonstrated loss of heterozygosity (LOH). The lengths of deleted chromosomal segments ranged from 2.3MB (for example, case 112) to the entire chromosome (for example, cases 26 and 91). The smallest common region of these chromosomal losses contained the EGFR gene. Our data indicate that the 7p12 locus is not only amplified but also is frequently deleted in breast tumors and EGFR most likely is a target of these rearrangements.
Fig. 1. Allelotyping of the breast tumors by multiplexed PCR. Top panel: microsatellite markers to be examined for gain/LOH. Bottom panel: microsatellite markers without allelic imbalances used as a reference (control). Names of the microsatellite markers analyzed are shown under the corresponding pictures. N, normal tissue; T, tumor tissue. Gain at the D7S499 in cases 84 and 108; at the D72550 loci in cases 48 and 120. LOH at the EGFR loci in cases 91, 26, 126 and 112; at the D72550 in case 115; at the D7499 in case 45.
Fig. 2. Map of deletions and gains at 7p12 chromosomal region in a set of breast tumors.

No correlation was found between allelic imbalances and clinical parameters such as histological grade, presence of the metastasis, tumor size or tumor reccurence. The average age of breast cancer patients with AI at EGFR locus was less than the age of patients without AI (51 years versus 57 years). This difference however was not significant when a t-test used.

*Evaluation of the MSI in breast cancer (task#2).*

The set of 202 breast tumors was analyzed for the presence of microsatellite instability using three mononucleotide repeats (BAT-26, BRCA1, BRCA2) and four dinucleotide repeats (D5S123, D2S346, D7S2550, EGFR-CA). None of the samples showed a frameshift mutation in the mononucleotide repeats including polyA sequences.
Fig. 3. Microsatellite analysis of one breast cancer sample with MSI. Electrophoregrams of the repeat-containing PCR fragments amplified from normal and tumor tissue DNA isolated from MSI-positive breast cancer patient are presented. Top panel: Mutations in dinucleotide markers. Bottom panel: mononucleotide microsatellite markers without mutations. Names of the microsatellite markers analyzed are shown under the corresponding pictures. N, normal tissue; T, tumor tissue.

in the coding regions of cancer susceptibility genes BRCA1 and BRCA2. One breast cancer tumor showed MSI at all four dinucleotide markers used for MSI status evaluation but not at the mononucleotide markers (Fig. 3). Our data convincingly demonstrated that microsatellite instability is very rare event in breast cancer tumors with a frequency of less than 0.5% of mutations in the dinucleotide repeats.
Key research accomplishments.

- We found that 22% of all breast cancer cases have allelic imbalances at least in one of the 14 microsatellite markers analyzed. These results suggest that chromosomal instability is a frequent event in breast cancer.
- The new region of frequent allelic imbalances at 7p12 was identified and the EGFR gene most likely is a target of these rearrangements. We showed that the EGFR locus is not only amplified in breast tumors, but it is also frequently deleted.
- Evaluation of the MSI status in the 202 samples showed that microsatellite instability is a very rare event in breast cancer tumors with a frequency of less than 0.5% of frameshift mutations in the dinucleotide repeats.

Reportable outcome.

Baranovskaya S, Malkhosyan SR. Frequent copy number alterations of chromosome 7p12 region in breast carcinomas (in preparation).

Conclusions.

Our data demonstrated that microsatellite instability is not relevant for the development of breast cancer in contrast to other genetic mechanisms, recurrent chromosomal imbalances that lead to losses or amplification of the genes controlling tumorigenesis.

Our study has found also that change in the EGFR gene copy number is a frequent event in breast cancer. Both the amplification and the deletion of the EGFR gene facilitate tumorigenesis in a set of breast tumors. This finding has a high importance, because epidermal growth factor receptor is a target for some chemotherapy drugs.

References.


ASSISTANCE AGREEMENT

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PRINCIPAL INVESTIGATOR:
Svetlana Baranovskaya, Ph.D.

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1. The basic grant Statement of Work is deleted. The revised 24 September 2002 Statement of Work submitted by the recipient is incorporated herein by reference.

2. The Principal Investigator is changed:

From: Izumi Nakamura, M.D.
To: Svetlana Baranovskaya, Ph.D

RECIPIENT

ACCEPTED BY: The 24 September 2002 letter request signed by Jean Freiser is incorporated herein by reference as agreement.

RECIPIENT SIGNATURE

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List of personnel receiving pay from the research effort:

Svetlana Baranovskaya, PhD

Publications:

Baranovskaya S., Perucho M., Malkhosyan S.R. Comparative Hybridization of AP-PCR Arrays (CHAPA), new method for detection of single copy number changes in cancer cell genome (estimated date of submission October, 2004)

Baranovskaya S., Falchetti M., Malkhosyan S.R. Modulation of EGFR expression by intron 1 dinucleotide repeat expansion in tumors of the microsatellite mutator phenotype (estimated date of submission September, 2004)

Baranovskaya S., Malkhosyan S.R. Frequent copy number alterations of chromosome 7p12 region in breast carcinomas (estimated date of submission September, 2004)


The paper was chosen to be included in the "highlights of exciting advances from the primary literature" published by the Nature Reviews (Greenwood E. A perfect mismatch. Nature Reviews 2:76-77, 2002)


Abstracts (selected)

Baranovskaya S., Malkhosyan S. High throughput analysis of genomic aberration in cancer”. The 4th Principal Investigator Meeting of the Innovative Molecular Analysis Technologies Program (June, 2003)


Papayn, L; Kobilyanskaya, V; Kapustin, Blinov, M; Baranovskaya, S; Schwartz, E; Tarkovskaya, L; Beliazo, O; Khrolova, P; Kargin, V; Saltikova, N. Factor V Leiden as important risk factor of hypercoagulation and thrombophilia. *Int. J. Haemost. Thromb. Res.* 28: Suppl. 2, 1998


