Microsatellite Markers in Breast Cancer Studies

Janatova M., Pohlreich P.
Department of Biochemistry and Experimental Oncology
of the First faculty of Medicine, Charles University in Prague, Czech Republic

Abstract: Microsatellites are tandem repeats of simple polymorphic sequences randomly distributed in non-coding regions of DNA. They can be used in cancer genetics and indirect cancer diagnosis and can help unraveling the genetic basis of tumor formation and progression of cancer. Breast cancer is a complex disease in which numerous genetic alterations occur. The knowledge of specific genetic changes and their biological consequences is critical to an understanding of breast cancer tumorigenesis, screening and treatment of patients. Microsatellites can undergo two events during tumor progression. Loss of heterozygosity indicates absence of one allele in a given locus, which is associated with the loss of a corresponding genes. Microsatellite instability reflects replication errors induced by defective function of mismatch repair genes and is demonstrated with the appearance of novel, noninherited alleles in tumor cells and represents a specific pathway of tumor development. Both events serve as prognostic markers, which can be correlated with clinicopathological features and can help exploring breast cancer formation.

Key words: Breast cancer – Loss of heterozygosity – Microsatellite instability

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Mailing Address: Janatová Markéta, M.A., Department of Biochemistry and Experimental Oncology of the First faculty of Medicine, Charles University, U Nemocnice 5, 128 53 Prague 2, Czech Republic, Phone +420 224 965 824, e-mail: mjana@lf1.cuni.cz

The analysis of common DNA sequence variations or polymorphisms is used for identification of human disease genes. The majority of human DNA is not involved in coding regions and sequence differences in this DNA between individuals occur very often. A genetic locus is considered polymorphic if the rare allele has a frequency of at least 0.01, with the result that heterozygotes carrying this allele occur at frequency greater than 2%. More than one third of human genetic loci coding for proteins has been found to be polymorphic. Polymorphisms can exist either as simple nucleotide changes or as repetitions of the same DNA sequence.

We recognize two types of DNA sequences. The single-copy DNA is more common type of DNA and composes about 75% of the genome. The rest of the genome consists of repetitive DNA. There are two classes of repetitive DNA sequences: dispersed repetitive DNA and satellite DNA. While dispersed repeats are scattered throughout the genome, satellite repeats are clustered together in tandem in head-to-tail fashion. They vary in the length of the repeats as well as in the number of the repeats. In contrast to restriction fragment length polymorphism (RFLP), which can be only dimorphic and therefore its applicability is limited through its low informativeness, the satellites, also called tandem repeat sequences, are better DNA markers. These polymorphisms can consist of a different number of tandem repeats and can occur in a high number of alleles. According to the length of the satellite repeats we recognize two types of markers.

Minisatellites, also called variable number of tandem repeats (VNTRs), are length polymorphisms. These loci contain repeats 20 to 70 nucleotides long. They are defined by restriction sites that flank the repeated region. VNTR locus can be several thousands base pairs long and Southern blot analysis is usually performed to characterize it.

Microsatellites, also called simple sequence repeats (SSRs), are much smaller. They contain only 1 – 6 base pairs and their total size does not exceed few hundreds of nucleotides. It is much easier to analyze them in laboratory in large scale by polymerase chain reaction technique. The tandem repeats of microsatellites have no known function but they are wide-spread throughout the whole genome of all vertebrates. Human genome is estimated to contain almost 100 000 microsatellite loci.

The major mechanism generating the number of variant alleles is a faulty DNA replication. The frequency of this kind of replication error is high enough to make alternative lengths at the polymorphic site common, but low enough that microsatellite changes will usually not occur within a few generations of a family [10].

Microsatellite markers in breast cancer

Microsatellites have such properties that make them a very useful tool for genetic mapping and linkage studies and thus can facilitate identification of disease genes. The variability in their length enables microsatellites to function as stable markers

in breeding studies and in exploring human pedigrees. Moreover, microsatellites can be well employed in cancer genetics and indirect cancer diagnosis. They can help unraveling the genetic basis of tumor formation and progression of cancer and can determine whether an individual has inherited chromosome containing a defective or normal gene.

Breast cancer is a malignancy with numerous genetic alterations. Microsatellite repeats can be affected with two events during tumorigenesis: loss of heterozygosity (LOH) and microsatellite instability (MSI).

Loss of heterozygosity

Loss of heterozygosity indicates absence of one allele at a given locus. To detect LOH, we compare the genotype of the tumor at a given polymorphic microsatellite marker with that of the matching normal tissue of the patient. DNA samples are usually obtained from peripheral lymphocytes and homogenized tumor tissue, respectively. In normal cells, both alleles are present in a 1:1 ratio. During tumorigenesis, one copy of the chromosomal region can be lost. Only one of the variable DNA fragments remains in the tumor [6]. A slight signal can be evident in tumor sample at the position characteristic for the chromosomal region that has been lost during tumorigenesis indicating contamination of the tumor sample with the normal cells. Thus, rather than detecting complete loss of an allele, in many cases we are actually observing a partial shift in the ratio of both parental alleles in the tumor DNA relative to that in normal DNA [11] (Fig. 1).

Loss of heterozygosity at various chromosomal regions (1p, 1q, 3p, 6q, 8p, 11p, 11q, 13q, 16q, 17p, 17q, 18q and 20q) has been reported in both familial and sporadic forms of breast cancer [reviewed 2,5]. In familial cases, the loss of a wild type allele detects complete inactivation of the predisposing tumor suppressor gene. The frequency of LOH in these specific regions ranges between 90%–95% in hereditary tumors [19, 21]. The genetic changes in sporadic forms of cancer are not exactly established yet and lower incidence of LOH in distinct loci is observed. It varies from 20%–60% [25, 7, 16, 13].

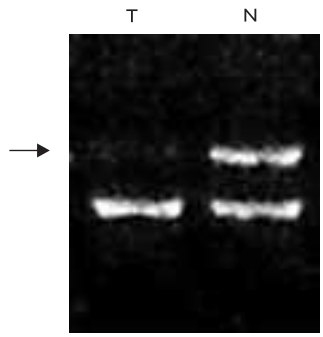

Fig. 1 – The highly polymorphic microsatellite marker distinguishes between normal (N) and tumor (T) tissue. Normal cells retain two copies of a chromosomal region. Arrow indicates loss of one copy during tumorigenesis

Initially, LOH studies have been used extensively to identify regions on chromosomes that may contain putative tumor suppressor genes. *RB1* gene was one of the first successes. In 1986, it was cloned taking advantage of genetic analysis of families and LOH studies of large numbers of tumors [9].

The lack of knowledge of the sequence of events during carcinogenesis remains still unclear. To solve this problem, genome-wide search for loss of

heterozygosity was performed [14]. DNA from 75 human primary breast carcinomas was tested with 184 microsatellite markers and 56 regions of the genome affected by deletions were found. No tumors with the same set of affected regions were found, all tumors had a different pattern of deletions and no highly preferential combination of events was defined. These results confirm the potential complexity of molecular processes involved in mammary carcinogenesis. However, the study of allelic losses enabled to identify preferentially affected regions of genomic DNA. Primary regions were defined by their high incidence and their involvement in tumors with low level of LOH. These primary regions may be early targets and their alteration may represent obligatory steps in the development of breast cancer. In contrast, alteration of the secondary regions may occur at random because they represent either optional targets or a result of genome instability.

Genetic alterations caused by the loss of one allele can serve as prognostic indicators, can predict postoperative prognosis and guide adjuvant therapy. Eiriksdottir *et al.* analyzed LOH at chromosome 13q in sporadic breast tumors and tried to determine their prognostic value. They analyzed 139 sporadic breast tumors using 18 microsatellite markers spanning the entire chromosome arm and correlated the results of LOH analysis with clinicopathological variables. Allelic losses were detected in 43% of tumors. The association of LOH with the high S phase fraction and aneuploidy of tumor cells suggests that one or more genes located at 13q are involved in the control of cell proliferation and maintenance of genome stability. The survival analysis showed that the patients with LOH detected in that region are at risk of 3–4 folds increased mortality and recurrence of a disease [7].

The analysis of allelic losses at 3p chromosome in sporadic invasive breast carcinoma was performed [17]. Specific regions of 3p were analyzed with 19 microsatellite markers in 40 tumors and the results of analysis were correlated with clinicopathological features. 22 tumors with LOH in one or more markers were found. Furthermore, a correlation between LOH and increased tumor grade was found. The obtained results suggest that there are genes on 3p that are involved in control of cell proliferation. Loss of these genes can result in the development of more aggressive tumors.

An extensive study was performed to explore chromosomal regions that are commonly deleted in breast cancer and to show that allelic losses at certain loci correlate with postoperative survival. Hirano *et al.* tested 504 breast tumors with 18 microsatellite markers in loci of tumor suppressor genes or regions where genetic alterations are frequent in breast tumors. They detected six loci (1p34, 3p25, 8p22, 13q12, 17p13.3 and 17q21.1) that served as negative prognostic markers for breast cancers. The patients with allelic losses at these regions had significantly higher risk of shorter postoperative disease-free interval than patients whose tumors retained both alleles [12].

LOH analysis can be used for an identification of inactivating mutations in familial breast cancers [21]. 47 breast tumors were analyzed from patients with high-risk familial history of the disease who had been completely screened for *BRCA1* and *BRCA2* genes and had shown either a germ line mutation, unclassified variant or polymorphism in either of the two genes. In familial breast cancers associated with germ line mutations in either *BRCA1* or *BRCA2*, the most common mechanism of the gene inactivation was complete loss of the wild-type allele. In contrast, in the familial cancers not associated with these genes, the rate of spontaneous LOH appeared to be very low. Thus, the analysis of allelic losses can be used to determine whether a germ line variant of unknown significance in *BRCA* genes is either a deleterious mutation or a common polymorphism.

Microsatellite instability

Microsatellite instability (MSI) is a novel molecular marker of carcinogenesis, reflecting replication errors induced by the defective function of the mismatch repair genes. It indicates the appearance of a novel, noninherited microsatellite allele in tumor cells with persistence of inherited alleles in the normal cell counterpart [24]. MSI can occur during replication of repetitive microsatellite sequences when two strands of DNA slip relative to one another, resulting in small loops of unpaired DNA. Slippage may occur with copies of the repeat unit being inserted or deleted, thereby altering the size of the locus. This results in occurrence of longer or shorter allele in the tumor relative to germ line lengths [3, 15] (Fig. 2).

Normally, the integrity of the genome is controlled by several mechanisms, one of which is the DNA mismatch repair (MMR) system involved in the detection and correction of mismatched base pairs [22]. MSI is tightly associated with MMR genes deficiency and was initially observed in cells from hereditary non-polyposis colorectal cancer (HNPCC), which is characterized by germ line mutations of major MMR genes *hMSH2* and *hMLH1*. The common characteristic of HNPCC is the presence of MSI in 90% of tumors [1]. Defective MMR genes have also been identified in some sporadic colorectal tumors and other types of cancers associated with MSI [26].

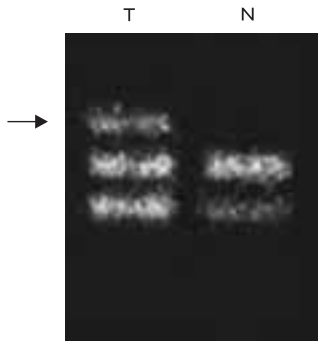

Fig. 2 – Comparison of microsatellite marker from normal (N) and tumor (T) tissue exhibiting microsatellite instability. In normal cells, two alleles are present. Arrow indicates the additional allele in tumor cells

In different studies, MSI has been observed in 0–50% of analyzed breast cancer cases [8, 30, 24, 4, 15], depending on the series of examined patients, on the used technical approach and on the selection of a marker. Such variability suggests that DNA mismatch repair defects, resulting in increased MSI, may play a role only in pathogenesis of particular breast cancers [29].

Siah et al. performed MSI analysis using 6 microsatellite markers in 66 breast tumors. They found all tumors microsatellite stable. They also reviewed data about MSI from other studies and found overall detection rate of MSI in breast cancer very low (2.9%). They concluded that it seemed unlikely that DNA mismatch repair defects, resulting in increased MSI, are major contributors in breast tumorigenesis [29].

To determine the incidence of MSI and its relationship with clinicopathological parameters and patient survival, 6 microsatellite markers in group of 101 cases of breast cancer were investigated [28]. MSI was detected in 11.9% of breast cancer cases. The patients with MSI had larger tumors, more distant metastases, more advanced stage of a disease at diagnosis and reduced survival in comparison to patients without MSI. In contrast, MSI status in HNPCC correlates with better prognosis of a disease [26]. These findings suggest the organ specificity of the tumorigenic pathways [28].

Some studies have demonstrated an association of MSI with defective MMR genes even in breast cancer. Van der Looij et al. studied occurrence of microsatellite instability of seven markers at chromosomes 13q and 17 in a serie of 41 breast tumors and determined their association with *BRCA1* and *BRCA2* gene mutations. 7% of breast tumors showed MSI, but no *BRCA* mutation. Absence of association between MSI and *BRCA1* mutation status suggests that MSI identified in a fraction of breast cancers may reflect possible mutations in one of the DNA mismatch repair genes [30].

Murata et al. in a set of 32 sporadic breast cancer cases with five microsatellite markers demonstrated significant association of MSI with reduced expression of the MMR genes (*hMLH1*, *hMSH2*). All cases with mutations in MMR genes exhibited MSI phenotype. These results indicate the contribution of defects in these genes for the development of sporadic breast cancer [20].

In conclusion, MSI seems to be marker of a wider genomic instability, possibly affecting not only short tandem repeats and other noncoding regions, but also target genes such as those involved in cell cycle control and/or cellular replication [23]. Molecular basis for the MSI observed in breast tumors might be different from mechanisms involved in the MSI in colorectal tumors [18]. However, combined effect of *BRCA1* and *hMLH1* dysfunction may be important in progression of a specific subtype of tumors [30].

Conclusion

Breast cancer is a disease with high heterogeneity of defects leading to tumor formation. The knowledge of specific genetic changes and their biological consequences is critical to an understanding the processes of tumorigenesis and for the development of prevention and treatment [3].

All presented results indicate that loss of heterozygosity and microsatellite instability are significant markers of tumor formation and progression and serve as

a perspective tool to improve our knowledge about breast carcinogenesis. An understanding of the genetic alterations involved in breast cancer development and progression may aid earlier detection and postoperative management [17].

References

1. AALTONEN L. A., PELTOMAKI P., LEACH F. S., SISTONEN P., PYLKKANEN L., MECKLIN J. P., JARVINEN H., POWELL S. M., JEN J., HAMILTON S. R., PETERSEN G., KINZLER K. W., VOGELSTEIN B., DE LA CHAPELLE A.: Clues to the pathogenesis of familial colorectal cancer. *Science* 1993, 260, p. 812–816.
2. BIECHE I., LIDERAU R.: Genetic alterations in breast cancer. Genes Chromosomes Cancer 1995, 14, p. 227–251.
3. DAHIYA R., DENG G.: Molecular prognostic markers in breast cancer. *Breast Cancer Res. Treat.* 1998, 52, p. 185–200.
4. DE MARCHIS L., CONTEGIACOMO A., D'AMICO C., PALMIROTTA R., PIZZI C., OTTINI L., MASTRANZO P., FIGLIOLINI M., PETRELLA G., AMANTI C., BATTISTA P., BIANCO A. R., FRATI L., CAMA A., MARIANI-COSTANTINI R.: Microsatellite instability is correlated with lymph-node-positive breast cancer. *Clin. Cancer Res.* 1997, 3, p. 241–248.
5. DEVILEE P., CORNELISSE C. J.: Somatic genetic changes in human breast cancer. *Biochim. Biophys. Acta* 1994, 1198, p. 113–130.
6. DEVILEE P., CLETON-JANSEN A. M., CORNELISSE C. J.: Ever since Knudson. *Trends Genet.* 2001, 17, p. 567–570.
7. EIRIKSDOTTIR G., JOHANNESDOTTIR G., INGVARSSON S., BJORNSDOTTIR I. B., JONASSON J. G., ARNARSSON B. A., HALLGRIMSSON J., GUDMUNDSSON J., EGILSSON V., SIGURDSSON H., BARKARDOTTIR R. B.: Mapping of loss of heterozygosity at chromosome 13q: Loss at 13q12-q13 is associated with breast tumour progression and poor prognosis. *Eur. J. cancer.* 1998, 34, p. 2076–2081.
8. FORMANTICI C., ORLANDI R., RONCHINI C., PILOTTI S., RANZANI G. N., COLNAGHI M. I., MENARD S.: Absence of microsatellite instability in breast carcinomas with both p53 and c-erbB-2 alterations. *J. Pathol.* 1999, 187, p. 424–427.
9. GELEHRTER T. D., COLLINS F. S., GINSBURG D.: Principles of medical genetics (2nd edition). Kelly P. J., Williams. & Wilkins, USA 1998, p. 261.
10. HARTWELL L. H., HOOD L., GOLDBERG M. L., REYNOLDS A. N., SILVER L. M., VERES R. C.: Genetics: from genes to genomes (1st edition). Smith J. M., McGraw-Hill, USA 2000, p. 312–314, 316.
11. HOUSMAN D.: Human DNA polymorphisms. *N. Engl. J. Med.* 332: 318–320, 1995.
12. HIRANO A., UTADA Y., HAGA S., KAJIWARA T., SAKAMOTO G., KASUMI F., NAKAMURA Y., EMI M.: Allelic losses as prognostic markers for breast cancer. *Int. J. Clin. Oncol.* 2001, 6, p. 6–12.
13. JOHNSON S. M., SHAW J. A., WALKER R. A.: Sporadic breast cancer in women: Prevalence of loss of heterozygosity at p53, BRCA1 and BRCA2. *Int. J. Cancer* 2001, 98, p. 205–209.
14. KERANGUEVEN F., NUGUCHI T., COULIER F., ALLIONE F., WARGNIEZ V., SIMONY-LAFONTAINE J., LONGY M., JACQUEMIER J., SOBOL H., EISINGER F., BIRNBAUM D.: Genome-wide search for loss of heterozygosity shows extensive genetic diversity of human breast carcinomas. *Cancer Res.* 1997, 57, p. 5469–5474.
15. LEE S. C., BERG K. D., SHERMAN M. E., GRIFFIN C. A., ESHLEMAN J. R.: Microsatellite instability is infrequent in medulary breast cancer. *Am. J. Clin. Pathol.* 2001, 115, p. 823–827.
16. LO Y. L., YU J. C., HUANG C. S., TSENG S. L., CHANG T. M., CHANG K. J., WU C. W.,

SHEN C. Y.: Allelic loss of the BRCA1 and BRCA2 genes and other regions on 17q and 13q in breast cancer among women from Taiwan. *Int. J. Cancer* 1998, 79, p. 580–587.
17. MARTINEZ A., WALKER R. A., SHAW J. A., DEARING S. J., MAHER E. R., LATIF F.: Chromosome 3p allele loss in early invasive breast cancer: detailed mapping and association with clinicopathological features. *Mol. Pathol.* 2001, 54, p. 300–306.
18. MENDEZ O., MANAS S., PEINADO M. A., FANRA A., ESCOBEDO A., MORENO A., SIERRA A.: Microsatellite instability is associated with loss of apoptosis in ductal breast carcinomas. *Breast cancer res. treat.* 2001, 65, p. 171–177.
19. MERAJVER S. D., FRANK T. S., XU J., PHAM T. M., CALZONE K. A., BENNET-BAKER P., CHAMBERLAIN J., BOYD J., GARBER J. E., COLLINS F. S.: Germline mutations and loss of the wild-type allele in tumors from families with early onset breast and ovarian cancer. *Clin. Cancer Res.* 1995, 1, p. 539–44.
20. MURATA H., KHATTAR N. H., KANG Y., GU L., LI G.: Genetic and epigenetic modification of mismatch repair genes hMSH2 and hMLH1 in sporadic breast cancer with microsatellite instability. *Oncogene* 2002, 21, p. 5696–5703.
21. OOSORIO A., DE LA HOYA M., RODRIGUEZ-LOPEZ R., MARTINEZ-RAMIREZ A., CAZORLA A., GRANIZZO J. J., ESTELLER M., RIVAS C., CALDES T., BENITEZ J.: Loss of heterozygosity analysis at the BRCA loci in tumor samples from patients with familial breast cancer. *Int. J. cancer* 2002, 99, p. 305–309.
22. OZER E., YUKSEL E., KIZILDAG S., SERCAN O., OZEN E., CANDA T., SAKIZLI M.: Microsatellite instability in early-onset breast cancer. *Pathol. Res. Pract.* 2002, 198, p. 525–530.
23. PIZZI C., PANICO L., DE MARCHIS L., MASTRANZO P., DI MAIO M., D'AMICO C., LIMITE G., PETTINATO G., COCOZZA S., BIANCO A. R., CONTEGIACOMO A.: p53 expression is decreased in primary breast carcinomas with microsatellite instability. *Breast Cancer Res. Treat.* 2002, 73, p. 257–266.
24. RICHARD S. M., BAILLIET G., PAEZ G. L., BIANCHI M. S., PELTOMAKI P., BIANCHI N. O.: Nuclear and mitochondrial genome instability in human breast cancer. *Cancer Res.* 2000, 60, p. 4231–4237.
25. RIO P. G., PERNIN D., BAY J. O., AALBUISSON E., KWIATKOWSKI F., DE LATOUR M., BERNARD-GALLON D. J., BIGNON Y. J.: Loss of heterozygosity of BRCA1, BRCA2 and ATM genes in sporadic invasive ductal breast carcinoma. *Int. J. Oncol.* 1998, 13, p. 849–853.
26. THIBODEAU S. N., BREN G., SCHAID D.: Microsatellite instability in cancer of the proximal colon. *Science* 1993, 260, p. 816–819.
27. TOKUNAGA E., OKI E., ODA S., KATAOKA A., KITAMURA K., OHNO S., MAEHARA Y., SUGIMACHI K.: Frequency of microsatellite instability in breast cancer by high-resolution fluorescent microsatellite analysis. *Oncology* 2000, 59, p. 44–49.
28. TOMITA S., DEGUCHI S., MIYAGUNI T., MUTI Y., TAMAMOTO T., TODA T.: Analysis of microsatellite instability and the transforming growth factor-beta receptor type II gene mutation in sporadic breast cancer and their correlation with clicinopathological features. *Breast Cancer Res. Treat.* 1999, 53, 33–39.
29. SIAH S. P., QUINN D. M., BENNETT G. D., CASEY G., FLOWER R. L. P., SUTHERS G., RUDZKI Z.: Microsatellite instability markers in breast cancer: A review and study showing MSI was not detected at 'BAT 25' and 'BAT 26' microsatellite markers in early-onset breast cancer. *Breast cancer Res. Treat.* 2000, 60, 135–142.
30. VAN DER LOOIJ M., PAPP J., SZTAN M., PULAY T., ELFADIL I., BESZNYAK I., TOTH J., DEVILEE P., OLAH E.: Allelic imbalance and microsatellite instability in BRCA1 associated breast and ovarian tumors. *Int. J. Oncol.* 2001, 18, 775–780.