

# Numerical aberrations of chromosome 17 and *TP53* in brain metastases derived from breast cancer

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**ABSTRACT.** Breast cancer is the second most common origin of brain metastases, after lung cancer, and represents 14-20% of all cases. Abnormalities of chromosome 17 are important molecular genetic events in human breast cancer, and several oncogenes and tumor suppressor genes are located on this chromosome. In about half of all human cancers, the tumor suppressor gene *TP53*, located at 17p13, is

either lost or mutated. Loss of p53 protein function influences not only cell cycle checkpoint controls and apoptosis, but also the regulation of other important stages of metastatic progression, such as cell migration and tissue invasion. The aim of our study was to identify numerical aberrations of chromosome 17 and *TP53* in 5 subjects with brain metastasis from breast cancer using dual-color fluorescence *in situ* hybridization experiments. Deletion of *TP53* was the most frequent alteration observed, suggesting that if this alteration is present in the primary tumors, breast tumors with loss of *TP53* copies have a poorer prognosis and a higher chance for metastasis. If this is true, the analyses of chromosome 17 and *TP53* in primary breast cancer could be important for predicting its metastatic potential.

**Key words:** Breast cancer; *TP53* gene; Chromosome 17; Metastasis; FISH

## INTRODUCTION

Tumor metastasis is a multistage process in which malignant cells spread from a primary tumor to distant organs via the blood and/or lymphatic system (Fidler, 2003; Talmadge and Fidler, 2010; Saunus et al., 2011). Of the approximately 1.3 million people diagnosed with cancer in the United States each year, approximately 100,000 to 170,000 will develop brain metastases; this corresponds to an annual incidence of around 4.1 to 11.1 per 100,000 individuals (Barnholtz-Sloan et al., 2004; Cambruzzi et al., 2011). Large autopsy studies suggest that approximately 20 to 40% of all patients with metastatic cancer have brain metastases (<http://www.cancer.org>; Weil et al., 2005).

The most common primary metastatic sites in adults are the lung (35%), central nervous system (CNS; 25-30%) and breast (14-18%; Weil et al., 2005; Cambruzzi et al., 2011; Saunus et al., 2011). This phenomenon is called organotropism, which means that the distribution of metastasis in different organs is nonrandom; i.e., the tumor cells could have specific affinity for the microenvironments of certain organs (Talmadge and Fidler, 2010; Saunus et al., 2011). Organotropism was first described by Paget (1889) after studying autopsy records of 735 women with breast cancer.

In Brazil, 52,680 new cases of breast cancer are estimated in 2012, corresponding to 52 cases in 100,000 women. In the northern region of Brazil, it is the second most frequent cancer (19/100,000 women) after cervical cancer (INCA, 2012). An immunohistochemical study of 100 Brazilian patients (Porto Alegre, RS) suggested breast cancer as the primary origin of brain metastasis in 16% of the studied cases (Cambruzzi et al., 2011); these results confirm data ascertained in other countries, although the overall incidence may be as high as 30% according to molecular studies (Marko et al., 2012). The outcome for patients with brain metastasis derived from breast tumors is poor, and the overall survival after diagnosis varies between 2-16 months, with only 20% of patients reaching the 12-month mark (Cheng and Hung, 2007). Hence, the search for biomarkers that could help in predicting or understanding the process of metastatic events is crucial, particularly for this type of cancer.

In addition to young age and estrogen-receptor negativity, high proliferation rates, p53

alterations, and genomic instability in the primary tumor were associated with an increased risk of CNS metastasis (Tham et al., 2006). After their spread from tumors, metastatic cells acquire the capacity to actively migrate and invade through the stroma (Muller et al., 2011). Several studies have shown that suppression of p53 can lead to more rapid migration of fibroblasts in scratch-wound assays and through 3-dimensional matrices (Guo and Zheng, 2004, Gadea et al., 2007). Other types of cells had increased growth cone motility associated with altered p53 function (Qin et al., 2009). Thus, in addition to affecting apoptosis and cellular senescence, p53 loss has recently been shown to influence cell motility, thus contributing to the invasive potential of tumors (Muller et al., 2011).

Herein, 5 breast cancer-derived brain metastases were evaluated for numerical aberrations of chromosome 17 and deletion or amplification of the tumor suppressor gene, *TP53*.

## MATERIAL AND METHODS

Samples of breast metastatic tumors submitted for surgical resection were obtained from 5 female patients at the Ophir Loyola Hospital (Belém, PA, Brazil). The ages of the patients and histopathology of the tumors are listed in Table 1. The patients did not undergo chemotherapy or radiotherapy prior to surgery. This study was approved by the Ethics Committee of Health Sciences Center, UFPA CCS/UFPA.

**Table 1.** Information about the subjects analyzed in this study.

Case No.	Gender	Age (year)	Histopathology
1	Female	23	Adenocarcinoma
2	Female	54	Adenocarcinoma
3	Female	45	Adenocarcinoma
4	Female	46	Adenocarcinoma
5	Female	50	Adenocarcinoma

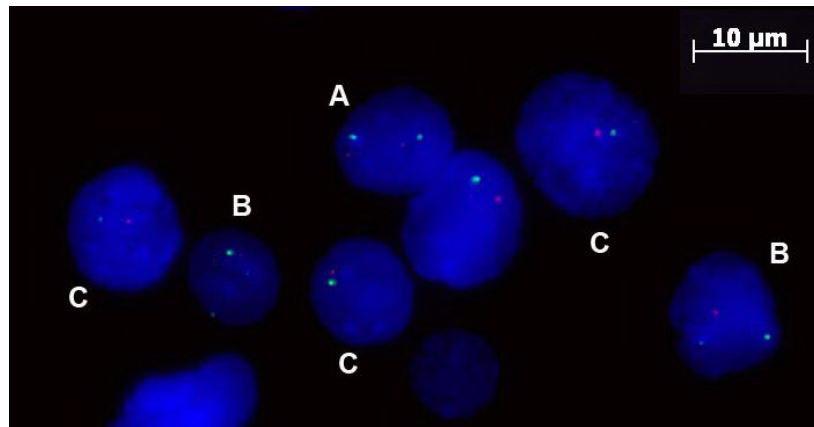
The samples were transferred to the laboratory in Roswell Park Memorial Institute medium, the material was dissociated, treated with KCl (incubated for 10 min at 37°C), and fixed in methanol-acetic acid. The suspension was used for preparing slides for use in subsequent experiments. A dual-color fluorescence *in situ* hybridization assay was performed using directly labeled fluorescent probes (SpectrumRed and SpectrumGreen) for chromosome 17. A centromere-specific probe (cep) and a locus-specific probe for the gene *TP53* on 17p13.1 were applied following manufacturer protocols (Abbott, Abbott Park, IL, USA; Vysis, Des Plains, IL, USA). After stringency washes, a drop of antifade solution (Vectashield, Vector Laboratories, Burlingame, CA, USA) containing 4',6-diamidino-2-phenylindole for counterstaining was added and each slide was covered with a 32 x 22 coverslip.

The slides were analyzed using a Zeiss Axio Imager epifluorescent microscope (Zeiss, Jena, Germany) at a magnification of 600X. Images were captured and analyzed using the Axiophot 4.1 software. For each subject, 160 to 200 interphase nuclei were evaluated. Counting was performed using criteria proposed by Hopman et al. (1994). We considered the occurrence of monosomy (1 signal for *TP53* and centromere 17), normal (2 signals for each probe) and polysomy (more than 2 signals for each probe). For the *TP53* gene, we considered the occurrence of deletions (less than 2 signals), normal (2 signals) or amplification (more than 2 signals).

For statistical analysis of the results, we used the non-parametric Mann-Whitney U-test, with  $P < 0.05$  considered significant (Espinosa et al., 2006).

## RESULTS

All 5 subjects presented to a certain extent with numerical alterations of chromosome 17 as well as copy number alterations (deletion or amplification) of *TP53* in the studied metastases (Figure 1). Three of the 5 cases showed 1 or no signal for *TP53* in 19.4 to 49% of cells, respectively, while 2 cep 17 signals were present in each sample. The other 2 cases had 2 signals for each probe in 26 and 50% of cells. For the latter 2 samples, a deletion of *TP53* was present in 33.7 and 34% of cells, respectively. In all 5 samples, gain of chromosome 17 was rare, varying from 0.5 to 8.4% of the analyzed cells. Statistical analyses are shown in Table 2.



**Figure 1.** Representative dual color fluorescence *in situ* hybridization experiments with centromeric probe of chromosome 17 (green) and locus-specific probe for *TP53* (17p13.1; red) in interphase nuclei: (A) nucleus, with two copies of chromosome 17 and *TP53*; (B) nuclei with two copies of chromosome 17 and deletion of one *TP53*; (C) nuclei with deletion of one chromosome 17, as one green and one red signal are absent.

**Table 2.** Main cytogenetics findings and statistical analyses.

Case No.	No alteration (%)	Trisomy 17 (%)	Monosomy 17 (%)	Deletion of p53 (%)	Number of analyzed cells
1	33.7	3.0	15.6	38.7	160
2	34.0	2.0	10.0	49.0	200
3	50.0	0.5	9.5	34.0	200
4	26.3	8.4	6.8	18.9	190
5	16.8	8.4	6.3	19.4	190

## DISCUSSION

Breast cancer is a heterogeneous disease with respect to molecular features, which is perhaps best exemplified by the molecular subgroups identifiable by gene expression profiling including basal-like, luminal A (hormone receptor positive), luminal B, and human epidermal growth factor receptor 2 (HER2) amplified/over-expressed (HER2+) subtypes (Saunus et al.,

2011). It has been demonstrated that primary breast cancer with a basal-like immunophenotype has the propensity to metastasize to the brain (Espinosa et al., 2006). Indeed, breast cancer is the second most common source of brain metastases after lung cancer, representing 14-20% of all cases (Fulford et al., 2007; Cambruzzi et al., 2011). Although the incidence of breast metastasis in the brain is high, and metastatic transition involves the occurrence of many chromosomal abnormalities (Lohr et al., 2001), little is known about the behavior of chromosomes in metastases.

The molecular basis for breast cancer metastasis to the brain is largely unknown (Gagos and Irminger-Finger, 2005). Some studies have shown that breast cancer metastasis to the brain involves mediators of extravasation through non-fenestrated capillaries, complemented by specific enhancers of blood-brain barrier crossing and brain colonization, involving cyclooxygenase 2 (COX2; also known as PTGS2), the epidermal growth factor receptor ligand HBEGF, and the  $\alpha$ 2,6-sialyltransferase ST6GALNAC5 as mediators of cancer cell passage through the blood-brain barrier (Chiang and Massagué, 2008). Moreover, the importance of mutations in p53 protein has also been highlighted due to the influence of these mutations in tumor cell migration and invasion (Muller et al., 2011). Studies involving the analyses of modifications in the copy number of the *TP53* gene, located at 17p13.1, have also demonstrated the occurrence of abnormalities in the number of copies of chromosome 17.

Two different studies focusing on breast metastases in lymph nodes have found loss of chromosome 17 to be the most common chromosomal abnormality (Pandis et al., 1998; Bos et al., 2009). Abnormalities of chromosome 17 are important molecular genetic events in human breast cancer as a whole, which include several widely studied oncogenes and tumor suppressor genes (Tsuda et al., 1998). Hence, the deletion of tumor suppressor genes or the amplification of oncogenes is an important step that can be crucial in the process of the development and progression of the primary tumor and its metastases. High rates of abnormalities involving chromosome 17 have been reported in some human cancers, including breast (Zhang and Yu, 2011), colon (Hopman et al., 1994), and bladder carcinoma (Rosenberg et al., 1994). This chromosome has a high content of guanine and cytosine, regions rich in important genes, a high number of short interspersed elements and a lack of long interspersed elements (Fadl-Elmula, 2005). Besides *TP53*, several oncogenes, such as HER2, topoisomerase (DNA) II alpha and microtubule-associated protein tau; other tumor suppressor genes (breast cancer 1 -BRCA1- and hypermethylated in cancer 1-HIC-1) or DNA double-strand break repair gene (*RDMI*) are located in chromosome 17 (Meszaros et al., 2010). Thus, abnormalities of chromosome 17 are important molecular genetic players in tumorigenesis, particularly in breast cancer (Reinholz et al., 2009).

Deletion of *TP53* was the most frequent alteration observed in our samples (Figure 1). If we consider that this alteration could be present in the primary tumors, it could be argued that primary tumors with loss of *TP53* gene copies had a poorer prognosis and a higher probability of metastasis. If this is true, analyses of chromosome 17 and *TP53* in primary breast cancer could be valuable in predicting its metastatic potential.

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