Investigating the presence of mutations in *TP53* and *PIK3CA* genes in free circulating DNA of patients with impalpable breast lesions

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INTRODUCTION AND OBJECTIVES

The introduction of so-called "liquid biopsy," has become a promising tool, it has made possible to investigate the free circulating DNA (cfDNA) in the blood stream, which may be coming from the tumor tissue. Some genes, involved in carcinogenesis are mutated, due to the functional role of protection of the human genome. The genes *TP53* and *PIK3CA* have high mutations rates in breast cancer. Thus, these genes become important research targets in free circulating DNA also. Recent studies have described that mutations found in cfDNA, may provide evidences to tumor micrometastasis and to the resistance to treatment. In this way, we evaluated the profile mutational of *TP53* and *PIK3CA* genes in circulating free DNA (cfDNA) of women with impalpable breast lesions.

MATERIAL AND METHOD

Thirty-six patients with impalpable breast lesions classified as BI-RADS 3 and 4 by imaging tests were included in this study. Blood collected before surgery in EDTA tubes was centrifuged at 3,000 g for 10 minutes, and the obtained supernatant was transferred and, centrifuged for 20 minutes at 16,000 g. The histopathological diagnosis was established at after the surgery.

The cfDNA in plasma was isolated by QIAamp® Circulating Nucleic Acid (Qiagen), according to manufacturer's instructions. Quantification of circulating DNA was performed by Qubit® 3.0 system (Invitrogen, USA). About 1 ng of genomic DNA was used for the amplification of the coding region of TP53 (exons 5 to 9). The purified products were sequenced in 3130 sequencer Genetic Analyzer from Applied Biosystems.

RESULTS AND CONCLUSIONS

Of the thirty-six selected patients, 6⁄36 (17%) were classified as fibroadenoma, 19⁄36 (53%) infiltrating ductal carcinoma (IDC) (grade I-III), 4⁄36 (11%) lobular carcinomas, 3⁄36 (8%) intraductal carcinoma (IC), 2⁄ 36 (5%) micropappilary carcinoma, 1⁄ 36 (3%) mucinous carcinoma, and 1⁄36 (3%) in situ ductal carcinoma. The evaluation of the cfDNA sequence revealed four mutations in *TP53* gene (exon 7). All the mutations were found in malignant cases (IDC and IC), and one case with involvement of lymph node (Fig 1). The four mutations found were missense type, with substitution of the encoded amino acid.

The presence of mutations in free circulating DNA may indicate tumor aggressiveness and⁄or the presence of tumor micrometastasis.

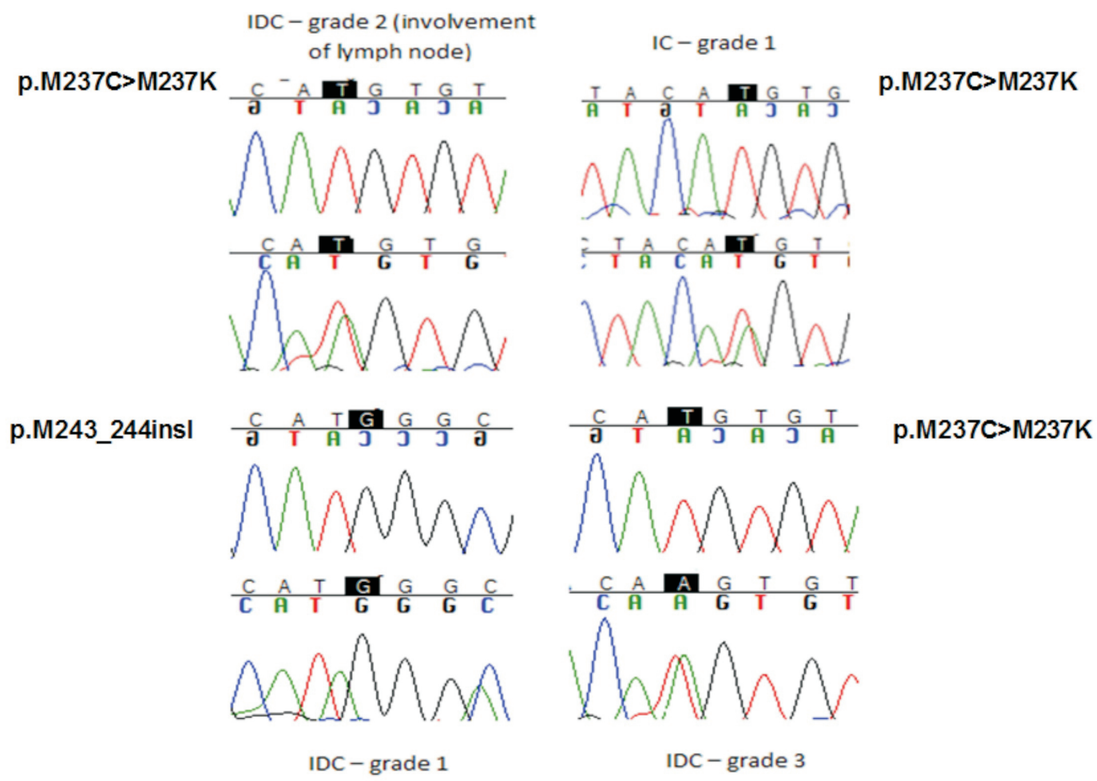

Figure 1 – Mutations in *TP53* gene

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