Comparative Analysis of Molecular Alterations in Fibroadenomas Associated or Not With Breast Cancer

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Hypothesis: The cause of breast cancer is linked to many macroscopic events, including benign breast disease. In this study we asked whether molecular changes could discriminate fibroadenoma, which is one of the most common benign breast disease lesions associated or not with breast cancer.

Design: Retrospective cohort study.

Setting: Anticancer medical center.

Subjects: Archival tissues in 32 cases of fibroadenoma, diagnosed in the same breast as a breast carcinoma, are compared with a control group of 26 cases of fibroadenomas unaffected by breast cancer.

Main Outcome Measures: Histological features are characterized in all samples. The epithelial and stromal components are analyzed for a loss of heterozygosity and a microsatellite instability using a polymerase chain reaction–based method with 11 polymorphic microsatellite markers at 7 chromosomal regions frequently altered in breast cancer. The p53 gene mutations were also determined at exons 5 to 9.

Results: The frequency of complex fibroadenomas was similar in both groups (P= .42). Only in the case group did we observe proliferative lesions confined in fibroadenomas, including atypical ductal hyperplasia (2 cases), lobular neoplasia (3 cases), or low-grade ductal carcinoma in situ (2 cases). There is no significant morphological difference between the 2 groups. Neither microsatellite alterations nor p53 gene mutations are present in the fibroadenoma components. Loss of heterozygosity is found only in the epithelial component of the 2 ductal carcinomas in situ confined in fibroadenomas.

Conclusions: Genetic alterations, which are most frequently involved in malignant breast carcinomas, are not present in fibroadenomas, regardless of their association with breast cancer or their histological complexity. These findings suggest that fibroadenomas are not associated with breast carcinogenesis.


The cause of breast cancer is linked to many macroscopic events that include a positive family history, the number of pregnancies, an exposure to estrogens, type of diet, and benign breast disease. Fibroadenoma is one of the most common benign breast lesions for women who are between 20 and 50 years old that has been associated with a risk of breast cancer by several investigators.1-3 The highest peak of incidence occurs at the third decade of life when breast cancer is an unusual event. The reported risk of developing breast cancer for patients previously diagnosed as having fibroadenomas varies from null to 3. The increase depends on the presence of complex features within the fibroadenomas (ie, cysts, sclerosing adenosis, epithelial calcifications, or papillary apocrine changes), a proliferative disease in the parenchyma adjacent to the fibroadenomas, or a family history of breast cancer. However, the family history of breast cancer may cause an overestimation of increased risk associated with fibroadenomas because women with a family history of breast cancer may more readily decide to undergo a surgical procedure.4 Thus, the association between fibroadenoma and breast cancer is still unclear. Progressive somatic genetic alterations are associated with the development of breast cancer. Genetic instabilities, manifested by a loss of heterozygosity (LOH) and/or microsatellite instability (MIN), are considered as early events in this type of lesion. Indeed, at least a subset of proliferative breast lesions is characterized by clonal genetic aberrations manifested by LOH and MIN. Some of these lesions, in-
In this study, we asked whether genetic alterations may also occur early in benign breast disease lesions. cluding usual ductal hyperplasia (UDH) and atypical ductal hyperplasia (ADH), are supposed to represent actual precursors of malignancy although histologically these lesions are considered benign. In addition, genetic instability detected by LOH6 and more recently by MIN5 occurs in multiple chromosomal regions, which are not only frequently altered in breast cancer but also reported to occur in fibroadenomas, as well as adjacent healthy tissue, when present. Parenchyma adjacent to the fibroadenoma was assessed by the presence of these proliferative diseases, that is, UDH, ADH, LN, or DCIS. Manual microdissection of epithelial and stromal components of fibroadenomas, as well as adjacent healthy tissue, was performed using a 30-gauge needle under microscopic visualization. The cases in which the fibroadenoma was inside or in contact with the breast cancer were excluded from the study. All selected cases for this study were either distant or separated from the associated carcinoma by at least a small rim of healthy tissue. Microdissection of the epithelial component was performed and included complex or proliferative lesions confined in the fibroadenoma, when present.

Molecular analyses were done without the knowledge of the fibroadenomas' histological characteristics. Microdissected cells were kept in 70% alcohol at 4°C until DNA extraction was performed; the cells were then washed with ultrapure water. Cells were incubated for 18 to 26 hours in 0.5% polysorbate 20 (Tween 20), 1mM EDTA (pH 8.0), 50mM Tris hydrochloride (pH 8.5), and 500mM potassium chloride (1X GeneAmp PCR Buffer II; Perkins-Elmer Inc, Applied Biosystems, Foster City, Calif). Polymerase chain reaction was carried out in a thermocycler (Hybaid PCR-Express Thermocycler Q; Ashford, England) and consisted of 10 minutes at 95°C, 45 cycles of 50 seconds at 94°C,
Thus, no significant difference was observed in the complexity of the 26 fibroadenomas were classified as complex.

In the hypothesis of an evolution of the fibroadenomas toward malignancy, it is important to determine which of the 2 cellular components (stromal or epithelial), if not both, is more prone to malignant transformation. The microdissection approach used was well adapted to the separate characterization of the distinct components of fibroadenoma.

In conclusion, the DNA from the epithelial, stromal, and adjacent normal tissue components was subjected to microsatellite analysis on 8 microsatellite loci (BAT26, D3S1514, D3S1244, D6S264, D10S197, D11S2179, TP53, and D17S855) for the case group. When sufficient material was available for the 3 components (16 of 32 cases), 2 additional loci were also analyzed (AR and TH01). D6S281 was analyzed only in noninformative cases for D6S264 since both markers screened the same region of interest (9q25-ter).

All microsatellite loci were analyzed for the control group. All loci were highly informative except for BAT26, which was used as a marker of MIN.

Loss of heterozygosity was detected in the epithelial component of only 2 fibroadenomas from the case group. Both fibroadenomas concern the 2 cases containing the low-grade DCIS, and they belonged to the subgroup concurrent to carcinomas. In these 2 cases, DCIS constituted an independent entity from the invasive carcinoma present in the same breast and were not an extension of the fibroadenomas (Figure 1). Two different loci, D3S1514 and TP53, were found altered in the epithelial component of these DCIS confined in the fibroadenomas (Figure 2). No MIN was detected in either the BAT26 locus or in the remaining loci of any of the analyzed samples. All of the other fibroadenomas from the case and the control groups showed normal patterns.

No mutation in the p53 gene at exons 5 through 9 was found in either the case or in the control groups. Only 1 fibroadenoma from the control group showed a polymorphism in exon 6 on the codon 213 (CGA/Arg:CGG/Arg). This polymorphism was found in the 3 cellular components obtained by microdissection (epithelium, stroma, and healthy adjacent tissues), as well as in lymphocyte blood cells.

Strong evidence proves that an essential feature of breast cancer malignancy resides in the accumulation of multiple genetic alterations. A subset of these aberrations may occur at early stages and some others at later stages of the disease. Thus, it is important to understand the
mutations. In this study we investigated whether fibroadenomas, microsatellite alterations, and fibroadenomas, including cytogenetic chromosomal aberrations, have emerged as markers of this region (6q spanning 6q25 to 6qter in 23 cases classified as complex). Thus, 2 markers of this region (D6S264 and D6S281) located at 6q25-27 and 6q27, respectively) were included in our study. Our results showed no LOH within these 2 loci, neither in the control nor in the case groups. Since the cytogenetic method requires a cellular incubation that may result in a cell selection during culturing, a comparison with cytogenetic results should be made cautiously. Cell cultures from tissues are made of different cell types. A bias of selection, which does not reflect the in vivo situation, may then be introduced.

The microsatellite analysis has reported LOH at D3S1514 in 1 of 39 cases analyzed, as well as 1 MIN and 1 LOH occurring at TH01 (11p15.5). In contrast to this, no alterations were found in another 8 loci determined in 7 fibroadenomas. A molecular-based study has also shown the presence of p53 gene mutations in some fibroadenomas. Three microsatellite loci spanning the 3p region, the TH01 marker, and the p53 gene mutations were analyzed in our study. Because MIN at BAT26, reflecting a dysfunction of the DNA mismatch repair system, was found in early stages of colon cancer progression, for example, in benign adenomas, we have also analyzed this marker. These data suggested a possible DNA mismatch repair system–defective mechanism in the origin of proliferative benign lesions. In addition, MIN was reported in fibroadenomas at breast cancer–related loci.

The results showed that neither microsatellite alterations (LOH or MIN), at the tested loci, nor p53 gene mutations were present in any of the fibroadenoma components regardless of their association with breast cancer or their histological complexity. A recurrent criticism in molecular studies is that the presence of normal tissue in the sample may mask abnormal clones. In our study, the fact that samples were obtained from microdissection, excludes risk of contamination with normal tissue.

Recently, it has been shown that atypia (atypical lobular or ductal hyperplasia) confined in fibroadenomas does not lead to an elevation of long-term breast cancer risk compared with fibroadenomas in general. The fact that the presence of complex features, including atypical hyperplasia, within fibroadenomas in our population did not correlate with an increased occurrence of genetic alterations at the examined loci is in agreement with this study.
typically, the only 2 alterations observed in the case group were related to the presence of a DCIS component within these fibroadenomas. However, although our results showed no genetic alterations in fibroadenomas at these loci, they did not exclude the fact that there may be some genetic changes, not analyzed in this study, leading to the initiation of these lesions.

The presence of neoplasia (LN, DCIS, and invasive carcinomas) within fibroadenomas is rare and is observed in patients 20 years older than patients without cancer. As our study found, LN is more frequent than DCIS, and invasive lesions confined in fibroadenomas are exceptional. Since such lesions are not frequent, there is no significance of these neoplasias confined in fibroadenomas because of the risk of developing invasive cancer in other areas of the breast is still unknown, and their gathering in the same area seems to be a fortuitous event. Although 2 cases of DCIS in fibroadenomas belong to the group with carcinomas, our series is too small to conclude that these DCIS increase the risk of subsequent breast cancer. Loss of heterozygosity has been interestingly reported in DCIS at several loci, including 3p21-22 and 17p13.1. Because no data on histological features were available,17188 molecular changes attributable to fibroadenomas may be due to the presence of these neoplasias confined in fibroadenomas. Together, our results suggest that genetic alterations, most frequently involved in malignant breast carcinomas, were not identified in fibroadenomas, regardless of their association with breast cancer or their histological complexity. These findings support the concept that fibroadenomas are not associated with breast carcinogenesis.

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