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Topoisomerase II alpha Expression in Breast Ductal Invasive Carcinomas and Correlation with Clinicopathological Variables

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Abstract. *Topoisomerase II alpha (topo IIα) is an enzyme that in normal cells is expressed predominantly in the S/G2/M-phase of the cell cycle. In malignant cells, in vitro studies have indicated that the expression of topo II alpha is both higher and less dependent on the proliferation state in the cell. To study the expression of topo IIα and the relationship between that expression and other variables in cases of breast ductal invasive carcinomas, 50 fine-needle aspiration biopsies were performed from the same number of female patients, diagnosed cytologically and confirmed histologically after surgery. The same cases were studied immunocytochemically using monoclonal antibodies to topo IIα and Her2/neu (CB11) by the alkaline phosphatase method (APAAP). Topo IIα was found in 32 cases (64%) of the carcinomas studied. An overexpression between topo IIα and Her2/neu was found ($p<0.005$). A relationship between topo IIα expression, histological grade and lymph node status (LNs) was also found ($p<0.005$). Increased topo IIα expression seems to be related to an aggressive form of breast cancer featuring Her2 amplification and lymph node metastasis.*

In human cells there are two closely but differently expressed topoisomerase II isoforms, designated topoisomerase II alpha and beta.

Topoisomerase IIα (topo IIα) is a component of the DNA machinery that is intricately involved at many levels of DNA metabolism (1-3). Its primary function is to alter DNA topology from its storage (supercoiled) form to a more exposed (partially uncoiled) form by inducing single-strand DNA breaks and simultaneously passing another intact double helix through the gap (4, 5).

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Exclusive of topo IIα's potential role as a target for anticancer drugs, few studies have analyzed if it is a potential prognostic marker in breast cancer. Of the limited data available, a common finding has been correlation with known proliferation markers such as MIB1 immunostaining and tumor S-phase fraction (6-8). These findings support the role of topo IIα as a proliferation marker in breast cancer.

An additional finding in some studies has been the coexpression of both topo II α and Her2/neu oncoprotein, a known poor prognostic indicator (9, 10).

The correlation of topo II α expression with other known outcome variables in breast cancer has been inconsistent. Studies have shown a significant correlation of topo II α expression with lymph node metastases, tumor grade, tumor size and distant metastases. Other studies have found an association of topo II α expression with cell proliferation markers only.

The aim of this study was to examine, on fine-needle aspiration biopsies (FNABs), the association between topo II α expression on the one hand and other clinico-pathological variables such as tumor grade, lymph node status (LNS) and Her2/neu expression on the other, in invasive ductal breast carcinomas.

Materials and Methods

Samples from 50 FNABs from the same number of female patients were studied. In all cases the slides (superfrost plus) were fixed in ethyl alcohol (96%) for Pap stain, while some others were air-dried and used for Giemsa stain and immunocytochemistry. All cases were diagnosed cytologically as invasive ductal breast carcinomas and confirmed by histological study after surgery. As verified by histological study, in this series there were 8 grade I carcinomas, 18 grade II and 21 grade III cases.

Immunocytochemisty. Monoclonal antibodies to topo IIα and Her2/neu (DAKO) were used at a 1/250, 1/50 dilution respectively, with an optimum incubation time of one hour. Immunostaining was performed using the alkaline phosphatase (APAAP) method . A step of microwave heating in a sodium citrate solution was

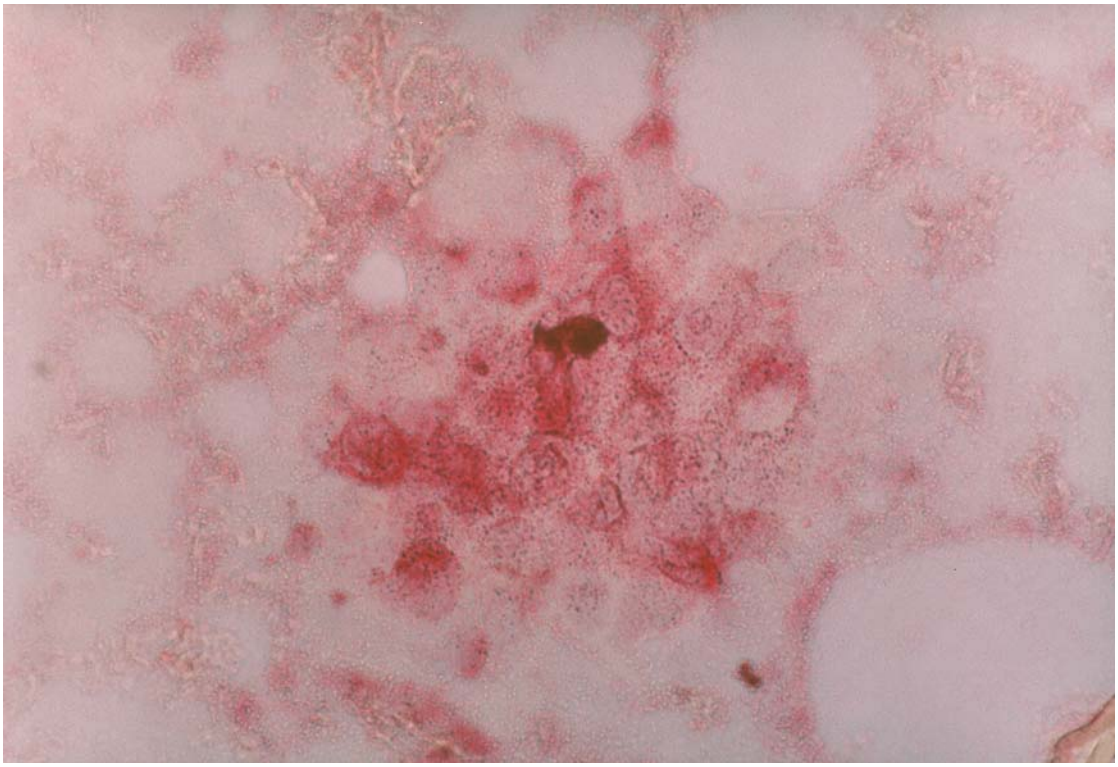

Figure 1. *Topo IIα expression on FNAB, in invasive ductal breast carcinoma (x250).*

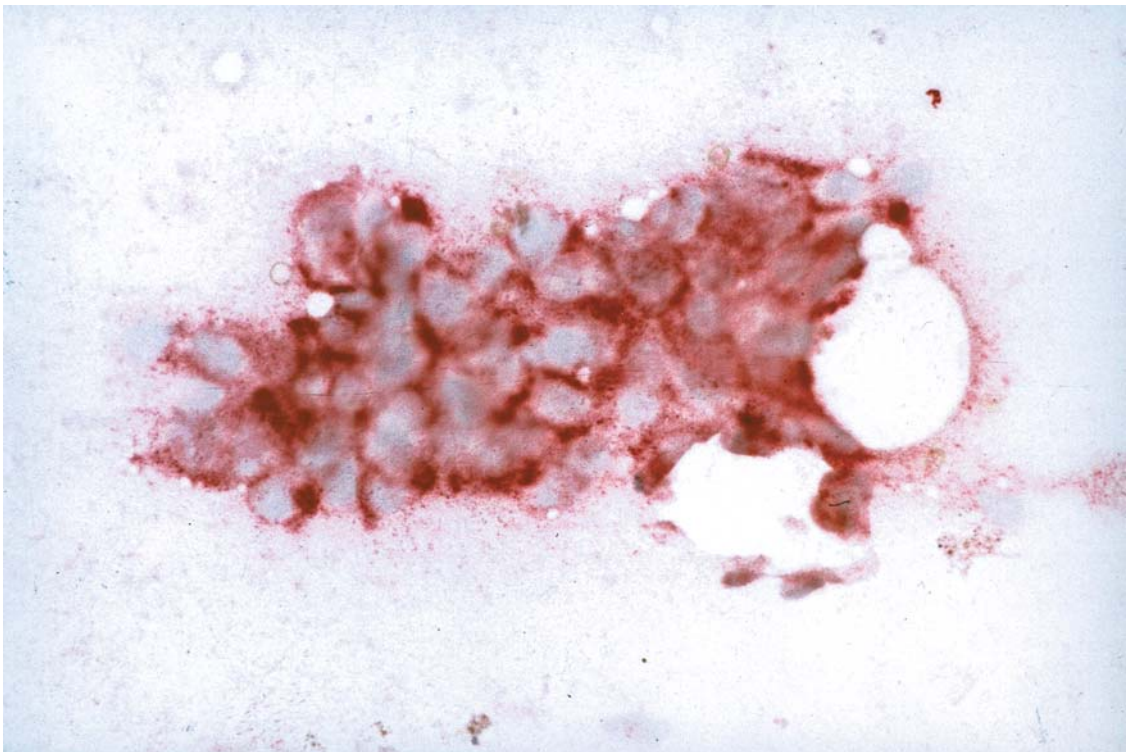

Figure 2. *Her2/neu expression on FNAB, in invasive ductal breast carcinoma (x250).*

Table I. *Topoisomerase II alpha expression and relationship with other clinicopathological variables in breast ductal invasive carcinomas.*

Tumor grade		
I	2/8 (25%)	
II	10/18 (55.6%)	$p<0.005$
III	20/24 (83.4%)	
Lymph node status		
LNs positive	25/29 (86.2%)	$p<0.005$
LNs negative	7/21 (33.3%)	
Her2/neu		
positive	28/30 (93.3%)	$p<0.005$
negative	4/20 (20%)	

Statistical analysis was performed using the χ^2 test.
P value < 0.005 was considered as significant.

performed prior to incubation. A very light hematoxylin counterstain was also performed.

Statistical analysis. The χ^2 test (Chi-square) was used. A *p* value <0.05 was considered as significant.

Evaluation of immunostaining. The slides were examined using an x40 objective lens.

Results

The results are summarized in Table I.

In all samples of invasive ductal breast carcinomas, neoplastic cells were seen either as small or large aggregates or separately. Topo IIα was distinct in the nucleus (Figure 1) of neoplastic cells in 2 (2/15) cases of grade I (25%) in 10 (10/18) cases of grade II (55.6%) and in 20 (20/24) cases of grade III (83.4%) $p<0.005$. Topo IIα was expressed in 25/29 (86.2%) cases of positive lymph node status $p<0.005$ and in 7/21 (33.3%) of negative lymph node status.

The expression of Her-2/ neu was transmembrane (Figure 2), found positive in 28/30 (93.3%) of positive topo IIα cases and negative in 4/20 (20%), and an overexpression between topo IIα and Her-2/neu was found ($p<0.005$).

Discussion

DNA topo IIα is a major component of interphase nuclear matrix fractions, present mainly in the S-phase of the cell cycle, playing a key role in DNA replication, and is a target for multiple chemotherapeutic agents.

In breast cancer, topo IIα expression has been linked to cell proliferation and Her2/neu protein overexpression. We evaluated primary breast invasive ductal cancer for topo IIα expression and correlated that expression with other variables. Of the few studies that have examined this association, most support our findings. Topo IIα expression was detected in 64% of the breast carcinomas we studied.

We found a significant association between topo IIα activity, tumor grade and lymph node status ($p<0.005$), in accordance with some recent studies (11, 12), but in contrast to other studies which failed to detect an association between topo IIα expression and tumor grade (1, 4).

In our series of invasive breast cancers, an important finding was the association of topo IIα expression with Her-2/neu oncoprotein overexpression. Of the limited number of reports that have evaluated this comparison, most found significant correlation between these two markers (11, 13-15). This suggests that topo IIα is preferentially expressed in a more aggressive subset of breast tumors (Her-2/neu-overexpressed).

In conclusion, the present study, the first on FNABs, showed significant correlation of increased topo IIα expression with known outcome variables, in ductal invasive breast carcinomas, indicating its potential role as a prognostic marker in these patients. We also reported a subset of tumors with concurrent topo IIα expression and Her-2/neu oncogene amplification, which may have therapeutic implications. Further study is warranted to better define the role of topo IIα in disease progression and therapy response.

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