Immunohistochemical study of topoisomerase II-α expression in primary ductal carcinoma of the breast

P Hellemans, P A van Dam, M Geyskens, A T van Oosterom, Ph Buytaert, E Van Marck

Abstract

Aims—To study the patterns of expression of topoisomerase II-α in primary invasive ductal breast carcinomas; to correlate this expression with clinicopathological data and prognosis.

Methods—Cryostat sections from 63 primary invasive ductal breast carcinomas were stained immunohistochemically for topoisomerase II-α. Nuclear immunoreactivity was quantified by counting at least 500 cells in different random fields and results were expressed as per cent of cells staining positively for topoisomerase II-α.

Results—Topoisomerase II-α nuclear immunoreactivity (median 14% of nuclei; range 2–62%) was detected in all tumours with highly variable intertumour and intratumour nuclear reactivity. Higher levels of topoisomerase II-α expression were strongly related to higher tumour grade, larger tumour size, nodal status, and the presence of distant metastases at diagnosis. No correlation was found with menopausal status, steroid hormone receptor status, disease free survival, or overall survival.

Conclusions—Expression of topoisomerase II-α is related to the presence of poor prognostic factors. Immunohistochemical assessment of topoisomerase II-α expression in breast cancer could be potentially useful for tailoring chemotherapy with topoisomerase II inhibitors.

(Keywords: Breast cancer, topoisomerase II-α, immunohistochemistry.)

Mammalian topoisomerase II enzymes are nuclear enzymes which play an important role in DNA replication, the formation of chromosome scaffolds, chromatin organisation, maintaining genomic stability, DNA recombination, and may be involved in DNA transcription and repair.12

Two forms of eukaryotic topoisomerase II have been identified: topoisomerase II-α has a molecular weight of 170 kilodaltons and is encoded by chromosome 17; topoisomerase II-β has a molecular weight of 180 kilodaltons and is encoded by chromosome 3.45 Topoisomerase II-α and II-β can be distinguished biochemically and pharmacologically, and their expression is regulated differentially.6 Topoisomerase II-α is not detectable in G0 cells, but its activity increases dramatically during S phase, peaks in G2-M, and then declines. By contrast, the β form remains constant throughout the cell cycle and is detectable in G0 cells.7 Immunocytochemical studies have shown that topoisomerase II-β is present almost exclusively in the nucleolus, whereas topoisomerase II-α is localised to the nucleoplasm.8 Topoisomerase II-β is thought to represent a structural element of the nucleolar remnant and to play a role in the regulation ribosomal gene transcription.9

Limited information is available on the activity of topoisomerase II in human neoplasms. Increased expression of topoisomerase II-α has been associated with the most aggressive and highly proliferative neoplasms.10-13 A correlation between in vitro resistance to chemotherapeutic agents and downregulation of topoisomerase II in human tumours has been demonstrated.14

Recently, several drugs have been developed which block topoisomerase II in vitro and in vivo.2 In vitro studies have shown that the cytotoxic activity of these drugs is dependent on the proliferative status of tumour cells, as they act predominately through inhibition of the alpha form.2,15-17 As some of these topoisomerase II inhibitors, such as epirubicin and doxorubicin, are currently used in the treatment of patients with breast cancer, the present study addresses the patterns of expression of topoisomerase II-α with respect to established risk factors in patients with invasive ductal breast cancer. Better knowledge of topoisomerase II-α expression in these patients may lead to more individualised use of topoisomerase II inhibitors.

Methods

Tumour specimens from 63 patients with primary invasive ductal breast cancer attending Antwerp University Hospital were collected prospectively between January 1990 and June 1992. All biopsy specimens were immediately snap frozen and stored in liquid nitrogen until sectioned.
The median age of the patients at diagnosis was 59 years (range 36–92 years). All patients had undergone preoperative chest x rays, bone scintigraphy, liver ultrasound scans, and blood tests, comprising full blood counts, liver function tests, and reactivity to carcinoembryonic antigen and CA15.3. If there was no evidence of metastatic disease, the patients were treated surgically by modified radical mastectomy or wide local excision of the tumour with axillary lymphadenectomy. All patients who underwent breast conserving surgery received adjuvant radiotherapy. Patients were pathologically staged according to the International Union Against Cancer (UICC) criteria. All sections were diagnosed as invasive ductal carcinoma and graded according to Bloom and Richardson. Data on tumour grade, tumour size, lymph node status, and presence or absence of metastases are presented in Table 1. Menopausal status was assessed using serum gonadotrophin and oestradiol measurements in perimenopausal patients. Patients with node negative disease were followed conservatively and received no adjuvant treatment. Postmenopausal patients with node positive disease received adjuvant endocrine treatment for five years (tamoxifen 20 mg/day by mouth). Node positive premenopausal patients underwent six cycles of CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) polychemotherapy. All patients underwent a follow up physical examination every three months and were investigated further if they developed symptoms or signs suggestive of recurrent or metastatic disease. The median time of observation was 34 months (range 16–45 months). No patients were lost to follow up.

Cryostat sections of the primary tumours were stained immunohistochemically. They were fixed for 10 minutes in a 3.7% neutral buffered formalin. After quenching endogenous peroxidase activity and a preincubation step with 10% normal swine serum (Dako, Glostrup, Denmark) in phosphate buffered saline (PBS), the cryostat sections were stained with a rabbit polyclonal antibody (diluted 1 in 80) against topoisomerase II-α (Cambridge Research Biochemicals, UK) and incubated overnight at 4°C. Subsequently, a standard double peroxidase antiperoxidase visualisation method was used. 3-3’ Diamobenzidine tetrahydrochloride (DAB) was used as the chromogen and light green as counterstain. For negative controls, rabbit anti-human IgM (Dako) (diluted 1 in 500) was used as the primary antibody. Spleen and placental tissue, which have high topoisomerase II activities, were used as positive controls.

Topoisomerase II-α nuclear immunoreactivity was quantified by counting at least 500 cells in different random fields, using a high power (×40) objective with a grid screen. Occasional cytoplasmic staining was not taken into account unless pronounced nuclear staining was also present. Results were expressed as per cent of cells staining positively for topoisomerase II-α. For further statistical analysis, two groups of tumours were defined: those with 14% or less (low expression) and those with over 14% of nuclei staining positively (high expression). This distribution was chosen because numerically comparable patient groups were obtained.

Oestrogen and progesterone receptor content were determined using an enzyme immunoassay (Abbott, Chicago, Illinois, USA). Results were expressed quantitatively as the amount of receptor protein per gram of tissue (fmol/g). Values greater than 20 fmol/g tissue protein were regarded as positive.

For statistical analysis, the χ2 test and Spearman rank regression analysis were used where appropriate. Disease free and actuarial overall survival estimates were calculated using the Kaplan–Meier life table method. Differences in survival curves were tested using the log rank test. Significance was set at the 5% level. Complete data sets, according to pathological variables for prognosis and clinical follow up data, were available for all patients.

### Results

Heterogeneously distributed nuclear immunoreactivity for topoisomerase II-α was observed in all invasive ductal carcinomas. Stromal cells did not stain for topoisomerase II-α. The percentage of positively staining tumour cells varied between 2 and 62% among different tumours (median 14%). No immunoreactivity for topoisomerase II-α was observed in normal mammary glandular tissue in those sections containing normal breast tissue adjacent to the tumour.

Low levels of topoisomerase II-α expression were found in 32 (51%) tumours and high levels in 31 (49%). Immunohistochemically detected topoisomerase II-α expression with respect to established risk factors is presented in Table 1. Higher tumour grade (p<0.001), larger tumour size (p<0.001), nodal status (p<0.01), and the presence of distant metastases at diagnosis (p<0.005) were significantly related to higher levels of topoisomerase II-α immunoreactivity. No correlation was detected.

### Table 1 Topoisomerase II-α immunoreactivity in 63 invasive ductal breast carcinomas in relation to tumour grade, pTNM staging (UICC criteria), and menopausal status

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Topoisomerase II-α nuclear immunoreactivity*</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤14%</td>
<td>&gt;14%</td>
</tr>
<tr>
<td>All tumours</td>
<td>32 (51%)</td>
<td>31 (49%)</td>
</tr>
<tr>
<td>Tumour grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17 (53%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>II</td>
<td>15 (47%)</td>
<td>14 (45%)</td>
</tr>
<tr>
<td>III</td>
<td>0 (0%)</td>
<td>15 (49%)</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>20 (63%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>pT2</td>
<td>10 (31%)</td>
<td>16 (52%)</td>
</tr>
<tr>
<td>pT3</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>pT4</td>
<td>1 (3%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>21 (66%)</td>
<td>12 (39%)</td>
</tr>
<tr>
<td>pN1</td>
<td>10 (31%)</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>pN2</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>pNX</td>
<td>0 (0%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Presence of metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>32 (100%)</td>
<td>22 (71%)</td>
</tr>
<tr>
<td>M1</td>
<td>0 (0%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10 (31%)</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>Post</td>
<td>22 (66%)</td>
<td>21 (68%)</td>
</tr>
</tbody>
</table>

*Expressed as per cent of positively staining cells. †χ2 test.
Topoisomerase II-α in breast cancer

Invasive ductal breast cancers. However, high levels of expression were found in only half of the patients studied. These results are very similar to those published by Tandon et al., who detected topoisomerase II-α overexpression in 36% of node negative primary breast tumour specimens using a semi-quantitative western blot procedure.

We demonstrated a statistically significant positive correlation between topoisomerase II-α immunoreactivity and tumour grade, tumour size, nodal status, and the presence of metastases. This confirms the recently published data of Tuccari et al., who studied a series of 80 breast carcinomas immunohistochemically. These authors also observed an inverse correlation between topoisomerase II immunoreactivity and oestrogen or progesterone receptor status. We did not find this association, which may be explained by differences in the characteristics of the patients studied or because different techniques were used to assess steroid receptor status. In this study steroid receptor expression was measured by an enzyme immunoassay, whereas Tuccari et al. used an immunohistochemical staining technique.

We present the first data on the prognostic value of topoisomerase II-α expression in breast cancer. As topoisomerase II-α expression is related to poor prognostic factors in breast cancer, such as tumour grade, tumour size, nodal status, and the presence of metastases at diagnosis, we would also expect high topoisomerase II-α expression in breast cancer to be a marker of poor prognosis. Given the limited number of patients and the short duration of follow up in our series, we could not show whether topoisomerase II-α expression has a prognostic value in invasive ductal breast carcinoma. Further studies are necessary to determine the exact prognostic value of this enzyme in breast cancer.

Topoisomerase II is an important cellular target for cytotoxic drugs in anticancer therapy. Antitumour topoisomerase II inhibitors have been subdivided into DNA intercalators, such as doxorubicin, amsacrine, and mitoxantrone, and DNA non-intercalators, such as teniposide and etoposide. New classes of inhibitors with higher selectivity and lower toxicity have recently been described. In vitro studies have revealed that the cytotoxic action of these drugs is highly dependent on the activity of proliferation dependent nuclear topoisomerase II-α. Conversely, reduced expression of topoisomerase II in tumour cell lines is one of the mechanisms involved in conferring resistance against antitumour topoisomerase II inhibitors.

Recent studies have demonstrated topoisomerase II-α co-amplification in a subset of c-erbB2 amplified breast tumours. The human breast cancer cell line SKBR III, with c-erbB2 amplification and topoisomerase II-α co-amplification, is more sensitive to topoisomerase II interactive drugs compared with other non-amplified breast cancer cell lines.

In human breast cancer cells oestrogen enhances the cytotoxicity of certain antitumour

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**Discussion**

In the present study we have shown that topoisomerase II-α expression can be detected immunohistochemically in virtually all primary

between topoisomerase II-α immunoreactivity and menopausal status. Spearman rank analysis did not reveal a correlation between topoisomerase II-α immunoreactivity and oestrogen (p>0.1) or progesterone receptor content (p>0.1).

Of the patients without distant metastases at diagnosis (M0 group, n = 54), 43 were disease free in September 1993. One patient had died of intercurrent disease. Details of follow up of the 10 patients in the M0 group who relapsed or developed distant metastases, or both, are presented in table 2. There was no significant difference in the nuclear expression of topoisomerase II-α between those who relapsed or developed distant metastases (n = 10; median 19.5) and disease free patients (n = 43; median 10.0). Kaplan–Meyer life table analysis did not reveal a difference between disease free survival of patients with low or high topoisomerase II-α expression. Of the patients with distant metastases at diagnosis (M1 group; n = 9), two patients died during follow up. One patient died of intercurrent disease, while another died of disseminated disease 23 months after the initial diagnosis.

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**Table 2** Clinical details of patients in the M0 group who relapsed or developed metastases, or both, during follow up

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Positively staining cells (%)</th>
<th>Relapse or distant metastases</th>
<th>Disease free survival (months)</th>
<th>Outcome at end of follow up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>DM</td>
<td>6</td>
<td>Died (16)</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Relapse</td>
<td>34</td>
<td>Survived (39)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Relapse</td>
<td>32</td>
<td>Survived (41)</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>DM</td>
<td>27</td>
<td>Survived (32)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>DM</td>
<td>20</td>
<td>Died (21)</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>DM</td>
<td>20</td>
<td>Survived (34)</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>DM</td>
<td>20</td>
<td>Survived (43)</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>DM</td>
<td>8</td>
<td>Survived (40)</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>DM</td>
<td>22</td>
<td>Survived (40)</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>DM</td>
<td>26</td>
<td>Survived (39)</td>
</tr>
</tbody>
</table>

DM = distant metastases.
This work was supported by grants from the "Belgian Association chemotherapeutic neoplasms using topoisomerase II-a in planning chemotherapy. Despite the use of taxol/doxorubicin combinations in patients with stage IV breast cancer is currently being studied. Although clinical studies are needed to assess the value of measuring topoisomerase II-α in planning chemotherapy for breast cancer, it is tempting to hypothesise that assessment of topoisomerase II-α activity in breast neoplasms using immunohistochemistry could help discriminate between tumours with high and low topoisomerase II-α activity, the former being more susceptible to topoisomerase II inhibitors. By distinguishing between tumoral types in this way, individualised chemotherapeutic treatment regimens can be devised.

This work was supported by grants from the "Belgian Cancer Association (Vereeniging voor Kankerbestrijding)" and "Door en Van Kanter.


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doi: 10.1136/jcp.48.2.147