Original Article

Topoisomerase II-alfa gene as a predictive marker of response to anthracyclines in breast cancer

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ABSTRACT

Amplification or deletion of the topoisomerase IIα (TOP2A) gene in breast cancer has been related with responsiveness to anthracyclines-based chemotherapy. The purpose of this study was to evaluate the predictive value of TOP2A gene for the efficacy of neo-adjuvant anthracycline in a population with locally advanced breast cancer. Sixty-two patients were included, and the status of TOP2A gene was determined by in situ hybridization method. Treatment efficacy was determined by clinical and pathological response and overall survival. TOP2A gene alterations were found in 22.6% (21.0% of cases with amplification and 1.6% with deletion), and these tumors were biologically more aggressive, with higher nuclear grade, more frequently with HER2 amplification and inflammatory type. Also in these tumors response to chemotherapy appeared to be increased. There was a higher clinical and pathological response rate (complete pathological response of 21.4% vs. 8.3%), a trend toward longer progression-free survival (82.51 vs. 63.12 months) and a trend to increased overall survival (92.08 months; 95% CI 82.81–101.35 vs. 73.40 months; 95% CI 63.44–83.36; $p = 0.113$). These results corroborate that the TOP2A gene alterations may play an important role in determining anthracycline sensitivity in breast cancer.

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Introduction

DNA repair pathways maintain genomic integrity and are among the therapeutic targets most frequently altered in cancer. The DNA repair machinery allows cells to repair DNA damage or induce apoptosis and cell cycle arrest if repair is not possible [9]. Many anticancer drugs are highly influenced by the integrity of this system and specific alterations in DNA repair pathways have been reported to be associated with differences in response to chemotherapy [25]. Anthracyclines (doxorubicin and epirubicin) emerged in the 1950s and remain as a main adjuvant treatment in breast cancer. However, anthracyclines are also associated with major toxicity, which includes acute (alopecia, gastrointestinal and hematological toxicity), and late (irreversible cardiotoxicity, myelodysplasia and acute leukemia) toxicity. This cytotoxic activity is due to their interaction with Topoisomerase IIα (TOP2A), a crucial enzyme in the replication, transcription and DNA repair. The drug binds to TOP2A forming a complex with DNA that prevents the action of RNA-dependent DNA polymerase and consequently gene transcription, with the fragmentation of double stranded DNA. Additionally, it induces the formation of free radicals inside the cell (membrane lipid peroxidation) leading to senescence and cell death by apoptosis or necrosis [23].

The benefit of anthracyclines in breast cancer has been widely demonstrated by prospective randomized clinical trials. However, probably due to the high biological diversity of these tumors, the benefit appears to be limited to only a subgroup of patients. The search of biomarkers predictive of response has been stepped up in an attempt to recognize the best candidates for treatment with these drugs. Several retrospective observational studies support that the HER-2 amplification, besides predicting response to anti-HER2 agents, also seems to confer sensitivity to anthracycline

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[16,18,21,28]. However, other published data do not support this association. More recently, it has been hypothesized that the amplification of *HER-2* is just a surrogate marker for the amplification of *TOP2A* gene, the real determinant of increased activity of anthracyclines [6,19,20,22].

The *TOP2A* gene is located on the long arm of chromosome 17 (17q21-22), in close proximity to HER2 gene (17q11.2-12). *In vitro* studies have shown that *TOP2A* alterations are frequent in tumor cells and determine a greater sensitivity to the action of anthracyclines [10–12,27]. Since then, it had been demonstrated in retrospective clinical trials a clinical benefit from the use of anthracyclines in tumors with *TOP2A* amplification, whose incidence varies between 10 and 25%. On the other hand, *TOP2A* deletion is documented in 5–15% of the breast cancer cases in different series [2–5,7,13,14,17]. There are contradictory results concerning the predictive value of *TOP2A* alterations, as well as their association with *HER2* amplification [1,15]. This inconsistency may be explained by different biological and technical causes, such as tumor heterogeneity, the lack of standard techniques for assessing the status of *TOP2A* and the type of sample used for the study. Moreover, for *TOP2A* the gene amplification is not directly related with the expression of mRNA or protein, as occurs with *HER*2 [24]. For these reasons, more validation studies are needed to establish *TOP2A* gene as a predictive marker for anthracycline response, optimizing its use in clinical practice.

The primary objective of this study was to evaluate the changes in the *TOP2A* gene copy number in breast cancer tissues and correlate them to clinical–pathological parameters, particularly the response to neo-adjuvant chemotherapy with anthracyclines, both in terms of clinical response, pathological response, progression-free survival and overall survival.

Materials and methods

Patient selection

Patients with locally advanced breast carcinoma who were treated with neoadjuvant, anthracycline-based chemotherapy at Medical Oncology Department, Centro Hospitalar de São João, Porto (Portugal) between 2004 and 2008 were included in this retrospective study. Patients received either doxorubicin or epirubicin, associated with other drugs, before surgical treatment.

The assessment of clinical response was made after completion of neoadjuvant chemotherapy, by physical examination and imaging studies. Complete clinical response (cCR) was defined by the total disappearance of the initial lesion (primary tumor and lymph nodes), partial clinical response (pCR) as a decrease of at least 50% of the initial tumor size, and no response in other cases.

The assessment of pathological response was performed in surgical specimens according to the degree of reduction in tumor size and degree of residual disease, either at the primary tumor and lymph node level. Complete pathological response (cPR) was defined as no residual tumor; partial pathologic response (pPR) as a reduction greater than 50% in tumor size or less than 50% of residual disease; the remaining cases were classified as absence of response.

TOP2A/CEP17 in situ hybridization

In situ hybridization of chromosome 17 (CEP17) and *TOP2A* gene was performed in 2 μm thick formalin-fixed, paraffin-embedded sections from diagnostic core biopsy using the technique of silver in situ hybridization (SISH). A slide stained with hematoxylin and eosin (H&E), prepared for each block, was used for identification of the invasive tumor component. Two color SISH was carried out using a double DNA probe: *TOP2A DNA Probe* (Ventana Medical

Systems Inc., Tucson, Arizona, USA), labeled with 2,4-dinitrophenol (DNP) which covers around 67,400 base pair (67 kb) of the region of TOP2A gene (17q21-22); and *Chromosome 17 DIG Probe* (Ventana) for the chromosome 17 centromere (17p11.1-q11.1). The entire procedure was carried out on the Ventana Benchmark automated staining system (BenchMark™ XT Staining System – Ventana).

Briefly, the sections were deparaffined and incubated in *CC1-Buffer* (Cell Conditioning solution; Ventana) for 28 min at 90 °C followed by enzymatic digestion with *ISH-Protease 3* (Ventana) at 37 °C for 12 min. The slides were washed with Reagent Buffer (pH 7.4; Ventana). This procedure was followed by incubation with the probe mixture consisting of *TOP2A DNA Probe* (Ventana) and *Chromosome 17 DIG Probe* (Ventana) and detection was performed with the *Ultraview SISH DNP Detection Kit* (Ventana). Codenaturation and hybridization occurred between 37 °C and 52 °C, over 6 h and after that the slides were stringently washed with 0.1× sodium chloride citrate (SCC – Ventana) (24 min at 72 °C). Finally the slides were incubated with *TOP2A* detection system *SISH DET HRP* (Ventana) for 16 min a 37 °C, followed by *ultraVIEW SISH Detection Kit* (Ventana), which included three sequential silver reagents (37 °C for 4 min each); and with the detection system for chromosome 17, *Ultraview RED ISH DIG detetion Kit* (Ventana) for 24 min at 37 °C. The slides were then counterstained using *Hematoxylin II* (Ventana), by incubation for 4 min at 37 °C. Outside the equipment the sections were washed with purified water and dehydrated with 100° alcohol and plates were assembled.

Histologic analysis was done with Axioscope2 Light microscope, using a 40× and 60× lens amplification (Carl Zeiss Microscopy, Jena, Germany). The number of *TOP2A* gene copies (each gene copy was labeled with silver and visualized as a black dot) and chromosome 17 (CEP17) (each copy was labeled with red dye and visualized as a red dot) was determined in a total of 40 non-overlapping nuclei of tumor cells. A ratio TOP2A/CEP17 of 2.0 or greater was considered *TOP2A* amplification; a ratio of 0.5 or lesser was considered deletion; and the remainder was considered normal. Regarding the number of copies of the CEP17, monosomy was defined as a ratio CEP17/tumor cell nuclei between 1.0 and 1.4; polysomy as a ratio >2.5; and the remainder were considered diploid [8].

Statistical analysis

Categorical data are presented as numbers and corresponding percentage, while continuous data are presented as median and range values. Univariate analysis was evaluated with Chi-squared test followed by a multivariable logistic regression analysis. The progression free survival (PFS) and overall survival (OS) were determined from the time of surgery to the date of progression and death, respectively. Kaplan–Meier curves and log-rank tests were performed for comparing time to event distributions, followed by Cox regression. A *P* value of 0.05 or less was considered to be significant, and the SPSS *software* was used for statistical analysis (SPSS Statistics v. 20.0).

Results

Patients and tumor characteristics

A total of 62 patients were included in this study (Table 1). Only one patient was male and the median age of diagnosis was 48.0 years (range: 23–74). All patients had biopsy-proven invasive breast carcinoma, the majority of which were ductal (85.5%), with high nuclear grade (51.6%) and luminal A or B type (66.1%). Mostly patients presented with stage II disease. Four patients with stage IV disease, with isolated bone metastases, were also included. Slightly more patients received doxorubicin than epirubicin (51.6%