Acta Histochemica 116 (2014) 740–746

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Co-expression of cancer testis antigens and topoisomerase 2-alpha in triple negative breast carcinomas

Ivana Mrklić[a,*], Giulio Cesare Spagnoli[b], Antonio Juretić[c], Zenon Pogorelić[d], Snježana Tomić[a]

[a] Department of Pathology, Forensic Medicine and Cytology, Split University Hospital Center, University of Split, School of Medicine, Spinčićeva 1, Split, Croatia
[b] Department of Surgery, Research Laboratory, University Hospital Basel, Basel, Switzerland
[c] Department of Oncology, Zagreb University Hospital Center, University of Zagreb, School of Medicine, Kispatićeva 12, Zagreb, Croatia
[d] Department of Pediatric Surgery, Split University Hospital Center, University of Split, School of Medicine, Spinčićeva 1, Split, Croatia

ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 2 January 2014
Accepted 6 January 2014

Keywords:
Basal like breast cancer
Cancer testis antigens
Combination treatments
Topoisomerase 2-alpha
Triple negative breast cancer
Human

ABSTRACT

Triple negative breast cancers (TNBC) are characterized by aggressive tumor biology, lack of targeted treatments and poor prognosis. Anthracyclins were shown to induce immunogenic death in target cells, potentially leading to "endogenous" vaccination. We comparatively assessed expression of cancer testis antigens (CTA) and topoisomerase 2-alpha (TOPO2A), a well defined molecular target of anthracyclins, in TNBC fully characterized for basal-like (BL) immunophenotype, BL morphology and conventional clinicopathological factors. The study included 83 patients undergoing surgery between January 2003 and December 2009. Tissue sections were stained with CK5/6, CK14, EGFR, Ki-67, TOPO2A, MAGE-A1, MAGE-A10, NY-ESO and multi-MAGE-A specific reagents. Of the 83 TNBC, >66.3% had BL immunophenotype and 48.2% had BL morphology. MAGE-A1 specific staining was most frequently detectable (69.2%), followed by multi-MAGE-A (58%), NY-ESO (27.1%) and MAGE-A10 (16%) specific staining. MAGE-A10 expression significantly correlated with tumor size ($p = 0.026$). Furthermore, MAGE-A1, MAGE-A10 and multi-MAGE-A specific stainings significantly correlated with advanced clinical stage ($p = 0.024$, $p = 0.041$, $p = 0.031$, respectively). We found no significant association between CTA expression and disease free (DFS) or overall survival (OS). Most interestingly, a significant correlation was observed between expression of MAGE-A10 and NY-ESO and expression of TOPO2A ($p = 0.005$, $p = 0.013$). Expression of defined CTA and TOPO2A are significantly correlated in TNBC. Considering the limited therapeutic options for TNBC, these findings might suggest novel forms of combination therapies that should be further explored.

Introduction

Triple negative breast cancers (TNBC) do not express estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor 2 (HER2). Since specific targeted therapies are ineffective, chemotherapy currently represents the only available treatment. Several studies have associated TNBC with aggressive clinical behavior, higher incidence of lung and brain metastases, and poor prognosis despite good responsiveness to conventional chemotherapy regimens (Nielsen et al., 2004; Lacroix et al., 2004; Rouzier et al., 2005; Hicks et al., 2006; Carey et al., 2007; Fulford et al., 2007).

Cancer testis antigens (CTA) are encoded by group of genes expressed physiologically in human germ line cells and aberrantly in various malignancies. To date, 153 CTA have been described: 83 of them are encoded on the X-chromosome and referred to as CT-X antigens (Simpson et al., 2005). Expression of CTA is highly variable and may be observed frequently in melanomas, bladder, lung, ovarian and hepatocellular carcinomas, but rarely in renal, colon, gastric cancers and hematological malignancies (Scanlan et al., 2004). A few studies exploring CTA expression in TNBC have reported a high incidence of CTA expression (Grigoriadis et al., 2009; Curigliano et al., 2011; Ademuyiwa et al., 2012; Badovinac Črnjević et al., 2012; Karn et al., 2012). Considering the limited therapeutic options for TNBC, expression of CTA antigens could provide the opportunity for targeted immunotherapies.

Topoisomerase 2 alpha (TOPO2A) is a type II DNA topoisomerase relaxing supercoiled DNA by transient double strand breaks (Wang, 2002; Petit et al., 2004). Most importantly, TOPO2A has been shown to represent a molecular target for anthracyclins. Effectiveness

* Corresponding author at: Department of Pathology, Forensic Medicine and Cytology, Split University Hospital Centre, Spinčićeva 1, 21000 Split, Croatia.
E-mail address: ivana.mrklic@mefst.hr (I. Mrklić).

0065-1281/$ – see front matter © 2014 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2014.01.003

of chemotherapy has recently been suggested to require the integrity of the immune system (Kepp et al., 2009; Kroemer et al., 2013). Indeed a number of treatments have been proposed to induce "immunogenic" cell death, consistent with calreticulin exposure on preapoptotic cell surfaces and the release by dying cells of molecules encompassing damage associated molecular patterns (DAMP). These stimuli concur in the activation of antigen presenting cells (APC), possibly triggering Toll-like receptors (TLR) (Kroemer et al., 2013). Based on this background and aiming at envisaging novel combination treatments, here we comparatively explored CTA and TOPO2A expression in TNBC.

Materials and methods

Patients

Case records of patients with breast cancer, undergoing surgery between January 2003 and December 2009, were retrospectively reviewed. Based on pathology reports, 124 TNBC cases were identified. 83 of these patients did not receive preoperative chemotherapy and had available paraffin embedded tissue blocks. Clinical information was collected through the breast cancer database.

46 (55.4%) of these patients, were treated with mastectomy, and 26 (31.3%) with quadrantectomy, following axillary lymph node dissection. For eleven (13.3%) patients with TNM stage III or IV, only biopsy was performed. All patients undergoing breast conserving surgery subsequently received postoperative radiotherapy. Systemic adjuvant chemotherapy was administered to all patients. Most of the patients, 56/83 (67%) were treated with anthracycline-based therapy, and the rest of the patients 27/83 (33%) were treated with other chemotherapy regimens. None was treated by hormonal or HER2 targeting therapy.

Complete follow-up was available for 81 patients, and only these patients were included in further analysis. Mean follow-up was 43 months (range 2–95 months). Disease-free survival (DFS) was defined as the interval from the date of primary surgery to the first locoregional recurrence or distant metastases. Overall survival (OS) was defined as the time from the date of primary surgery to the time of breast cancer-related death.

All histological and IHC tumor slides were evaluated by two pathologists (S.T., I.M.) and graded according to Elston and Ellis (1991). Histological types were determined according to WHO and staging was based on TNM Classification (Ellis, 2003; Sobin et al., 2009).

Histopathology and immunohistochemistry

Sections from fixed, paraffin embedded, cancer tissues were stained by hematoxylin/eosin with additional immunostains for ER (1:200, Dako, Glostrup, Denmark), PR (1:100, Dako), and HER2/neu (HercepTest assay, Dako), CK5/6 (1:100, Dako), CK14 (1:25, Novocastra, Leica Microsystems, UK), EGFR (1:40, Dako), Ki-67 (1:200, Dako), and topoisomerase 2-alpha (1:75, Dako).

As primary reagents, monoclonal antibodies (mAb) recognizing the following CTA were used: mAb 77B (MAGE-A1), mAb 57B (multi-MAGE-A), mAb 3GA11 (MAGE-A10) and D8.38 (NY-ESO-1) (Schultz-Thater et al., 1994, 2011; Jungbluth et al., 2000, 2001). Immunoassays were performed on Ventana BenchMark Ultra autostainer (Roche, Tucson, AZ, USA). HER2 status was evaluated by IHC (Hercept Test, Dako, Glostrup, Denmark) or by chromogenic in situ hybridization (SPOT-Light® HER2 CISH Kit, Invitrogen/Zymed, Camarillo, CA, USA). Tests were scored according to ASCO/CAP guidelines (Wolff et al., 2007). ER and PR were considered positive if at least 1% of the invasive tumor cells nuclei in the sample were positive (Hammond et al., 2010). To minimize the issue of tumor heterogeneity, whole sections were used to determine the frequency of CTA expression by IHC. MAGE-A1, NY-ESO and multi-MAGE-A specific stainings were considered positive if a cytoplasmic and/or nuclear reaction was detectable in ≥10% tumor cells. MAGE-A10 specific staining was considered positive if there was nuclear reactivity in ≥10% tumor cells (Schultz-Thater et al., 2011). CK5/6, CK14 and EGFR were considered positive if ≥10% tumor cells showed positive membranous expression. BL immunophenotype was defined by ER/PR/HER2 negativity, and positivity to one or more basal cell markers: CK5/6, CK14 or EGFR (Ho-Yen et al., 2012).

BL morphology was considered positive if characteristic features such as syncytial growth pattern, high mitotic index, large central acellular/necrotic zone, pushing borders, dense lymphocytic infiltrate at the periphery of the invasive component and the presence of metaplastic and medullary elements were present (Fulford et al., 2006).

Ki-67 and TOPO2A expression were scored by counting 1000 tumor cells using the Olympus Image Analyser (magnification 400×), at the hot spots and at the periphery of the invasive component. Data are expressed as percentages of positive cells (Dowsett et al., 2011).

Cut-offs were established by ROC curve analysis (see below). In particular, optimal cut-off for Ki-67 staining was 61%, with sensitivity 56% and specificity 85% (area 0.657, SE 0.060, 95% CI: 0.539–0.776, $p = 0.020$). Optimal cut-off for TOPO2A was 36%, with sensitivity 65.5% and specificity 57% (area 0.647, SE 0.065, 95% CI: 0.520–0.774, $p = 0.030$). Optimal cut-off for mitotic score was 21, with sensitivity 64% and specificity 86% (area 0.672, SE 0.061, 95% CI: 0.551–0.792, $p = 0.011$).

Table 1
Histopathological factors and biomarkers of 83 patients with TNBC.

Variables	*N* (%)
Age	
Years old	60.7 (32–97)
Histological subtype	
IDC NOS	60 (72.3%)
Other	23 (27.7%)
Tumour size (cm)	2.7 (0.3–12%)
Clinical stage	
I	21 (25.3%)
II	30 (36.1%)
III	26 (31.3%)
IV	6 (7.2%)
Mitotic count	
Number/10 HPF	25.9 (2–110)
BL immunophenotype	
No	28 (33.7%)
Yes	55 (66.3%)
BL morphology	
No	43 (51.8%)
Yes	40 (48.2%)
Histological grade	
I	1 (1.2%)
II	13 (15.7%)
III	69 (83.1%)
Vascular invasion	
No	53 (63.9%)
Yes	30 (36.1%)
Ki-67 (%)	53.7 (3–95)
TOPO2A (%)	41.7(5–97)

Statistical analysis

Data were analyzed using Statistics for Windows Release 12.0 (Statsoft, Tulsa, OK, USA). All p-values <0.05 were considered statistically significant. All statistical tests were two-sided, with 95% confidence interval. Correlations between categorical variables