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

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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

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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

Table 2
Correlation between expression of MAGE-A1, multi-MAGE, NY-ESO, MAGE-A10 and histopathological parameters of 81 patients with TNBC.

	MAGE-A1			multi-MAGE			NY-ESO			MAGE -A10		
	Negative *N* (%) 25 (30.9%)	Positive *N* (%) 56 (69.1%)	*p*	Negative *N* (%) 34 (42%)	Positive *N* (%) 47 (58%)	*p*	Negative *N* (%) 59 (72.8%)	Positive *N* (%) 22 (27.2%)	*p*	Negative *N* (%) 68 (84%)	Positive *N* (%) 13 (16%)	*p*
Age												
<52	5 (20)	10 (17.8)	0.171	8 (23.5)	7 (14.9)	0.735	13 (22)	2 (9.1)	0.378	11 (16.2)	4 (30.8)	0.466
52–58	9 (36)	16 (28.6)		9 (26.5)	16 (34)		19 (32.2)	6 (27.3)		23 (33.8)	2 (15.4)	
59–71	9 (36)	14 (25)		9 (26.5)	14 (29.8)		16 (27.1)	7 (31.8)		19 (27.9)	4 (30.8)	
>71	2 (8)	16 (28.6)		8 (23.5)	10 (21.3)		11 (18.6)	7 (31.8)		15 (52.1)	3 (23.1)	
Histological subtype												
IDC NOS	17 (68)	42 (75)	0.517	23 (67.6)	36 (76.6)	0.373	40 (67.8)	19 (86.4)	0.080	47 (69.1)	12 (92.3)	0.057
Other	8 (32)	14 (25)		11 (32.4)	11 (23.4)		19 (32.2)	3 (13.6)		21 (30.9)	1 (7.7)	
Tumor size #												
<1.5	4 (19)	11 (22.4)	0.860	10 (34.5)	5 (12.2)	0.056	13 (25)	2 (11.1)	0.551	15 (25)	0 (0)	0.026
1.5–2.5	9 (43)	17 (34.7)		11 (37.9)	15 (36.6)		18 (34.6)	8 (44.4)		24 (40)	2 (20)	
2.5–3.0	4 (19)	8 (16.3)		2 (6.9)	10 (24.4)		8 (15.4)	4 (22.2)		9 (15)	3 (30)	
>3.0	4 (19)	13 (26.5)		6 (20.7)	11 (26.8)		13 (25)	4 (22.2)		12 (20)	5 (50)	
Clinical stage												
I	7 (28)	12 (21.4)	0.024	12 (35.3)	7 (14.9)	0.031	16 (27.1)	3 (13.6)	0.195	19 (27.9)	0 (0)	0.041
II	11 (44)	19 (33.9)		7 (20.6)	23 (48.9)		18 (30.5)	12 (54.5)		24 (35.3)	6 (46.1)	
III	3 (12)	23 (41.1)		13 (38.2)	13 (27.7)		21 (35.6)	5 (22.7)		21 (30.9)	5 (38.5)	
IV	4 (16)	2 (3.6)		2 (5.9)	4 (8.5)		4 (6.8)	2 (9.1)		4 (5.9)	2 (15.4)	
Mitotic count												
≤21	9 (36)	26 (46.4)	0.379	17 (50)	18 (38.3)	0.294	27 (45.8)	8 (36.4)	0.445	29 (42.6)	6 (46.2)	0.815
>21	16 (64)	30 (53.6)		17 (50)	29 (61.7)		32 (54.2)	14 (63.6)		39 (57.4)	7 (53.8)	
BL morphology												
No	10 (40)	31 (55.4)	0.201	19 (55.9)	22 (46.8)	0.420	28 (47.5)	13 (59.1)	0.351	35 (51.5)	6 (46.2)	0.725
Yes	15 (60)	25 (44.6)		15 (44.1)	25 (53.2)		31 (52.5)	9 (40.9)		33 (48.5)	7 (53.8)	
BL imuno-phenotype												
No	6 (24)	21 (37.5)	0.226	12 (35.3)	15 (31.9)	0.750	20 (33.9)	7 (31.8)	0.859	24 (35.3)	3 (23.1)	0.380
Yes	19 (76)	35 (62.5)		22 (64.7)	32 (68.1)		39 (66.1)	15 (68.2)		44 (64.7)	10 (76.9)	
Histological grade												
I	0 (0)	1 (1.8)	0.639	1 (2.9)	0 (0)	0.397	1 (1.7)	0 (0)	0.539	1 (1.5)	0 (0)	0.101
II	4 (16)	7 (12.5)		5 (14.7)	6 (12.8)		9 (15.2)	2 (9.1)		11 (16.2)	0 (0)	
III	21 (84)	48 (85.7)		28 (82.4)	41 (87.2)		49 (83.1)	20 (90.9)		56 (82.3)	13 (100)	
Vascular invasion												
No	16 (64)	35 (62.5)	0.897	19 (55.9)	32 (68.1)	0.263	35 (59.3)	16 (72.7)	0.259	43 (63.2)	8 (61.5)	0.908
Yes	9 (36)	21 (37.5)		15 (44.1)	15 (31.9)		24 (40.7)	6 (27.3)		25 (36.8)	5 (38.5)	
Ki-67												
≤61	15 (60)	33 (58.9)	0.928	21 (61.8)	27 (57.4)	0.696	37 (62.7)	11 (50)	0.303	42 (61.8)	6 (46.2)	0.298
>61	10 (40)	23 (41.1)		13 (38.2)	20 (42.6)		22 (37.3)	11 (50)		26 (38.2)	7 (53.8)	
TOPO2A												
≤36	11 (44)	21 (37.5)	0.582	15 (44.1)	17 (36.2)	0.471	28 (47.5)	4 (18.2)	0.013	31 (45.6)	1 (7.7)	0.005
>36	14 (56)	35 (62.5)		19 (55.9)	30 (63.8)		31 (52.5)	18 (81.8)		37 (54.4)	12 (92.3)	
MAGE-A10												
neg.	23 (92)	45 (80.4)	0.164	32 (94.1)	36 (76.6)	0.025	53 (89.8)	15 (68.2)	0.025			
pos.	2 (8)	11 (19.6)		2 (5.9)	11 (23.4)		6 (10.2)	7 (31.8)				
NY-ESO												
neg.	23 (92)	36 (64.3)	0.005	31 (91.2)	28 (59.6)	0.001				53 (77.9)	6 (46.2)	0.025
pos.	2 (8)	20 (35.7)		3 (8.8)	19 (40.4)					15 (22.1)	7 (53.8)	
multi- MAGE												
neg.	11 (44)	23 (41.1)	0.805				31 (52.5)	3 (13.6)	0.001	32 (47.1)	2 (15.4)	0.025
pos.	14 (56)	33 (58.9)					28 (47.5)	19 (86.4)		36 (52.9)	11 (84.6)	
MAGE-A1												
neg.				11 (32.4)	14 (29.8)	0.805	23 (39)	2 (9.1)	0.005	23 (33.8)	2 (15.4)	0.164
pos.				23 (67.6)	33 (70.2)		36 (61)	20 (90.9)		45 (66.2)	11 (84.6)	

Table 3
Log rank analysis of disease-free survival (DFS) and overall survival (OS) according to CT antigen expression of 81 patients with TNBC.

Variables	OS		DFS	
	LR	*p*	LR	*p*
MAGE-A1				
neg.	0.34	0.562	1.81	0.178
pos.				
MAGE-A10				
neg.	0.17	0.681	0.67	0.413
pos.				
NY-ESO				
neg.	0.37	0.543	0.04	0.849
pos.				
multi-MAGE				
neg.	0	0.991	0	0.957
pos.				

Abbreviations: DFS, disease free survival; OS, overall survival; LR, log rank.

Table 4
Univariate Cox regression analysis of disease-free survival (DFS) and overall survival (OS) of 81 patients with TNBC.

Variables	OS		DFS	
	RR (95% CI)	*p*	RR (95% CI)	*p*
MAGE-A1				
neg.	0.95 (0.36–2.5)	0.922	0.567 (0.245–1.31)	0.185
pos.				
MAGE-A10				
neg.	0.84 (0.19–3.6)	0.813	1.56 (0.528–4.64)	0.419
pos.				
NY-ESO				
neg.	1.6 (0.64–4.1)	0.307	1.1 (0.428–2.8)	0.849
pos.				
multi-MAGE				
neg.	1.1 (0.44–2.8)	0.825	1.02 (0.441–2.37)	0.957
pos.				

Abbreviations: DFS, disease free survival; OS, overall survival; RR, risk ratio; CI, confidence interval.

were studied using chi-square test. For univariate analysis, survival time was analyzed by the Kaplan–Meier method, and the log-rank test was used to assess differences among groups. For disease-free survival and overall survival, survival time was censored at death if the cause was not breast cancer or if the patient was alive without relapse on March 1, 2011. For multivariate analysis Cox proportional hazard regression model was used to simultaneously examine all factors predictive of survival in univariate analysis. Optimal cut-off values for Ki-67, TOPO2A and mitotic score were selected by using ROC (receiver operating characteristic) method, by minimizing the sum of the observed false-positive and false-negative errors with bootstrapping methodology.

Results

Out of 83 TNBC, 60 (72.28%) were invasive ductal carcinomas not otherwise specified (IDC NOS), 13 (15.66%) metaplastic carcinomas, 2 (2.4%) invasive lobular carcinomas, 3 (3.61%) invasive mixed carcinomas, 3 (3.61%) medullary carcinomas and 2 (2.4%) apocrine carcinomas. In agreement with previous studies (Nielsen et al., 2004; Ho-Yen et al., 2012; Mrklić et al., 2013a), a majority of TNBC were associated with high histological grade (84.3%), mitotic counts (25.9; range 2–110) and proliferative activity, as measured by Ki-67 antigen (53.7; range 3–95). According to expression of basal markers, 66.3% of tumors showed BL immunophenotype (Table 1).

CTA were expressed at different frequencies in these cancers. MAGE-A1 specific staining was most frequently detectable (56/81 TNBC, 69.2%), followed by multi-MAGE (47/81, 58.0%), NY-ESO-1 (22/81, 27.1%) and MAGE-A10 specific staining (13/81, 16.0%) (Table 2). Detection of NY-ESO-1 specific staining significantly correlated with expression of MAGE-A1, MAGE-A10 and detection of multi-MAGE specific staining ($p = 0.005$, $p = 0.025$, $p = 0.001$, respectively) (Table 2). A trend hinting to a correlation between MAGE-A10 expression and histological type was also observed, since all but one tumor expressing MAGE-A10 were IDC NOS ($p = 0.057$) (Table 2).

Analysis of the relationship occurring between CTA expression and clinicopathological parameters revealed that tumors of >2.5 cm width expressed MAGE-A10 at a higher frequency than tumors of smaller size ($p = 0.026$). Expression of MAGE-A1 and MAGE-A10 and multi-MAGE-A positivity were significantly correlated with clinical stage ($p = 0.024$, $p = 0.041$, $p = 0.031$, respectively) (Table 2). No significant association was found between CTA expression and age at diagnosis, histological grade, mitotic counts, vascular invasion, BL morphology, BL immunophenotype and Ki-67 (Table 2).

Univariate survival analysis revealed that CTA expression was not associated with DFS and OS (Table 3). The results were confirmed by univariate Cox regression analysis for DFS and OS (Table 4).

Expression of TOPO2A in TNBC was previously assessed and described elsewhere (Mrklić et al., 2013b). When correlated with CTA, a significant correlation between expression of MAGE-A10 and NY-ESO and expression of TOPO2A was observed ($p = 0.005$, $p = 0.013$) (Table 2). In order to obtain evidence supporting the association of TOPO2A and CTA in the same cancerous tissues, we stained serial TNBC sections with specific reagents (Fig. 1). Our data clearly indicate that TOPO2A and NY-ESO-1 and MAGE-A10 are frequently co-expressed in the same cancer areas.

Additionally, in order to confirm co-expression of NY-ESO and TOPO2A double immunostaining was performed (Fig. 2). Since MAGE-A10 shows nuclear reaction, similar to that of TOPO2A, double immunostaining was not applicable.

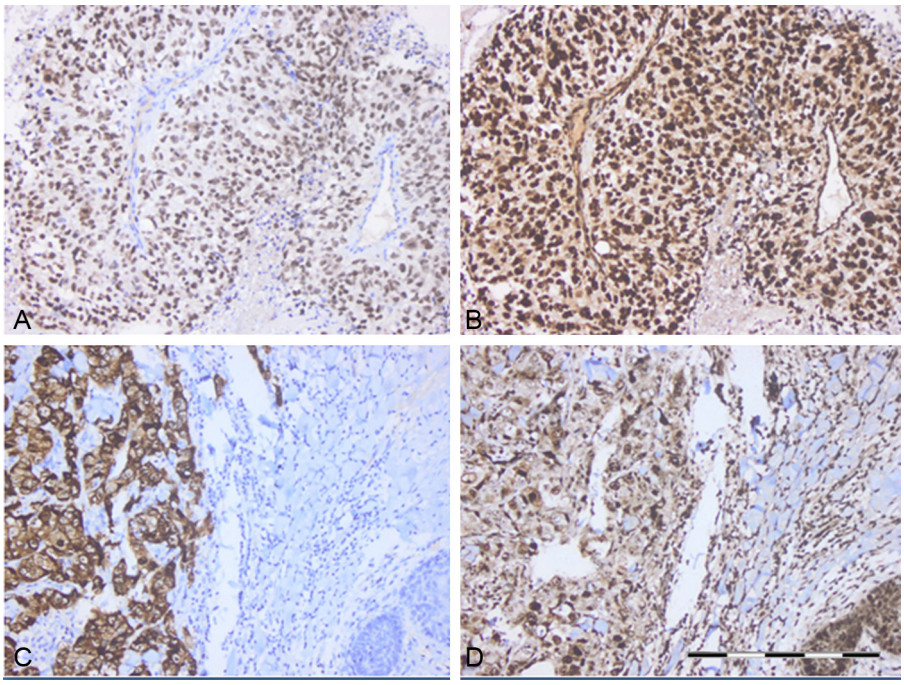

Fig. 1. Immunohistochemical staining on serial TNBC sections. Representative staining with reagents specific for (A) MAGE-A10, (B) TOPO2A, (C) NY-ESO, and (D) TOPO2A are reported.

Discussion

TNBC are characterized by poor prognosis, due to aggressive tumor biology, and lack of targeted treatments (Gluz et al., 2009). Several reports suggest that TNBC are heterogeneous and comprise subtypes with different clinical outcomes, including more aggressive basal and less aggressive non-basal subtype, as identified by the expression of basal cytokeratins and EGFR (Nielsen et al., 2004; Cheang et al., 2008).

CTA expression was previously detected at the gene and protein level to highly variable degrees in breast cancer (Sugita et al., 2004; Mischo et al., 2006; Theurillat et al., 2007; Chen et al., 2011). The heterogeneity of these results could be explained by different cohort designs in these studies, since unselected breast cancers would consist mainly of ER-positive tumors showing low levels of CTA expression as compared to ER-negative tumors (Grigoriadis et al., 2009; Chen et al., 2011; Curigliano et al., 2011).

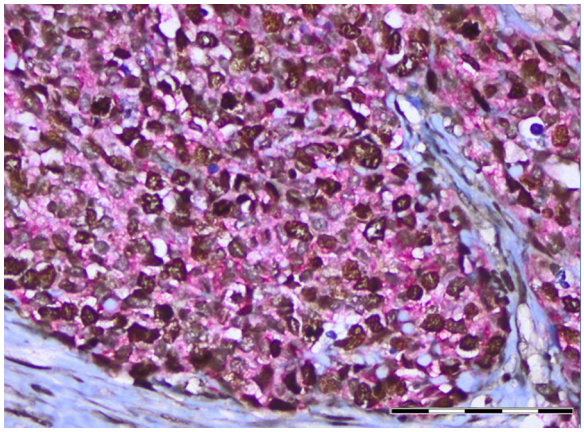

Fig. 2. Co-expression of NY-ESO and TOPO2A shown by double immunostaining, positive nuclear staining for TOPO2A is shown using brown chromogen and positive cytoplasmic staining for NY-ESO is shown using red chromogen.

Immunohistochemical studies have shown that CTA are frequently expressed in TNBC, thus envisaging specific immunotherapy as a potential novel therapeutic approach to these cancers (Grigoriadis et al., 2009; Curigliano et al., 2011; Ademuyiwa et al., 2012; Badovinac Črnjević et al., 2012). Our results are largely in agreement with these data and with those by Adams et al. (2011) who investigated CTA expression in BRCA-associated breast cancer consisting predominantly of TNBC, and reported MAGE-A and NY-ESO-1 expression in 50% and 38% of tumors, respectively.

TOPO2A protein represents an intracellular target for anthracycline-based therapy. High expression of TOPO2A protein was first described in HER2 and TOPO2A amplified tumors. According to meta-analysis by Di Leo et al. (2011) high TOPO2A expression was recently observed in TNBC. They confirmed that patients with HER2 gene amplification show greater benefit from anthracycline-based adjuvant therapy, but also suggested that differential benefit from anthracyclines might exist in patients with triple negative and moderately hormone-sensitive tumours (Di Leo et al., 2011).

Importantly, anthracyclines were shown to induce “immunogenic” cell death, characterized by the release of damage associated molecular patterns (DAMP) capable of TLR-mediated activation of different types of potential antigen presenting cells (APC) and by calreticulin exposure on plasma membranes (Kroemer et al., 2013).

To the best of our knowledge, correlation between CTA and TOPO2A expression has not been investigated in TNBC. We report that a significant association between selected CTA and TOPO2A expression is frequently detectable in TNBC.

The number of patients treated with anthracycline-based therapy in our study is too small to draw general conclusions. Although we did not find significant correlation between CTA expression and DFS or OS, significant correlation observed between expression of MAGE-A10 and NY-ESO and expression of TOPO2A may suggest that clinical outcome of anthracyclin treatments in these cancers might be in part related to the release of potentially antigenic determinants by tumor cells and to the ensuing induction of specific immune responses. Future studies are warranted to verify whether

and to what extent anthracyclin treatment induces CTA specific immune responses in patients with CTA+ breast cancers.

Within this context, it is of interest that at least NY-ESO-1 is known to relatively frequently induce humoral immune responsiveness. Therefore, serological studies might provide important information on NY-ESO-1 specific immune responsiveness in anthracyclin treated patients.

Considering the limited therapeutic options for TNBC, these findings might suggest novel forms of combination therapies that should be further explored.

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