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HER2 expression in Brazilian patients with estrogen and progesterone receptor-negative breast carcinoma

Susana Ramalho[a], Katia Piton Serra[a], Jose Vassallo[b], Fernando Augusto Soares[c], Glauce Aparecida Pinto[b], Luiz Carlos Teixeira[a], Isabela Werneck da Cunha[c], Sophie F.M. Derchain[a,*], Gustavo de Souza[a]

[a] Department of Obstetrics and Gynecology, Universidade Estadual de Campinas – UNICAMP, School of Medicine, Campinas, São Paulo, Brazil
[b] Department of Pathological Anatomy, Universidade Estadual de Campinas – UNICAMP, School of Medicine, Campinas, São Paulo, Brazil
[c] Department of Pathology, Hospital do Cancer A.C. Camargo, Fundação Antônio Prudente, São Paulo, Brazil

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ABSTRACT

The aim of the study was to evaluate the relationship between clinical and pathological factors and survival in patients with double negative HER2-overexpressing carcinoma and triple negative carcinoma. One hundred and sixty-one (161) patients diagnosed with breast cancer negative for estrogen receptor (ER) and progesterone receptor (PR) were included. Of the total, 58 patients had double negative HER2-overexpressing (ER/PR-negative and HER2-positive) and 103 had triple negative (ER-negative, PR-negative and HER2-negative). ER and PR expression was assessed through immunohistochemistry (IHC) and HER2 expression was measured by immunohistochemistry and Fluorescent in situ Hybridization (FISH) analysis in tissue microarray. More than 80% had stages II and III disease and histologic grade III and nuclear grade 3. Patients with triple negative breast carcinoma had undifferentiated histologic types in 11% of cases and vascular invasion in 14.5%. Both groups had more than 50% visceral metastases. HER2 expression ($p = 0.42$) and vascular invasion ($p = 0.05$) did not interfere with survival. Survival of patients with Stages I-II disease was significantly longer than in those with Stage III disease both for double negative HER2-overexpressing carcinomas ($p < 0.0001$) and triple negative carcinomas ($p = 0.03$). The study shows that hormone receptor-negative breast carcinomas were undifferentiated and diagnosed at advanced stages and that HER2 expression was not associated with overall survival.

Introduction

Breast cancer is the most common malignancy among women. Approximately 1.38 million new cases of breast cancer were predicted to occur worldwide in 2008, accounting for 23% of all types of cancer (Ferlay et al., 2011). In Brazil, the incidence of breast cancer for the year 2012 is expected to be 52.608 new cases, with an estimated risk of 52 cases per 100,000 women (INCA, 2011). It is the leading cause of cancer death in women. Some estimates put the number of deaths at 269,000 in developed countries and 189,000 in developing countries (Ferlay et al., 2011).

Breast cancer is a heterogeneous disease. Its origin is closely related to alterations derived from signaling pathways of breast epithelial cells due to genetic and epigenetic changes in tumor suppressor genes, oncogenes and DNA repair genes (Pelekanou and Leclercq, 2011). Molecular biology studies (Perou et al., 2000; Sørlie et al., 2001) have identified four subtypes of breast carcinomas: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-overexpressing and basal-like. These subgroups were correlated with immunohistochemical phenotype. Luminal A is ER-positive and/or PR-positive/HER2-negative. Luminal B is ER-positive and/or PR-positive/HER2-positive. HER2-overexpressed molecular subtype is ER-negative/PR-negative/HER2-positive. Basal-like subtype does not express ER, PR or HER2.

ER and PR are members of the nuclear receptor superfamily that act as nuclear transcription factors modulated by ligands. Estrogen binding at the receptor domain signals growth factor receptor pathways, activating this receptor. Once activated, their dimers recruit coactivators and mediate gene transcription by binding estrogen response elements to target genes (Hewitt and Korach, 2002). ER/PR-positive breast carcinomas have a better prognosis than tumors that do not express these markers with a 5–10% increase in disease-free survival rate (DFS) in five years (Grann et al., 2005). These tumors respond to hormone manipulation aimed at preventing breast cancer cells from receiving estrogen endogenous stimulation.

* Corresponding author at: Departamento de Tocoginecologia da Faculdade de Ciências Médicas, Caixa Postal 61, Universidade Estadual de Campinas – UNICAMP, CEP 13083-970, Campinas, SP, Brazil.
E-mail addresses: susana_ramalho@hotmail.com (S. Ramalho), derchain@fcm.unicamp.br (S.F.M. Derchain).

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Overexpression of HER2 is due to HER2 gene amplification and is present in approximately 20% of breast carcinomas. This condition is associated with a more aggressive tumor phenotype frequently exhibiting high levels of tumor proliferation markers. HER2 overexpression is also associated with a high risk of recurrence and death in the absence of adjuvant systemic therapy (Slamon et al., 1987; Dowsett et al., 2000).

Determination of ER/PR positivity and human epidermal growth factor receptor 2 (HER2) oncogene amplification are currently used as prognostic and predictive factors for response to systemic treatment (Goldhirsch et al., 2009). Breast carcinomas that do not express ER and PR are more undifferentiated, exhibit a more aggressive behavior and a worse prognosis (Gruvberger et al., 2001; Grann et al., 2005; Hu et al., 2006). These tumors are termed triple negative (TN) when they fail to express HER2 and double negative with HER2-overexpression when this receptor is positive (Hu et al., 2006). No clear consensus exists about the prognostic effect of HER2 expression on these patients (Harris et al., 2007; Brown et al., 2008). Therefore, the aim of this study was to compare the relationship between clinical and pathological factors and survival in patients with double negative HER2-overexpressing carcinoma and triple negative breast carcinoma.

Materials and methods

Patient selection

Patients selected for the study had been diagnosed with invasive non-metastatic breast carcinoma and treated from April 2004 to October 2008 in the Prof. Dr. Jose Aristodemo Pinotti Women's Hospital (Integrated Healthcare Center of the Universidade Estadual de Campinas), São Paulo, Brazil, with follow-up until October 2010. Paraffin-embedded blocks were identified and clinical–pathological and follow-up data were obtained after reviewing patient medical records. ER and PR expression was evaluated by immunohistochemistry (IHC) in tissue microarray (TMA). IHC analysis of HER2 expression was performed in all cases. Fluorescent in situ Hybridization (FISH) was used in patients with HER2 2+ IHC results (equivocal). One hundred and sixty-one (161) patients were included in the study. Fifteen cases with paraffin-embedded blocks considered inadequate for analysis were excluded from the study. The following variables were assessed: age at diagnosis, tumor stage at diagnosis (Sobin et al., 2009), nuclear grade, histologic grade and presence of vascular invasion. Histologic type was classified according to criteria of the World Health Organization (Tavassoli and Devilee, 2003). The site of first metastasis was also evaluated. Adjuvant chemotherapy given after surgery and neoadjuvant chemotherapy given before surgery were analyzed according to drugs used. All patients who received neoadjuvant chemotherapy used anthracyclines (doxorubicin or epirubicin) combined or not with taxanes (paclitaxel or docetaxel). Adjuvant chemotherapy was performed with anthracycline (doxorubicin or epirubicin) or a combination of cyclophosphamide, methotrexate and 5-fluorouracil. The benefit of chemotherapy was the same whether it is administered before or after surgery, as previously reported in a study by Rastogi et al. (2008). Therefore, patients receiving adjuvant and neoadjuvant chemotherapy were assigned together in the same group. For statistical analysis, the patients were divided into groups receiving or not receiving anthracycline, irrespective of whether the drug was used as an adjuvant or neoadjuvant agent. This study was approved by the Research Ethics Committee under number CEP 009/2010.

Specimens

Slides stained with hematoxylin and eosin (H&E) from the original paraffin blocks were analyzed for the selection of representative tumor regions. A tissue microarray (TMA, Beecher Instruments Microarray Technology, Silver Spring, CA, USA) was built and sections from the TMA were placed on electrostatic charged slides for immunohistochemical and FISH procedures.

Assay methods

Immunohistochemistry (IHC)

Sections were deparaffinized with xylene and dehydrated in an alcohol series. Washes in hydrogen peroxide were performed, followed by distilled water washes. For antigen retrieval, we used a commercially available pressure cooker (T-fal®), in which slides were immersed in citrate buffer pH 6.0 for 30 min. The slides were dried at room temperature and washed in distilled water. After that, the sections were incubated in a moist chamber, with the specific primary antibodies at 4 °C, overnight (HER2 dilution 1/800: clone c-erbB2 Oncoprotein, Dako; ER dilution 1/800: clone 1D5, Dako; PR dilution 1/1000: clone PgR 636, Dako, Glostrup, Denmark). The slides were then washed in PBS, pH 7.4. As detection system, the slides were incubated in ADVANCE™ HRP Detection System (Dako, Glostrup, Denmark) at 37 °C for 1 h, and washed in PBS. After, DAB chromogenic substrate (3′-diaminobenzidine, Sigma–Aldrich, St. Louis, MO, USA) was applied at a proportion 0.06–100 mL of PBS, 500 μL hydrogen 3% peroxide and 1 mL dimethylsulfoxide (DMSO) at 37 °C for 5 min. Finally, the slides were washed in tap water and counterstained with Harris' hematoxylin for 30–60 s. After being dehydrated, the slide was mounted in Entellan® (Merck, Darmstadt, Germany). Internal/external, positive/negative controls were used for validation of the reactions.

Fluorescent in situ hybridization (FISH)

After deparaffinization, the slides were incubated at 56 °C and dehydrated in an alcohol series. The slides were washed in alcohol and incubated in 2× SSC at 75 °C for 20 min. Proteinase K (0.25 mg/mL) was used for digestion at 45 °C for 20 min. The slides were washed in tap water and dehydrated in an alcohol series. The HER2/neu (VYSIS 36-161060) probe and slides were denatured at 75 °C and at 80 °C, respectively, for 5 min. Dehydration was performed. The probe was applied to the slides, which were sealed with rubber cement and placed in an oven at 37 °C overnight. Post-hybridization washes were performed in 1.5 M urea/1× SSC for 30 min and 2× SSC for 5 min. After dehydration, the slides were counterstained with DAPI and visualized under fluorescence microscopy.

Image analysis

IHC staining was assessed by a single observer who was blinded to clinical characteristics and tumor histology. For ER/PR analysis, nuclear staining was considered, using the criteria of staining intensity and percentage of stained cells (Hammond et al., 2010). Only patients who scored 0–2 were included in this study (Fig. 1). For IHC analysis of HER2, membrane staining was considered and reactivity scores were classified as 0 = negative without staining of invasive tumor cells; 1 = weak and incomplete staining of the membrane in any proportion of invasive tumor cells or weak and complete staining in less than 10% of these cells; 2 = complete membrane staining that is not uniform or weak staining, but with obvious circumferential distribution, in at least 10% of cells or intense and complete membrane staining in 30% or less of invasive tumor cells and 3 = uniform and intense membrane staining in more than 30% of invasive tumor cells (Middleton et al., 2009). HER2 IHC scoring