The Breast 22 (2013) 1066–1071

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Therapeutic strategies in male breast cancer: Clinical implications of chromosome 17 gene alterations and molecular subtypes

Hans-Ulrich Schildhaus [a, 1], Lars Schroeder [b, 1], Sabine Merkelbach-Bruse [a], Elke Binot [a], Reinhard Büttner [a], Walther Kuhn [b], Christian Rudlowski [b, *]

[a] Institute of Pathology, University Hospital Cologne, Cologne, Germany
[b] Department of Gynecology and Obstetrics, University Hospital Bonn, Sigmund-Freud-Str. 25, 53125 Bonn, Germany

ARTICLE INFO

Article history:
Received 20 January 2013
Received in revised form
4 August 2013
Accepted 30 August 2013

Keywords:
Topoisomerase II-α
Male breast cancer
Prognosis
Subtypes

ABSTRACT

Male breast cancer (MBC) is a rare disease. To date, therapy is mainly based on studies and clinical experiences with breast cancer in women. Only little is known about molecular typing of MBC, particularly with regard to potential biological predictors for adjuvant therapy. In female breast cancer tumors with chromosome 17 centromere (CEP17) duplication, HER2 and/or Topoisomerase II alpha (Topo II-α) gene alterations have been suggested to be associated with poor prognosis and increased sensitivity to anthracycline-containing regimens.

In a well characterized cohort of 96 primary invasive MBC, we studied CEP17, HER2 and Topo II-α alterations by fluorescence in-situ hybridization (FISH), and expression of hormone receptors (HR), HER2 and Ki67 by immunohistochemistry to define molecular subtypes. Tumor characteristics and follow-up data were available and correlated with molecular findings.

HER2 amplification and Topo II-α amplification/deletion were exceptionally rare in MBC (6.3% and 3.1%, respectively). CEP17 polysomy were found in 9.4% of tumors. HER2, Topo II-α and CEP17 gene alterations were not correlated to patients outcome. 96.9% of our cases were HR positive. Triple negative tumors were found in only 3.1% of the cases. In nodal negative tumors luminal A subtypes were significantly associated with better overall survival.

Our results provide evidence for a predominant male breast cancer phenotype, characterized by HR expression and a lack of HER2/Topo II-α alterations and CEP17 duplicates. Therefore, the impact of anthracycline sensitivity linked to HER2/Topo II-α alterations as found in female breast cancer has low clinical significance for this specific male breast cancer phenotype.

Introduction

Male breast cancer (MBC) is an uncommon disease with a raising incidence, accounting for approximately 1% of all breast cancer cases [1]. Its phenotypic alterations are not well studied, and therapy is mainly based on experiences with female breast cancer. Although both diseases share similarities [2], there are notable differences in risk factors, prognosis, and survival [3]. The relatively unfavorable outcome in male breast cancer has been attributed to more advanced local tumor stage and high incidence of lymph node invasion at the time of diagnosis [4]. Data on molecular markers and their prognostic and predictive significance are limited compared to female breast cancer patients [5].

In female breast cancer, gene expression profile studies identified molecular subtypes that are associated with clinical outcome [6–8]. Recently, molecular subtyping in MBC revealed substantial differences compared to females suggesting MBC to be quite different disease [9,10]. However, as gene expression analysis by microarray is not routinely feasible, immunohistochemical surrogates have been used for breast cancer classification [11]. Investigation of such markers may provide insights into the biologic differences between male and female breast cancer patients and could improve the clinical management.

To date, tailored therapy of MBC is suggested for two modalities: anti-estrogen therapy with tamoxifen for hormone receptor positive tumors, and trastuzumab for HER2 positive tumors [12]. However, HER2 positivity in MBC seems to be a rare event, as studies have

Abbreviations: MBC, male breast cancer; Topo II-α, topoisomerase II-α; HR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor; CEP 17, chromosome 17 centromere; n.s., not significant.

* Corresponding author. Tel.: +49 228 287 15447; fax: +49 228 287 16091.
E-mail address: rudlowski@t-online.de (C. Rudlowski).

[1] Both authors contributed equally and should be considered as first authors.

0960-9776/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.breast.2013.08.008

reported 3–11% of the cases to be HER2 amplified [10,13–15] compared to approximately 15–20% gene amplifications in female breast cancer [14,16,17]. Given the established benefit of chemotherapy in women and the suggested effect in men, most clinicians use similar guidelines for adjuvant chemotherapy in male and female patients [18]. To date, a significant number of male breast cancer patients receive chemotherapy, e.g. anthracycline-based protocols, in accordance to the current guidelines for female breast cancer [19]. This is due to the fact that male breast cancer is diagnosed in more advanced tumor stages often related to unfavorable tumor characteristics. However, anthracyclines are not devoid of acute and potentially fatal long-term side effects [20] and currently no data are available demonstrating survival benefit for these regimens in male breast cancer [21].

A number of recent studies suggests a potential correlation between the HER2 amplification, Topo II-α gene alterations and chromosome 17 centromere (CEP17) duplication and the efficacy of adjuvant anthracyclines in female patients with breast cancer [22–25]. HER2 and Topo II-α genes are located close to each other near the centromeric region of chromosome 17. Because Topo II-α is a target of anthracyclines this gene might be the link between HER2-positive disease and anthracycline responsiveness [26]. Both genes are often co-amplified and may be associated with the underlying mechanism of anthracycline sensitivity which is still unknown to date [27,28]. Data from the literature suggests that patients whose tumors do not exhibit Topo II-α gene alterations or lack amplification of *HER2* could potentially be treated with a less toxic regimen, whereas patients whose tumors Topo II-α show alterations or *HER2* amplification should receive dose-intensive anthracycline-containing regimens [29].

The aim of this study was to analyze potential therapeutic targets in male breast cancer including hormone (estrogen and progesterone) receptors, Ki67 as well as CEP17, HER2 and Topo II-α aberrations.

Patients and methods

Breast cancer specimens and tissue microarrays

Two tissue microarrays (TMA) were constructed with replicate 1.2 mm cores of 96 invasive male breast carcinomas. They were collected consecutively for this retrospective study by the following criteria: (a) treatment with primary surgical excision and histopathological examination within a period from the year 1998–2008 in two German medical centers (University Hospitals of Bonn and Marburg, Germany) and (b) histologically confirmed primary invasive male breast cancer. With the approval of the appropriate institutional review boards, paraffin-embedded tissue samples were obtained from the hospital archives. Covariables including tumor stage, differentiation, histological subtype and nodal stage were available. Tumor grading was carried out according to the criteria recommended by the WHO. Data regarding adjuvant therapy were extracted from patients' charts. The study was approved by the Ethic Committee of the University of Bonn.

Fluorescence in situ hybridisation (FISH) analysis

4 μm thick sections of the tissue microarrays were hybridized by using the PathVysion™ and LSI TOP2A/CEP 17 DNA probe kits (Abbott, Wiesbaden, Germany) in two different hybridisations, as recommended by the manufacturer. Hybridisation signals were visualized with a Leica DM 5500 fluorescence microscope and appropriate filter sets (Leica Inc., Wetzlar, Germany).

Calculation of FISH indices was done as follows: 60 individual, non-overlapping tumor cell nuclei were counted and the numbers of HER2, TOPO IIα and CEP17 were recorded for each nucleus. Ratios were calculated by dividing the total number of gene counts (HER2, TOPO IIα) for the entirety of 60 cells by the total number of CEP 17. Average number of CEP17 copies/tumor cell was calculated by dividing the total number of CEP 17 by 60. Cases were scored as HER2 or Topo II-α amplified if the ratio was 2.0 or higher. A Topo II-α deletion was assumed if the ratio was less than 0.8. CEP17 gain was noticed if the average number of CEP17 copies/tumor cell was ≥3.

Immunohistochemistry (IHC)

The estrogen, progesterone and HER2 receptor status of the paraffin-embedded tumor tissue was determined by IHC and was performed as previously described [14]. Estrogen (ER) and progesterone (PR) receptor stainings were evaluated semi-quantitatively using the All red score, which is defined by the sum of proportion score (proportion of immunopositive tumor cells) and intensity score (intensity of the staining) [30]. Cases with a score >2 were considered hormone receptor positive (ER+/PR+). HER2 protein expression was determined with the HercepTest™ (DAKO Corp., Hamburg, Germany) according to the FDA approved protocol as described by the manufactures. HER2 membrane staining intensity and pattern were scored according to the FDA approved criteria: 3+ immunostaining was considered positive (over-expressed), and 0/1+ immunostaining was considered to be negative. 2+ staining was considered as equivocal. For further statistical analyses, HER2 positivity was defined by the gene amplification status (FISH result).

Immunohistochemistry for Ki67 was performed according to a previously described method [31] with mouse monoclonal antibody (MIB-1 (1:200) provided by DAKO) by using the Envision kit (DAKO). The percentages of Ki67 immunopositive tumor cells were scored semi-quantitatively by two of the authors (C.R. & H.U.S.) on a multi-headed microscope. Ki67 protein expression were classified (high vs. low expression) for statistical considerations on the basis of the median percentage of stained tumor cells. Therefore, high Ki67 expression was defined by >30% positive tumor cells.

The immunohistochemical stainings were used to classify the breast cancer cases into four different subtypes: luminal type A (ER+ and/or PR+, and HER2− and/or Ki67low), luminal B (ER+ and/or PR+, and HER2+ and/or Ki67high), HER2 driven (HER2+ and ER−/PR−) and triple negative (ER−/PR− and HER2−) [11].

Statistical analysis

The SPSS-software for windows (version 17.0) was used for all calculations. Correlations between categorical variables were performed using the chi-square test and Fisher's exact test. Overall survival (OS) was expressed as the number of months from diagnosis to patients' death. Cumulative survival probabilities were calculated using the Kaplan–Meier method. Differences between survival rates were tested with the log-rank test and Cox-regression analyses. All tests were two-tailed, with a confidence interval of 95%.

Results

Clinical, pathological and tumor biological characteristics of the patients are listed in Table 1. The median age at diagnosis was 68 years (mean 67.4 years). It is notable that only 25.0% of the tumors were staged as pT1 carcinomas and 34.4% of the patients showed tumor infiltration in the surrounding tissue (pT4) at the time of diagnosis. 38.6% of the tumors were poorly differentiated and 38.5% of the patients had axillar lymph node metastasis. Due to the retrospective character of the study data regarding adjuvant