Annals of Diagnostic Pathology 34 (2018) 170–174

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Expression of p27 and c-Myc by immunohistochemistry in breast ductal cancers in African American women

Farhan Khan[a,*], Luisel J. Ricks-Santi[c], Rabia Zafar[a], Yasmine Kanaan[b], Tammey Naab[a]

[a] Department of Pathology, Howard University College of Medicine, Washington, DC, United States
[b] Department of Microbiology, Howard University College of Medicine, Washington, DC, United States
[c] Department of Biological Sciences, Hampton University, Hampton, VA, United States

ARTICLE INFO

Keywords:
p27
c-Myc
Cyclin and cyclin dependent kinase
Axin I tumor suppressor gene
Triple negative breast cancer
African American

ABSTRACT

Objectives: Proteins p27 and c-Myc are both key players in the cell cycle. While p27, a tumor suppressor, inhibits progression from G1 to S phase, c-Myc, a proto-oncogene, plays a key role in cell cycle regulation and apoptosis. The objective of our study was to determine the association between expression of c-Myc and the loss of p27 by immunohistochemistry (IHC) in the four major subtypes of breast cancer (BC) (Luminal A, Luminal B, HER2, and Triple Negative) and with other clinicopathological factors in a population of 202 African-American (AA) women.

Materials and methods: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary ductal breast carcinomas in 202 AA women. Five micrometer sections were stained with a mouse monoclonal antibody against p27 and a rabbit monoclonal antibody against c-Myc. The sections were evaluated for intensity of nuclear reactivity (1–3) and percentage of reactive cells; an H-score was derived from the product of these measurements.

Results: Loss of p27 expression and c-Myc overexpression showed statistical significance with ER negative ($p < 0.0001$), PR negative ($p < 0.0001$), triple negative (TN) ($p < 0.0001$), grade 3 ($p = 0.038$), and overall survival ($p = 0.047$). There was no statistical significant association between c-Myc expression/p27 loss and luminal A/B and Her2 overexpressing subtypes.

Conclusion: In our study, a statistically significant association between c-Myc expression and PTEN loss and the triple negative breast cancers (TNBC) was found in AA women. A recent study found that constitutive c-Myc expression is associated with inactivation of the axin 1 tumor suppressor gene. p27 inhibits cyclin dependent kinase2/cyclin A/E complex formation. Axin 1 and CDK inhibitors may represent possible therapeutic targets for TNBC.

1. Introduction

Breast cancer is the most common cause of cancer morbidity and the second most common cause of cancer mortality in women worldwide. Histologically, breast neoplasia is divided into two major types, ductal and lobular. Molecular classification of ductal breast cancer by gene expression profiling has identified five major subgroups (Luminal A, Luminal B, Her-2, Normal breast like and basal phenotype) that differ in clinical behavior [1-3]. Luminals A and B, are estrogen and/or progesterone receptors(ER/PR) hormone receptor positive. They are generally low grade cancers with good prognosis, increased overall survival and can be treated with hormone receptor inhibitors [1,2]. Her2 overexpressing tumors are aggressive, and carry poor prognosis [1,2]. The treatment of these tumors with trastuzumab (HER2 inhibitor) has significantly improved prognosis. The triple negative breast cancers (TNBC), tumors lacking expression of ER, PR and HER2 receptors, are generally high grade ductal cancers with established aggressive clinical course, high proliferative index, decreased overall survival and increased incidence of distant metastasis; they are often resistant to currently available chemotherapy and carry poor prognosis [1,2]. The basaI-like TNBC subtype expresses CK5. However, all the TNBC are not basal type and vice versa.

Recent studies have shown that cell cycle dysregulation plays an important role in the pathogenesis of TNBC [17,18]. Still, the significance of c-Myc expression, p27 loss and cell cycle dysregulation in breast carcinogenesis is poorly understood. The high proliferative activity of TNBC supports the upregulation of cell cycle driver genes and the downregulation of cell cycle inhibitors as potential pathogenetic

* Corresponding author at: Department of Pathology, Howard University College of Medicine, 2041 Georgia Avenue, NW, Washington, DC, 20060, United States.
E-mail address: farhan.khan@wustl.edu (F. Khan).

https://doi.org/10.1016/j.anndiagpath.2018.03.013

mechanisms.

In particular, c-Myc is a proto-oncogene, located on chromosome 8, that regulates the expression of many target genes involved in cell growth, cell cycle regulation, and apoptosis [14,32]. Constitutive expression of c-Myc can result in uncontrolled cell proliferation. C-Myc activation promotes formation of cyclin A/E and cyclin dependent kinase 2 complex (CDK2 and cyclinA/E), which are critical for progression from the G1 to the S phase of the cell cycle. It also downregulates p21; this inhibits progression from the G2 to the M phase [17]. A recent study has found that c-Myc stabilization by selective phosphorylation results in c-Myc with enhanced oncogenic activity due to inactivation of the axin 1 tumor suppressor gene, an important regulator of survival, growth, and stress pathways [40,41]. Protein p27 (cyclin-dependent kinase inhibitor 1B) is a tumor suppressor protein, encoded by the CDKN1B gene. It inhibits formation of CDK2/cyclin A/E complex and prevents progression of the cell cycle from the G1 phase to the S phase [9].

Notably, Breast cancers in African American (AA) women present at a younger age, have a higher grade and stage at diagnosis, and are associated with a higher mortality [38,39]. We hypothesize that the inverse expression of c-myc with p27 could be associated with more adverse and aggressive clinical phenotypes. However, no study has specifically investigated the combined expression of c-Myc and p27 in breast cancer in AA women, who manifest an increased incidence of TNBCs. For this study, we compared the immunohistochemical expression of c-Myc and p27 in the four major subtypes of breast cancer (BC) (Luminal A, Luminal B, HER2, and Triple Negative), and determined that association with other clinicopathological features including grade, stage, disease-free, and overall survival in a population of 202 AA women.

2. Materials and methods

2.1. Tissue samples

This study was reviewed and formally exempted by the Howard University Institutional Review Board (IRB-10-MED-24). We analyzed invasive breast ductal carcinomas (IDCs) from 202 AA women diagnosed and treated at the Howard University Hospital between 2000 and 2010. Demographic and clinical information was obtained through the Howard University Cancer Center Tumor Registry.

2.2. Tissue microarrays

A series of tissue microarrays (TMAs) was constructed containing the consecutive primary IDCs (Pantomics, Inc., Richmond, CA). The TMAs consisted of 10 × 16 arrays of 1.0-mm tissue cores from well preserved, morphologically representative tumor cells in archived, formalin-fixed, paraffin-embedded (FFPE) surgical blocks. A precision tissue arrayer (Beecher Instruments, Silver Spring, MD) with two separate core needles for punching the donor and recipient blocks was used. The device also had a micrometer-precise coordinate system for tissue assembly on a multitissue block. Two separate tissue cores of IDC represented each surgical case in the TMA. Each tissue core was assigned a unique TMA location number, which was subsequently linked to an Institutional Review Board-approved database containing demographic and clinical data. Using a microtome, 5-μm sections were cut from the TMA blocks and mounted onto Superfrost Plus microscope slides.

2.3. Immunohistochemistry

Immunohistochemistry (IHC) was performed on TMA sections of FFPE tumor tissue. The polymer-HRP system was utilized for immunostaining. Following deparaffinization and rehydration of the tissue sections, heat-induced epitope retrieval at pH 9.0 was performed. Five micrometer sections were stained with mouse monoclonal antibodies against p27Kip1 (SX53G8, Cell Marque, Rocklin, CA). Additional five micrometer sections were stained with a rabbit monoclonal antibody against c-Myc (EP121, Cell Marque, Rocklin, CA). Primary antibody detection was carried out using a polymer-based detection system with staining development achieved by incubation with 3,3′-diaminobenzadine (DAB) and DAB Enhancer (Envision Plus, DAKO, Carpinteria, CA). IHC staining was performed at Quest Diagnostics (Chantilly, VA).

Immunohistochemical stains were scored by two independent observers (TN and FK) blinded to the clinical outcome. The sections were evaluated for intensity of nuclear reactivity (1–3) and percentage of reactive cells. The results were entered into a secure research database. An H-score was derived from the product of these measurements. Cases were categorized as having decreased (score ≤ 50) or increased (score > 50) nuclear expression.

Breast subtypes were defined using immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 based on criteria established in the literature. Luminal A was characterized by strong expression of ER and PR (H-score ≥ 200), Ki-67 proliferation < 14%, and HER2 negativity. Luminal B was characterized by Ki-67 proliferation ≥14% and often weaker expression of ER/PR (H-score < 200). Luminal B cases were HER2-negative or HER2 positive. The HER2 subtype was hormone receptor-negative with only HER2 positivity. The triple-negative subtype lacked expression of ER, PR, and HER2.

2.4. Statistical analysis

To determine the association between c-MYC, p27 and ER, PR, HER2, subtype, grade, stage, age, overall survival, and recurrence, bivariate analysis was performed using the chi square test or Fisher's exact test, as appropriate. To examine the correlation between variables, overall survival, and disease-free survival, Kaplan-Meier survival analyses were carried out. Estimates were considered statistically significant for two-tailed values of $p < 0.05$. All analyses were carried out using the SPSS 22.0 statistical program (SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of the study population

Clinical and pathological characteristics of the study population are summarized in Table 1. There were 202 patients diagnosed with infiltrating ductal carcinomas, with adequate FFPE tumor tissue for analysis, at our institution from 2000 to 2010. The majority of the tumor blocks (67.8%) came from women over the age of 50 (mean = 57.65, SD = 13.03), most of whom (76.2%) had no cancer recurrence. In this population, 43%, 47.7%, and 13.9% of tumors were ER-, PR-, and HER2-positive, respectively. The most prevalent breast cancer subtype was luminal A (ER+ or PR+, HER2−; 43.5%), followed by triple-negative (ER−, PR−, HER2−; 33.7%). Greater than 70% of all tumors were stage I and II; however, they tended to be of higher grade, with Grade 3 tumors comprising 67.4% of the tumors in the study population.

3.2. Immunohistochemistry and analysis

Because some cores were lost in deeper TMA sections, only 197 cases were included in the analysis. The association between clinicopathological features with c-Myc and p27 expression (no expression/co-expression, c-Myc positive/p27 negative and c-Myc negative/p27 positive) can be found in Table 2. Loss of p27 expression and c-Myc overexpression showed statistical significance with ER negative ($p < 0.0001$), PR negative ($p < 0.0001$), triple negative (TN) ($p < 0.0001$) and grade 3 ($p = 0.038$) breast cancers in AA women. There was no statistical significant association between c-myc