Int J Clin Exp Pathol 2010;3(6):600-607
www.ijcep.com /IJCEP1006002

*Original Article*

# p16 INK4a expression in basal-like breast carcinoma

Olga L. Bohn[1,2], Mariana Fuertes-Camilo[2], Leticia Navarro[2], Jesus Saldivar[2], Sergio Sanchez-Sosa[2]

*[1]Departments of Pathology, MetroHealth Medical Center-Case Western Reserve University, Cleveland, OH, USA; [2]Department of Pathology, UPAEP University Hospital-Grupo Christus Muguerza, Puebla, Mexico.*

Received June 7, 2010, accepted June 26, 2010, available online June 30, 2010

**Abstract**: BLBC represents a distinctive group of invasive breast carcinomas with specific genotype and immunoprofile. BLBC is usually defined by gene expression profiling and is currently associated with poor outcome. BLBCs are estrogen receptor (ER) negative, progesterone receptor (PgR) negative, HER2 negative, and usually show a variable expression of basal cytokeratins (CKs), EGFR and CD117. p16 INK4a is a tumor suppressor protein, encoded by the *CDKN2A* gene, which regulates cell cycle. The reported association of abnormalities in the p16/Rb pathway with increased risk of malignancy prompted us to determine the expression of p16INK4a in a group of BLBC; the results were compared with a group of high-grade invasive carcinoma (HG-IC) of breast. Tissue microarrays (TMA) were constructed in triplicate including 18 BLBC and 18 HG-IC. All BLBC cases were ER-/PgR-/HER2-. Seventeen (94%) BLBC were CK 5/6+/CK 14+; 14 (78%) BLCB showed EGFR expression and 13 (72%) were CD117 positive. BLBCs showed a strong positive reaction with p16 INK4a antibody in 16 of 18 (89%) cases. Although the significance of p16 INK4a expression in breast cancer is not fully understood, we have shown that p16INK4a is strongly expressed in breast cancers with basal-like phenotype. Since it is known that p16INK4a is associated with aggressive behavior in human carcinomas, these data suggest that p16INK4a play a role in the poor prognosis of BLBC.

**Keywords**: Breast cancer, basal cancer, immunohistochemistry, HER2, estrogen receptor, progesterone receptor, p16 INK4a

## Introduction

Microarray studies have allowed the identification of molecular breast cancer subtypes, distinguished by differences in their gene expression profile, which usually correlate with prognosis and response to the therapy [1]. Triple-negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) [2]. The basal-like breast carcinoma (BLBC) class is a molecular subtype of breast cancer that usually shows a triple-negative phenotype [3] and constitute about 15% of invasive cancers [4]. No current targeted therapies are available for BLBC. Although BLBC and TNBC are not synonymous, their morphological features overlap and are not enough to provide a distinction between the tumors. BLBCs usually show a variable expression of basal cytokeratins (CKs) (CK5/6, CK14, CK17) and/or overexpression of human epidermal growth factor receptor 1 (HER-1/EGFR) and CD117 (c-Kit) [4-5]. The purpose of identifying BLBCs is to provide insight in terms of prognosis, treatment and response to therapy.

p16/Retinoblastoma (Rb) pathway abnormalities have been widely reported in human neoplasm and associated with increased risk of malignancy [6-10]. Rb protein is a tumor suppressor that arrests cell cycle at G1. p16 INK4a is a tumor suppressor protein, located at chromosome 9p21, encoded by the *CDKN2A* gene, which regulates the transition from the G1 to the S phase of the cell cycle [11]. p16 INK4a protein is a cyclin-dependent kinase (cdk) inhibitor that decelerates the cell cycle, inactivating cdks that phosphorylate Rb. Inactivation of p16 INK4a results in loss of inhibition of Rb phosphorylation, facilitating loss of control of cell cycle arrest [12]. Interestingly, p16INK4a overexpression has been found in high-grade carcinomas of the oropharynx, genital and genitourinary tract, in association with human papilloma virus (HPV). The reported association of abnormalities in the

p16/Rb pathway with increased risk of malignancy prompted us to determine the expression of $p16^{INK4a}$ in a group of BLBC; the results were compared with a group of high-grade invasive carcinoma (HG-IC) of breast. We examined the significance of $p16^{INK4a}$ positivity in carcinomas of the breast, and its utility for identification of BLBC.

## Materials and methods

*Case selection*

Cases were selected based on the availability of paraffin blocks. In total, 18 cases of high-grade invasive carcinoma (HG-IC) and 18 cases of BLBC were retrieved from the files of the Department of Pathology of UPAEP University Hospital, Mexico, after approval from the IRB. The patients were diagnosed at the hospital between 2006 and 2008. All tumor slides were reviewed, and morphological parameters including mitotic activity, necrosis and Nottingham Grade were recorded. Donor blocks were prepared after evaluation of Hematoxylin-eosin (H&E) slides. Tissue microarrays (TMA) were constructed in triplicate for both HG-IC and BLBC by extracting cores of invasive breast carcinoma from the original paraffin blocks and re-embedding the cores into a receptor block. Tissue controls (smooth muscle and liver) were included in the blocks. In addition, unstained whole tissue sections were also prepared for BLBC.

*Immunohistochemistry*

Each set of tissue sections (5 μm) were deparaffinized and rehydrated through a series of graded ethanols. The slides were stained with commercially available antibodies for ER, PgR, HER2, CK5/6, CK14, EGFR, CD117, p53, Ki67 and $p16^{INK4a}$. Immunohistochemistry for HER2 was performed using A0485 antibody (prediluted, DakoCytomation, Carpinteria, CA, USA), according to manufacturer's instructions. HER2 expression was scored according to membranous staining of tumor cells: negative (0/1+), equivocal (2+) and positive (3+). ER (clone SP1, 1:100, NeoMarkers, Fremont, CA, USA) and PgR (clone SP2, 1:150, NeoMarkers, Fremont, CA, USA) antibodies were used. Tumors were considered positive if there are at least 1% positive tumor nuclei. Internal and external controls were used. Tumors were also stained with CK5/6 (clone D5/16B4, 1:100, DakoCytomation, Carpinteria, CA, USA), CK 14 (clone LL002, 1:20, NeoMarkers, Fremont, CA, USA), EGFR (clone H11, prediluted, DakoCytomation, Carpinteria, CA, USA), CD117 (polyclonal, 1:100, DakoCytomation, Carpinteria, CA, USA), Ki67 (Clone MIB-1, 1:150, DakoCytomation, Carpinteria, CA, USA) and p53 (clone Rabbit SP3, 1:100, NeoMarkers, Fremont, CA, USA). CK5/6 and CK14 were scored positive if any cytoplasmic and/or membrane staining was observed and a percentage was given. EGFR was considered positive if any strong membranous staining was seen, and CD117 was scored positive if any cytoplasmic staining was present. $p16^{INK4a}$ antibody was used (1:20, DakoCytomation, Carpinteria, CA, USA); cases were scored based on percentage of positive invasive tumor cells showing nuclear and/or cytoplasmic staining. Positive and negative controls were applied. To detect $p16^{INK4a}$, CK5/6, CK14, ER and PgR, tissue sections were pretreated with Trilogy retrieval buffer in sequence in steam heat; for HER2 and EGFR, Citrate buffer and proteinase K were used, respectively. The sections were stained using DAKO autostainer. The slides were reviewed by two pathologists (OB, SS) and consensus was achieved.

*Statistical analyses*

Statistical analyses were performed using the Fisher exact test. A 2-sided P value of $<0.05$ was considered as significant.

## Results

*Basal like-breast cancer*

The majority of BLBC tumors were high grade as expected, with Nottingham grade 3 in 14 cases (78%) (**Figure 1A**) and grade 2 in the remainder cases as shown in **Table 1**. Necrosis was seen in 10 of 18 cases (55.5%) (**Figure 1B**). p53 expression was seen in 14 of 14 cases (100%), ranging from 5 to 100% (**Figure 1C**), and Ki67 showed a high proliferative index in 13 of 15 cases (86.6%), ranging from 10 to 90% (**Figure 1D**). IHC results are summarized in **Table 2** and **Table 3**. All eighteen (100%) BLCB cases lacked of expression for ER, PgR and HER2. Seventeen (94%) BLBC demonstrated membranous and cytoplasmic staining co-expression for CK 5/6 and CK 14 (94%) (**Figure 2A** and **2B**); fourteen

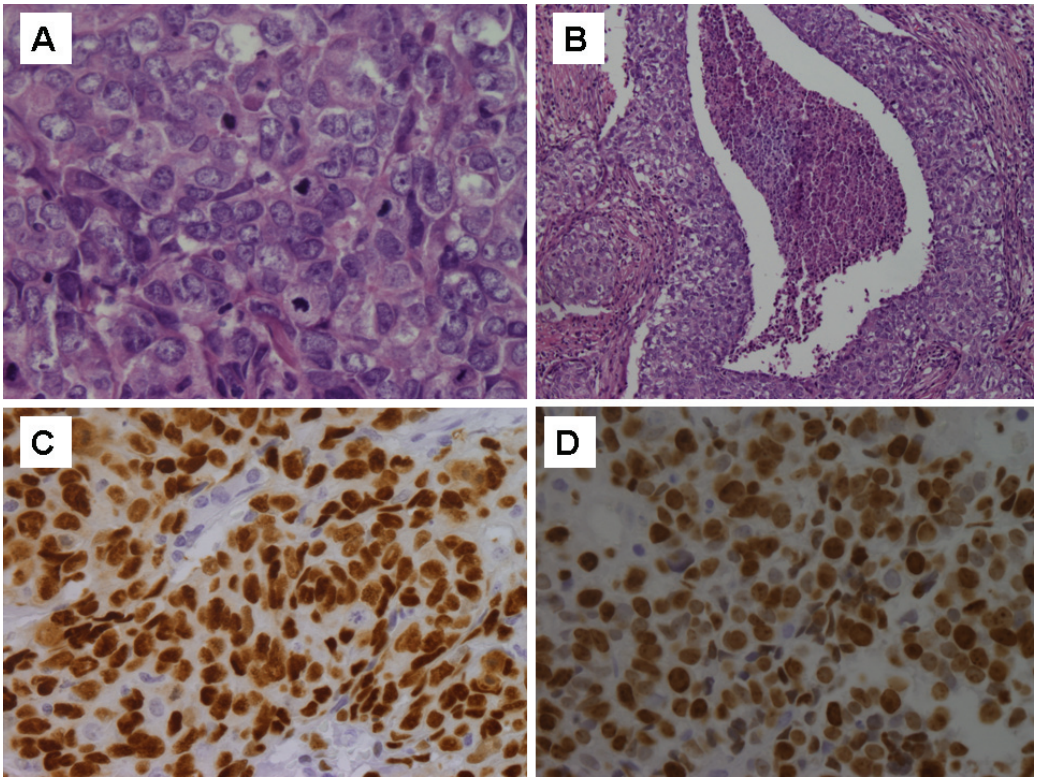


**Figure 1.** Basal-like breast carcinomas demonstrating multiple mitotic figures. Hematoxylin-eosin (A), necrosis is present (B), strong p53 nuclear immunoreactivity (C) and high proliferative Ki67 index (D).

**Table 1.** Morphologic features and Immunohistochemical findings in Basal-Like Breast carcinomas

| Case | Necrosis | MF/10HPF | Nottingham grade | p53 (%) | Ki67 (%) |
|---|---|---|---|---|---|
| 1 | Present | 10 | 2 | 5 | 90 |
| 2 | Present | >20 | 3 | NA | NA |
| 3 | Absent | >20 | 3 | NA | NA |
| 4 | Absent | 14 | 2 | 5 | 80 |
| 5 | Absent | 13 | 3 | NA | NA |
| 6 | Present | 18 | 3 | 80 | 40 |
| 7 | Present | 11 | 3 | 90 | 40 |
| 8 | Absent | 10 | 2 | 5 | 70 |
| 9 | Present | >20 | 3 | 10 | 60 |
| 10 | Absent | 10 | 3 | 40 | 90 |
| 11 | Present | >20 | 3 | 90 | 90 |
| 12 | Absent | 11 | 3 | NA | 10 |
| 13 | Present | >20 | 3 | 100 | 90 |
| 14 | Present | >20 | 3 | 90 | 90 |
| 15 | Present | >20 | 3 | 90 | 90 |
| 16 | Absent | 10 | 3 | 10 | 20 |
| 17 | Absent | 11 | 2 | 95 | 90 |
| 18 | Present | >20 | 3 | 95 | 90 |

MF indicates mitotic figures; HPF, high power field.

**Table 2.** Immunohistochemistry-High grade invasive carcinomas and Basal-Like carcinomas of the breast

| Case | Cancer type | ER (%) | PR (%) | HER2 | p16 (%) | EGFR (%) | CK5/6 (%) | CK14 (%) | CD117 (%) |
|---|---|---|---|---|---|---|---|---|---|
| HG-IC 1 | Metaplastic | 0 | 0 | 1+ | 0 | 0 | 90 | 90 | 0 |
| HG-IC 2 | IDC NOS | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 |
| HG-IC 3 | IDC NOS | 90, s | 80, s | 0 | 0 | 0 | 0 | 0 | 0 |
| HG-IC 4 | IDC NOS | 0 | 0 | 0 | <10 | 0 | 50 | 0 | 0 |
| HG-IC 5 | IDC NOS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HG-IC 6 | Metaplastic | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 |
| HG-IC 7 | IDC NOS | 0 | 0 | 0 | 0 | 10 | 50 | 0 | 0 |
| HG-IC 8 | IDC NOS | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 10 |
| HG-IC 9 | IDC NOS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HG-IC 10 | IDC NOS | 0 | 0 | 1+ | 0 | 0 | <10 | 0 | 0 |
| HG-IC 11 | IDC NOS | 80, s | 80, s | 1+ | 80 | 0 | 0 | 0 | 0 |
| HG-IC 12 | IDC NOS | 0 | 0 | 3+ | 100 | 0 | 0 | 0 | 0 |
| HG-IC 13 | IDC NOS | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| HG-IC 14 | IDC NOS | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 |
| HG-IC 15 | IDC NOS | 0 | 30, m | 3+ | 0 | 0 | 0 | 0 | 0 |
| HG-IC 16 | IDC NOS | 0 | 0 | 0 | 50 | 0 | 0 | 20 | 0 |
| HG-IC 17 | IDC NOS | 0 | 0 | 1+ | 10 | 0 | 0 | 0 | 0 |
| HG-IC 18 | IDC NOS | 0 | 0 | 3+ | 0 | 5 | 10 | 5 | 0 |
| BLCB1 | Basal-like | 0 | 0 | 0 | 0 | 0 | 10 | 5 | 5 |
| BLCB2 | Basal-like | 0 | 0 | 0 | 80 | 10 | 50 | 30 | 0 |
| BLCB3 | Basal-like | 0 | 0 | 0 | 90 | 10 | 10 | 30 | 10 |
| BLCB4 | Basal-like | 0 | 0 | 0 | 85 | 0 | 15 | 10 | 10 |
| BLCB5 | Basal-like | 0 | 0 | 0 | 100 | 5 | 10 | 20 | 0 |
| BLCB6 | Basal-like | 0 | 0 | 0 | 90 | 10 | 60 | 20 | 0 |
| BLCB7 | Basal-like | 0 | 0 | 0 | 0 | 90 | 50 | 40 | 5 |
| BLCB8 | Basal-like | 0 | 0 | 0 | 80 | 10 | 50 | 50 | 10 |
| BLCB9 | Basal-like | 0 | 0 | 0 | 85 | 10 | 40 | 90 | 10 |
| BLCB10 | Basal-like | 0 | 0 | 0 | 100 | 10 | 10 | 10 | 10 |
| BLCB11 | Basal-like | 0 | 0 | 0 | 100 | <10 | 50 | 90 | 10 |
| BLCB12 | Basal-like | 0 | 0 | 0 | 90 | <10 | 40 | 40 | 10 |
| BLCB13 | Basal-like | 0 | 0 | 0 | 90 | 10 | 20 | 10 | 0 |
| BLCB14 | Basal-like | 0 | 0 | 0 | 100 | 10 | 0 | 0 | 10 |
| BLCB15 | Basal-like | 0 | 0 | 0 | 100 | 0 | 30 | 20 | 5 |
| BLCB16 | Basal-like | 0 | 0 | 0 | 90 | 5 | 50 | 50 | 0 |
| BLCB17 | Basal-like | 0 | 0 | 0 | 80 | 0 | 30 | 80 | 5 |
| BLCB18 | Basal-like | 0 | 0 | 0 | 90 | 5 | 50 | 50 | 5 |

HG-IC indicates high grade invasive carcinoma; BLBC, basal-like breast carcinomas; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK, cytokeratin.; s, strong; m, moderate.

(78%) BLBC showed EGFR expression and thirteen (72%) were CD117 positive. Sixteen BLBC showed diffuse nuclear and cytoplasmic expression of $p16^{INK4a}$ (89%), in 80% to 100% of the tumor cells (mean 80.5%) (**Figure 2C** and **2D**). Either basal CKs, EGFR or both were expressed

**Table 3.** Immunohistochemical expression. Basal-like carcinomas and high grade invasive carcinomas

| Biomarker | Result | BLBC | HG-IC |
|---|---|---|---|
| | Positive | 16 (89%) | 10 (55.5%) |
| p16 | Negative | 2 (11%) | 8 (44.5%) |
| | Positive | 17 (94%) | 5 (28%) |
| CK5/6 | Negative | 1 (5.6%) | 13 (72%) |
| | Positive | 17 (94%) | 3 (17%) |
| CK14 | Negative | 1 (5.6%) | 15 (83%) |
| | Positive | 14 (78%) | 2 (11%) |
| EGFR | Negative | 4 (22%) | 16 (89%) |
| | Positive | 13 (72%) | 2 (11%) |
| CD117 | Negative | 5 (28%) | 16 (89%) |
| | Positive | 0 (0%) | 2 (11%) |
| ER | Negative | 18 (100%) | 16 (89%) |
| | Positive | 0 (0%) | 3 (17%) |
| PR | Negative | 18 (100%) | 15 (83%) |
| | Positive | 0 (0%) | 3 (17%) |
| HER2 | Negative | 18 (100%) | 15 (83%) |

HG-IC indicates high grade invasive carcinoma; BLBC, basal-like breast carcinomas; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK, cytokeratin.

in all p16 $^{INK4a}$ cases. In order to exclude the possibility of immunophenotypic heterogeneity that have been previous described with regard to basal CKs, c-Kit and EGFR, we decided to use whole tissue sections in BLBC. The results using whole tissue were consistent to those found in the TMA.

*High-grade invasive carcinoma*

The results are presented in **Table 2** and **Table 3**. Among eighteen invasive carcinomas, two were considered metaplastic-type and the remainder of cases, invasive ductal carcinoma Grade III, NOS type. Thirteen cases (72.2%) were TNBC (ER-/PR-/HER2-), including the two metaplastic carcinomas. Twelve of 18 cases (66.6%) lacked of immunoreactivity for CK5/6 and CK14. Tumors expressing CK 5/6 or CK14 were ER negative. Sixteen of 18 (88.8%) lacked of CD117 expression. p53 expression was seen in 16 of 16 cases (100%), ranging from 15 to 90%, and Ki67 showed a high proliferative index in 16 of 16 cases (100%), ranging from 20 to 90%. Diffuse nuclear and cytoplasmic p16$^{INK4a}$ staining was present in 10 of 18 (55.5%) HG-IC, in 5% to 100% of the tumor cells (mean 26.3%). Only one of the three HER2 positive cases (3+) showed reactivity for p16 $^{INK4a}$. Eight of 10 cases expressing p16 $^{INK4a}$ were negative for EGFR, CK5/6, CK14 and CD117.

BLBC had higher and stronger p16 $^{INK4a}$ expression (89%) compared with the HG-IC (55%). The difference was at borderline statistical significance ($p$=0.05). Similarly, BLBC cases showed higher expression of CK5/6, CK14, EGFR and CD117 than HG-IC, and it was statistically significant ($p$<0.0001).

## Discussion

Gene profiling studies have provided new insights into the classification of invasive breast cancer, prognosis and benefit to therapy [1, 13-15]. To the date, five molecularly distinct breast cancer subtypes have been identified, including Luminal A, Luminal B, HER2 group, normal breast-like and the basal-like group. Currently, BLBC is defined by gene profiling (which is considered the gold standard for identification of basal-like cancers [16] and/or immunohistochemistry [17]. Basal-like cancer is a heterogeneous group that do not cluster with ER or HER2

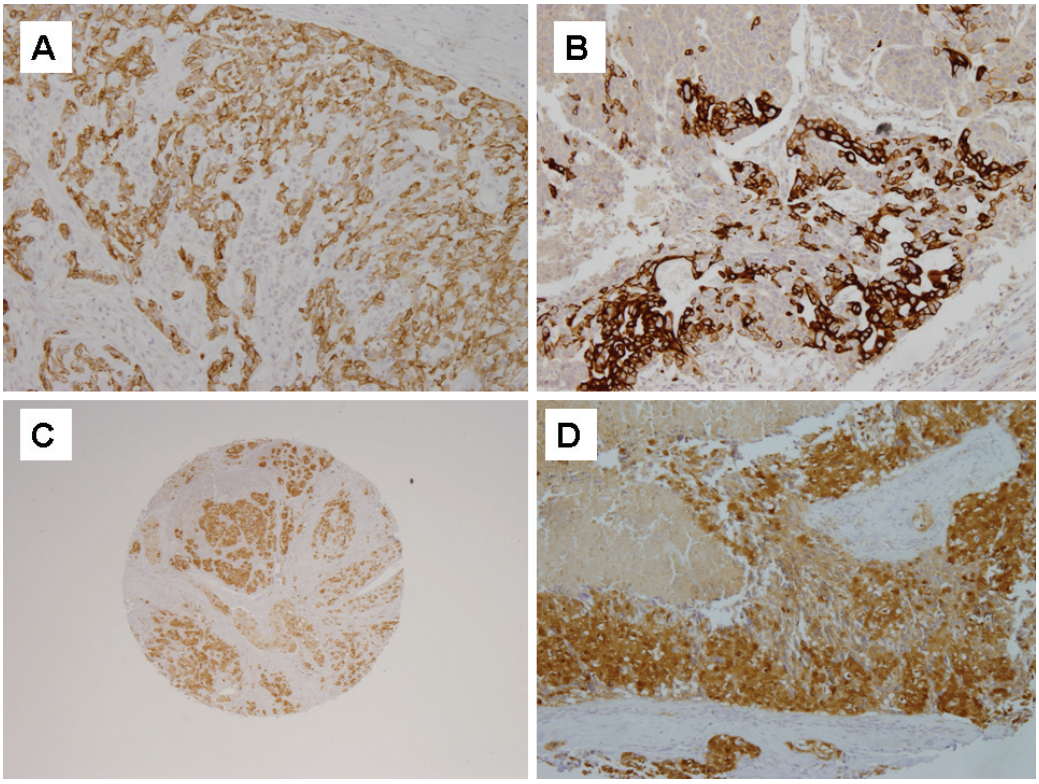


**Figure 2.** Basal-like breast carcinomas demonstrating CK5/6 immunoreactivity (A), CK14 immunoreactivity (B), TMA section showing p16 expression (C) and diffuse cytoplasmic/nuclear p16 expression (D). CK indicates cytokeratin; TMA, tissue microarray.

tumors [1], associated with poor outcome and limited therapeutic tools [18-19]. In a study, Fulford *et al* [20] reported that the specific morphologic features including the presence of a central scar, tumor necrosis and high mitotic count are strongly associated with BLBC. Although these findings are in agreement with our observations (certain histological features may strongly suggest the basal-type), the phenotype is not pathognomonic of BLBC-subtype, and we did not identified specific histological features that distinguish basal-like cancers from high grade invasive carcinomas. Because of the poorly-differentiated morphology associated with BLBC, the use of morphological features alone may misclassify cases as such, affecting the results of many studies in relationship to prognosis and therapy. Interestingly, recent studies have shown that BLBC possess the worse overall survival and disease free survival when compared to the other molecular subtypes [2, 17].

We selected a group of basal CKs usually expressed in BLBC (CK5/6 and CK14), and CD117 and EGFR, because these biomarkers have been used in previous studies as part of the immunohistochemical definition of BLBC. [19, 21] These CKs have been strongly associated with poor prognosis [22-23]. Bryan *et al* [4] have reported immunophenotypic heterogeneity with basal CKs, CD117 (c-Kit) and EGFR. To avoid discrepancies in the results, we assessed whole tissue sections, in addition to the breast cancer TMA slides; the results were consistent, validating the higher expression of CKs in basal cancer subtype when compared to HG-IC. We have also found that the majority of BLBC showed high proliferative Ki67 index and demonstrated p53 overexpression, but the differences between BLBC and HG-IC were not statistically significant.

We evaluated p16 $^{INK4a}$ expression by immunohistochemistry on TMAs. This study has shown that strong p16 $^{INK4a}$ positivity coupled with co-

expression of basal CKs (CK5/6 and CK14) is seen in a high number of BLBC cases. Interestingly, the ratio of p16-positive cases was higher in the BLBC group than in HG-IC group. The importance of p16 $^{INK4a}$ expression is not fully understood in breast cancer. p16 $^{INK4a}$ expression has been studied in human carcinomas. For instance, in a study, Hui *et al* [24] demonstrated an inverse relationship between p16$^{INK4a}$ and ER mRNA levels in cell lines and primary breast cancers, suggesting that p16$^{INK4a}$ inactivation by hypermethylation and overexpression is a marker of poor prognosis. Similary, Milde-Langosch *et al* [25] have described high p16 $^{INK4a}$ reactivity (both nuclear and cytoplasmic) as indicative of a more undifferentiated phenotype in mammary carcinomas. The reciprocal correlation between p16/Rb has been observed in several human HPV-associated malignancies. The functional loss of Rb1 has been found in basal-like tumors, playing a possible key role in their aggressive behavior and metastatic potential [22]. Subhawong *et al* [26] demonstrated that some breast carcinomas lacking of Rb expression and positive immunoreactivity for p16 $^{INK4a}$, showed histological features similar to HPV-related squamous cell carcinomas. However, we did not identify morphologic parameters that suggest HPV infection. Although our study lacks of HPV infection data, several studies have shown the utility of p16 $^{INK4a}$ to distinguish HPV-related lesions in different organs including the cervix and the urinary tract, and further studies regarding this issue are required.

Finally, we propose p16 $^{INK4a}$ as a biomarker for identification of truly basal-like cancers. We are aware that the limitations of this study are due to the number of cases, however, but it raises the possibility that TNBC with basal CKs and p16 $^{INK4a}$ co-expression may adequately identify BLBC, and serve as a potential diagnostic/prognostic biomarker. p16 $^{INK4a}$ overexpression is easily detectable with immunohistochemical stains; therefore is a feasible and simple tool for routine practice.

In summary, although many phenotypic features of BLBC are commonly seen in other high grade breast cancers, BLBC represents a distinctive group of invasive breast carcinomas with specific genotype and immunoprofile. Although the significance of p16 $^{INK4a}$ expression in breast cancer is not fully understood, we have shown that p16$^{INK4a}$ is strongly expressed in breast cancers with basal-like phenotype. Since it is known that p16$^{INK4a}$ is associated with aggressive behavior in human carcinomas, these data suggest that p16$^{INK4a}$ play a role in the poor prognosis of BLBC; therefore, the presence of this abnormality may be used in the development of a targeted therapy in such group.

**Please address correspondence to:** Olga L. Bohn, MD, Department of Pathology, MetroHealth Medical Center-Case Western Reserve University, 2500 MetroHealth Drive, Cleveland, OH 44109, USA. Fax: 216-778-5151, E-mail: olga.bohn@gmail.com

## References

[1] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA *et al*: Molecular portraits of human breast tumours. Nature 2000; 406 (6797):747-752.

[2] Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, Symmans WF, Gonzalez-Angulo AM, Hennessy B, Green M *et al*: Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008; 26(8):1275-1281.

[3] Reis-Filho JS, Tutt AN: Triple negative tumours: a critical review. Histopathology 2008; 52(1):108-118.

[4] Bryan BB, Schnitt SJ, Collins LC: Ductal carcinoma in situ with basal-like phenotype: a possible precursor to invasive basal-like breast cancer. Mod Pathol 2006; 19(5):617-621.

[5] Pintens S, Neven P, Drijkoningen M, Van Belle V, Moerman P, Christiaens MR, Smeets A, Wildiers H, Vanden Bempt I: Triple negative breast cancer: a study from the point of view of basal CK5/6 and HER-1. J Clin Pathol 2009; 62 (7):624-628.

[6] Beasley MB, Lantuejoul S, Abbondanzo S, Chu WS, Hasleton PS, Travis WD, Brambilla E: The P16/cyclin D1/Rb pathway in neuroendocrine tumors of the lung. Hum Pathol 2003; 34(2):136-142.

[7] Brambilla E, Moro D, Gazzeri S, Brambilla C: Alterations of expression of Rb, p16(INK4A) and cyclin D1 in non-small cell lung carcinoma and their clinical significance. J Pathol 1999; 188 (4):351-360.

[8] Brambilla E, Gazzeri S, Moro D, Lantuejoul S, Veyrenc S, Brambilla C: Alterations of Rb pathway (Rb-p16INK4-cyclin D1) in preinvasive bronchial lesions. Clin Cancer Res 1999; 5(2):243-250.

[9] Yin M, Bastacky S, Parwani AV, McHale T, Dhir R: p16ink4 immunoreactivity is a reliable marker for urothelial carcinoma in situ. Hum Pathol 2008; 39(4):527-535.

[10] Santos M, Montagut C, Mellado B, Garcia A, Ramon y Cajal S, Cardesa A, Puig-Tintore LM, Ordi J: Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. Int J Gynecol Pathol 2004; 23(3):206-214.
[11] Ortega S, Malumbres M, Barbacid M: Cyclin D-dependent kinases, INK4 inhibitors and cancer. Biochim Biophys Acta 2002; 1602(1):73-87.
[12] Sherr CJ: Cancer cell cycles. Science 1996; 274 (5293):1672-1677.
[13] Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS *et al*: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98(19):10869-10874.
[14] Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J *et al*: Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 2006; 24 (23):3726-3734.
[15] Lin Y, Lin S, Watson M, Trinkaus KM, Kuo S, Naughton MJ, Weilbaecher K, Fleming TP, Aft RL: A gene expression signature that predicts the therapeutic response of the basal-like breast cancer to neoadjuvant chemotherapy. Breast Cancer Res Treat 2009.
[16] Rakha E, Reis-Filho JS: Basal-like breast carcinoma: from expression profiling to routine practice. Arch Pathol Lab Med 2009; 133(6):860-868.
[17] Fadare O, Tavassoli FA: The phenotypic spectrum of basal-like breast cancers: a critical appraisal. Adv Anat Pathol 2007; 14(5):358-373.
[18] Da Silva L, Clarke C, Lakhani SR: Demystifying basal-like breast carcinomas. J Clin Pathol 2007; 60(12):1328-1332.
[19] Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L *et al*: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10(16):5367-5374.
[20] Fulford LG, Easton DF, Reis-Filho JS, Sofronis A, Gillett CE, Lakhani SR, Hanby A: Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. Histopathology 2006; 49(1):22-34.
[21] van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J, Torhorst J, Sauter G, Zuber M, Kochli OR *et al*: Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol 2002; 161(6):1991-1996.
[22] Herschkowitz JI, He X, Fan C, Perou CM: The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas. Breast Cancer Res 2008; 10(5):R75.
[23] Malzahn K, Mitze M, Thoenes M, Moll R: Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas. Virchows Arch 1998; 433(2):119-129.
[24] Hui R, Macmillan RD, Kenny FS, Musgrove EA, Blamey RW, Nicholson RI, Robertson JF, Sutherland RL: INK4a gene expression and methylation in primary breast cancer: overexpression of p16INK4a messenger RNA is a marker of poor prognosis. Clin Cancer Res 2000; 6(7):2777-2787.
[25] Milde-Langosch K, Bamberger AM, Rieck G, Kelp B, Loning T: Overexpression of the p16 cell cycle inhibitor in breast cancer is associated with a more malignant phenotype. Breast Cancer Res Treat 2001; 67(1):61-70.
[26] Subhawong AP, Subhawong T, Nassar H, Kouprina N, Begum S, Vang R, Westra WH, Argani P: Most basal-like breast carcinomas demonstrate the same Rb-/p16+ immunophenotype as the HPV-related poorly differentiated squamous cell carcinomas which they resemble morphologically. Am J Surg Pathol 2009; 33(2):163-175.