Expression of stem cell marker Bmi1 in invasive breast cancer and correlation with estrogen receptor, progesterone receptor, HER2/*neu*, and ki67

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Background
Worldwide, breast cancer is the most common cancer in women. Currently, Bmi1 has been linked to a stem cell-like 11 gene expression microarray signature, predictive of tumor relapse, metastasis, and resistance to therapy in multiple human cancers.
Aim
The aim of this study was to evaluate immunohistochemical expression of Bmi1 in invasive breast cancer, and its correlation with the clinicopathological features, hormone receptor status [estrogen receptor (ER) and progesterone receptor (PR)], HER2/*neu* score, Ki67 proliferation index, and molecular subtypes.
Patients and methods
Fifty invasive breast carcinomas were studied for immunohistochemical demonstration of Bmi1, ER, PR, HER2/*neu*, and Ki67. Cases were classified into four molecular subtypes (luminal A, luminal B, Her2-enriched, and triple negative).
Results
Bmi1 expression was detected in 37 (74%) breast carcinoma cases, and a significant positive association with tumor size (P=0.03) and lymph node metastasis (P=0.01) was reported in this study. No significant correlation was detected between Bmi1 expression and other variables such as age, histologic type, grade, hormone receptor status, Her2 status, Ki67, and molecular subtypes (P>0.05).
Conclusion
Bmi1 stem cell marker was detected in a high percentage of breast cancer cells, and there was a significant positive association with tumor size and lymph node metastasis, which confirms its role in aggressiveness and dissemination of cancer cells. However, no correlations with ER, PR, Her2, Ki67 expressions, or molecular subtyping were found. Further studies are required to rule out the prognostic value of cancer stem cell marker Bmi1 and its therapeutic targeting.

Keywords:
Bmi1, breast cancer, estrogen receptor and progesterone receptor, HER2/*neu*, Ki67

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Introduction

Worldwide, breast cancer is the most common cancer in women [1]. Despite major progress in surgery, adjuvant chemotherapy, endocrine therapy, and targeted therapy, a percentage of patients with advanced-stage breast carcinoma have poor outcome and early metastasis. It has been reported that 11% of patients with invasive duct carcinoma will develop local relapse in a period of 5 years following surgery, involving 15% with triple-negative carcinomas and 8% with luminal A subtype [2,3].

Increasing evidence supports the role of cancer stem cells (CSCs) in multiple tumor types. There is wide agreement that a distinct subpopulation of cells that is more resistant to chemotherapy and radiotherapy exists in many tumor types and may lead to tumor relapse and metastasis. Targeting these CSCs holds great promise in cancer treatment [4].

B-lymphoma Moloney murine leukemia virus insertion region-1 (Bmi1) is a member of the polycomb proteins that was first identified as an oncogene that cooperates with c-myc in the induction of mouse B-cell lymphoma [5,6].

Currently, Bmi1 has been linked to a stem cell-like 11 gene expression microarray signature, predictive of tumor relapse, metastasis, and resistance to therapy in multiple human cancers, including prostate, lung, ovarian, urinary bladder, lymphoma, mesothelioma, medulloblastoma, glioma, acute myeloid leukemia, and breast cancer [7,8].

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The role of Bmi1 in maintaining self-renewal capacity is attributed to repressing the INK4A locus encoding p16INK4A and p19ARF, which are responsible for arresting growth potential, induction of cellular senescence, and programmed cell death [9–11].

In breast cancer, studies conducted to reveal the role of Bmi1 expression yielded conflicting results as some studies linked it to poor prognostic parameters such as lymph node metastasis and poor survival rates and others to favorable outcome. Moreover, investigations have suggested that it is estrogen α-coupled receptor [7,8,12–14].

In this study, we aimed to reveal the correlations between Bmi1 expression and the different clinicopathologic factors, estrogen receptor (ER), progesterone receptor (PR), Her2, Ki67 proliferation index, and molecular subtypes in invasive breast carcinomas to verify the role in cancer progression and the feasibility of therapeutic targeting.

Patients and methods

Patients and specimens

This retrospective cross-sectional study included 50 cases of invasive breast carcinomas obtained through collection of archived paraffin blocks of surgical resection specimens from the Department of Pathology, Faculty of Medicine, Cairo University, during the period from January 2013 to September 2014. Patients' ages ranged from 29 to 73 years. The patients' medical records were revised and clinicopathologic data were retrieved. Clinicopathological characteristics of these patients are summarized in Table 1. The patients were informed of the purpose of the study and gave their informed consent. The institutional review board of Kasr Al-Ainy School of Medicine approved this study

Immunohistochemical staining

Briefly, 5-μm-thick tissue sections were deparaffinized in xylene and rehydrated in graded alcohol, and subsequently microwave-treated in sodium citrate buffer (pH 6.0) twice. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 15 min, followed by washing with Tris-buffered saline. The sections were then incubated with diluted anti-Bmi1 monoclonal mouse antibody (NBP2-22204; Novus Biologicals, Littleton, USA). Thereafter, the sections were refrigerated at 4°C overnight in a humid closed chamber. The sections were again washed in Tris-buffered saline and incubated with avidin–biotin–peroxidase system (Dako, Dako Corporation, Carpinteria, CA, USA) for 30 min. The diaminobenzidine was used as a chromogen and hematoxylin as a counterstain. Known rectal carcinoma-positive slides were used as a positive control. Commercially available ER (1 : 50; Dako), PR (1 : 10; Dako), HER2/*neu* (1 : 10; Dako), and Ki67

Table 1 Bmi1 expression profiles in relation to patients' clinicopathologic characteristics

	Bmi1 [*N* (%)]					
	Negative	Weak positive	Moderate positivity	Strongly positive	Total	*P*-value
Age (years)						
≤45	3 (6)	3 (6)	9 (18)	2 (4)	17 (34)	0.138
>45	10 (20)	7 (14)	7 (14)	9 (18)	33 (66)	
Grade						
II	12 (24)	8 (16)	14 (28)	8 (16)	42 (84)	0.578
III	1 (2)	2 (4)	2 (4)	3 (6)	8 (16)	
Tumor size (cm)						
<2	4 (8)	0 (0)	2 (4)	0 (0)	6 (12)	**0.032**
2–5	8 (16)	9 (18)	8 (16)	7 (14)	32 (64)	
>5	1 (2)	1 (2)	6 (12)	4 (8)	12 (24)	
Histologic subtype						
Duct	8 (16)	10 (20)	11 (22)	7 (14)	36 (72)	0.440
Lobular	1 (2)	0 (0)	1 (2)	2 (4)	4 (8)	
Mixed	3 (6)	0 (0)	4 (8)	2 (4)	9 (18)	
Papillary	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	
Intraductal carcinoma component (%)						
≥25	3 (6)	1 (2)	0 (0)	1 (2)	5 (10)	0.235
<25	10 (20)	9 (18)	16 (32)	10 (20)	45 (90)	
Lymph node metastasis						
Negative	6 (12)	4 (8)	3 (6)	0 (0)	13 (26)	**0.015**
Positive	7 (14)	6 (12)	13 (26)	11 (22)	37 (74)	

P value less than 0.05 [statistically significant].

(1 : 300, Cat. #RB-9043-P; Lab Vision, Thermo Fisher Scientific, Fremont, USA) were used as primary antibodies and steps of immunostaining were routinely performed as previously described.

Evaluation of the staining

Bmi1 nuclear and cytoplasmic immunostaining were scored semiquantitatively as a percentage of positively stained tumor cells as follows: negative, less than 5% positive tumor cells; mild, 5–25% positive tumor cells; moderate, 25–50% positive tumor cells; and marked, >50% positive tumor cells. For ER and PR, a nuclear staining in 1% of the cells was considered positive [15]. Her2 scores were assessed as follows: 0, no staining or membrane staining in less than 10% of tumor cells; 1+, faint membrane staining in greater than 10% of tumor cells and only a part of membrane is stained; 2+, weak/moderate complete membrane staining in greater than 10% of tumor cells; and 3+, strong complete membrane staining in greater than 10% of tumor cells [16]. Her2 scores 2+ and 3+ were considered positive and Her2 score 0 and 1+ were considered negative. Ki67 proliferation index was scored as low if less than 14% and as high if equal to or more than 14% [17].

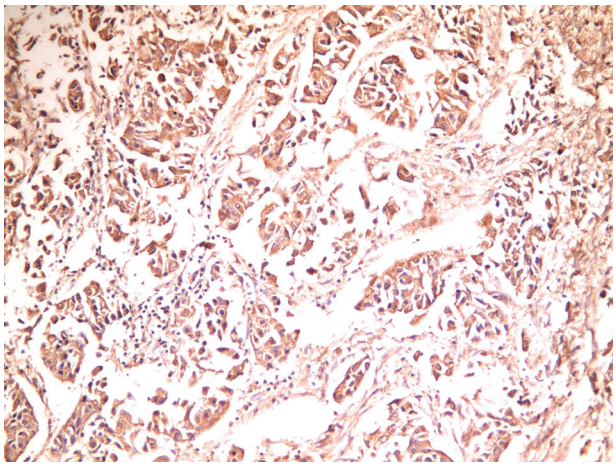
Statistical analysis

SPSS (statistical product for services solutions, version 22.0, IBM corporation, New York, USA) was used and correlations were determined using the χ^2-test. The P value of less than 0.05 was chosen to represent statistical significance.

Results

We conducted this study on 50 invasive breast carcinoma patients between 29 and 73 years, with a mean of 55.22±11.298 years.

Figure 1



Marked Bmi1 overexpression in invasive duct carcinoma (original magnification, Bmi1, ×200).

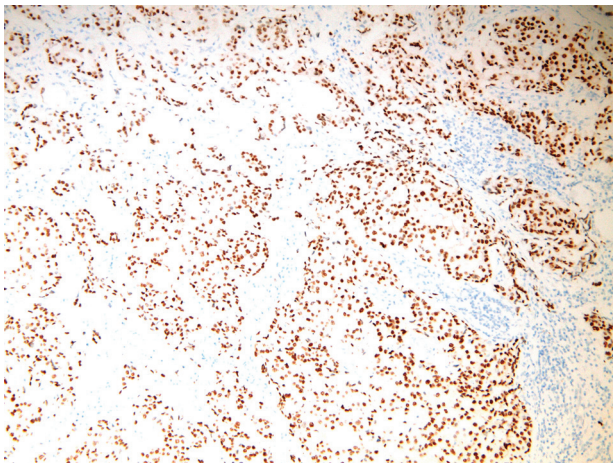
Cytoplasmic and nuclear Bmi1 immunostaining was seen in 37 cases (Fig. 1): 10 (20%) were weakly positive, 16 (32%) were moderately positive, and 11 (22%) were strongly positive; however, 13 (26%) cases were negative.

ERs were expressed in 37 (74%) of 50 cases (Fig. 2), whereas PRs were expressed in 28 (56%) cases (Fig. 3). HER2/*neu* receptors were scored as 0 (33/50; 66%), 1+ (4/50; 8%), 2+ (4/50; 8%), and score 3+ (9/50; 18%) (Fig. 4).

As regards Ki67 expression, high proliferation (≥14%) was seen in 23 (46%) of 50 cases, whereas 27 (54%) cases showed low proliferation (<14%) (Fig. 5).

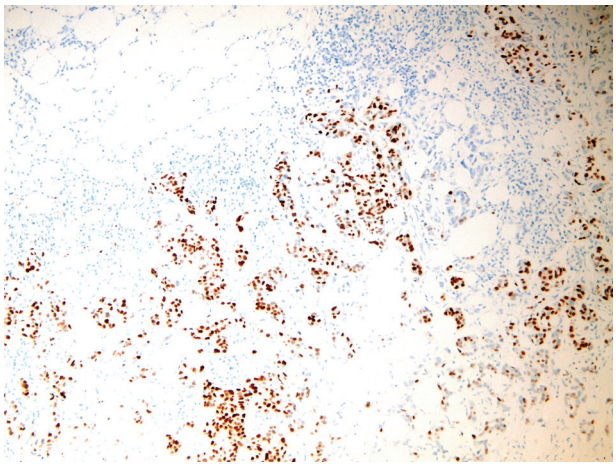
Possible correlations between Bmi1 expression profiles and the patients' clinicopathologic characteristics are

Figure 2



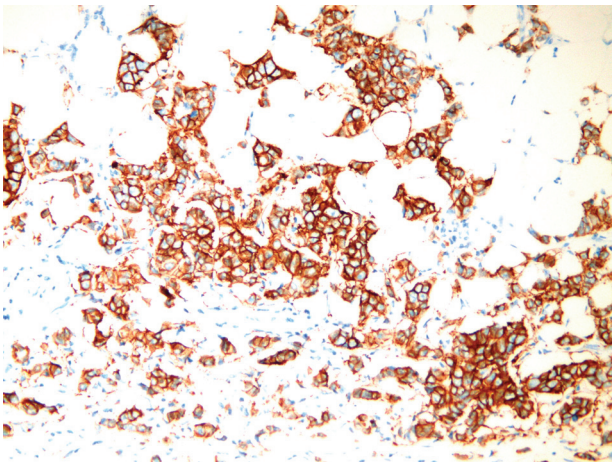
Estrogen receptor (ER)-positive nuclear staining in invasive duct carcinoma (original magnification, ER, ×100).

Figure 3



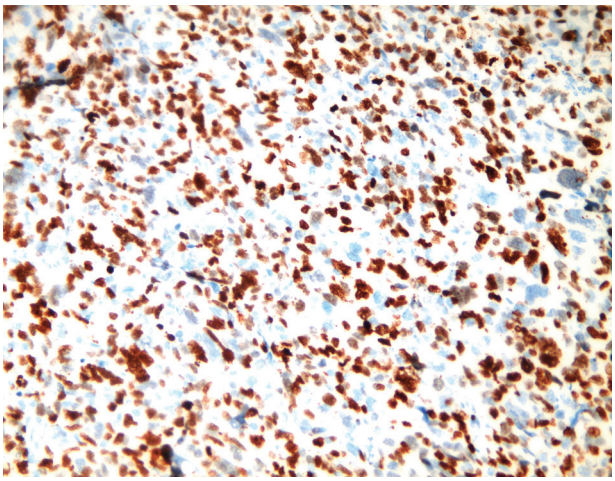
Progesterone receptor (PR)-positive nuclear staining in invasive duct carcinoma (original magnification, PR, ×100).

Figure 4



Strong (3+) membrane immunoreactivity for HER2/*neu* in high-grade invasive duct carcinoma (original magnification, HER2/*neu*, ×200).

Figure 5



Ki67-positive nuclear staining in invasive duct carcinoma with high proliferation index (original magnification, Ki67, ×200).

presented in Table 1. A significant correlation was detected between Bmi1 expression profiles and size of tumor (P=0.032) and between Bmi1 expression in tumor cells and status of nodal metastasis (P=0.015) as Bmi1 was expressed in 74% of patients with positive nodal tumor deposits. These observations suggested a correlation between increased Bmi1 expression and clinical progression in breast cancer. However, no evident correlations were observed between Bmi1 expression profiles and other clinicopathologic features, including age, grade, histopathologic subtype, and intraductal component.

Associations between expression profiles of Bmi1 in relation to other immunohistochemical markers and molecular subtypes of breast cancer cases are displayed in Table 2.

Discussion

For developing novel treatments of breast cancer, it is essential to address the factors underlying tumorigenesis, invasion, and metastasis [12]. In this study, we identified Bmi1 as an important factor in breast cancer progression. We first illustrated the expression of Bmi1 in primary breast cancer tissues,

Table 2 Bmi1 expression profiles in relation to other marker staining and molecular subtypes of breast cancer

	Bmi1 [*N* (%)]					
	Negative	Weak positive	Moderate positivity	Strongly positive	Total	*P*-value
ER status						
Negative	3 (6)	2 (4)	4 (8)	4 (8)	13 (26)	0.833
Positive	10 (20)	8 (16)	12 (24)	7 (14)	37 (74)	
PR status						
Negative	6 (12)	4 (8)	6 (12)	6 (12)	22 (22)	0.835
Positive	7 (14)	6 (12)	10 (20)	5 (10)	28 (56)	
Her2 score						
0	10 (20)	6 (12)	10 (20)	7 (14)	33 (6)	0.960
1+	1 (2)	1 (2)	1 (2)	1 (2)	4 (8)	
2+	1 (2)	1 (2)	2 (4)	0 (0)	4 (8)	
3+	1 (2)	2 (4)	3 (6)	3 (6)	9 (18)	
Ki67 (%)						
≥14	6 (12)	5 (10)	6 (12)	6 (12)	23 (46)	0.837
<14	7 (14)	5 (10)	10 (20)	5 (10)	27 (54)	
Molecular subtype						
Luminal A	7 (14)	5 (10)	10 (20)	5 (10)	27 (54)	0.772
Luminal B	3 (6)	3 (6)	2 (4)	2 (4)	10 (20)	
HER2/*neu*	0 (0)	1 (2)	3 (6)	2 (4)	6 (12)	
Triple negative	3 (6)	1 (2)	1 (2)	2 (4)	7 (14)	

ER, estrogen receptor; PR, progesterone receptor.

followed by demonstrating the association between Bmi1 expression and clinicopathologic parameters and then addressed the role of Bmi1 in breast cancer new molecular classification.

In the current study, we found Bmi1 immunostaining positivity in 37 (74%) of 50 cases. Our results are nearly similar to those of Guo *et al.* [12], who reported that 72.2% of their cases were Bmi1 positive. This incidence of high Bmi1 expression was much higher than previously demonstrated in the Korean study presented by Choi *et al.* [13], who reported that 53.2% of their cases were Bmi1 positive. Such differences may be attributed to genetic and geographic variability, differences in tissue processing and immunohistochemical techniques, different primary antibodies used, and scoring with a different setting of threshold scores among different studies. However, to further confirm Bmi1 expression in breast cancers, multicenter studies are required.

A significant correlation was detected between Bmi1 expression profiles in this study and size of tumor (P=0.032), and between Bmi1 expression in tumor cells and status of nodal metastasis (P=0.015). Bmi1 was expressed in 74% of patients with positive nodal tumor deposits. These observations suggested a correlation between increased Bmi1 expression and clinical progression in breast cancer. Moreover, Guo *et al.* [12] in their analysis found that high Bmi1 expression showed an obvious correlation with larger tumor size, lymph node involvement, organ metastasis, and advanced clinical stage. Kima *et al.* [18] identified the fact that Bmi1 expression was positively correlated with axillary lymph node metastases. Our results supported by previous ones revealed that higher Bmi1 expression was related to more aggressive behavior. Therefore, these findings suggest that Bmi1 may be one of the novel prognostic markers available in invasive breast cancer.

In our study, Bmi1 was not significantly correlated with other immunohistochemical marker expressions, nor the molecular subtyping of breast cancer cases, which is consistent with previous reports indicating that Bmi1 expression had no significant correlation with ER or PR expression [12,19], but it is inconsistent with other previously published data [13,16,20]. These findings indicate that hormonal therapy did not affect the prognostic role of Bmi1. In addition, a possible correlation between Bmi1 expression and outcome after hormonal therapy and chemotherapy needs more investigations requiring a large number of samples.

Conclusion

Bmi1 stem cell marker was detected in a high percentage of breast cancer cells and there were statistically significant relationships as regards association with tumor size and lymph node metastasis, which confirms its role in aggressiveness and dissemination of cancer cells, but no correlations were found with ER, PR, Her2, Ki67 expressions, or molecular subtyping. Further studies are required to rule out the prognostic value of CSC marker Bmi1 and its therapeutic targeting.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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