Original Article

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Loss of Nuclear ARID-1A Expressions Is Associated with Hormone Receptor Status in Breast Cancers

Melek Ünçel, Gülden Diniz, Gamze Aköz, Zübeyde Yıldırım Ekin, Sevil Sayhan, Serdar Yardım, Semra Salimoğlu

Department of Pathology and Surgery, Tepecik Training and Research Hospital, İzmir, Turkey

ABSTRACT

Objective: Breast cancer is the most common cancer among women worldwide. Adenine thymine-rich interactive domain 1A (ARIDIA) is a tumor suppressor gene involved in chromatin remodeling and it encodes the ARIDIA protein. Recent studies have shown the loss of ARIDIA protein expression in different carcinomas may have a prognostic significance. In the present study, we aimed to evaluate the interactions between ARIDIA loss and molecular subtypes of breast carcinomas.

Materials and Methods: ARIDIA expressions were studied in 292 formalin- fixed, paraffin- embedded breast carcinoma specimens and its association with different pathological and clinical parameters was evaluated.

Results: Loss of ARIDIA expression was detected in 123 cases. There was no statistically significant association between ARID-1A expression and molecular subtype of breast carcinomas (p=0.110) or HER2 amplification (p=0.909). Contrarily, there was a significant association between ARIDIA expression and presence of estrogen (p=0.047) or progesterone receptors (p=0.023). Besides a statistically significant relationship was found between loss of ARID1A, and the presence of both in situ component (p=0.016) and lymph node metastasis (p=0.001).

Conclusion: In this study, we have demonstrated that loss of ARID1A expression positively correlates with hormone receptor status as well as tumor aggressiveness.

Keywords: Breast cancer, HER2, ARID1A, molecular subtype

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Introduction

Breast cancer is the most frequently seen cancer type in women, and it is the second most frequent cause of cancer-related deaths (1). Multiple number of factors play important roles in its etiopathogenesis including mainly hormonal factors followed by family history, advanced age, alcohol consumption, obesity, dietary habits, and genetic factors (2). While traditional classification of malignant breast tumors by World Health Organization (WHO) was made based on histological features of the tumor, nowadays some subtypes have been described according to molecular characteristics of the tumors (1, 3). Firstly, in the year 2000, subtypes of breast cancers was described based on the presence of estrogen receptor (ER) in the light of gene expression studies (4). According to this still currently valid classification, ER-positive tumors demonstrate gene expression from luminal cells of breast glands, cytokine profile, and markers associated with other luminal cells. On the contrary in immunohistochemical analyses, some of ER negative tumors demonstrate positivity for human growth factor-2 receptor (CerbB2) and show amplification of human epidermal growth factor receptor-2 gene (HER2). These tumors are known as HER2 positive tumors. HER2 negative non-luminal tumors demonstrate gene expression and immunoreactivity similar to normal basal cells of breast glands. Since these cells generally manifest both ER, and PR negativities, this group is termed as basal-like or triple negative tumor group (5-7). As a result of studies and meta-analyses performed, it has been understood that 75% of breast tumors contain ER and/ or progesterone (PR) receptors, in other words they belong to the luminal group (6). However, since tumors in the luminal group manifest diverse behaviours, this group is divided into luminal A, and B subgroups. Luminal A group which has the highest prevalence among breast cancers consists of HER2 negative tumors with lower proliferative activity, decreased rates of mitosis, and histological grade. Prognosis of the patients with luminal A tumor is very good, and most of the metastases are confined to bones. Luminal B

Corresponding Author :
Gülden Diniz, e-mail: gulden.diniz@idu.edu.tr

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tumors have higher proliferation rates, and they lead a more aggressive course. Nowadays immunohistochemically cut-off value discriminating between luminal A, and luminal B tumors is accepted as nuclear Ki67 expression demonstrated by less than 14% of tumor cells. Besides immunohistochemically nearly 30 % of HER2 positive tumors have luminal B phenotype (7).

Adenine thymine-rich interactive domain 1A (ARIDIA) gene is a non-catalytical unit of SwItch/Sucrose nonfermenting (SWI/SNF) chromatin-remodeling complex, which encodes the BRG1-related factor 250a (BAF250a) (8). ARID1A, which is localized on chromosome 1, plays a role as a tumor suppressor gene (9). It has been shown that mutations of ARID1A gene contribute to carcinogenesis, and cause transformation of cells in association with the PI3K/AKT pathway (8- 12). Besides C- terminal of protein which is encoded by ARID1A stimulates activation of glucocorticoid receptor- dependent transcription factors (10). In different studies it has been associated with prognosis of multiple number of cancer types (8-12). Also, in breast cancers loss of ARID1A expression is associated with poor prognosis (12). Therefore, loss of ARID1A expression which can be detected using immunohistochemical techniques or molecular methods may be a prognostic factor, and it will be used as a target biomarker in the treatment of breast cancer in the future (8-12).

Our objective in this study is to examine the relationship between loss of ARIDIA expression, and HER2 status, and their correlations with clinicopathological parameters in cases of breast carcinomas, and to investigate the potential role of ARIDIA as a target marker in the treatment of breast carcinoma.

Materials and Methods

A total of 292 women, who underwent mastectomy, and excisional breast biopsy between the years 2011 and 2014, and histopathologically diagnosed with breast carcinoma in the Pathology Laboratory of the Tepecik Training and Research Hospital were included in the study. Demographic data, and medical information, including age of the patients, tumor location, diameter, TNM stage of the tumor, and overall survival were retrospectively evaluated. All cases were also investigated as type, and grade of the tumor, lymphovascular, and perineural invasion, and lymph node involvement. The clinical features of the patients are also evaluated (Table 1). This study was approved by the Tepecik Training and Research Hospital Local Ethics Committee (24.11.2015/15/2) and Informed Consents were be provided for each patient.

Hematoxylin-Eosin (H&E) stained archived slides were re-evaluated based on 2012 breast tumor classification of the the World Health Organization. For immunohistochemistry (IHC), hematoxylin - eosin staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. The paraffin block most suitable for immunohistochemical evaluation was selected, and labeled firstly on the slide, and then the block, and 2 mm thick cylindrical paraffined tissue samples were harvested from donor blocks. Then multiple blocks were prepared using mapping, and addressing techniques, then IHC was performed using streptavidin- biotin- peroxidase method (85-9043 CA; Invitrogen). Serial 5-µm sections were obtained and these slides were baked over-night at 60^{0}C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat- induced epitope retrieval in the microwave (in 10mM/L citrate buffer, pH 6.0, for 20 minutes, followed by cooling at room temperature for 20 minutes) and blocked for endogenous peroxidase and biotin. The purified monoclonal mouse antibodies against ARIDIA (HPA005456; Sigma-Aldrich) were used at a dilution of 1: 200.

In the evaluation of immune reactivity for ARIDIA, percentage, and intensity (mild, moderate, strong) of nuclear staining in the tumoral area were evaluated (Figure 1). Accordingly, staining percentage of 60 % was calculated using ROC curve analyses, and below this limit was evaluated as loss of ARIDIA expression.

In the statistical analysis, for the comparison of the quantitative data *chi*-square test was used. In the comparison of parametric data independent groups T test, and for non-parametric data Mann-Whitney U test were used. For the comparison of the measurements in more than two groups non-parametric Kruskal-Wallis test was utilized. $p \leq 0.05$ was accepted as the level of significance.

Results

Median age of the patients at the time of diagnosis was 55.4 years. Mean ages of the patients in different groups were close to each other (p=0.836). Luminal A (n=90/30.8%), luminal B (n=87/29.8%), HER2- positive (n=78/26.7%), and triple negative (n=37/12.7%) subtypes were detected in respective number of patients. Mean follow-up period was 22.5±10.9 months (range, 8-77 months). During follow-up period 7.8%, and 21.6% of the patients exited in luminal A, and triple-negative groups, respectively. Overall mortality rate was 10.6% (n=31). The lowest survival rate was detected among triple negative patients, and only 29 (78.4%) patients survived. The highest survival rate was detected in luminal A group in compliance with the literature findings (n=83, 92.2%). HER2- positive cases had achieved the second highest survival rates thanks to targeted therapies (n=71, 91%). However, a significant correlation was not found between molecular subtypes, and survival rates (p=0.090). The axillary dissection rate var-

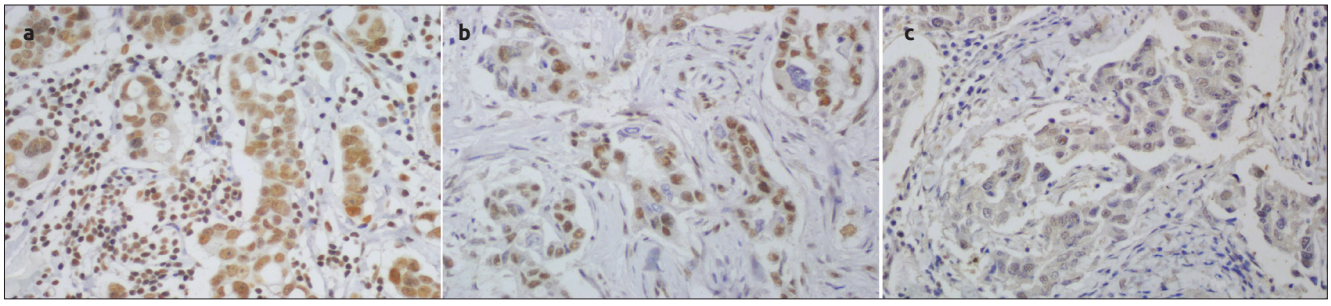

Figure 1. Nuclear ARID1A expression in three different invasive ductal carcinomas: A) Nearly all tumor cell and stromal lymphocytes were expressed strong nuclear ARID1A expressions. B) Moderate loss of nuclear ARIDIA expressions and C) Severe loss of ARIDIA expressions (DABX 200)

Table 1. Features of the cases according to the ARID1A expression status

	ARID1A Normal	ARID1A Absent or Decreased	p
Patients N=292	169	123	-
Mean±SD age (years)	55.45±13.10	55.46±12.73	0.839
Molecular subtype (%)			0.110
Luminal A	51%	49%	
Luminal B	49.6%	50.4%	
HER2 positive	53.4%	46.6%	
Triple negative	46.2%	53.8%	
Estrogen Receptor negativity	20%	30.8%	0.047
Progesterone receptor negativity	27.5%	39.8%	0.027
HER2 positivity	29.8%	29.2%	0.909
Presence of in-situ component	35%	65%	0.016
Lymph node metastasis	46.5%	56%	0.001
Overall survive	21.71±10.9	23.71±10.9	0.831

ied between 62.2% and 75.6% compared to the molecular groups. The mean lymph node metastasis rate was found as 50.9 % in these patients. Using immunohistochemical method according to determined cut-off value, loss of ARID1A expression was detected in 123 (42.1%) of the patients. Mild, moderate, and intense ARID1A immunoreactivity were detected in 32.8%, 47.3%, and 19.9% of the cases, respectively. A statistically significant correlation was found between loss of ARIDIA expression, ER- (p=0.047), and PR- negativity (p=0.027). Besides a statistically significant difference was found between loss of ARID1A, and the presence of both in situ component (p=0.016) and lymph node metastasis (p=0.001). Clinical, histopathological, and immunohistochemical features of the patients are summarized in Table 1.

Discussion and Conclusion

Up to the beginning of the 21th century, breast cancers were classified as for their histological types, and grades, and similarities in their gene expression profiles occurring with time were used to develop molecular classification (1, 4) In the practice of pathology, these molecular subtypes which were also determined using immunohistochemical markers as ER, PR antibodies, Ki67, HER2/neu, CK5/6, and EGFR had significant effects on the development of new treatment approaches in breast cancer (4-7). When incidence, and mortality rates were taken into consideration, nowadays, in order to be able to categorize breast cancer patients into different risk groups more accurately, new markers are needed in addition to clinicopathologic factors used (6). Thus, patients in the lower risk groups are saved from adverse effects of unnecessary treatments, and more aggressive treatment modalities may be applied for previously identified high-risk patients (7). The mutation, and deficient expression of ARID1A protein are rather frequently encountered in ovarian, and uterine carcinomas, and also in a significant number of cases with breast carcinoma (9-12). Presence of mutation is strongly associated with loss of ARIDIA expression (9). Decrease in ARIDIA expression has been associated with poor prognosis, and metastatic disease (11). A significant correlation was found between deteriorated clinical course, and loss of ARIDIA expression. Loss of ARIDIA expression is associated with highly malignant clinical phenotypes, and poor prognosis which signifies tumor suppressor role of ARIDIA in carcinogenesis (13). In our study, detection of ARID1A tissue expression in the luminal group breast cancers which is especially associated with better prognosis supports the prognostic role of ARID1A mutations in breast cancer.

Terminal ductal lobular unit (TDLU) gives rise to breast tumors, and contains acini, and ducti which constitute secretory part of mammary glands. Majority (85-90%) of invasive breast cancers arise from ductal epithelium. Invasive ductal carcinoma (IDC) is the most frequently seen breast cancer type (1-3). In our study, the patients were diagnosed as IDC (62.5%), and IDC with dominant *in situ* component (22.5%). In compliance with literature data, IDC was found in 85% of the cases. Grade of invasive breast cancer is important regarding prognosis of the tumor. Loss of differentiation was detected in line with increasing grade of the tumor, and relapses were more frequently seen in grade 3 tumors (7). In our study 24-month overall survival rates in grades 1, 2, and 3 patients were detected as 93.3%, 91.1%, and 87.6%, respectively. However, the difference between groups was not statistically significant (p=0.443). Whereas an association between molecular subtype, and histological grade was found in accordance with literature data and tumors in the luminal-A group had the lowest histological grade (p=0.004). However contrary to some studies in the literature, a statistically significant correlation was not found between ARID1A expression, and molecular subgroups (p=0.110), grades (p=0.332) or histological types (p=0.637) of the tumors (14-16).

Spread into axillary lymph nodes is the most important prognostic factor in cases with breast cancer after presence of metastases (1, 3, 6, 14). In our study, overall 24-month survival rates of the patients with and without axillary lymph node involvement were 85.7, and 97.4%, respectively with a statistically significant difference between groups (p=0.04). However, the incidence of metastases did not change based on molecular subtypes. In addition, a statistically significant difference was found between loss of ARID1A, and the presence of lymph node metastasis (p=0.01). This finding was thought the presence of relationship between the ARID1A deficiency and aggressive behaviour of tumor.

Thanks to development of medical treatments, ER- positivity in tumors has gained importance. In cases with ER- positive breast cancers some drugs with anti-estrogenic activity have been used with very good responses especially in luminal tumors. In the literature ER-positivity has been reported in 60- 65% of breast cancers. (17-19). ER-negative breast cancers are generally high-grade tumors with worse prognosis (20). Hypotheses proposed related to the development of ER-negative breast cancers may be summarized as downregulation of ER expression during tumoral development, development of tumor through differentiation of non-ER expressing cells or from non-ER expressing myoepithelial cells (20). In this study ER-positivity was found in 76.8% of the cases. Overall 24-month survival rates were 92.5%, and only 81.9% in ER-positive, and negative breast cancers, respectively without any statistically significant intergroup difference (p=0.079). However, a statistically significant correlation existed between loss of ARID1A expression and ER (p=0.047), or PR- negativities (p=0.027).

Overexpression of HER2 expression in breast cancers is another treatment-altering parameter. In the treatment of breast cancer with HER2 overexpresssion, recently developed special, targeted HER2 receptor blocking agents have been used (19, 21). The incidence of HER2 overexpression in the literature has been reported to range between 15, and 25%, and higher incidence rates have been indicated in relatively younger patients (21, 22). In our study we detected HER2- positivity in 26.7 % of the patients. In our series, the reason why we found relatively higher rates of HER-positivity despite our younger patient population is related to our inability to achieve complete randomization when we were forming our study group, and the need to gather this subgroup in our center with a laboratory where we could perform the FISH method. Although HER2- positive disease has been known to have an aggressive course, recently developed targeted treatments today seem to eliminate this handicap. In our study, the overall 24-month survival rates were 91%, and 88.3% in HER2- positive, and negative groups which supports this information.

In conclusion, we basically investigated loss of ARID1A expression, and molecular groups, and based on *chi*-square test results, any statistically significant difference was not found between these groups (p=0.110). Loss of ARID1A expression was found in respective percent of luminal A (49%), and B (50.6%), HER2 expressing (46.6%), and triple-negative tumors (53.6%). A significant difference was detected between groups with and without loss of ARID1A as for expressions of ER, and PR. In the group with ER –positivity, loss of ARID1A expression was detected in 37.9 % of the cases, while 52% of ER-negative patients demonstrated loss of ARID1A expression. When mechanisms of their activities were considered, since hormone receptors effective in the cell nucleus, and ARID1A tumor suppressor gene presumably have an impact on similar pathways, it can be said that detection of loss of ARID1 expression in breast cancers that are induced by hormonal factors convey importance in the development of novel diagnostic, and therapeutic alternatives.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Tepecik Training and Research Hospital (24.11.2015/15-2).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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