Original paper

Aldehyde dehydrogenase-1 positivity is associated with ER negativity in patients with invasive ductal carcinoma of the breast

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Cancer stem cells (CSCs) are self-renewable and can be differentiated into different cell types. They play an important role in oncogenic signaling pathways, tumor cell heterogeneity, metastasis, and therapeutic resistance. Aldehyde dehydrogenase 1 (ALDH1) was identified as a specific marker for breast CSCs. The study included a total of 105 patients with a diagnosis of invasive ductal carcinoma (IDC) who underwent mastectomy and with sufficient pathology material for histopathological examination. Patient demographics, tumor location, tumor diameter, the presence of lymphovascular and perineural invasion and lymph node metastasis, surgical margin status, and immunohistochemistry (IHC) staining results were obtained from patients’ records. The tumors were classified into IHC-based molecular subtypes according to the St. Gallen Consensus Conference in 2013. A four-tiered scoring system was used based on ALDH1 staining percentage in tumor cells. The tumor was determined as positive if the score was 2 or higher. Clinical, histopathological findings, and ALDH1 staining results were correlated. Twenty-five cases (23.8%) were ALDH1 positive. The ALDH1 positive group compared to the negative group was found to be associated with ER negativity (p = 0.044), but there was no correlation with other clinical and histopathological findings. ALDH1-positive IDCs may be less sensitive to hormonal therapy and associated with aggressive behavior.

Key words: aldehyde dehydrogenase 1, stem cell, breast, invasive ductal carcinoma, pathology.

Introduction

Cancer stem cells (CSCs), which are self-renewable and can be differentiated into different cell types, play an important role in oncogenic signaling pathways, tumor cell heterogeneity, metastasis, and therapeutic resistance [1, 2, 3]. The presence of CSCs has been shown in various types of malignancies, such as colon, brain, lung, and breast tumors [2, 4, 5, 6, 7]. In 2003, the breast CSCs were first isolated by their CD44+CD24low surface marker expression [6]. In 2007, expression of a single marker, aldehyde dehydrogenase 1 (ALDH1), was shown to be specific for normal and malignant human breast stem cells [7]. Aldehyde dehydrogenase functions to catalyze the oxidation of aldehydes to their relevant carboxylic acids [8]. To date, 19 different ALDH functional genes and multiple splice variants have been described [9]. Aldehyde dehydrogenase 1 is one of the isoforms playing an important role in the retinoic
acid pathway through the catalysis of retinaldehyde to retinoic acid [10]. Isolated cancer cells with relatively high ALDH1 activity were shown to have in vitro features of CSCs, including capabilities of proliferation, self-renewal, and differentiation, and resistance to chemotherapy [7, 11, 12].

In this study, we aimed to determine the clinical and pathological value of immunohistochemical (IHC) staining of ALDH1 in tumor cells as well as peritumoral and intratumoral stromal cells in a series of invasive ductal carcinomas (IDCs).

Material and methods

This study was approved by the non-interventional clinical research ethical board (No: 2016/462).

Patients and clinical information

We retrospectively identified the patients diagnosed with IDC in the pathology laboratory between 2010 and 2016. We included all the patients who underwent mastectomy and with sufficient pathology material for histopathological examination. Clinical data were retrieved from the electronic medical records.

Review of histopathological and immunohistochemical findings

Two pathologists (YK and ÜT) reviewed all hematoxylin-eosin slides received from the pathology archive and determined the Nottingham histological grade of each tumor [13]. Patient demographics, tumor location, tumor diameter, the presence of lymphovascular and perineural invasion, surgical margin status, the presence of lymph node metastasis, and IHC staining results were obtained from patients’ records and pathology reports. The status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) was determined according to the American Society of Clinical Oncology/College of American Pathologists guidelines [14, 15]. The Ki-67 proliferation index was regarded as high if there was more than 14% staining in tumor cells [16]. All cases with available IHC results were divided into molecular subtypes according to the International Breast Cancer Conference in St Gallen in 2013 [16]. Microscopic examination was performed using an Olympus brand MDOB3 model 8H16329 serial microscope.

Aldehyde dehydrogenase 1 staining and evaluation method

One representative block from each case was selected for staining with ALDH1 (polyclonal antibody, IgG isotype, dilution 1:150; GeneTex, Irvine, CA, USA). Sections with a thickness of four microns were taken to positive-charged slides. The sections were left for 60 minutes at a temperature of 60°C. Then, the sections were kept at room temperature for 10 minutes and automatically stained with the XT DAB V3 protocol based on multimer technology in the Ventana BenchMark XT model. After the staining process, the sections were washed in soapy water for 5-10 minutes. After that, the slides were dried, placed in 96% alcohol and xylene, then they were closed in the Sakura Tissue Single Film model automatic film closure device.

We evaluated and scored ALDH1 immunostaining in the tumor cells as follows: score 0, no staining in the tumor cells; score 1, staining in less than 10% of the tumor cells; score 2, staining in more than 10% but less than 50% of the tumor cells; score 3, staining in more than 50% of the tumor cells. In the following analysis, we determined the tumor as ALDH1 positive if the tumor had a score of 2 or higher as previously described [17]. We also examined the stromal cell staining using a 10% cutoff value for positivity [18]. Adult liver tissue was used as a positive control.

Statistical analysis

Histopathological and clinical findings and IHC results were tabulated. All statistical analyses were carried out using the IBM SPSS Statistics 22.0 package program (IBM Corp., Armonk, New York, USA). The frequencies of clinical and histopathologic variables were presented by using cross-tabulations. A two-sided Fisher’s χ² exact test for rxc tables was applied to compare the differences between groups for categorical variables. The normal distribution of variables was examined visually (histogram and probability plots) and with analytical methods (Shapiro-Wilk tests). A p value of less than 0.05 was considered significant.

Results

Patients and clinical information

There were a total of 105 patients including two males with a diagnosis of IDC. All cases had sufficient pathology material and clinicopathologic data.

The median age was 50 (range 24-85). Fifty-two (49.5%) of the tumors were located in the right breast, 48 (45.7) were in the left, and five (4.8%) involved both. The cases predominantly underwent modified radical mastectomy (n = 70, 66.7%, including two cases with bilateral involvement), 24 (22.9) cases had a partial mastectomy, and eight cases (7.6%) had a simple mastectomy. Three out of five cases with...
bilateral breast carcinoma had simple mastectomy and modified radical mastectomy during the same operation.

**Review of histopathological and immunohistochemical findings**

The median diameter of the tumors was 25 mm (range 5-70 mm). Eighty-four tumors (80%) had a single focus, 16 (15.2%) were multifocal, and the remainder were bilateral. According to the histological grading system, 78 cases (74.3%) were grade 3, 26 cases (24.8%) were grade 2, and one case (1%) was grade 1. Lymphatic invasion was present in 66 cases (62.9%) and vascular invasion was seen in 25 cases (23.8%). Lymph node metastasis was detected in 70 cases (66.7%). Ninety cases (85.7%) were ER positive, 80 (76.2%) were PR positive, 28 out of 81 cases (34.6%) were HER2 positive, and Ki-67 was high in 73 (69.5%) cases. Classification of the tumors based on the IHC-based molecular subtypes (n = 100) revealed that 62 cases (62%) were in luminal B, 24 cases (24%) were in luminal A, 12 cases (12%) were in HER2, and 2 cases (2%) were in triple-negative breast carcinoma (TNBC) subtype.

**Aldehyde dehydrogenase 1 staining in tumor and stromal cells**

Aldehyde dehydrogenase 1 staining in IDCs is displayed in Fig. 1. Aldehyde dehydrogenase 1 was recorded as score 0 in 21 cases (20%), score 1 in 59 cases (56.2%), score 2 in 18 cases (17.1%), and score 3 in 7 cases (6.7%). The tumor cells in 25 cases (23.8%) were ALDH1 positive. All cases showed positivity in peritumoral stromal areas. All cases but one showed positivity in intratumoral stromal areas (Fig. 2). In addition, the concentration of ALDH1-positive stromal cells was more prominent in the peritumoral areas compared to the stromal cells surrounding benign ducts within tumor cells.

**Correlation of aldehyde dehydrogenase 1 staining in tumor cells with clinical and histopathological features**

The ALDH1 positive group compared to the negative group was found to be associated with ER negativity (p = 0.044). Aldehyde dehydrogenase 1 staining and IHC-based molecular subtypes were found to have a significant association (p = 0.025). A pairwise z-test post hoc analysis with Bonferroni correction revealed that only for TNBC was there a significant
Table I. Correlation of ALDH1 staining in the tumor cells with the clinicopathological parameters in invasive ductal carcinomas

| Clinical and Pathological Parameters               | ALDH1 in Tumor | P Value |
|---------------------------------------------------|----------------|---------|
|                                                  | Negative | Positive |         |
|                                                  | # | % | # | % |
| **Age group (years)**                             |         |         |         |         |
| ≤ 40                                              | 17 | (77.3) | 5 | (22.7) |
| 40-55                                             | 31 | (79.5) | 8 | (20.5) |
| ≥ 55                                              | 32 | (72.7) | 12 | (27.3) |
| **Diagnosis**                                     |         |         |         |         |
| IDC                                               | 74 | (75.5) | 24 | (24.5) |
| IDC with micropapillary component                 | 6 | (85.7) | 1 | (14.3) |
| **Laterality**                                    |         |         |         |         |
| Right                                             | 40 | (76.9) | 12 | (23.1) |
| Left                                              | 36 | (75) | 12 | (25) |
| Bilateral                                         | 4 | (80) | 1 | (20) |
| **Operation**                                     |         |         |         |         |
| PM                                                | 20 | (83.3) | 4 | (16.7) |
| SM                                                | 6 | (75) | 2 | (25) |
| MRM                                               | 51 | (72.9) | 19 | (27.1) |
| SM + MRM                                          | 3 | (100) | 0 | (0) |
| **Number of foci**                                |         |         |         |         |
| Single focus                                      | 67 | (79.8) | 17 | (20.2) |
| Multifocal                                        | 9 | (56.3) | 7 | (43.8) |
| Bilateral                                         | 4 | (80) | 1 | (20) |
| **Diameter**                                      |         |         |         |         |
| ≤ 2 cm                                            | 23 | (85.2) | 4 | (14.8) |
| 2-5 cm                                            | 53 | (73.6) | 19 | (26.4) |
| ≥ 5 cm                                            | 4 | (66.7) | 2 | (33.3) |
| **Histological grade**                            |         |         |         |         |
| Grade I-II                                        | 20 | (74.1) | 7 | (25.9) |
| Grade III                                         | 60 | (76.9) | 18 | (23.1) |
| **Lymph node metastasis**                         |         |         |         |         |
| Absent                                            | 26 | (74.3) | 9 | (25.7) |
| Present                                           | 54 | (77.1) | 16 | (22.9) |
| **Lymphatic invasion**                            |         |         |         |         |
| Absent                                            | 29 | (74.4) | 10 | (25.6) |
| Present                                           | 51 | (77.3) | 15 | (22.7) |
| **Vascular invasion**                             |         |         |         |         |
| Absent                                            | 63 | (78.8) | 17 | (21.3) |
| Present                                           | 17 | (68) | 8 | (32) |
| **ER**                                            |         |         |         |         |
| Negative                                          | 8 | (53.3) | 7 | (46.7) |
| Positive                                          | 72 | (80) | 18 | (20) |
difference between the ALDH1 positive and negative cases (p < 0.05). There was no significant relationship between ALDH1 positivity and the other clinicopathologic parameters (Table I).

**Discussion**

Aldehyde dehydrogenase 1 was identified as a reliable stem cell marker of the normal human breast and breast carcinomas [7]. In this study, our purpose was to appraise the clinical and pathological value of ALDH1 staining in patients with a diagnosis of IDC, no special type. Our results showed that ALDH1-positive IDCs are associated with ER negativity. Hormone receptor negativity in invasive breast carcinomas is a well-known factor associated with treatment resistance and poor prognosis; hence, our results suggested that ALDH1-positive IDCs may represent a biologically aggressive phenotype [4, 19].

In breast carcinomas, ALDH1 positivity has ranged from 18% to 56% in different studies [7, 20, 21, 22, 23]. Likewise, 23.8% of IDCs in our series were positive for ALDH1. Various studies have aimed to determine the clinical and pathological differences between ALDH1-positive and negative breast carcinomas. In accordance with our results, some of these studies have shown that ALDH1 positivity is associated with ER negativity [7, 17, 18, 24, 25]. Some studies have found that ALDH1 positive breast carcinomas are also more likely to be PR negative and/or HER2 positive, and they are associated with a high Ki67 proliferative index; but some others including our study have found no such correlation [7, 17, 18, 24, 25, 26, 27]. Several studies have demonstrated that ALDH1-positive invasive breast carcinomas are associated with TNBC and/or HER2(+) subtype [17, 18, 26, 27]. In our series, ALDH1 positivity was observed more frequently in TNBCs as well; however, the number of TNBC patients was too small to determine an exact association. There are discrepancies in studies correlating ALDH1 positivity and histological grade, tumor size, and patient outcome [6, 7, 17, 18, 24, 25, 26, 27, 28, 29]. Most studies have reported no value of finding of ALDH1-positive tumor cells in predicting lymph node metastasis [7, 17, 18, 24, 26, 27]. In our study, ALDH1 positivity was observed more frequently in TNBCs as well; however, the number of TNBC patients was too small to determine an exact association. There are discrepancies in studies correlating ALDH1 positivity and histological grade, tumor size, and patient outcome [6, 7, 17, 18, 24, 25, 26, 27, 28, 29]. Most studies have reported no value of finding of ALDH1-positive tumor cells in predicting lymph node metastasis [7, 17, 18, 24, 26, 27]. In our study, we did not find an association with ALDH1 positivity and several significant prognostic factors, such as tumor diameter, histological grade, lymph node metastasis, lymphatic or vascular invasion.

Aldehyde dehydrogenase 1 staining in intratumoral stromal and peritumoral stromal cells in breast cancers has received special attention. One study reported high levels of ALDH1 staining in stromal cells in breast cancers that develop sporadically or in patients with BRCA1 mutations. Peritumoral stromal staining in the BRCA1-mutated group was significantly higher than the non-BRCA1-mutated group. According to this study, peritumoral stromal ALDH1 staining was associated with ER and PR negativity, basal-like phenotype, and high mitotic activity [30].
In our study, ALDH1 staining in the peritumoral stromal cells was observed in all but one case. Our findings are incompatible with the above study. Another observation of ours was that ALDH1-positive stromal cells were more concentrated in the peritumoral areas compared to the stromal cells surrounding benign ducts within tumor cells. In one study, ALDH1, which takes part in the retinoic acid synthesis pathway, was shown to have a role in normal epithelial cell proliferation and their ability to acquire progenitor/stem cell differentiation properties [31]. In another study, retinoic acid-producing ALDH1-positive dendritic cells in the intestines were shown to activate immune system cells. In addition, it has been found that the presence of ALDH1-positive stromal cells in tumors increases retinoic acid synthesis and secretion, thus increasing tumor cell differentiation and reducing the aggressiveness of the tumor [18]. These findings have suggested that activated immune system cells may play a role in preventing the development of malignancy in the areas where ALDH1-positive stromal cell density is high around the tumor, as seen in our cases.

There were a few limitations of our study. The number of patients included in this study was relatively small. This was particularly a drawback in the correlation analysis between ALDH1 staining and molecular subtypes. Survival analyses were not carried out in this study; hence we have limited results regarding the prognostic value of ALDH1.

In conclusion, our findings suggest that stem cell-like features are more prominent in IDGs with ER negativity. Hence, ALDH1-positive IDCs may be more aggressive and less sensitive to hormonal therapy. However, further studies would be needed to reveal whether there is any prognostic significance of ALDH1. It is plausible that the peritumoral concentration of ALDH1-positive stromal cells may generate a reactive stromal response against the development of malignancy or may be protective.

The authors declare no conflict of interest.

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