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ALDH1 immunohistochemical expression and its significance in salivary adenoid cystic carcinoma

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Abstract

Background—Adenoid cystic carcinoma (ACC) cells with a high level of ALDH1, a known cancer stem cell (CSC) marker, had higher tumorigenic, invasive, and metastatic abilities. We examined the immunohistochemical expression of ALDH1 in ACC and its correlation with survival.

Methods—Archival paraffin blocks of ACC were analyzed. A tissue microarray was constructed and immunohistochemical expression of ALDH1 was analyzed using anti-ALDH1 monoclonal antibody. Correlations between ALDH1 expression and clinical and histological parameters were assessed by chi-square tests. Survival was assessed by the Kaplan–Meier method and log-rank test.

Results—Most of the tumors (63%) showed stromal staining only, 11% of the tumors showed both epithelial and stromal expression, and 26% of the tumors did not show either epithelial or stromal staining. Statistical analyses did not show any correlation between the pattern of ALDH1 expression and tumor histology, tumor size, or perineural invasion. There were no significant differences in survival among the 3 patterns of ALDH1 expression.

Conclusion—Other factors, besides CSCs, may play important roles in tumorigenesis, cell differentiation, and tumor progression in these tumors.

Keywords

ALDH1 expression; adenoid cystic carcinoma; tissue microarray; predictive marker

INTRODUCTION

Accumulating evidence supports the concept of cancer stem cells (CSCs).¹⁻⁶ The CSC hypothesis states that a subpopulation of tumoral cells is capable of propagating the tumor; this hypothesis relies on the hierarchical model to explain tumor heterogeneity and behavior.

ALDH1 is considered a marker for normal stem cells as well as CSCs in several tissue types.⁷⁻¹³ ALDH1 is a member of the aldehyde dehydrogenase superfamily, which consists of multifunctional enzymes that catalyze the oxidation of various aldehydes to carboxylic acids. The ALDH1 enzyme mediates progenitor cell chemoresistance and has been used to identify highly enriched progenitor stem cell population in bone marrow and umbilical cord blood.

Currently, 2 hypotheses of tumorigenesis exist. One proposes that tumor cells are heterogeneous, but all have the potential of acquiring mutations that lead to tumor growth; the other hypothesis states that only a small, distinctive subset of tumor cells, referred to as CSCs, are capable of self-renewal and differentiation leading to tumor growth. The latter hypothesis gained support with the discovery of CSCs in leukemia in the 1990s and in breast cancer in 2003. It is further supported by the fact that current therapeutic drugs can seemingly cure cancer, but recurrence and metastasis still occur. It has been postulated that a proportion of CSCs are insensitive to the current therapeutic drugs, and the repopulation and migration of these cells results in recurrence and metastasis.

With the belief that CSCs exist came the search for a marker that can identify these cells for research and possible therapeutic applications. ALDH1 was first found in higher amounts in normal hematopoietic stem cells than in more differentiated cells. Recently, there have been reports of ALDH1 as a CSC marker in cancers of the lung,¹¹ breast,¹⁴ colon,¹² prostate,¹³ ovary,⁹ head and neck squamous cell carcinoma,¹⁵ and adenoid cystic carcinoma of the salivary gland.¹⁵ Studies by Clay et al¹⁵ showed that a subpopulation of cancer cells in head and neck squamous cell carcinoma express high levels of ALDH1, and very small numbers of these cells were capable of producing tumors. However, this study did not address the issue of whether high levels of ALDH1 were also expressed in normal squamous cells.

Adenoid cystic carcinoma (ACC) is composed of luminal epithelial cells, abluminal myoepithelial cells, and stromal cells. The abluminal myoepithelial cells are immunohistochemically positive for p63, and it has been suggested that these cells are the stem cells that drive the growth of the neoplasm.¹⁴ Sun et al¹⁶ have shown, using a xenograft model, that ALDH1^{high} cells in adenoid cystic carcinoma display CSC properties.

To date, most of the studies of CSCs have used Alde-fluor assay to detect ALDH1 in cancer cells. Aldefluor assay uses cell labeling and flow cytometry technology, which is excellent for sorting out cells of interest but does not identify the location of the cells within the tissue. Immunohistochemical analysis is able to pinpoint the locations of specific cells within a tissue section, but there have been no reports of the immunohistochemical expression of ALDH1 in adenoid cystic carcinoma.

In this study, we examined the expression of ALDH1 in 216 adenoid cystic carcinoma specimens using tissue microarray and immunohistochemical analyses. We then correlated the expression of ALDH1 with survival using data from a 12-year follow-up cohort.

MATERIALS AND METHODS

Patient selection and tissue microarray construction

Archival paraffin blocks of adenoid cystic carcinomas obtained between 1988 and 2006 from patients undergoing surgery at The University of Texas MD Anderson Cancer Center were used for this study, with the approval of the institutional review board. The tissue microarrays were constructed using two 1.0-mm diameter cores of spatially different areas of representative tumor from each paraffin block. Normal salivary gland and splenic tissue were used as controls.

Immunohistochemical analysis

Immunohistochemical expression of ALDH1 was analyzed using mouse monoclonal ALDH1 antibody (BD Biosciences, San Jose, CA). Briefly, after dewaxing and hydration, 4-mm sections of the formalin-fixed, paraffin-embedded tissues were treated with Target Retrieval (Dako, Carpinteria, CA) at pH 6.00 under high pressure in a pressure cooker for 15 minutes. Endogenous peroxidase activity was blocked by Peroxo-block (Invitrogen Corporation, Carlsbad, CA). The slides were then incubated with ALDH1 antibody (1:200) for 30 minutes at room temperature. After washing, the sections were incubated with Envision + Rabbit Polymer (Dako) for 30 minutes. The stain was visualized using DAB Plus (Dako) and hematoxylin QS (Vector Laboratories, Burlingame, CA) counterstain. The specificity of staining was confirmed in liver tissue samples (positive control), and negative controls consisted of replacing the primary antibody with either phosphate-buffered saline or isotype-specific immunoglobulin G controls.

Immunoreactivity evaluation and analysis

The immunoreactivity of ALDH1 was evaluated in both stromal and epithelial tissue on each microsection of tumor and the controls. The pattern of ALDH1 expression was recorded as either positive or negative in stromal tissue, epithelial tissue, or both. Histologic patterns and the phenotypic expression of ALDH1 were evaluated independently. The patterns of ALDH1 expressions were correlated with clinical and histological parameters, including patient sex and age, size, location, and histological pattern of tumor, presence of perineural invasion, and survival.

Statistical analysis

Descriptive statistics for scaled values and frequencies of study subjects within the categories for each of the parameters were enumerated with the assistance of commercial statistical software. Correlations between ALDH1 expression and clinical and histological parameters were assessed using Pearson chi-square and M-L chi-square tests. Survival curves were generated using the Kaplan–Meier method. The statistical significance of differences between the actuarial curves was evaluated by using the log-rank test. Follow-up time was from the first appointment at MD Anderson Cancer Center for the primary tumor

of concern until the date of last contact or death. Calculated p values $< .05$ were considered significant. These statistical tests were performed using the Statistica (StatSoft, Tulsa, OK) and SPSS (SPSS for Windows, SPSS, Chicago, IL) statistical software applications.

RESULTS

Patient demographics

The patient population comprised 113 women (52%) and 103 men (48%), a total of 216 patients. The mean age $\pm$ SD at the time of tissue acquisition was 52 ± 14 years (median, 52 years; range, 15–86 years). Seventy-three patients (34%) were older than 60 years. One hundred sixty patients (74%) were white, 26 (12%) were Hispanic, and 16 (7%) were black, with all the other racial groups making up about 7%.

Tumor characteristics

The mean tumor size $\pm$ SD was 3 ± 1.77 cm (median, 3.0 cm; range, 0.02–15 cm). The most frequent location was the maxilla (22%), followed by the parotid glands (12%), the orbit (6%), the palate (5%), the submandibular glands (5%), the floor of mouth (5%), the tongue (5%), and the mandible (4%), with the remainder of the tumors in various locations such as the trachea, larynx, buccal mucosa, and nasal sinuses. One hundred three tumors (56%) were of cribriform type, 52 (28%) were tubular type, and 29 (16%) were solid type. Not all data points were available for every sample. Only the samples for which the type was known were included in this tabulation (this accounted for 184 samples). One hundred thirty-one tumors (94%) exhibited perineural invasion.

ALDH1 expression and correlation with tumor characteristics

The tumors showed 3 distinct patterns of ALDH1 expression. Most of the tumors (116; 63%) had stromal staining only, 21 (11%) had both epithelial and stromal expression, and 47 (26%) did not have either epithelial or stromal staining (Figure 1). The normal salivary glands showed epithelial expression only.

Statistical analyses did not show any correlation between the different patterns of ALDH1 expression and tumor pattern, tumor size, or the presence of perineural invasion (Table 1).

ALDH1 expression and correlation with survival

The survival analysis using the Kaplan–Meier method and log-rank test did not show any significant differences in survival among patients with the 3 patterns of ALDH1 expression (Figure 2).

DISCUSSION

In this study, we found that ALDH1 was expressed in stromal cells in the majority (63%) of the adenoid cystic carcinomas and in epithelial cells in all of the normal salivary glands. The pattern of ALDH1 expression did not affect survival.

A recent study¹⁶ of adenoid cystic carcinoma showed that 1.04% to 9.76% of cells expressed a high level of ALDH1 activity using the Aldefluor assay. Moreover, the adenoid

cystic carcinoma cells with high levels of ALDH1 had higher tumorigenic, invasive, and metastatic abilities compared with tumor cells expressing low levels of ALDH1. However, the authors stated that they could not determine whether the CSCs arose from mutated reserve cells or uncommitted stem cells in the salivary gland. In our study, we found that ALDH1 was expressed predominantly in the stromal cells of adenoid cystic carcinomas and in epithelial cells of the normal salivary gland. One explanation is that adenoid cystic carcinoma stems from stromal cells under appropriate conditions. A study using a colony-forming assay¹⁸ and electron microscopy found that pleomorphic adenomas were monoclonal neoplasms with multidirectional differentiation. It is possible that the stromal cells in adenoid cystic carcinoma can differentiate into epithelial and myoepithelial cells. The other possibility is that some of the epithelial cells have undergone epithelial-mesenchymal transition. Chen et al¹⁹ found that a subpopulation of the CSCs in head and neck squamous cell carcinoma had acquired features of myofibroblasts and may have undergone epithelial-mesenchymal transition.

Limited data address the usefulness of ALDH1 as a prognostic/predictive marker. Ginestier et al¹⁴ and Neumeister et al²⁰ concluded in separate studies that the expression of ALDH1 or its coexpression with CD44+/ CD24- in breast cancer correlated with poor survival. However, Resetkova et al²¹ found that tumors with high stromal ALDH1 expression were significantly associated with better disease-free survival and a trend toward longer overall survival in triple-negative breast cancer cohorts. In addition, they did not find any correlation between tumoral ALDH1 expression and nodal status, expression of prognostic markers, response to neoadjuvant therapy, or disease-free or overall survival after adjuvant/neoadjuvant therapy. Other reports have linked low expression of ALDH1A1 with poor survival in pancreatic cancer²²; high expression of ALDH1A1 with advanced disease stage, high histologic grade, and worse outcome in bladder urothelial carcinomas²³; and high expression of ALDH1 with endometrioid adenocarcinoma, early stage disease, complete response to chemotherapy, and longer overall and disease-free survival.²⁴ A study by Chen et al²⁵ found that high ALDH1 expression correlated positively with stage and grade of head and neck squamous cell carcinoma but negatively with outcome in 226 patients. The article did not specify how the ALDH1 expression was measured. We did not find any correlation between the various patterns of ALDH1 expression and survival. Although the sample size of tumors with both epithelial and stromal expression (11%) may be too small to discern a difference in survival, these results suggest that other unknown factors, besides CSCs, may play important roles in tumorigenesis, cell differentiation, and tumor progression in adenoid cystic carcinoma.

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FIGURE 1.

ALDH1 staining patterns in the adenoid cystic carcinoma. (A) Low-power ($\times 4$) micrograph of positive and negative staining on the tissue microarray. (B) High-power ($\times 10$) micrograph of both epithelial and stromal staining. (C) High-power ($\times 10$) micrograph of negative staining. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

Kaplan–Meier curve shows no statistical significance in survival with different ALDH1 expression patterns. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1

ALDH1 expression and patient/tumor characteristics.

Patient and tumor characteristics (n = 216)	No. of patients (%)			Pearson chi-square test
	ALDH1 stromal expression	ALDH1 epithelial-stromal expression	ALDH1 negative	
Age				.019
60 (n = 143)	83 (45)	9 (5)	27 (15)	
>60 (n = 73)	33 (18)	12 (6)	20 (11)	
Sex				.871
Female (n = 113)	60 (33)	11 (6)	25 (14)	
Male (n = 103)	56 (30)	10 (5)	22 (12)	
Tumor size				.828
<4 cm (n = 91)	58 (48)	9 (7)	24 (20)	
4 cm (n = 31)	18 (15)	4 (3)	9 (7)	
Perineural invasion				.440
Yes (n = 116)	74 (60)	10 (8)	32 (26)	
No (n = 7)	5 (4)	1 (1)	1 (1)	
Histologic type				.620
Cribriform (n = 103)	52 (33)	13 (8)	25 (16)	
Tubular (n = 52)	28 (18)	5 (3)	11 (7)	
Solid (n = 29)	18 (11)	1 (0.6)	6 (3.4)	