Antizyme inhibitor 2 (AZIN2) associates with better prognosis of head and neck minor salivary gland adenoid cystic carcinoma

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The key regulator of the polyamine biosynthetic pathway is ornithine decarboxylase (ODC). ODC is activated by antizyme inhibitor 1 (AZIN1) and 2 (AZIN2). AZIN1 and recently AZIN2 have been related to cancer; however, their functions in adenoid cystic carcinoma (ACC) have not been studied. We performed immunohistochemical study on minor salivary and mucous gland ACC tissue samples of patients treated at the Helsinki University Hospital (Helsinki, Finland) during 1974–2012. We scored AZIN1 and 2 immunoexpression in 42 and 45 tumor tissue samples, respectively, and correlated them with clinicopathological factors and survival. Enhanced AZIN2 expression was associated with better survival. In addition, both AZINs were seen more commonly in cribriform and tubular than in solid growth patterns. AZIN1 expression did not correlate with the studied clinicopathological factors. It seems that AZIN2 expression is higher in cancer tissue with secretory functions. In ACC tissue, high AZIN2 expression could be related to well-differentiated histological type which still has a functioning vesicle transportation system. Thus, AZIN2 could be a prognostic factor for better survival of ACC patients.

Key words: Adenoid cystic carcinoma; ornithine decarboxylase; antizyme inhibitor; prognosis; histopathology; pathology of tumours; prognostic markers.

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Among salivary gland malignancies, adenoid cystic carcinoma (ACC) is a common type according to the World Health Organization (WHO) [1]. Sixty percent of ACCs occur in salivary glands although ACC could occur in glandular structures containing areas such as nose, sinonasal cavities, and ear (10%), breast (13%), skin (5%), lung and bronchus (4%), trachea (2%), female genital system (2%), eye (2%), and larynx (1%) [2].

Unfortunately, the long-term prognosis of salivary gland ACC has remained poor despite treatment by radical surgical resection and postoperative radiotherapy [1]. ACC has polymorphic histological growth patterns—cribriform, tubular, solid, and their combinations [1]. Tumors with more than 30% solid pattern are histologically considered as high-grade tumors with more aggressive behavior [1]. Many immunohistochemical studies have sought to reveal immunomarkers to better understand ACC and to predict its behavior. Well-

known immunomarkers of ACC are CD117 which usually stains inner epithelial tumor cells, and p63 and SMA which stain outer myoepithelial tumor cells [1, 3, 4]. ACC tissue remains histologically interesting due to the presentation of various morphologies, and the same immunomarkers may be differently expressed in ACC tissue. The recently discovered ACC-specific MYB-NFIB fusion gene aids in the diagnosis of the otherwise challenging ACC cases by MYB1-immunohistochemistry [1]. For example, small samples which do not demonstrate typical histology could now be verified by immunohistochemistry [5–7].

Polyamines are organic cations that are essential for cell functions, such as cell growth, proliferation, differentiation, apoptosis, and gating of ion channels [8–10]. Dysregulation of polyamines in the cell has been related to cancer and neurological disorders [8, 10, 11]. The key regulator of the polyamine biosynthetic pathway is ornithine decarboxylase (ODC), the activity of which is mediated by transcriptional, post-transcriptional, translational, and post-translational mechanisms [10]. ODC is down-regulated post-translationally by antizymes (AZs) and up-regulated by antizyme inhibitors (AZINs) [10]. Interaction of AZ1 with ODC prevents the formation of active ODC homodimers and directs ODC to proteosomal degradation independently of ubiquitination [10]. In addition, AZs inhibit the polyamine transporter system at the cell membrane by an unknown mechanism [10]. Activation of ODC is maintained by antizyme inhibitors (AZINs), which are antizyme antagonist proteins that bind AZs with stronger affinity than ODC [9, 10].

In mammalians, there are two forms of AZINs, namely AZIN1 [12] and 2 [13]. All cell types express AZIN1, and it is essential for cell survival. Overexpression of AZIN1 stimulates cell proliferation with oncogenic potential in both tumor and non-tumor cells [10, 14–17]. Overexpression of AZIN1 is detected in various malignancies, such as lung and colorectal cancer [18, 19]. Furthermore, AZIN1 may induce cell proliferation by preventing degradation of cell-cycle regulator cyclin D1 by a mechanism that does not include binding to AZ1 [15]. Contrary to the ubiquitous AZIN1, a high amount of AZIN2 expression is seen in terminally differentiated cells, such as type-2 pneumocytes in the lungs, adipocytes, neurons, mast cells, megakaryocytes in normal bone marrow, and acinar cells of sweat glands [20]. AZIN2 has been shown to regulate intracellular vesicle transport and the mast cell degranulation process [9, 21]. Elevated expression of AZIN2 has been detected in neurons affected by Alzheimer's disease [22]. Although the AZIN2 gene is not usually up-regulated in cancer

[10], we have recently shown AZIN2 to act as a prognostic predictor in colorectal cancer [23]. AZINs have not been investigated previously in salivary gland tissue or salivary gland cancer.

The aim of our study was to evaluate the immunoexpression of AZIN1 and 2 in a series of head and neck ACCs of minor salivary and mucous gland tissue and to compare the immunoexpression with clinical and clinicopathological features and patient survival.

MATERIALS AND METHODS

Patients

The study population consisted of 68 patients with ACC of the minor salivary gland treated at the Helsinki University Hospital (Helsinki, Finland) between the years 1974 and 2012. Our previous study shows the clinical data of this series [24], and Table 1 shows the main patient and tumor characteristics of the current study. To this study, we have included all the patient cases with tumor tissue available. For AZIN1 immunohistochemical staining, we obtained samples from 42 patients including 35 primary tumors and 7 recurrent tumors. For AZIN2 immunohistochemical staining, we obtained samples from 45 patients including 37 primary tumors and 8 recurrent tumors. Tumor staging was performed according to the WHO 2005 and 2017 criteria [1, 25]. The institutional Research Ethics Board approved the study concept (Dnro 31/13/03/02/2010, 01 February 2010).

Immunohistochemistry

For immunohistochemistry, formalin-fixed and paraffin-embedded blocks were freshly cut into 4 μm thick sections. Deparaffinization was performed in xylene and rehydration in graded ethanol and distilled water. The slides were heated in a PreTreatment module (Agilent Dako, Santa Clara, CA, USA) in antibody-specific buffer, pH = 9, for 20 min at 98°C for antigen retrieval. Endogenous peroxidase activity was blocked by incubation of the slides with EnVision Flex peroxidase-blocking reagent for 15 min. The primary antibody was diluted in Dako REAL Antibody Diluent. The primary antibodies used were rabbit polyclonal AZIN1 antibody (1:500) (Biorbyt Ltd, Cambridge, UK) and K3 antibody (1:600) [23]. The incubation times were overnight at +4°C and one hour at room temperature, respectively, followed by detection with Dako REAL Detection System (Peroxidase/DAB+, Rabbit/Mouse, Dako, Glostrup, Denmark). Finally, slides were visualized by Dako REAL DAB+ Chromogen for 10 min and counterstained with hematoxylin (Mayer's Hematoxylin Dako, Glostrup, Denmark). As a positive control, we used skin tissue for AZIN1 and gastric tissue for AZIN2. A slide without primary antibody served as the negative control.

Immunoscoring

Two independent researchers (HH and LCA) performed scoring of the slides blinded to the clinical data. We

Table 1. Patient and tumor characterization of the 45 (AZIN2) and 42 (AZIN1) patients with an adenoid cystic carcinoma of minor salivary and mucous glands

Tumor site	Oral cavity	Oropharynx	Nasopharynx	Paranasal cavities	Larynx	Trachea	Esophagus	Ear
AZIN2								
Number of cases (n = 45)	23 (51.1%)	3 (6.7%)	4 (8.9%)	3 (6.7%)	2 (4.4%)	5 (11.1%)	1 (2.2%)	4 (8.9%)
Age								
<65 years	16 (35.6%)	2 (4.4%)		3 (6.7%)	1 (2.2%)	5 (11.1%)	1 (2.2%)	3 (6.7%)
>65 years	7 (15.6%)	1 (2.2%)	4 (8.9%)		1 (2.2%)			1 (2.2%)
Gender								
Male	12 (26.7%)		1 (2.2%)	2 (4.4%)			1 (2.2%)	1 (2.2%)
Female	11 (24.4%)	3 (6.7%)	3 (6.7%)	1 (2.2%)	2 (4.4%)	5 (11.1%)		3 (6.7%)
T class								
T1	7 (15.6%)	3 (6.7%)			1 (2.2%)			2 (4.4%)
T2	4 (8.9%)							
T3	1 (2.2%)		2 (4.4%)				1 (2.2%)	1 (2.2%)
T4	9 (20%)		1 (2.2%)	3 (6.7%)	1 (2.2%)			1 (2.2%)
N/D	2 (4.4%)		1 (2.2%)					
N class								
N0	19 (42.2%)	3 (6.7%)	3 (6.7%)	3 (6.7%)	2 (4.4%)			4 (8.9%)
N1	1 (2.2%)							
N2	1 (2.2%)						1 (2.2%)	
N/D	2 (4.4%)		1 (2.2%)					
M class								
M0	19 (42.2%)	3 (6.7%)	3 (6.7%)	3 (6.7%)	2 (4.4%)		1 (2.2%)	4 (8.9%)
M1	2 (4.4%)							
N/D	2 (4.4%)		1 (2.2%)					
Stage								
I	5 (11.1%)	3 (6.7%)			1 (2.2%)			2 (4.4%)
II	5 (11.1%)							
III	2 (4.4%)		2 (4.4%)				1 (2.2%)	1 (2.2%)
IV	9 (20%)		1 (2.2%)	3 (6.7%)	1 (2.2%)			1 (2.2%)
N/D	2 (4.4%)		1 (2.2%)					
Neural invasion								
No	4 (8.9%)	1 (2.2%)			1 (2.2%)	3 (6.7%)	1 (2.2%)	
Yes	16 (35.6%)	2 (4.4%)	1 (2.2%)	2 (4.4%)		1 (2.2%)		4 (8.9%)
N/D	3 (6.7%)		3 (6.7%)	1 (2.2%)	1 (2.2%)	1 (2.2%)		
Primary recurrence								
No	17 (37.8%)	3 (6.7%)	2 (4.4%)	2 (4.4%)	2 (4.4%)	4 (8.9%)		2 (4.4%)
Yes	6 (13.3%)		2 (4.4%)	1 (2.2%)		1 (2.2%)		2 (4.4%)
N/D							1 (2.2%)	
Regional recurrence								
No	20 (44.4%)	3 (6.7%)	4 (8.9%)	3 (6.7%)	2 (4.4%)	4 (8.9%)		4 (8.9%)
Yes	3 (6.7%)					1 (2.2%)		
N/D							1 (2.2%)	
Distant recurrence								
No	9 (20%)	2 (4.4%)	3 (6.7%)	3 (6.7%)	1 (2.2%)	4 (8.9%)		4 (8.9%)
Yes	14 (31.1%)	1 (2.2%)	1 (2.2%)		1 (2.2%)	1 (2.2%)	1 (2.2%)	
N/D								
IHC								
0	1 (2.2%)			1 (2.2%)				
1	4 (8.9%)		2 (4.4%)	2 (4.4%)	1 (2.2%)	1 (2.2%)		1 (2.2%)
2	14 (31.1%)	2 (4.4%)	2 (4.4%)		1 (2.2%)	2 (4.4%)		3 (6.7%)
3	4 (8.9%)	1 (2.2%)				2 (4.4%)	1 (2.2%)	
AZIN1								
Number of cases (n = 42)	23 (54.8%)	3 (7.1%)	2 (4.8%)	2 (4.8%)	2 (4.8%)	5 (11.9%)	1 (2.4%)	4 (9.5%)
Age								
<65 years	16 (38.1%)	2 (4.8%)	2 (4.8%)	2 (4.8%)	1 (2.4%)	5 (11.9%)	1 (2.4%)	3 (7.1%)
>65 years	7 (16.7%)	1 (2.4%)			1 (2.4%)			1 (2.4%)
Gender								
Male	13 (31%)			2 (4.8%)			1 (2.4%)	1 (2.4%)
Female	10 (23.8%)	3 (7.1%)	2 (4.8%)		2 (4.8%)	5 (11.9%)		3 (7.1%)
T class								
T1	7 (16.7%)	3 (7.1%)			1 (2.4%)			2 (4.8%)
T2	4 (9.5%)							
T3	1 (2.4%)		1 (2.4%)				1 (2.4%)	1 (2.4%)
T4	10 (23.8%)			2 (4.8%)	1 (2.4%)			1 (2.4%)
N/D	1 (2.4%)		1 (2.4%)					

Table 1 *(continued)*

Tumor site	Oral cavity	Oropharynx	Nasopharynx	Paranasal cavities	Larynx	Trachea	Esophagus	Ear
N class								
N0	20 (47.6%)	3 (7.1%)	1 (2.4%)	2 (4.8%)	2 (4.8%)			4 (9.5%)
N1	1 (2.4%)							
N2	1 (2.4%)						1 (2.4%)	
N/D	1 (2.4%)		1 (2.4%)					
M class								
M0	20 (47.6%)	3 (7.1%)	1 (2.4%)	2 (4.8%)	2 (4.8%)		1 (2.4%)	4 (9.5%)
M1	2 (4.8%)							
N/D	1 (2.4%)		1 (2.4%)					
Stage								
I	5 (11.9%)	3 (7.1%)			1 (2.4%)			2 (4.8%)
II	5 (11.9%)							
III	2 (4.8%)		1 (2.4%)				1 (2.4%)	1 (2.4%)
IV	10 (23.8%)			2 (4.8%)	1 (2.4%)			1 (2.4%)
N/D	1 (2.4%)		1 (2.4%)					
Neural invasion								
No	4 (9.5%)	1 (2.4%)			1 (2.4%)	3 (7.1%)	1 (2.4%)	
Yes	17 (40.5%)	2 (4.8%)		1 (2.4%)		1 (2.4%)		4 (9.5%)
N/D	2 (4.8%)		2 (4.8%)	1 (2.4%)	1 (2.4%)	1 (2.4%)		
Primary recurrence								
No	17 (40.5%)	3 (7.1%)	1 (2.4%)	1 (2.4%)	2 (4.8%)	4 (9.5%)		2 (4.8%)
Yes	6 (14.3%)		1 (2.4%)	1 (2.4%)		1 (2.4%)		2 (4.8%)
N/D							1 (2.4%)	
Regional recurrence								
No	20 (47.6%)	3 (7.1%)	2 (4.8%)	2 (4.8%)	2 (4.8%)	4 (9.5%)		4 (9.5%)
Yes	3 (7.1%)					1 (2.4%)		
N/D							1 (2.4%)	
Distant recurrence								
No	15 (35.7%)	2 (4.8%)	1 (2.4%)	2 (4.8%)	1 (2.4%)	4 (9.5%)		4 (9.5%)
Yes	8 (19.0%)	1 (2.4%)	1 (2.4%)		1 (2.4%)	1 (2.4%)	1 (2.4%)	
N/D								
IHC								
0	8 (19.0%)			1 (2.4%)		3 (7.1%)		1 (2.4%)
1	10 (23.8%)	2 (4.8%)	1 (2.4%)	1 (2.4%)	1 (2.4%)	2 (4.4%)	1 (2.4%)	
2	4 (9.5%)	1 (2.4%)	1 (2.4%)					3 (7.1%)
3	1 (2.4%)				1 (2.4%)			

Five (11.1%) tracheal tumors were excluded from the TNM classification.
IHC, immunohistochemistry; M, metastasis; N, node; N/D, not done; T, tumor; 0, Negative or very mild; 1, Mild; 2, Moderate; 3, Strong.

analyzed the location of immunoexpression on the different growth patterns (tubular, cribriform, and solid) and estimated the percentage of positively stained tumor cells. For statistical analysis, we grouped the immunoscores as follows: 0 for 0–10% (negative or very mild), 1 for 11–40% (mild), 2 for 41–70% (moderate), and 3 for 71–100% (strong). Grouping was modified from an earlier publication [26]. We immunoscored the normal salivary gland and apocrine gland tissue outside the tumor to validate expression in normal-appearing tissues.

Statistical analysis

For statistical analysis, staining results were grouped into three categories: 0 (negative or very mild), 1 (mild), and 2–3 (moderate to strong). The associations of AZIN1 and 2 immunoexpression with clinicopathological factors were evaluated by chi-square test and Fisher's exact test. Overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) between AZIN1 and 2 were analyzed by Kaplan–Meier method and log-rank test. p-values below 0.05 were considered statistically significant, and no adjustments were made for multiple testing. Statistical analyses were performed with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

RESULTS

Immunohistochemistry

AZIN2 immunopositivity was cytoplasmic and strongest in the tumors with a tubular growth pattern, followed by cribriform and solid morphology. It seemed that the histologically poorly differentiated tumors (solid growth pattern) showed less immunoexpression. In the normal structures, positivity was seen in mast cells, type-2 pneumocytes, neural structures, acinar structures of the sweat gland, and ductal components of the minor salivary glands. We evaluated AZIN2 immunostaining in 45 tumors; two were scored as negative or very mild, 11 as mild, 24 as moderate, and 8 as strong (Fig. 1).

AZIN1 immunostaining showed cytoplasmic immunopositivity in tumor cells, which was
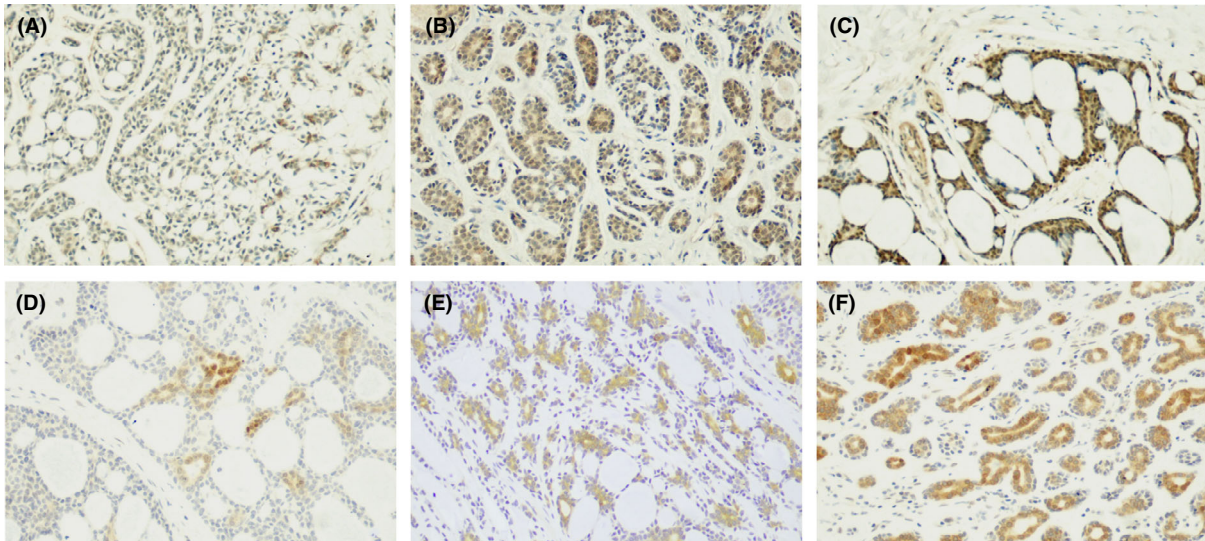
Fig. 1. (A–C) Immunoexpression of AZIN1. (A) Mild immunoexpression of cribriform ACC of the oral cavity; (B) Moderate immunoexpression of tubular ACC of the oral cavity; (C) Strong immunoexpression of cribriform ACC of the ear. (D and E) Immunoexpression of AZIN2. (D) Mild immunoexpression of cribriform ACC of the larynx; (E) Moderate immunoexpression of cribriform ACC of the trachea; (F) Strong immunoexpression of tubular ACC of the trachea. Magnification A–F 200×. ACC, Adenoid cystic carcinoma; AZIN, Antizyme inhibitor.

strongest in the cribriform growth pattern, followed by tubular and solid patterns, but with lower rate of total immunopositivity compared to the AZIN2 staining. Similarity to AZIN2 immunostaining, the poorly differentiated tumors (solid growth pattern) showed less immunoexpression. In the normal tissues, AZIN1 immunopositivity was seen in acinar structures of apocrine glands and in myoepithelial cells, in the tubular structures of the sweat gland, in skeletal muscle cells, in hair follicles, and in the germinal centers of lymphoid tissue. We evaluated AZIN1 immunostaining in 42 tumors; 13 were scored as negative or with very mild expression, 18 as mild, 9 as moderate, and two as strong (Fig. 1).

Clinicopathological parameters and survival analysis

In statistical analysis, stronger immunoexpression of AZIN2 was associated with better OS and DSS in Crosstabs and with better DSS and DFS in Kaplan–Meier (Table 2, Figs 2 and 3). In addition, AZIN2 reached significant correlation with T class when we separated T4a and T4b into subclasses (p = 0.018), although due to the low number of T4b cases we did not include this to Table 2. AZIN1 immunostaining showed no statistically significant association with clinicopathological parameters (Table 2). Age, gender, T class, N class, M class, tumor stage, neural invasion, or recurrent

tumors did not associate significantly with the immunoexpression of AZIN1 and 2 (Table 2).

DISCUSSION

In this pilot study on AZINs in ACC, we found AZIN2 immunoexpression to associate with better prognosis of patients with minor salivary and mucous gland ACC in the head and neck region.

In the seminal work of AZIN2 in colorectal cancer tissue, Kaprio et al. [23] linked high AZIN2 expression to poor prognosis of colorectal cancer patients. However, the specific molecular events remained unclear. In their study, higher expression of AZIN2 was detected in cancers that originated from proximal colon which have more secreting mucinous histology [23]. AZIN2 may be especially important in secreting cancer tissue due to its role in intracellular vesicle transport [9, 20].

ACC originates from glandular tissues such as salivary, mucous, lacrimal, and mammary glands that mainly function to produce secretion [7, 27]. In our study, the histological samples of ACC consisted mostly of cribriform and tubular types. The cribriform growth pattern presents nests of tumor cells and round spaces filled with basophilic myxoid matrix, whereas tubular form is characterized by bilayered tubules with true lumina [1]. Cribriform

Table 2. The evaluation of the association of antizyme inhibitor (AZIN) 1 and 2 immunoexpression with clinicopathological factors by chi-square test or Fisher's exact test

	AZIN2 expression (n (%))				AZIN1 expression (n (%))			
	Negative	Mild	Moderate to Strong	p-Value	Negative	Mild	Moderate to Strong	p-Value
Age								
<65	2 (100.0)	9 (81.8)	24 (75.0)	1.000	12 (92.3)	14 (77.8)	6 (54.5)	0.108
>65	0 (0.0)	2 (18.2)	8 (25.0)		1 (7.7)	4 (22.2)	5 (45.5)	
Gender								
Male	2 (100.0)	4 (36.4)	11 (34.4)	0.229	6 (46.2)	7 (38.9)	4 (36.4)	0.874
Female	0 (0.0)	7 (63.6)	21 (65.6)		7 (53.8)	11 (61.1)	7 (63.6)	
T class								
T1	0 (0.0)	0 (0.0)	13 (48.1)	0.055	2 (22.2)	5 (33.3)	6 (54.5)	0.447
T2	0 (0.0)	1 (12.5)	3 (11.1)		2 (22.2)	2 (13.3)	0 (0.0)	
T3	0 (0.0)	2 (25.0)	3 (11.1)		0 (0.0)	2 (13.3)	2 (18.2)	
T4	2 (100.0)	5 (62.5)	8 (29.6)		5 (55.6)	6 (40.0)	3 (27.3)	
N class								
N0	2 (100.0)	8 (100.0)	24 (88.9)	1.000	9 (100.0)	13 (86.7)	10 (90.9)	0.773
N+	0 (0.0)	0 (0.0)	3 (11.1)		0 (0.0)	2 (13.3)	1 (9.1)	
M class								
M0	2 (100.0)	8 (100.0)	25 (92.6)	1.000	9 (100.0)	14 (93.3)	10 (90.9)	1.000
M+	0 (0.0)	0 (0.0)	2 (7.4)		0 (0.0)	1 (6.7)	1 (9.1)	
Stage								
I	0 (0.0)	0 (0.0)	11 (40.7)	0.126	2 (25.0)	4 (26.7)	5 (45.5)	0.766
II	0 (0.0)	1 (12.5)	4 (14.8)		2 (25.0)	2 (13.3)	1 (9.1)	
III	0 (0.0)	2 (25.0)	4 (14.8)		0 (0.0)	3 (20.0)	2 (18.2)	
IV	2 (100.0)	5 (62.5)	8 (29.6)		4 (50.0)	6 (40.0)	3 (27.3)	
Neural invasion								
No	0 (0.0)	2 (22.2)	8 (30.8)	1.000	3 (25.0)	6 (37.5)	1 (14.3)	0.628
Yes	1 (100.0)	7 (77.8)	18 (69.2)		9 (75.0)	10 (62.5)	6 (85.7)	
Primary recurrence								
No	1 (50.0)	6 (54.5)	24 (77.4)	0.249	10 (76.9)	12 (70.6)	8 (72.7)	1.000
Yes	1 (50.0)	5 (45.5)	7 (22.6)		3 (23.1)	5 (29.4)	3 (27.3)	
Regional recurrence								
No	2 (100.0)	10 (90.9)	28 (90.3)	1.000	11 (84.6)	15 (88.2)	11 (100.0)	0.544
Yes	0 (0.0)	1 (9.1)	3 (9.7)		2 (15.4)	2 (11.8)	0 (0.0)	
Distant recurrence								
No	1 (50.0)	5 (45.5)	23 (71.9)	0.220	8 (61.5)	13 (72.2)	8 (72.7)	0.835
Yes	1 (50.0)	6 (54.5)	9 (28.1)		5 (38.5)	5 (27.8)	3 (27.3)	
OS								
Alive	0 (0.0)	2 (18.2)	21 (65.6)	0.006	6 (46.2)	10 (55.6)	7 (63.6)	0.690
Dead	2 (100.0)	9 (81.8)	11 (34.4)		7 (53.8)	8 (44.4)	4 (36.4)	
DSS								
Alive	0 (0.0)	3 (27.3)	24 (75.0)	0.004	8 (61.5)	11 (61.1)	9 (81.8)	0.500
Dead	2 (100.0)	8 (72.7)	8 (25.0)		5 (38.5)	7 (38.9)	2 (18.2)	
DFS								
No	0 (0.0)	3 (27.3)	17 (53.1)	0.151	7 (53.8)	8 (44.4)	6 (54.5)	0.823
Yes	2 (100.0)	8 (72.7)	15 (46.9)		6 (46.2)	10 (55.6)	5 (45.5)	

Total number of cases submitted to immunohistochemistry (IHC) for AZIN2 is 45. Age, gender, distant recurrence, OS, DSS, DFS: IHC = 45/45; TNM and stage: IHC = 37/45, N/D = 8; Neural invasion: IHC = 36/45, N/D = 9; Primary and regional recurrence: IHC = 44/45, N/D = 1. Total number of cases submitted to immunohistochemistry (IHC) for AZIN1 is 42: Age, gender, distant recurrence, OS, DSS, DFS: IHC = 42/42; TNM and stage: IHC = 35/42, N/D = 7; Neural invasion: IHC = 35/45, N/D = 10; Primary and regional recurrence IHC = 41/45, N/D = 4.
DFS, disease-free survival; DSS, disease-specific survival; M, metastasis; N, node; OS, overall survival; T, tumor.

and tubular ACC tissues are reported as highly differentiated as they have secretory functions. In our material, cribriform and tubular tumor tissue showed high expression of AZIN2, which could be related to active vesicle transport in tumor cells. On the other hand, solid ACC consists of sheets of tumor cells without lumen formation and is graded as poorly differentiated tumor with more aggressive behavior [1]. The solid ACC samples showed no or modest immunoexpression of AZIN2, and it seems that the tumor tissue might have lost the main function of the originating tissue. Overall, histologically it seems that a high amount of AZIN2 expression is beneficial for patients since high
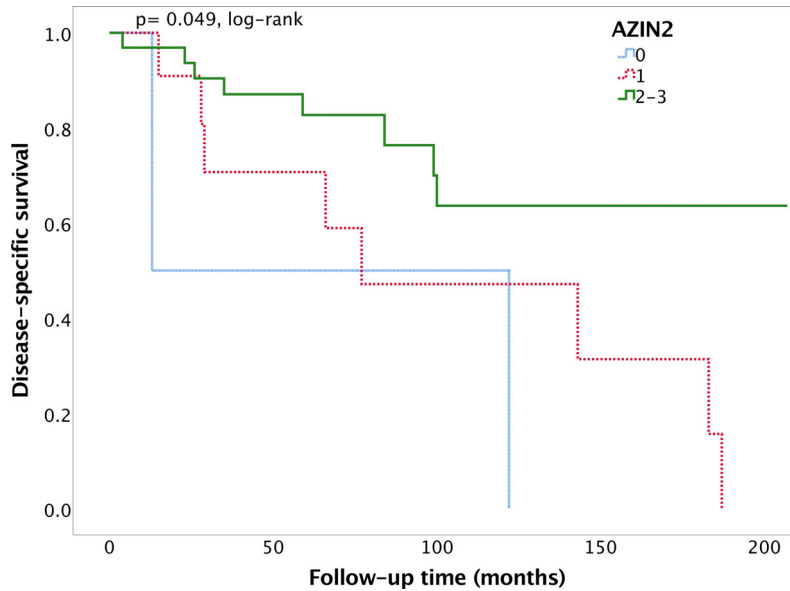
Fig. 2. Disease-specific survival for adenoid cystic carcinoma patients. Kaplan–Meier plot showing better prognosis for tumors with moderate to strong AZIN2 immunopositivity. Total number of cases is 45. 0 as negative or very mild = 2/45; 1 as mild = 11/45; 2–3 as moderate to strong = 32/45.
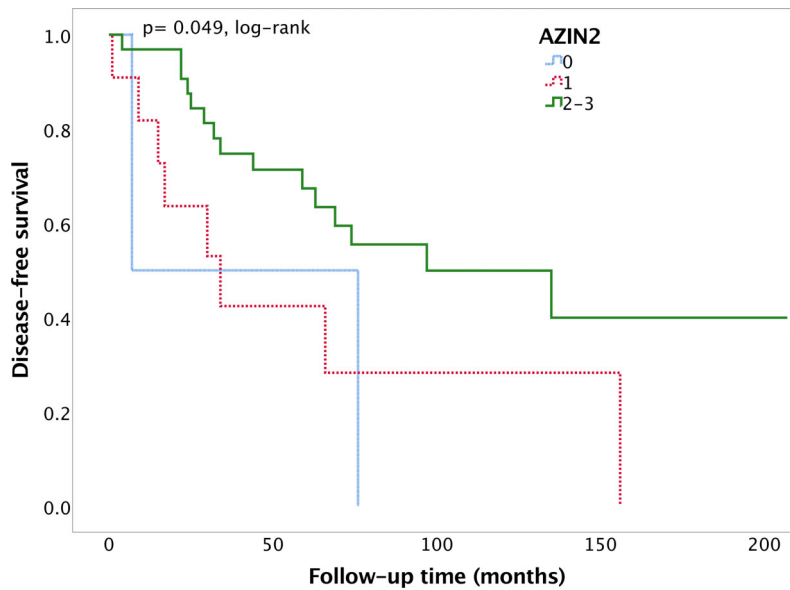

Fig. 3. Disease-free survival for adenoid cystic carcinoma patients. Kaplan–Meier plot showing better prognosis for tumors with moderate to strong AZIN2 immunopositivity. Total number of cases is 45. 0 as negative or very mild = 2/45; 1 as mild = 11/45; 2–3 as moderate to strong = 32/45.

immunoexpression of AZIN2 was related to smaller tumor size and better survival. Nonetheless, further studies are needed to reveal the ultimate functions of AZIN2 in ACC.

Disturbance in RNA editing has been shown to take part in carcinogenesis [28]. In normal cells, AZIN1 regulates the cell cycle and stimulates cell proliferation and edited gene levels of AZIN1 have

been detected in cancer tissue [28]. Thus, disturbances in the AZIN1 gene may induce cell proliferation and enhance tumorigenesis [10, 18, 28]. Upregulation of AZIN1 has been shown in various cancers such as gastric, ovarian, breast, prostate, lung, and colorectal cancers [17-19]. Lung cancer study demonstrated that both *in vitro* and *in vivo* AZIN1 RNA editing induced proliferation, invasion, and migration [18]. In addition, colorectal cancer tissue showed higher levels of edited AZIN1 RNA compared with normal tissue, which might contribute to poorer patient survival [19]. Further, dysregulation of edited AZIN1 seemed to predict lymph node metastasis, hepatic metastasis, and distant metastasis in colorectal cancer [19].

In our study, AZIN1 showed no association with clinical parameters or survival of ACC. Like most salivary gland tumors, ACC is a slow-growing tumor and has several histological growth patterns. High mitotic activity has been linked to unfavorable prognosis in ACC patients [29]. Cell proliferation in solid-type tumors has been shown to be higher, and behavior more aggressive, compared with cribriform and tubular types that might partly explain our results [30]. Only a small number of solid tumors (8.8%) were present in the present ACC series, and the growth pattern did not affect patient survival in our previous report [24]. Although we expected to find the opposite behavior of AZIN1 compared to AZIN2, the expression of AZIN1 was not associated with clinical parameters or patient survival. In the vicinity of ACC tissue, the normal-appearing salivary gland tissue did not immunoexpress AZINs. However, we did not study any salivary gland tissue samples which would have been totally free of any carcinomatous signs.

Among the genes related to the development of cancer, the *MYC* gene family (*c-MYC, MYCN, and MYCL*) is one of the most studied, and their oncogenic functions correlate with tumor growth and poor prognosis [31]. In addition, the C-myc signaling pathway drives carcinogenesis of ACC, and a recent study has shown promising therapeutic results by controlling this pathway [32]. *MYC* upregulates ODC activity and polyamine levels and *MYC* oncogenes may contribute to tumor hyperproliferation, especially of *MYC*-driven cancers [31]. It seems that AZ2 may control the proteasomal degradation of MYC and ODC, which is connected to tumor formation [31].

LIMITATIONS AND STRENGTHS

One limitation of the current study is that the tissue material is partly old and it has been used a lot. The study comprises all minor salivary and mucous gland ACC of 38-year period in the Helsinki University Hospital district which we consider as a strength of the study.

CONCLUSIONS

Although AZIN1 has been shown to have a role in several cancers, our present study adds knowledge to the behavior of AZIN2 in ACC tissue. AZs and AZINs may have therapeutic potential in the future as these enzymes are linked to tumorigenesis. It seems that AZIN2 expression is higher in cancer tissue with secretory functions. In ACC tissue, high AZIN2 expression could be related to a well-differentiated histological type which still has a functioning vesicle transportation system. Thus, AZIN2 could be a prognostic factor for better survival of ACC patients.

CONFLICT OF INTEREST

None.

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