



Ataxia-Telangiectasia-Mutated Protein Expression as a Prognostic Marker in Adenoid Cystic Carcinoma of the Salivary Glands

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Purpose: Adenoid cystic carcinoma (ACC) is a high-grade malignant tumor of the salivary glands, clinically characterized by multiple recurrences and late distant metastasis. Biological markers for assessing the prognosis of ACC have remained elusive. The purpose of this study was to investigate whether the protein expressions of ataxia telangiectasia mutated (ATM), p53, and ATM-mediated phosphorylated p53 are related to patient survival in ACC.

Materials and Methods: In this study, 48 surgical samples were used to assess the expressions of ATM and its downstream target p53. Fisher's exact test and Kaplan-Meier analysis were conducted to evaluate the role of ATM, p53, and phospho-p53 (S15) protein expressions in predicting patient survival and distant metastasis.

Results: Myb expression was positive in 85.4% of ACCs, but did not reflect patient survival rate. In contrast, low expression of ATM in cancer cells was significantly correlated with poor survival rate ($p=0.037$). Moreover, under positive p53 expression, low expression of ATM was highly predictive of poor survival in ACC ($p=0.017$).

Conclusion: These data indicate that combined assessment of ATM and p53 expression can serve as a useful prognostic marker for assessing survival rate in patients with ACC of the salivary glands.

Key Words: Adenoid cystic carcinoma, ataxia telangiectasia mutated (ATM), p53, prognostic marker, salivary gland

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•The authors have no financial conflicts of interest.

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INTRODUCTION

Adenoid cystic carcinoma (ACC) is a locally aggressive salivary gland neoplasm characterized by its long clinical history with multiple recurrences and late distant metastasis.¹ Distant metastasis can develop over more than 10 years after the initial treatment, and 10-year survival has been shown to decrease by 30% in ACC patients with distant metastasis. Distant metastasis has often been associated with advanced stage tumors, although one study reported occurrence of distant metastasis in 47% of patients with early stage ACC, suggesting that distant metastasis can readily occur in ACC patients regardless of the tumor stage.²

One of the main oncogenic drivers of ACC is the tumor type-

specific chromosomal translocation t(6;9) (q22-23;p23-24) resulting in *Myb-NFIB* fusion oncogene. *Myb-NFIB* fusion contributes to Myb overexpression and activation of critical Myb target genes, including those associated with apoptosis, cell cycle control, cell growth, angiogenesis, and cell adhesion.³ Recent studies in surgical pathology have shown that overexpression of Myb occurs in most ACCs (89%), including ACCs without *Myb-NFIB* fusion, suggesting Myb expression as a useful marker for ACC.^{4,5} Despite Myb activation being an important diagnostic marker of ACC, its value as a prognostic marker was rather limited.³

Ataxia telangiectasia mutated (ATM) is a 370-kd protein belonging to a family of phosphoinositide 3-kinases with roles in DNA processing, regulation of the cell cycle, and control of telomere length. The principal function of ATM is the integration of cellular responses to DNA double-strand breaks.⁶ Homozygous mutation of the *ATM* gene results in the loss of detectable protein, triggering ataxia-telangiectasia (A-T),⁷ an autosomal recessive disorder characterized by neurological and immunological symptoms, radiosensitivity, and predisposition to cancer, particularly of the lymphoid system.^{8,9} Accumulating evidence indicates that A-T heterozygotes also have increased risk of developing cancer, breast cancer in particular, for which female ATM carriers have increased risk, compared with the general population.¹⁰ Intriguingly, a recent study demonstrated that ATM expression in cancer-associated stroma, as well as cancer cells, can serve as an independent prognostic marker in early stage breast cancer.¹¹ Loss of ATM expression has also been associated with poor prognosis in pancreatic cancer and gastric cancer.^{12,13}

As a downstream target protein of ATM, p53 is recognized as the most common tumor suppressor in human malignancies. The mutational status of p53 is an independent unfavorable prognostic marker in many malignancies.¹⁴ Due to the lack of biological markers for assessing the prognosis of ACC patients, this study attempted to investigate the role of ATM and p53 expressions in predicting the survival of ACC patients. The prognostic biomarkers obtained in this study will likely facilitate more accurate assessment of ACC patients, thereby allowing more timely application of proper treatments and improving prognosis.

MATERIALS AND METHODS

Human tissue samples

This study included 48 surgical samples identified as ACC of the salivary glands at the Department of Oral Pathology, Dental Hospital, Yonsei University from 1990–2015. This study was approved by the Institutional Review Board of the Faculty of Dental Sciences, Yonsei University, South Korea (2-2015-0031). The clinical information of all patients was collected retrospectively through medical records (Table 1). All cases were con-

firmed histologically in accordance with the guidelines of the latest World Health Organization classification of salivary gland tumors, based on hematoxylin and eosin-stained tissue sections.¹⁵ There was no age, sex, ethnicity, or tumor stage restriction on patient enrollment. Follow-up was calculated as the time from the first visitation at the institution for the primary tumor to the latest follow-up or deceased date.

Immunohistochemical staining

The staining was performed on 4- μ m-thick sections using an EnVision-HRP detection system (Dako, Carpinteria, CA, USA). All procedures were performed at room temperature. Sections were deparaffinized through a series of xylene baths and rehydrated in graded concentrations of alcohol. To retrieve antigenicity, the slides were steamed with 10 mmol/L of citrate buffer (pH 6.0; DakoREAL™). Tissue sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity, followed by incubation in 5% bovine serum albumin. The sections were then incubated with primary antibodies diluted with phosphate buffered saline for 90 min in a humid chamber. All information on primary antibodies is listed on Supplementary Table 1 (only online). The slides were then incubated with secondary EnVision reagent (Dako) for 30 min, followed by incubation with diaminobenzidine chromogen. Next, the sections were counterstained with Mayer hematoxy-

Table 1. Clinico-Pathological Characteristics of Patients

Parameter	No. of patients	Myb positivity n (%)	p value
Sex			0.175
Woman	23 (47.9)	18 (78.3)	
Man	25 (52.1)	23 (92)	
Age (yr)			0.403
<55	26 (54.2)	23 (88.5)	
≥55	22 (45.8)	14 (63.6)	
Tumor site			0.523
Parotid gland	3 (6.3)	3 (100)	
Submandibular gland	8 (16.7)	5 (62.5)	
Sublingual gland	1 (2.1)	1 (100)	
Palate	23 (47.9)	17 (73.9)	
Upper lip	2 (4.2)	1 (50)	
Lower lip	1 (2.1)	1 (100)	
Tongue	2 (4.2)	1 (100)	
Buccal	1 (2.1)	0 (0)	
Retromolar area	1 (2.1)	0 (0)	
Floor of mouth	6 (12.5)	5 (83.3)	
Histology			0.011
Solid	13 (27.1)	8 (61.5)	
Tubular and cribriform	35 (72.9)	33 (94.3)	
Perineural invasion	29 (60.4)	21 (72.4)	0.739
Total no. of patients	48 (100)	41 (85.4)	

p values from Fisher's exact test are provided.

lin for visualization. The phosphate-buffered saline solution without primary antibody treatment was applied as the negative control for all antibody staining. Human lymphocytes and normal salivary glands were used as positive controls for ATM staining as recommended by Villaruz, et al.¹⁶ A paraffin-embedded breast cancer cell line was used to determine appropriate dilution ratio and as a positive control for p53 and phospho-p53 (S15).

With a cutoff of >25% positive cells, positivity of nuclear Myb expression was determined according to previously reported guidelines,¹⁷ whereas “loss of ATM expression” was determined as tumors showing >25% ATM-negative cells.¹⁸ Because mutant p53 proteins generally have longer half-lives than wild-type p53 protein, which leads to their nuclear accumulation, tumors showing >20% nuclear p53 or phospho-p53 (S15)-positive tumor cells were determined as having positive p53 or phospho-p53 (S15) expression.¹⁹

Statistical analysis

Correlation between clinical findings and patient survival was analyzed by Fisher’s exact test. Cumulative survival probabilities were estimated using the Kaplan-Meier method. All statistical analyses were performed using statistical software (SPSS version 21.0, IBM Corp., Armonk, NY, USA), and the level of statistical significance was set at $p < 0.05$.

RESULTS

Characteristics of the patients

The clinicopathological characteristics of the patients are listed in Table 1. The age range of the patients was 26–71 years; median age at diagnosis was 55 years. Among the 48 patients, 25 (52.1%) were male; 26 (54.2%) were younger than 55 years old. Thirty-six tumors (75%) were found in minor salivary glands, and most of them (47.9%) were in the palate, followed by the submandibular gland (16.7%), floor of the mouth (12.5%), and parotid glands (6.3%). Histologically, 13 tumors (27.1%) were diagnosed as ACC solid type; 35 tumors (72.9%) were diagnosed as ACC tubular and cribriform type. perineural invasion was found in 29 cases (60.4%) of ACC samples.

The median follow-up period for the patient population was 5 years. During that time, local recurrence and distant metastasis were identified in two (4.2%) and 18 (37.5%) of 48 patients, respectively. Five (10.4%) of the 48 patients died during follow-up (Table 2). Of the deceased patients, four (80%) were older than 55 years, and all tumors were found in minor salivary glands: two of them were solid type, three of them had perineural invasion, and four of them showed distant metastasis. Since the hallmarks of poor prognosis are solid histological type, perineural invasion, and distant metastasis,² Kaplan-Meier analysis was performed to evaluate whether these factors af-

Table 2. Clinico-Pathological Characteristics of Patients Showing Poor Prognosis

Parameter	No. of patients (%)		
	Recurrence	Distant metastasis	Death due to ACC
Sex			
Woman	-	8 (44.4)	3 (60)
Man	2 (100)	10 (55.6)	2 (40)
Age (yr)			
<55	-	11 (61.1)	1 (20)
≥55	2 (100)	7 (38.9)	4 (80)
Tumor site			
Parotid gland	-	2 (11.1)	-
Submandibular gland	-	1 (5.6)	-
Sublingual gland	-	1 (5.6)	-
Palate	2 (100)	10 (55.6)	2 (40)
Upper lip	-	-	-
Lower lip	-	-	-
Tongue	-	1 (5.6)	-
Buccal	-	-	1 (20)
Retromolar area	-	-	-
Floor of mouth	-	3 (16.7)	2 (40)
Histology			
Solid	2 (100)	5 (27.8)	2 (40)
Tubular and cribriform	-	13 (72.2)	3 (60)
Perineural invasion	2 (100)	14 (77.8)	3 (60)
All patients	2 (100)	18 (100)	5 (100)

ACC, adenoid cystic carcinoma.

ected prognosis. Histological type and perineural invasion were not significantly correlated with survival rate, whereas distant metastasis was found to be significantly correlated with poor survival ($p=0.044$) (Supplementary Fig. 1A-C, only on-line).

Myb expression pattern in ACC

All 48 ACC patient samples were screened for Myb staining. Myb expression was not detected in nontumoral salivary gland parenchyma. Overall, 41 of 48 ACC samples (85.4%) were positively stained for Myb. Myb expression was detected in the nucleus and ranged from negative to strongly positive in intensity. In tubular foci, positive signals were predominantly found in myoepithelial cells (Fig. 1). Myb expression did not significantly differ depending on the anatomic site of tumors or clinical features of the patients. However, Myb expression differed significantly based on the histological type of ACC (Table 1). Thirty-three cases of tubular and cribriform ACC

(94.3%) showed Myb positivity, whereas eight cases of solid type ACC (61.5%) showed Myb positivity ($p=0.011$), according to Fisher’s exact test. Myb expression had no significant effect on survival rate when Kaplan-Meier analysis was performed (Supplementary Fig. 1D, only online).

Relationship between ATM levels and patient outcomes in ACC

Antibody specificity was validated using human lymph node tissue as a positive control. For negative control, the entire procedure was conducted with the omission of ATM antibody (Fig. 2A and B). For normal salivary glands, ATM protein was detected in the nucleus of acinar and ductal cells (Fig. 2C). ATM expression was reduced/absent in cancer cells and stromal fibroblasts in 58.3% and 60.4% of patients, respectively, compared with that in adjacent normal tissue (Fig. 2D-G). ATM levels in cancer cells were negatively correlated with age ($p=0.003$). Moreover, perineural invasion was significantly associ-

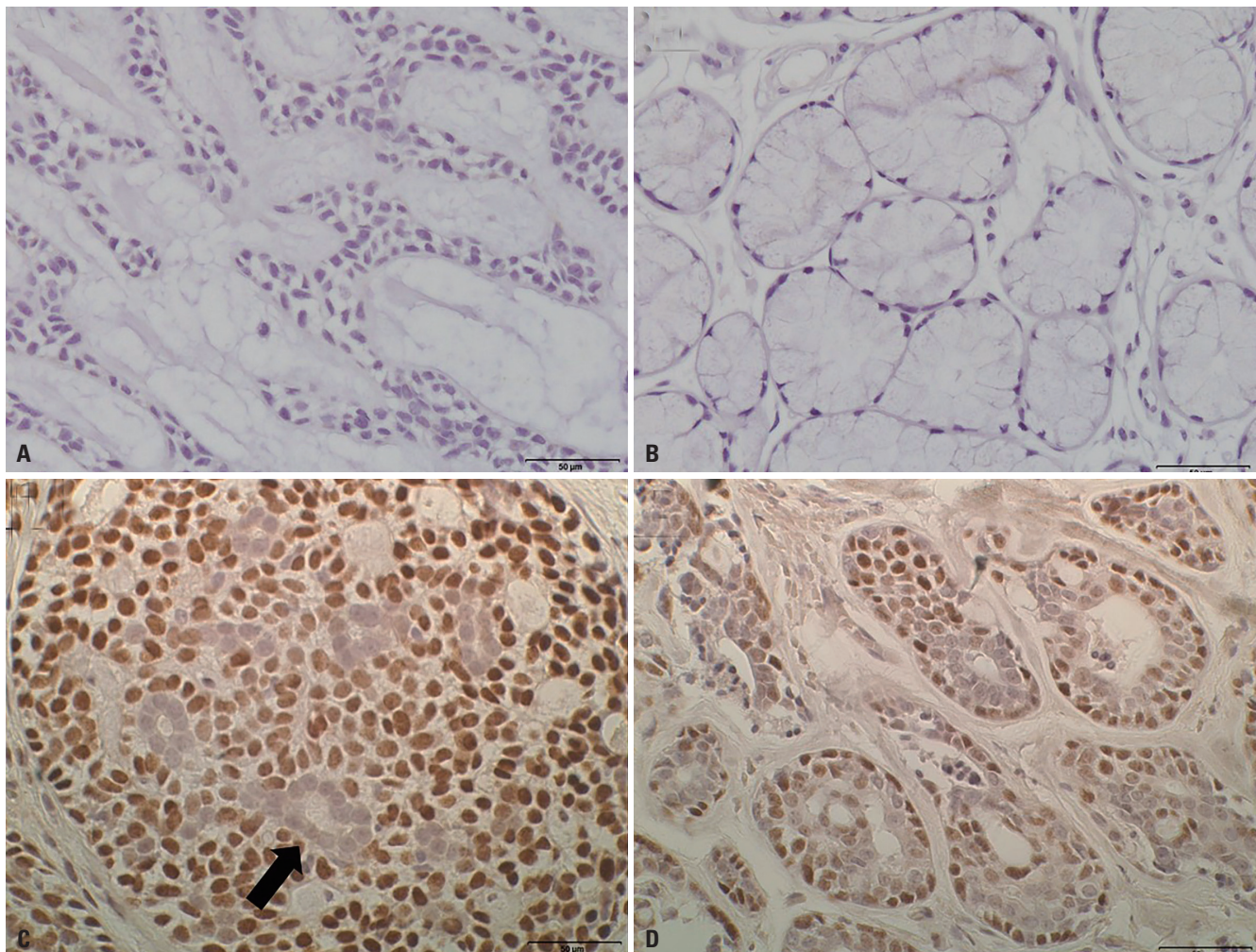


Fig. 1. Myb expression in ACC. (A) Negative control was obtained by the omission of Myb antibody in ACC. (B) Myb expression was not detected in non-tumoral salivary gland parenchyma. (C) Myb expression in ACC, solid type. Myb expression was detected in myoepithelial cells, but not in ductal cells (indicated by the black arrow). (D) Myb expression in ACC, tubular and cribriform types. Original magnification of all figures: $\times 400$. ACC, adenoid cystic carcinoma.

ated with the loss of ATM expression in stromal fibroblasts ($p=0.008$), according to Fisher's exact test, as shown in Table 3. Kaplan-Meier analysis revealed that low ATM expression in cancer cells was correlated with poor survival ($p=0.037$). Low ATM expression in stromal fibroblasts, however, was not significantly related to patient survival (Fig. 3A and B). Furthermore, low ATM expression, either in cancer cells or stromal fibroblasts, was not significantly correlated with metastasis-free survival (Fig. 3C and D).

Relationship among p53, phospho-p53 (S15), and patient outcomes in ACC

Of the 48 patients, 38 (79.2%) had p53 expression and 30 (62.5%) had phospho-p53 (S15) expression (Fig. 4). No positive correlation was observed between the expression patterns of these proteins and clinical findings, except for histological type, according to Fisher's exact test ($p=0.016$) (Table 3). Although statistical significance was not attained, positive p53 expression or negative phospho-p53 (S15) expression

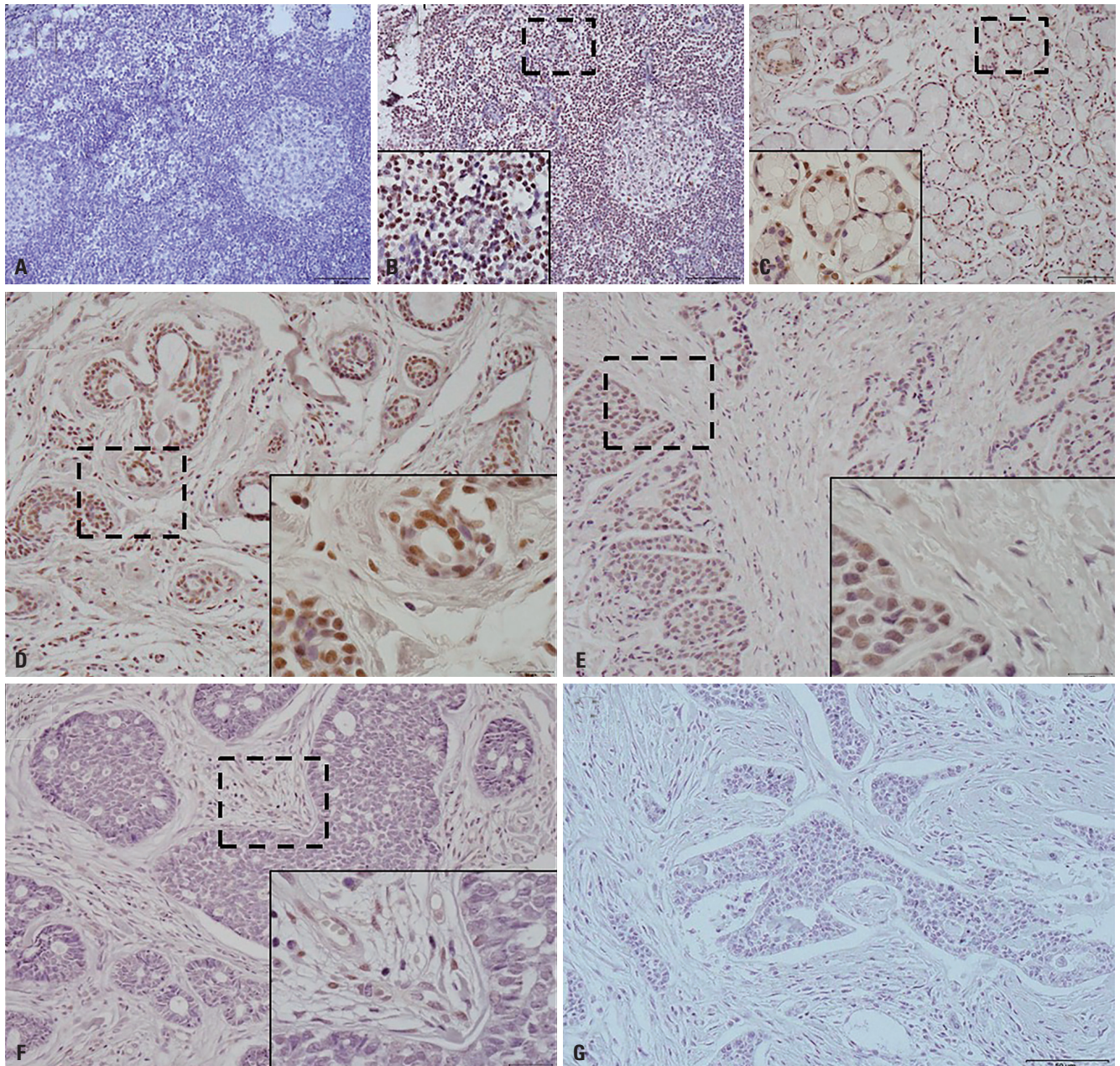


Fig. 2. ATM expression in ACC. (A) Negative control was obtained by the omission of ATM antibody. (B) Positive control: ATM antibody-specific staining was performed in human lymph nodes. (C) ATM expression was observed in the nuclei of acinar and ductal cells in normal salivary glands. (D) ATM positivity in cancer cells and stromal fibroblasts. (E) ATM was positive in cancer cells and negative in stromal fibroblasts. (F) ATM was positive in stromal fibroblasts and negative in cancer cells. (G) ATM was negative in both cancer cells and stromal fibroblasts. Original magnification of all figures: $\times 200$. Inset original magnification $\times 1000$. ATM, ataxia telangiectasia mutated; ACC, adenoid cystic carcinoma.

Table 3. Relationships among ATM, p53, and Phospho-p53 (S15) Expressions and Patient Outcomes in ACC

Parameter	No. of patients	ATM loss in cancer	ATM loss in stroma	Positive expression of p53 expression	Positive expression of phospho-p53 (S15)
	n (%)	n (%)	n (%)	n (%)	n (%)
Sex					
Woman	23 (47.9)	15 (65.2)	16 (69.6)	16 (69.6)	9 (39.1)
Man	25 (52.1)	13 (52)	13 (52)	22 (88)	9 (36)
<i>p</i> value		0.263	0.172	0.112	0.529
Age (years old)					
<55	26 (54.2)	10 (38.5)	14 (53.8)	21 (80.8)	9 (34.6)
≥55	22 (45.8)	18 (81.8)	15 (68.2)	17 (77.3)	9 (40.9)
<i>p</i> value		0.003	0.238	0.521	0.440
Tumor site					
Parotid gland	3 (6.3)	1 (33.3)	2 (66.7)	3 (100)	0 (0)
Submandibular gland	8 (16.7)	4 (50)	5 (62.5)	6 (75)	6 (75)
Sublingual gland	1 (2.1)	1 (100)	1 (100)	1 (100)	1 (100)
Palate	23 (47.9)	14 (60.9)	15 (65.2)	17 (73.9)	8 (34.8)
Upper lip	2 (4.2)	1 (50)	1 (50)	2 (100)	1 (50)
Lower lip	1 (2.1)	0 (0)	0 (0)	1 (100)	0 (0)
Tongue	2 (4.2)	2 (100)	1 (50)	2 (100)	0 (0)
Buccal	1 (2.1)	0 (0)	0 (0)	1 (100)	0 (0)
Retromolar area	1 (2.1)	0 (0)	0 (0)	1 (100)	0 (0)
Floor of mouth	6 (12.5)	5 (83.3)	4 (66.7)	4 (66.7)	2 (33.3)
<i>p</i> value		0.475	0.863	0.992	0.219
Histology					
Solid	13 (27.1)	10 (76.9)	8 (61.5)	7 (53.8)	4 (30.8)
Tubular and cribriform	35 (72.9)	18 (51.4)	21 (60)	31 (88.6)	14 (40)
<i>p</i> value		0.102	0.597	0.016	0.406
Perineural invasion	29 (60.4)	17 (58.6)	22 (75.9)	23 (79.3)	11 (37.9)
		0.597	0.008	0.624	0.592
Distant metastasis	18 (37.5)	13 (72.2)	14 (77.8)	13 (72.2)	6 (33.3)
		0.113	0.053	0.287	0.442
Death due to ACC	5 (11.6)	5 (100)	3 (60)	5 (100)	0 (0)
		0.057	0.667	0.293	0.083
Total patients	48 (100)	28 (58.3)	29 (60.4)	38 (79.2)	18 (37.5)

ATM, ataxia telangiectasia mutated; ACC, adenoid cystic carcinoma. *p* values from Fisher’s exact test are provided.

showed tendencies of being correlated with poor survival rate (Fig. 5A and B).

Upon analyzing ATM expression stratified by positive p53 expression or negative phospho-p53 expression, loss of ATM and positive p53 expression significantly reduced overall survival ($p=0.017$) (Fig. 5C). Loss of ATM and negative phospho-p53 expression was associated with worse prognosis, although statistical significance was not attained (Fig. 5D). In addition, loss of ATM, given positive p53 or negative phospho-p53 expression, reduced metastasis-free survival, although statistical significance was not attained (Supplementary Fig. 2, only online). As shown in Table 3, all five deceased patients exhibited a loss of ATM expression, as well as positive p53 and negative phospho-p53 expression, confirming that these markers can

predict poor prognosis in ACC patients.

DISCUSSION

ATM is activated within seconds to minutes upon exposure to ionizing radiation and can phosphorylate various downstream substrates, some of which play crucial roles in regulating cell cycle arrest, DNA repair, and apoptosis.²⁰ To illustrate, ATM is an upstream regulator of p53, a well-known tumor suppressor: ATM modulates progression of the cell cycle and apoptosis via activation and stabilization of p53.²¹ In response to ionizing radiation, the kinase activity of ATM is enhanced, leading to phosphorylation of p53 on serine 15 and activation of the

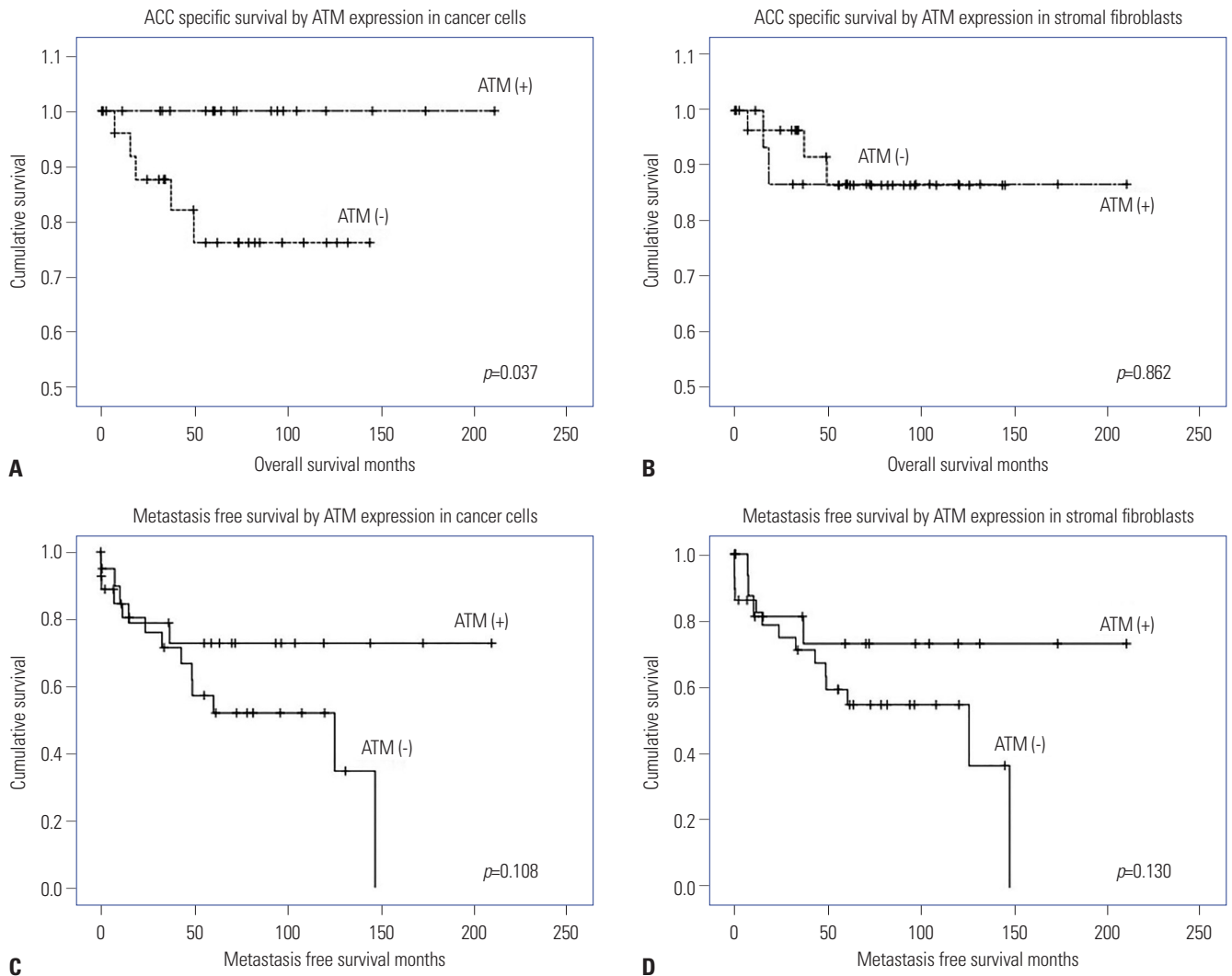


Fig. 3. Overall survival rates according to ATM expression in cancer cells (A) and stromal fibroblasts (B). Metastasis-free survival rates according to ATM expression in cancer cells (C) and stromal fibroblasts (D). ATM, ataxia telangiectasia mutated; ACC, adenoid cystic carcinoma.

checkpoint kinase Chk2, which, in turn, phosphorylates p53 on serine 20. These interactions result in stabilization and activation of p53:^{22,23} serine 15, threonine 18, and serine 20, being key phosphorylation sites of p53, are involved not only in stimulating p53's interaction with the transcriptional machinery but also in inhibiting MDM-mediated repression of p53.²⁴

Mutated p53 is often characterized by increased half-life, as it inhibits its own degradation by the ubiquitin complex.²⁵ Therefore, the level of p53 can be modulated both by the ATM-dependent pathway and the mutational status of p53 itself. The mutational status of p53 itself is an independent unfavorable prognostic marker in many malignancies.¹⁴ In terms of ACC, patients with p53-positive expression had a greater chance of developing metastasis, as well as poorer 5-year overall survival.²⁶ In our study, positive p53 expression showed a tendency of being correlated with poor survival, even though statistical significance was not attained.

This study focused on investigating whether the expression

patterns of ATM and p53 and its ATM-mediated phosphorylated form are associated with patient survival. The prognostic role of ATM and p53 has previously been demonstrated in human malignancies, including breast,²² pancreas,¹² lung,¹⁶ and stomach cancer,²⁷ but data thereof in ACC are lacking. All these studies have shown that ATM expression, either independently or in combination with p53 expression, is correlated with patient outcome. In our study, loss of ATM expression in ACC cancer cells was significantly associated with reduced patient survival. In addition, p53-positive expression or phospho-p53 (S15)-negative expression was weakly correlated with poor survival. Interestingly, combined analysis of ATM and p53 had better predictability than individual analysis of each protein. Given positive p53 or negative phospho-p53 (S15) expression, loss of ATM was associated with worse survival. The loss or reduced expression of ATM may render cells susceptible to irreversible DNA damage and subsequent cancerization, ultimately facilitating cancer progression as the cells lose control of differenti-

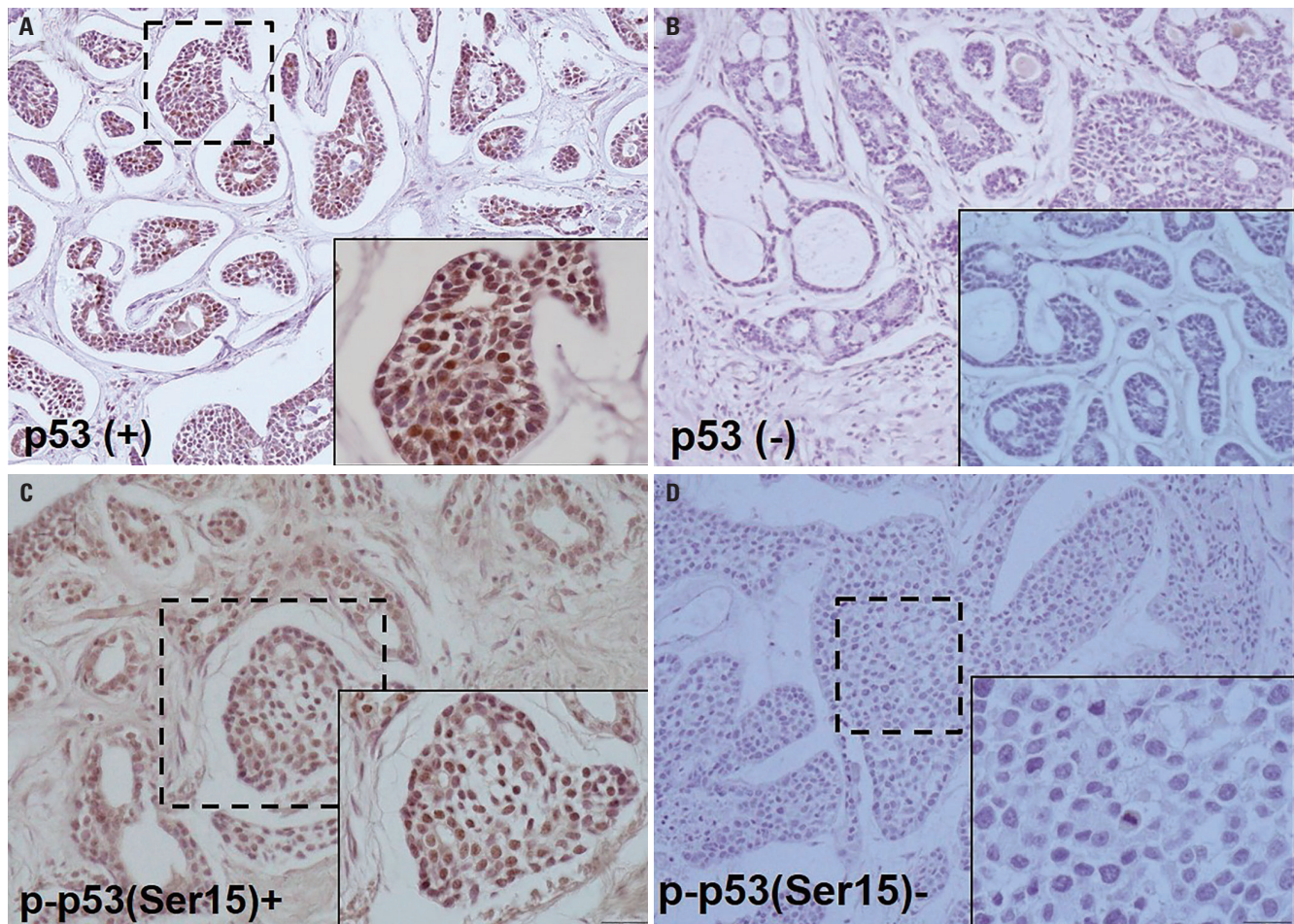


Fig. 4. Expression of p53 and phospho-p53 (S15) in adenoid cystic carcinoma. Positive expression of p53 (A) and negative expression of p53 (B). Positive expression of phospho-p53 (S15) (C) and negative expression of phospho-p53 (S15) (D). Original magnification of all figures: $\times 200$. Inset: original magnification $\times 1000$.

ation and proliferation.²⁸

Several researchers have retrospectively studied the clinicopathological features of ACC, attempting to identify significant prognostic factors; however, findings remain controversial. Solid histological type and perineural invasion have been considered as important indicators of poor prognosis in ACC patients.² In particular, the presence of perineural invasion can result in higher rates of recurrence.^{29,30} In our study, however, the prognostic value of perineural invasion was not significant, possibly due to a small sample size limited to minor salivary glands and relatively short follow-up period.

Of note, ATM loss in stroma was strongly correlated with perineural invasion in our study, even though ATM loss in stroma was not correlated with patient survival. As ATM loss in stroma can serve as an independent prognostic marker in early stage breast cancer,¹¹ further study needs to be conducted to unravel the mechanism underlying the association between the loss of ATM in stromal fibroblasts and perineural invasion.

In accordance with previous studies, *Myb-NFIB* fusion was not expressed in non-ACC neoplasms of the head and neck,

confirming the high specificity of *Myb-NFIB* fusion for ACC.⁴ All 48 ACC patient samples were screened for Myb staining: 85.4% were positively stained for Myb, whereas Myb staining was negative in nontumoral salivary gland parenchyma. Myb expression was not significantly associated with patient survival, confirming that Myb is a suitable diagnostic marker for ACC, but not a prognostic marker.

Taken together, expression levels of ATM in cancer cells alone or in combination with positive p53 expression hold strong prognostic value for predicting the overall survival of ACC patients, and thus could serve as promising biomarkers to assess patient outcomes. Prognostic values of well-known clinical factors, perineural invasion, and histological types were not evident in this study, possibly due to small sample size and relatively short follow-up period. Additional studies with a larger sample are warranted.

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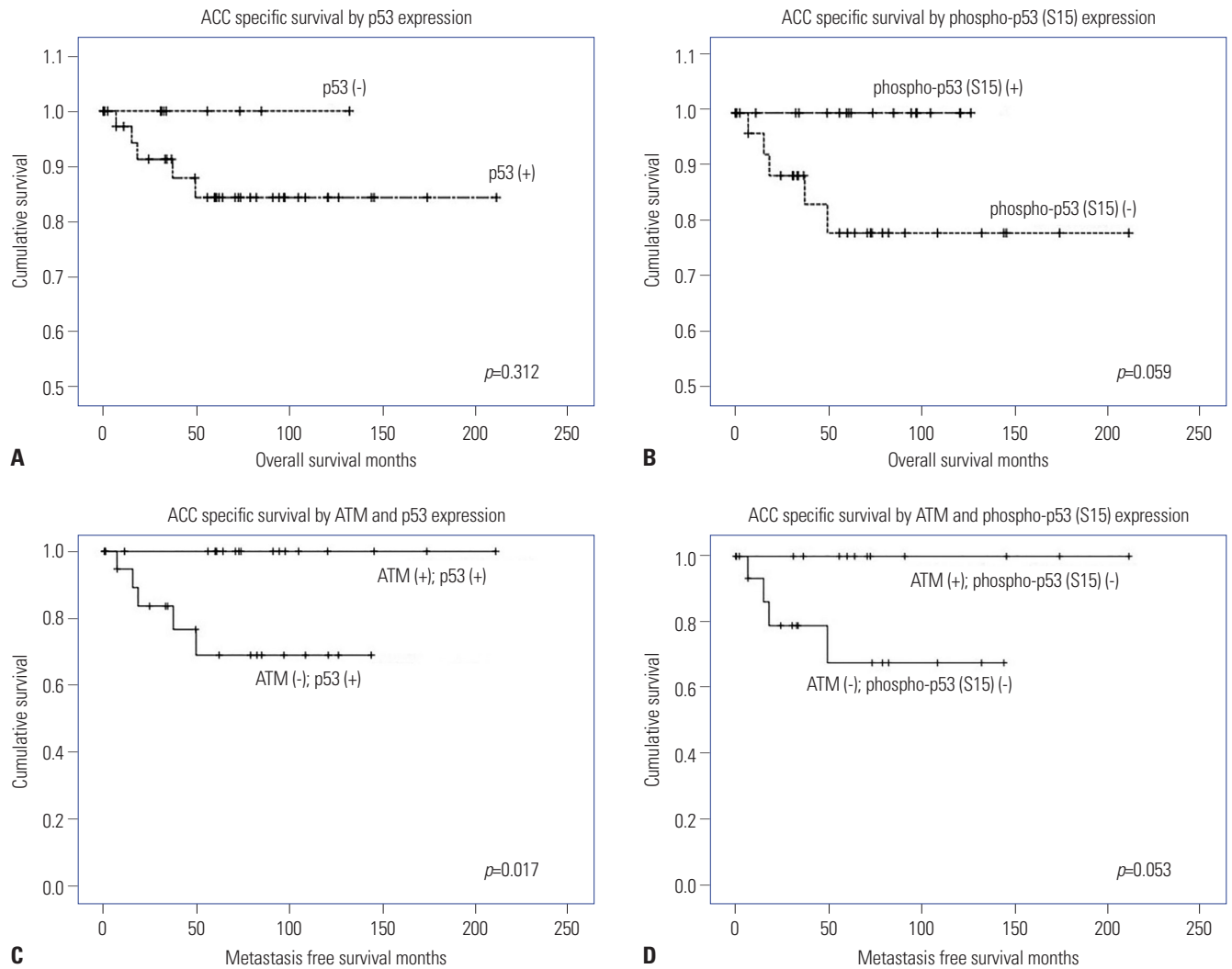


Fig. 5. Overall survival rates of ACC patients according to the expression of p53, phospho-p53 (S15), ATM given positive p53 expression, and ATM given negative phospho-p53 (S15) expression. (A) Overall survival according to p53 expression. (B) Overall survival according to phospho-p53 (S15) expression. (C) Overall survival according to the expression of ATM given positive p53 expression. (D) Overall survival according to the expression of ATM given negative phospho-p53 (S15) expression. ATM, ataxia telangiectasia mutated; ACC, adenoid cystic carcinoma.

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REFERENCES

- Nagao T, Gaffey TA, Serizawa H, Sugano I, Ishida Y, Yamazaki K, et al. Dedifferentiated adenoid cystic carcinoma: a clinicopathologic study of 6 cases. *Mod Pathol* 2003;16:1265-72.
- Bhayani MK, Yener M, El-Naggar A, Garden A, Hanna EY, Weber RS, et al. Prognosis and risk factors for early-stage adenoid cystic carcinoma of the major salivary glands. *Cancer* 2012;118:2872-8.
- Bell D, Roberts D, Karpowicz M, Hanna EY, Weber RS, El-Naggar AK. Clinical significance of Myb protein and downstream target genes in salivary adenoid cystic carcinoma. *Cancer Biol Ther* 2011;12:569-73.
- Brill LB 2nd, Kanner WA, Fehr A, Andr n Y, Moskaluk CA, L ning T, et al. Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms. *Mod Pathol* 2011;24:1169-76.
- West RB, Kong C, Clarke N, Gilks T, Lipsick JS, Cao H, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. *Am J Surg Pathol* 2011;35:92-9.
- Shiloh Y. ATM and ATR: networking cellular responses to DNA damage. *Curr Opin Genet Dev* 2001;11:71-7.
- Zhao D, Wang SH, Feng Y, Hua CG, Zhao J, Tang XF. Intratumoral c-Met expression is associated with vascular endothelial growth factor C expression, lymphangiogenesis, and lymph node metastasis in oral squamous cell carcinoma: implications for use as a prognostic marker. *Hum Pathol* 2011;42:1514-23.
- Guarini A, Marinelli M, Tavolaro S, Bellacchio E, Magliozzi M, Chiaretti S, et al. ATM gene alterations in chronic lymphocytic leukemia patients induce a distinct gene expression profile and predict disease progression. *Haematologica* 2012;97:47-55.
- Gilad S, Khosravi R, Shkedy D, Uziel T, Ziv Y, Savitsky K, et al. Pre-dominance of null mutations in ataxia-telangiectasia. *Hum Mol Genet* 1996;5:433-9.
- Janin N, Andrieu N, Ossian K, Laug  A, Croquette MF, Griscelli C, et al. Breast cancer risk in ataxia telangiectasia (AT) heterozy-

- gotes: haplotype study in French AT families. *Br J Cancer* 1999; 80:1042-5.
11. Feng X, Li H, Dean M, Wilson HE, Kornaga E, Enwere EK, et al. Low ATM protein expression in malignant tumor as well as cancer-associated stroma are independent prognostic factors in a retrospective study of early-stage hormone-negative breast cancer. *Breast Cancer Res* 2015;17:65.
 12. Kim H, Saka B, Knight S, Borges M, Childs E, Klein A, et al. Having pancreatic cancer with tumoral loss of ATM and normal TP53 protein expression is associated with a poorer prognosis. *Clin Cancer Res* 2014;20:1865-72.
 13. Kim JW, Im SA, Kim MA, Cho HJ, Lee DW, Lee KH, et al. Ataxia-telangiectasia-mutated protein expression with microsatellite instability in gastric cancer as prognostic marker. *Int J Cancer* 2014; 134:72-80.
 14. Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A* 2005;102:13550-5.
 15. El-Naggar AK, Chan JKC, Takata T, Grandis JR, Slootweg PJ. The fourth edition of the head and neck World Health Organization blue book: editors' perspectives. *Hum Pathol* 2017;66:10-2.
 16. Villaruz LC, Jones H, Dacic S, Abberbock S, Kurland BF, Stabile LP, et al. ATM protein is deficient in over 40% of lung adenocarcinomas. *Oncotarget* 2016;7:57714-25.
 17. North JP, McCalmont TH, Fehr A, van Zante A, Stenman G, LeBoit PE. Detection of MYB alterations and other immunohistochemical markers in primary cutaneous adenoid cystic carcinoma. *Am J Surg Pathol* 2015;39:1347-56.
 18. Abdel-Fatah TM, Powe DG, Hodi Z, Reis-Filho JS, Lee AH, Ellis IO. Morphologic and molecular evolutionary pathways of low nuclear grade invasive breast cancers and their putative precursor lesions: further evidence to support the concept of low nuclear grade breast neoplasia family. *Am J Surg Pathol* 2008;32:513-23.
 19. Abdel-Fatah TM, Powe DG, Agboola J, Adamowicz-Brice M, Blamey RW, Lopez-Garcia MA, et al. The biological, clinical and prognostic implications of p53 transcriptional pathways in breast cancers. *J Pathol* 2010;220:419-34.
 20. Cremona CA, Behrens A. ATM signalling and cancer. *Oncogene* 2014;33:3351-60.
 21. Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 1998;281:1677-9.
 22. Angèle S, Treilleux I, Tanière P, Martel-Planche G, Vuillaume M, Bailly C, et al. Abnormal expression of the ATM and TP53 genes in sporadic breast carcinomas. *Clin Cancer Res* 2000;6:3536-44.
 23. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science* 2000;287:1824-7.
 24. Meek DW. Tumour suppression by p53: a role for the DNA damage response? *Cancer* 2009;9:714-23.
 25. Maki CG, Huijbregtse JM, Howley PM. In vivo ubiquitination and proteasome-mediated degradation of p53(1). *Cancer Res* 1996;56: 2649-54.
 26. Li Q, Huang P, Zheng C, Wang J, Ge M. Prognostic significance of p53 immunohistochemical expression in adenoid cystic carcinoma of the salivary glands: a meta-analysis. *Oncotarget* 2017;8: 29458-73.
 27. Kang B, Guo RF, Tan XH, Zhao M, Tang ZB, Lu YY. Expression status of ataxia-telangiectasia-mutated gene correlated with prognosis in advanced gastric cancer. *Mutat Res* 2008;638:17-25.
 28. Han M, Ma L, Qu Y, Tang Y. Decreased expression of the ATM gene linked to poor prognosis for gastric cancer of different nationalities in Xinjiang. *Pathol Res Pract* 2017;213:908-14.
 29. Min R, Siyi L, Wenjun Y, Ow A, Lizheng W, Minjun D, et al. Salivary gland adenoid cystic carcinoma with cervical lymph node metastasis: a preliminary study of 62 cases. *Int J Oral Maxillofac Surg* 2012;41:952-7.
 30. Chen AM, Bucci MK, Weinberg V, Garcia J, Quivey JM, Schechter NR, et al. Adenoid cystic carcinoma of the head and neck treated by surgery with or without postoperative radiation therapy: prognostic features of recurrence. *J Radiat Oncol Biol Phys* 2006;66: 152-9.