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Abnormal expression of Rb pathway–related proteins in salivary gland acinic cell carcinoma

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Summary Salivary gland acinic cell carcinoma (ACC) is a relatively rare neoplasm, and limited information is available regarding its molecular pathogenesis. Because the deregulation of Rb pathway is common to most human tumors, we immunohistochemically investigated the expression of Rb pathway–related proteins, including Rb, Rb proteins phosphorylated at serine 780 and 795 (pRb-S780 and pRb-S795, respectively), cyclin D1, and $p16^{INK4a}$ in 18 cases of ACC. The expression of topoisomerase II–α and Ki-67 was also examined to evaluate cell proliferation. All the ACCs exhibited substantial numbers of positive cells against Rb antibody that recognizes both unphosphorylated and phosphorylated Rb proteins. The numbers of positive cells for pRb-S795 and cyclin D1 significantly increased in ACCs as compared with normal salivary glands. Double immunofluorescent staining demonstrated that pRb-S795 was colocalized with cyclin D1 in most tumor cells. However, neither significant change of the expression of Rb protein phosphorylated at serine 780 nor its colocalization with cyclin D1 was observed. The loss of $p16^{INK4a}$ is infrequent, but its expression was correlated with phosphorylated Rb proteins. Our results suggest that serine 795 but not serine 780 is the preferred phosphorylation site induced by cyclin D1. This phosphorylation appeared to be critical for inactivation of Rb-mediated growth suppression and may play an important role in the pathogenesis of ACC.

1. Introduction

Acinic cell carcinoma (ACC) of salivary gland origin is a relatively rare neoplasm that is defined by cytological differentiation toward serous acinar cells [1]. ACC occurs in patients of all age groups and affects women more commonly than men. It mainly occurs in the parotid gland with only occasional occurrence in the submandibular and minor salivary glands. The histological growth patterns can be categorized as solid, microcystic, papillary cystic, and follicular. Despite being regarded as low-grade malignancies, most ACCs are infiltrative and are sometimes associated with

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recurrence and metastasis [2,3]. However, neither any of the 4 histological growth patterns nor the degree of acinar cell differentiation has been found to be reliably predictive of a more favorable or worse clinical course. Increased knowledge and understanding of the basic biology of tumorigenesis in ACC may provide important information for predicting the response of individual patients to treatment and for identification of the prognosis of the patients, using molecular alterations as clinical biomarkers. Although several clinicopathologic and immunohistochemical studies of ACC have been reported [2-5], limited information is available regarding its molecular pathogenesis.

Recent advances in cell cycle research and cancer research reveal that tumor cells typically sustain damages to genes that regulate G_1-S progression [6]. The retinoblastoma gene product Rb protein serves as a checkpoint that restricts entry into the S phase by binding to transcription factor E2F and blocks the transcription of the S phase genes. Normally, this inhibition by Rb is relieved at the appropriate time by phosphorylation of the Rb protein, a process initially triggered by cyclin D–Cdk4/Cdk6 complexes [6-8]. The activities of cyclin D–Cdk4/Cdk6 complexes are constrained by inhibitors such as $p16^{INK4a}$ [9,10]. Thus, the loss of $p16^{INK4a}$ and Rb and overexpression of cyclin D have similar effects on G_1 progression and represent a common pathway to tumorigenesis. Abnormalities in the Rb pathway can lead to uncontrolled cell proliferation and tumorigenesis in various types of cell lines, animal models, and human tumors [11-17].

Rb is inactivated either by mutation of the *RB* gene or by hyperphosphorylation of the protein as a result of mutations of other components of the Rb pathway, for example, loss of $p16^{INK4a}$ and overexpression of cyclin D1 or Cdk4. Current studies on the Rb pathway in salivary gland tumors have been focused on only adenoid cystic carcinomas and pleomorphic adenomas [18-22]. In this study, we investigated the status of the Rb pathway in salivary gland ACC.

Topoisomerase II–α (topo II–α) is an essential nuclear enzyme for chromosome segregation during mitosis [23]. Previous investigation suggested that the values of topo II–α index paralleled those of Ki-67 index in salivary gland adenoid cystic carcinoma [24]. In our study, therefore, the immunohistochemical detection of topo II–α and Ki-67 was used to evaluate cell proliferative capacity.

In the present study, we examined the expression of Rb protein, cyclin D1, and $p16^{INK4a}$ by immunohistochemistry to evaluate whether these molecular events play a role in the tumorigenesis of salivary gland ACC. The functional status of Rb protein was investigated in situ by immunohistochemical analysis using 2 antibodies that detect phosphorylated Rb proteins. In addition, we analyzed whether there is a correlation among these molecular events and whether it is associated with cell proliferative capacity or clinicopathologic features. Here, we suggest that serine 795 but not serine 780 of Rb protein is the preferred site for phosphorylation induced by cyclin D1, and this phosphorylation may be critical for inactivation of Rb-mediated growth suppression in ACC.

2. Materials and methods

2.1. Patients and tissue samples

Tumor specimens from 18 patients with ACC who had undergone curative resection between 1983 and 2002 were studied (Table 1). The patients had received no chemotherapy

Table 1 Clinical and histopathologic features of 18 salivary gland ACCs

Case	Age (y)	Sex	Site	Growth pattern	Follow-up period (y)	Follow-up status
1	70	F	Buccal	Follicular	3	No, A
2	74	F	Buccal	Microcystic	3	No, A
3	44	F	Buccal	Solid	4	No, DOD
4	64	F	Buccal	Solid	3	No, A
5	44	M	Buccal	Solid	5	No, A
6	51	M	Labial	Solid	4	No, A
7	34	F	Labial	Microcystic	3	No, A
8	55	F	Palatal	Microcystic	18	No, A
9	46	M	Palatal	Solid	19	RE, ME, D
10	22	F	Parotid	Solid	22	No, A
11	54	M	Parotid	Papillary cystic	6	RE 2 y
12	61	M	Parotid	Solid	18	RE 13 y, DOD
13	18	F	Parotid	Papillary cystic	3	No, A
14	31	F	Parotid	Microcystic	3	No, A
15	16	F	Parotid	Solid	3	No, A
16	49	F	Parotid	Solid	3	No, A
17	59	M	Parotid	Microcystic	0	UN
18	56	M	Parotid	Solid	4	RE 4 y, A

NOTE. We categorized the cases by their predominant growth patterns, although two or more patterns can coexist in a single tumor.

Abbreviations: F, female; M, male; No, no recurrence or metastasis; A, alive; DOD, dead of other disease; RE, recurrence; ME, metastasis to cervical lymph node; D, dead of ACC; UN, unknown.