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Estrogen receptor expression in adrenocortical carcinoma

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Abstract: Objective: Adrenocortical carcinoma (ACC) is a rare but highly malignant tumor, and its diagnosis is mostly delayed and prognosis is poor. We report estrogen receptor (ER) expression in this tumor and our clinical experiences with 17 ACC cases. Methods: The data of the 17 patients (9 females and 8 males, age range from 16 to 69 years, mean age of 42.6 years) with ACC were reviewed, and symptoms, diagnostic procedures, treatment, and results of follow-up were evaluated. Immunohistochemistry was used to detect ER expression in tumor samples from the 17 patients. Results: At the time of diagnosis, 4 tumors were classified as Stage I, 4 as Stage II, 3 as Stage III, and 6 as Stage IV. Eight patients demonstrated positive nuclear immunostaining of ER. The prognosis of patients with ER positive was significantly better ($P<0.05$) than that of patients with ER negative, with 1- and 5-year survival rates at 86% and 60% for ER-positive patients, and 38% and 0% for ER-negative patients, respectively. Conclusion: ER-positivity may be one of the factors associated with a worse prognosis of ACC.

Key words: Adrenocortical carcinoma (ACC), Estrogen receptor (ER), Diagnosis, Treatment
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INTRODUCTION

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with an incidence of approximately 0.02% (Dackiw *et al*., 2001), and ranks among the least common malignant endocrine tumors. It tends to be slightly more common in women than in men. The carcinoma tends to be highly aggressive, with approximately 75% being metastatic at the time of diagnosis, and the 5-year mortality rate is approximately 75%~90% (Demeure and Somberg, 1998). The main treatment of ACC is the complete surgical removal of the tumor. The poor prognosis of ACC is explained in part by its relative unresponsiveness to chemotherapy and external irradiation.

The adult adrenal cortex, embryologically derived from mesoderm, displays histological and functional zonation, and produces hormones including aldosterone, cortisol, and androgens. In the fetus, an innermost fetal zone of the cortex is observed; it secretes large amounts of precursors that are converted to estrogen in the placenta (Lingappa and Farey, 2001).

In this study, we reported our experience of the treatment of ACC patients. We retrospectively reviewed the data of 17 cases with histologically confirmed ACC from January 1987 to December 2005. The clinical symptoms, treatment, and outcome of these patients were presented and compared with data from the literature. We applied immunohistochemistry to detect estrogen receptor (ER) in the tissues from those ACC patients. To our knowledge, it is the first study looking for ER in ACC tissue.

MATERIALS AND METHODS

Patients' characteristics and clinical presentation

Clinical data of 17 patients (8 males and 9 females) with ACC treated at the Department of Urology, the Second Affiliated Hospital, School of

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Medicine, Zhejiang University, China, between January 1987 and December 2005, were retrospectively reviewed. Since children are not treated in our institution, the patients included in the current study were solely adult patients. Mean age of the patients at the time of diagnosis was 42.6 (16~69) years. The diagnosis of ACC was based on the histological criteria of Medeiros and Weiss (1991). And according to the criteria of MacFarlane (1958) as modified by Sullivan (1978), at the time of diagnosis, 4 tumors were classified as Stage I, 4 as Stage II, 3 as Stage III, 6 as Stage IV.

All the patients underwent appropriate clinical and hormonal investigation (Table 1). Five of 17 patients (29.4%) had functioning tumor, and were associated with Cushing's syndrome (n=2), Cushing's syndrome with virilization (n=1), Conn's syndrome (n=1), and feminization (n=1). The tumors in the remaining 12 patients were not functional, without any clinical or biochemical evidence of hypersecretion. Eight of these 12 patients were presented with abdominal pain, 1 with fever and abdominal mass, and 3 asymptomatic and with their tumors found during routine clinical examinations.

Ultrasonography and computed tomography (CT) were performed in all patients. All CT scans were reviewed by a single radiologist to determine the size and radiological features of the tumors.

Primary treatment

All the 17 patients had surgeries to remove the tumor, except 4 patients who received CT-guided percutaneous core biopsy only, because of the size of the tumor and the presence of multiple (hepatic and pulmonary) metastases. Adrenalectomy with regional lymphadenectomy was performed in 9 patients, adrenalectomy with resection of a single liver metastasis in 2 patients, and adrenalectomy with nephrectomy in 1 patient. Tumor thrombi were removed from the inferior vena cava in 1 patient. The incision of the surgeries included two types, one was open surgery via anterior subcostal approach and the other was lateral approach in which incision was made between the 10th and 11th ribs. During all the surgeries, only one surgical complication happened: an intra-operative hemorrhage requiring blood transfusion. None of the patients died during the surgeries, nor during radiation therapy pre- or post-operatively. Two patients underwent a re-operation 10 and 17 months after the original procedures, respectively. The characteristics of patients at diagnosis were summarized in Table 1.

Table 1 Clinical data of 17 patients with adrenocortical carcinoma

Patient No.	Sex	Age (year)	Location	Tumor size (cm×cm×cm)	Hormone levels above normal range	Stage	Surgery	ER	Follow-up (month)	Status
1	F	33	Right	5×5×6	None	II	L, AE+LA	+	65	NED
2	M	19	Right	6×7.5×10	SEL	II	A, AE+LA	+	120	NED
3	M	56	Left	10×10×15	None	II	A, AE+LA	+	25	AWD
4	M	63	Right	3×3.5×3	None	I	L, AE+LA	+	36	NED
5	M	26	Right	4×5×5	None	I	L, AE+LA	+	42	NED
6	F	43	Right	15×15×20	None	III	A, AE+LA	−	14	NED
7	F	39	Right	8×8×8	SC, UHK	II	A, AE+LA	−	12	DOD
8	M	28	Right	2.5×2.5×3	PA	I	L, AE+LA	−	18	DOD
9	F	38	Right	25×25×30	None	IV	A, AE+LA+LLE	−	12	DOD
10	F	16	Right	22×25×30	None	IV	Biopsy	−	6	DOD
11	F	34	Left	5×5×6	ST, SC, UHK	IV	L, AE+LA+T	−	16	DOD
12	M	60	Right	5×5×4.5	None	I	L, AE+LA	+	66	NED
13	M	65	Left	10×6×6	None	III	Biopsy	−	3	DOD
14	F	26	Left	7×6×6	SC, UHK	IV	Biopsy	+	6	DOD
15	F	50	Left	12×6×9	None	IV	A, AE+LA+N	−	15	DOD
16	M	69	Right	5×5×5	None	IV	A, AE+LA+LLE	−	2	DOD
17	F	60	Right	6×6×9	None	III	Biopsy	+	10	DOD

M: male; F: female; SEL: serum estrogen levels; SC: serum cortisol; UHK: urinary 24-h 17-hydroxycorticosteroid and 17-ketosteroid; PA: plasma aldosterone; ST: serum testosterone; L: lateral, the 11th supracostal approach; A: anterior subcostal approach; T: thrombectomy; N: nephrectomy; AE: adrenalectomy; LA: lymphadenectomy; LLE: liver lobectomy; +: positive; −: negative; NED: no evidence of disease; AWD: alive with disease; DOD: dead of disease

Immunohistochemistry

Paraffin-embedded and formalin-fixed ACC tissue samples from all the 17 patients were obtained. Serial sections of each sample were cut, and slides were stained with haematoxylin and eosin (H&E) for routine histological evaluation. Suitable blocks were selected and examined by an experienced pathologist. The diagnosis of each block was confirmed by the examination of a routinely stained H&E section juxtaposed to the section used for estrogen receptors immunostaining.

Sections (4-μm thick) were de-waxed, rehydrated in xylol and alcohol, and then stained using an avidin-biotin technique with a primary antibody to ER (mouse monoclonal, clone 1D5, 1:50 dilution). Slides were microwaved in citrate buffer (pH 6.0) for 20 min prior to staining. The immunostaining was developed using 3,3′-diaminobenzidine tetrahydrochloride as the chromogen. The slides were counterstained with Mayer's hematoxylin. The negative control was processed in the same manner as the test staining, with omission of the primary antibody. A specimen with known strong expression of ER from breast cancer served as positive controls.

A positive result for estrogen receptors was defined as more than 5% tumor nuclei stained.

Statistical analysis

Follow-up was calculated from the time of the first operation for the primary tumor to the date of the last follow-up. The statistical analysis was performed by SPSS package. Statistical significance was set at 0.05. Statistical results are expressed as mean± standard deviation (*SD*). Correlation analysis was made by measuring the Spearman coefficient. Univariate influence of prognostic factors on survival rates was analyzed by the log-rank test. Multivariate analysis based on Cox's proportional hazards regression model was used to associate covariates to the 1- and 5-year survival rates.

RESULTS

Eight patients (8/17, 47.1%) demonstrated positive nuclear immunostaining of ER (Fig.1), which was strongly correlated with low tumor stage with Spearman's test (P=0.005). The incidence of metastasis at the time of the first surgery was 55.6% (5/9) in immuno-negative patients and 12.5% (1/8) in immuno-positive patients (P<0.01).

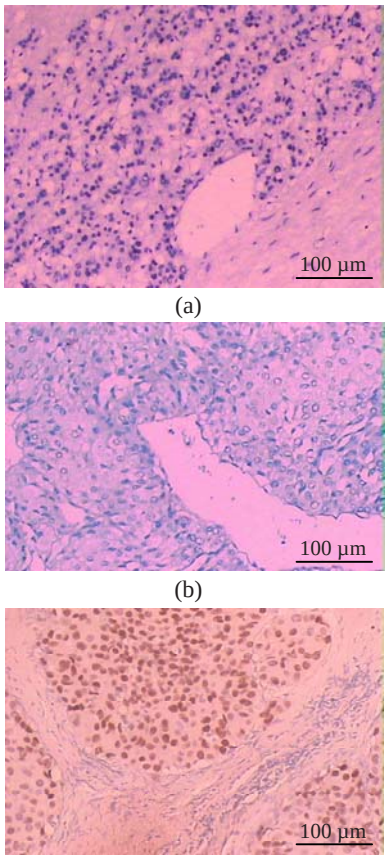

Fig.1 Positive and negative staining of estrogen receptor (ER) in adrenocortical carcinoma (ACC) by haematoxylin and eosin staining

(a) Positive staining; (b) Negative staining; (c) Breast cancer with positive ER

Clinical follow-up, telephone interview, and mailed questionnaires were used to update the data. The median follow-up was 27.5 months (ranged 2 to 120 months). No patient was lost to follow-up. The most frequent sites of metastasis were the liver (n=4) and the lung (n=4), followed by bone (n=1). Out of the 9 patients with localized disease who were operated, 2 had a recurrence (22.2%) with a median disease-free interval of 12.5 months (ranged from 8 to 17 months). Ten patients died of progressive tumor during follow-up, of which 4 patients who did not have surgery died at 3, 6, 6, and 10 months, respectively, with the median postoperative survival time of 10 months. Seven patients were still alive with their

median follow-up period being 52.5 months.

The results of univariate influence of prognostic factors analyzed by the log-rank test and multivariate analysis based on Cox's proportional hazards regression model are shown in Tables 2 and 3, respectively. Significant unfavorable prognostic factors on univariate analysis for overall survival included advanced stage, nonoperative management, and ER negative. There was no significant survival difference between functioning and non-functioning patients or between men and women. However, only the tumor stage was a prognostic factor in multivariate analysis. The prognoses of patients with Stages I and II were significantly better (P<0.05) than those of patients in Stages III and IV, with 1- and 5-year survival rates of 86% and 75% (Stages I and II) and 33% and 0% (Stages III and IV), respectively.

Table 2 Analysis of 1- and 5-year survival rates in the 17 patients with ACC

Characteristic	Survival rate (%)		Log-rank value	P
	1-year	5-year		
Sex				
Male (n=8)	75±15	63±17	2.74	0.0976
Female (n=9)	67±16	15±13		
Age				
>40 years (n=9)	56±17	33±16	0.03	0.8700
<40 years (n=8)	63±17	47±19		
Location				
Right (n=12)	75±13	49±15	1.07	0.3005
Left (n=5)	60±22	20±18		
Tumor size				
≤10 cm (n=11)	58±14	42±14	0.04	0.8474
>10 cm (n=6)	60±22	30±24		
ER				
Positive (n=8)	86±13	60±15	11.47	0.0032
Negative (n=9)	38±20	0		
Stage				
I and II (n=8)	86±12	75±15	11.42	0.0007
III and IV (n=9)	33±16	0		
Functioning				
Function (n=5)	60±22	20±18	0.27	0.6000
Non-function (n=12)	58±14	49±15		

Table 3 Multivariate analysis based on Cox's proportional hazards regression model

Factor	β	SE	Wald	P	Exp(β)
Stage	2.649	1.141	5.386	0.02	14.141

β: regression coefficient; SE: standard error

DISCUSSION

ACC is a rare malignancy with dismal prognosis. The tumors are usually defined, on the basis of biochemical and clinical findings, as either "functioning" or "non-functioning". Different to other reports (Ng and Libertino, 2003), we found that functioning tumors were less than hormonally inactive ones in the current study. The number of functional tumors may actually be higher than that reported because some nonfunctional tumors may secret hormones not enough for the clinical symptoms. The most common clinical presentation of ACC in this study was abdominal discomfort or pain because of tumor extension. Our study did not show a statistically significant difference in survival rates based on functionality, which was similar to the review by Ng and Libertino (2003).

Unfortunately, most ACC patients were seen in advanced stages of the disease, which sometimes makes complete resection difficult or impossible (Pommier and Brennan, 1992; Chen *et al*., 2004). In out study, 4 patients lost the possibility of operations and died soon (3~10 months) after the diagnosis. More than half (53%) patients were presented with Stages III and IV tumors. Most lesions had already reached a considerable size.

In our study, tumor size was not a prognostic factor for ACC. The 1- and 5-year survival rates of tumors with size ≤10 cm and >10 cm were similar. The results were similar to several previous studies that have suggested that tumor size alone could not be used to determine the biological behavior of adrenal tumors (Weiss, 1984).

The mainstay of therapy for ACC is radical surgery, i.e., complete surgical extirpation of the tumor and adrenal gland, en-bloc resection of invaded organs and, if necessary, peri-aortic retroperitoneal lymphadenectomy. In our study, the resection rate was 76.4%, and 58.8% of the patients underwent en-bloc resections. The patients who did not have surgery died 3~10 months after diagnosis. The incisions should permit wide exposure, minimize chances of capsule rupture and tumor spillage, and allow vascular control of the aorta, inferior vena cava, and renal vessels, as needed. There were two kinds of incisions in this study, open surgeries via anterior subcostal approach and lateral approach, in which

incision was made between the 10th and 11th ribs. The first approach is the standard incision for ACC; however, we found that the 11th rib supracostal incision could provide adequate exposure for unilateral adrenalectomy for a localized tumor of moderate size, with few complications.

Other approaches to treat ACC, such as radiation therapy and chemotherapy, are not very encouraging (Ng and Libertino, 2003; Schteingart *et al.*, 2005; Allolio and Fassnacht, 2006). ACC has been reported to be resistant to radiation therapy, which only brings transient reduction of local disease. The role of chemotherapy is still a matter of debate. Several combinations of cytotoxic agents have been used. However, available evidence does not allow any conclusions on the efficacy of these agents in ACC. High response rates to mitotane treatment were reported several decades ago. However, mitotane has a narrow therapeutic window and significant side effects, such as nausea, vomiting, diarrhea, and depression. In addition, the toxic effect on the unaffected adrenal gland may make lifelong steroid replacement therapy necessary. Blood levels of the drug have to be controlled carefully and the therapy should be discontinued, if no response is documented. There are some strategies of immunotherapy and gene therapy currently being tried in animal models or in human adrenocortical cancer cells in vitro. First trials in humans with adrenal cancer applying dendritic cell therapy have been started, but it is still too early to comment on its efficacy.

In light of the unsatisfactory clinical situation in the management of patients with ACC, the search for better medical treatment protocols is a continuing challenge. However, there are currently no well-established prognostic criteria based on histological or immunohistochemistry analysis of the primary tumors. Our analysis shows there was significant correlation between ER status and clinical stages and survival rate in ACC. Estrogens are believed to modulate cell growth by causing an increase in stimulatory growth factors and a decrease in inhibitory growth factors (Dickson and Lippman, 1987). These growth factors are thought to initiate, prevent, or progress through the cell cycle by the interactions with their respective membrane receptors. Antiestrogens negate the stimulatory effects of estrogen by blocking the ER, causing the cell to be held at the G1 phase of the replicative cycle (Osborne *et al.*, 1983). Colletti *et al.*(1989) reported that tamoxifen causes a decrease in the circulating levels of insulin-like growth factor-1 (IGF-1). IGF gene over-expression has been shown to be strongly related to ACC. Both IGF-1 and IGF-2 are involved in differentiation of the adrenal cortex. High levels of these factors may play a role in tumorigensis and dedifferentiation (Schulick and Brennan, 1999). The discovery that ER-positivity is strongly correlated to the low stage of ACC may help us to find out a new method of treatment.

The difficulty in assessing the effectiveness of published treatment protocols stems from the fact that most studies were limited in the number of patients studied. Because adrenal cancer is rare, worldwide and multi-center controlled collaborative studies will be necessary in order to reach consensus on the efficacy and the safety of ACC treatment protocols.

CONCLUSION

ACC is a rare aggressive tumor that is usually diagnosed in an advanced stage. Stage at diagnosis is the most significant prognostic factor, while age, sex, tumor size, and tumor functionality are not. ER-positivity is strongly correlated to the low stage of ACC.

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References

Allolio, B., Fassnacht, M., 2006. Adrenocortical carcinoma: clinical update. *J. Clin. Endocrinol. Metab.*, **91**(6): 2027-2037. [doi:10.1210/jc.2005-2639]

Chen, D.Y., Sosa, R.E., Scherr, D.S., 2004. Treatment of adrenocortical carcinoma: contemporary outcomes. *Current Urology Reports*, **5**(1):65-72. [doi:10.1007/s11934-004-0014-x]

Colletti, R.B., Roberts, J.D., Devlin, J.T., Copeland, K.C., 1989. Effects of tamoxifen on insulin-like growth factor 1 in patients with breast cancer. *Cancer Res.*, **49**(7): 1882-1884.

Dackiw, A.P., Lee, J.E., Gagel, R.F., Evans, D.B., 2001. Adrenal cortical carcinoma. *World J. Surg.*, **25**(7):914-926.

[doi:10.1007/s00268-001-0030-7]

Demeure, M.J., Somberg, L.B., 1998. Functioning and nonfunctioning adrenocortical carcinoma: clinical presentation and therapeutic strategies. *Surg. Oncol. Clin. N. Am.*, **7**(4):791-805.

Dickson, R.B., Lippman, M.E., 1987. Estrogenic regulation of growth and polypeptide growth factor secretion in human breast carcinoma. *Endocr. Rev.*, **8**(1):29-43.

Lingappa, V.R., Farey, K., 2001. Physiological Medicine: A Clinical Approach to Basic Medical Physiology. McGraw-Hill, p.584.

MacFarlane, D.A., 1958. Cancer of the adrenal cortex: the natural history, prognosis and treatment in the study of fifty-five cases. *Ann. R. Coll. Surg. Engl.*, **23**(3):155-186.

Medeiros, L.J., Weiss, L.M., 1991. New development in the pathologic diagnosis of adrenal cortical neoplasms: a review. *Am. J. Clin. Pathol*., **97**(1):73-83.

Ng, L., Libertino, J.M., 2003. Adrenocortical carcinoma: diagnosis, evaluation and treatment. *J. Urol.*, **169**(1):5-11. [doi:10.1016/S0022-5347(05)64023-2]

Osborne, C.K., Boldt, D.H., Clark, G.M., Trent, J.M., 1983. Effects of tamoxifen on human breast cancer cell kinetics: accumulation of cells in early G1 phase. *Cancer Res.*, **43**(8):3583-3585.

Pommier, R.F., Brennan, M.F., 1992. An eleven-year experience with adrenocortical carcinoma. *Surgery*, **112**(6): 963-970.

Schteingart, D.E., Doherty, G.M., GAuger, P.G., Giordano, T.J., Hammer, G.D., Korobkin, M., Worden, F.P., 2005. Management of patients with adrenal cancer: recommendations of an international consensus conference. *Endocr. Relat. Cancer*, **12**(3):667-680. [doi:10.1677/erc.1.01029]

Schulick, R.D., Brennan, M.F., 1999. Adrenocortical carcinoma. *World J. Urol.*, **17**(1):26-34. [doi:10.1007/s003450050101]

Sullivan, M., 1978. Adrenal cortical carcinoma. *Urology*, **120**(6):660-665.

Weiss, L.M., 1984. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am. J. Surg. Pathol*., **8**(3):163.