Original Article

Thrombospondin Expression in Aldosterone-Producing Adenomas

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Thrombospondin (TSP) 1 and 2 are extracellular matrix proteins that appear to play a role in cell adhesion and cell migration. It has been demonstrated that the pattern of TSP expression is shifted from TSP1 to TSP2 under adrenocorticotropic hormone treatment in bovine adrenocortical cells. We investigated the expression in human adrenal tissues by Northern blot analysis and correlated these data with the expression of the adrenocorticotropic hormone-receptor (ACTH-R). All adrenal tissues (control adrenals, nonfunctional adenomas, and ACTH-dependent aldosterone-producing adenomas (APA)) expressed both TSP1 and TSP2 mRNAs. Compared to control adrenals (TSP1 and TSP2 expression = 100 ± 12%, respectively), TSP1 expression was negatively (51 ± 10%, p < 0.01) and TSP2 expression was positively (289 ± 36%, p < 0.01) regulated in APA. No significant differences in TSP1 and TSP2 expressions were found between control adrenals and nonfunctional adenomas. In APA, TSP1 (r = -0.86, p < 0.01) and TSP2 (r = 0.88, p < 0.01) expressions correlated closely with the expression of ACTH-R. These results suggest that ACTH activity plays an important role in regulating the expression of TSPs in human adrenal tissues. We speculate that the shift of expression observed in APA may be associated with the phenotype of the tumors. (Hypertens Res 2002; 25: 523–527)

Key Words: thrombospondin, aldosterone-producing adenomas, adrenocorticotropic hormone receptor, centripetal migration

Introduction

Adrenocortical masses are the most common tumors in humans. However, only a small proportion of these tumors cause endocrine diseases (such as primary hyperaldosteronism, hypercortisolism, hyperandrogenism, or hyperestrogenism). Primary hyperaldosteronism, which is characterized by excessive production of aldosterone, potassium loss and suppressive renin activity, is an uncommon cause of hypertension. Its frequency among the hypertensive population is around 2%. Nevertheless, it is crucial to distinguish the main cause, aldosterone-producing adenoma (APA), from other causes of this disorder, because removal of the adenoma may result in cure of the hypertension.

In recent years, several of the molecular and cellular mechanisms involved in adrenal tumorigenesis have been revealed. As a result, alterations in the activation of protooncogenes (1) and tumor suppressor genes (2, 3) and changes in adrenocortical tissue-specific factors (such as steroidogenic factor 1) have been implicated in adrenal cell growth and tumor formation (4). However, the importance of hormonal factors and intercellular communication factors has not been studied in detail.

Thrombospondin (TSP) was initially characterized as a glycoprotein released by platelets in response to the activation by thrombin (5, 6). It was later shown to be synthesized in a wide variety of cells. TSPs are multimodal extracellular...
lar matrix proteins and their distinct modules trigger distinct biological effects through their interaction with module-specific receptors. TSPs appear to play roles in cell adhesion, cell migration (7) and angiogenesis (8). To date, five distinct genes encoding members of the TSP family have been identified (9). Among these five proteins, TSP1 and TSP2 have been found to be expressed in bovine adrenal cells under the control of adrenocorticotrophic hormone (ACTH). In addition, anti-TSP2 antibodies inhibit the sustained rounding-up of adrenocortical cells induced by ACTH, suggesting a prominent role of TSP2 in the migration of adrenocortical cells (10). Several reports have demonstrated a positive correlation between cell migration and cell proliferation. Therefore, in order to examine the role of TSPs in adrenal tumorigenesis, especially that of APA, we investigated the expression of TSP1 and TSP2 in adrenal tissues (control adrenals, nonfunctional adenomas and APA) and correlated these data with the endocrine profiles of the tumors.

Methods

Patients

Adrenal tissues from 8 patients with adrenal disease were collected on their surgical resection after obtaining informed consent, and studied with the approval of the Ethical Committee of the Hospital of Fukui Medical University. The clinical and pathological diagnosis was made according to the established criteria. The basal hormone data from the patients are shown in Table 1. The daily rhythm of ACTH secretion was assessed by assaying plasma ACTH concentrations every 4 h (4:00, 8:00, 12:00, 16:00, 20:00 and 24:00) for 1 day. The plasma aldosterone concentrations in all patients with APA (n = 5) showed an ACTH-dependent diurnal rhythm. Patients with nonfunctional adenomas (n = 3) had no signs or symptoms of hormonal excess and a normal suppression of serum cortisol (below 83 nmol/l) after low-dose dexamethasone (1 mg). Control adrenals (n = 5) were obtained from the adjacent normal cortex of patients with APA. Only central parts of adrenal tissues were used, avoiding necrotic areas and contaminations with normal adjacent tissue. The tissues were snap-frozen and stored at -80°C until analyzed.

Northern Blot Analysis

Total RNAs (30 µg) isolated from adrenal tissues were electrophoresed on 1.5% agarose gel containing 1.1 mol/l formaldehyde, blotted onto Hybond N+ nylon membrane (Amersham, Buckinghamshire, UK), and hybridized at 68°C for 1 h in Quickhybri Solution (Stratagene, La Jolla, USA) containing human TSP1, TSP2 and adrenocorticotrophic hormone receptor (ACTH-R) cDNAs, which had been labeled with [α-32P]dCTP to a specific activity of ca. 1 x 10^9 cpm/µg. The cDNA probes were originally obtained by reverse transcriptase-polymerase chain reaction with adrenal gland total RNA as described previously (11). The sequences were verified by the dideoxy chain termination method. After hybridization, the membranes were washed twice with 2 x standard saline citrate and 0.1% sodium dodecyl sulfate (SDS) at room temperature and then twice with 0.1 x standard saline citrate and 0.1% SDS at 60°C and autoradiographed. The hybridized signals were analyzed with a BAS 1500 Bioimaging Analyzer (Fuji, Tokyo, Japan). For standardization the signals were rehybridized with a GAPDH cDNA probe.

Statistical Analysis

All values are expressed as mean ± SD. Significance of differences between group means was assessed by 1-way analysis of variance (ANOVA) followed by Scheffe’s multiple comparison test. From the daily profile of ACTH secretion, the average area under the curve (AUC) was calculated using...
trapezoidal integration. The Mann-Whitney’s U test was used to evaluate differences between nonfunctional adenomas and APA groups with respect to basal ACTH concentrations and the mean AUC values. P values less than 0.05 were considered to indicate statistical significance. Correlations between TSPs and ACTH-R mRNA were assessed by linear regression analysis and expressed as Pearson’s correlation coefficient. P values less than 0.05 were considered to indicate statistical significance.

Results

We investigated the expression of TSP1 and TSP2 in control adrenals, nonfunctional adenomas and APA. The control adrenals were obtained from the adjacent normal cortex of APA. Mean serum ACTH concentrations were not different between the nonfunctional adenomas (38.3 ± 13.6) and APA (32.6 ± 6.3) groups (p = 0.47; Table 1). Although the mean AUC of ACTH in the nonfunctional adenomas group was larger than that in the APA group, there were no significant differences in the values (359.7 ± 15.4 vs. 316.3 ± 38.6, p = 0.06; Table 1). Northern blot analysis confirmed the existence of TSP1 and TSP2 mRNAs at a size of 6.0 kb in all samples. Representative data are shown in Fig. 1. Despite evidence for individual heterogeneity, mean TSP1 and TSP2 mRNA levels showed significant differences among groups. The mean TSP1 mRNA level was significantly lower in the APA group (51 ± 10%, p < 0.01) than in the control adrenals or nonfunctional adenomas (Fig. 2A). However, the mean TSP2 mRNA level was significantly higher in the APA group (289 ± 36%, p < 0.01) (Fig. 2B). No significant differences in TSP1 or TSP2 expressions were found between nonfunctional adenomas and control adrenals. Mean ACTH-R mRNA levels also showed significant differences among groups (Fig. 2C). These levels were significantly higher in APA (137 ± 23%, p < 0.01) and lower in nonfunctional adenomas (51 ± 17%, p < 0.01). A close negative correlation between ACTH-R and TSP1 mRNA (r = - 0.86, p < 0.01) (Fig. 3A) and a positive correlation between ACTH-R and TSP2 mRNA (r = 0.88, p < 0.01) were present only in the APA group (Fig. 3B). No significant correlations between the expressions of ACTH-R and TSPs were observed in control adrenals or nonfunctional adenomas.

Discussion

In the present study, we confirmed for the first time the ex-
expression of TSP1 and TSP2 in human adrenal tissues. We demonstrated that TSP1 expression is negatively and TSP2 expression is positively regulated in ACTH-dependent APA, and close correlations between the expressions of TSPs and ACTH-R exist in the adenomas. It has been postulated that the pattern of TSP expression is shifted from TSP1 to TSP2 under ACTH treatment in bovine adrenocortical cells (10). Although the significance of this finding is still obscure, we observed the same shift in ACTH-dependent APA, suggesting that ACTH activity plays a prominent role in regulating the expression of TSPs in vivo. We also confirmed the overexpression of ACTH-R in APA. Since mean serum ACTH concentrations and the mean AUC of ACTH values were not different between nonfunctional adenomas and APA, the ACTH-R overexpression may support the enhanced ACTH action.

The adrenal cortex is composed of three morphologically and functionally distinct zones (the glomerulosa, fasciculata and reticularis). It has been postulated that cell proliferation is restricted to the zona glomerulosa, and that mitotic pressure causes centripetal migration of cells from the glomerulosa to the inner compartments, where they differentiate into fasciculata and reticularis cells and eventually die by apoptosis (12). It is also known that ACTH is the main stimulator of this whole process (13). Since TSP2 has been shown to be distributed in the glomerulosa and fasciculate zones, and plays an important role in the migration of adrenocortical cells (14), it is tempting to speculate that this protein could contribute to the mechanism of the centripetal migration. It is difficult to correlate this in vitro effect of TSP2 on adrenocortical cell morphology with precise tumorigenesis of APA. However, we speculate that modifications of the expression patterns of TSPs could contribute to the changes in the pattern of expression of steroidogenic enzymes involved in the steroid biosynthesis pathway. Cheng and Hornsby (15) observed such an extracellular matrix-mediated regulation of the differentiated steroidogenic function. In their study, expression of steroid 11β-hydroxylase and 21-hydroxylase in bovine adrenocortical cells was greatly enhanced when cells were grown on Matrigel, a commercial preparation of extracellular matrix.

TSPs have also been shown to be involved in tumor angiogenesis. An important function recently assigned to TSP1 is its capacity to inhibit angiogenesis in vivo and to block the migration of endothelial cells to the angiogenic factor basic fibroblast growth factor (16). The potential role of TSP1 in the angiogenic process in the adrenal cortex would appear to be of primary interest, since the adrenal cortex consists of highly vascularized tissue in which cross-talking between steroidogenic cells and endothelial cells is known to take place. It is possible, therefore, that altered expression of TSP1 in APA plays some roles in the formation of tumor vessels, resulting in tumor progression.

In conclusion, TSP1 and TSP2 mRNAs are expressed in human adrenal tissues, and the pattern of expression may be under the control of ACTH. We speculate that the overexpression of TSP2 observed in APA tissues in the present study may be associated with the phenotype of the tumors.

References


