Expression of Alpha-1-antichymotrypsin in Prostate Carcinoma

Prostate specific antigen (PSA) is a glycoprotein with the enzymatic activity of serine protease, the gene which is encoded in the human glandular kallikrein gene locus. Catalytically active PSA released into serum may be inactivated by a complex formation with α1ACT (ACT) and α2MG (MG), two major protease inhibitors. The serum complex-to-total PSA ratio can be used as a marker for the differentiation between prostate carcinoma (PCa) and a benign lesion because of a significant elevation of PSA binding to ACT in PCa. Apparently higher immunohistochemical expressions of PSA and ACT have been reported in PCa of low Gleason scores when compared with benign lesions. The fact that only normal secretory epitheliums are capable of producing ACT was recently proved by immunohistochemical and in situ hybridization methods. Our immunohistochemical study of ACT showed a tendency toward stronger expression in high Gleason grade PCa than in low Gleason grade PCa. Prostate intraepithelial neoplasia (PIN), as well as BPH, seldom react to ACT. Expression of ACT in normal ducts or acini was influenced by their location. In a normal prostate, expression of ACT was predominantly in secretory epithelial cells, with a minority of basal cells and rarely in the interdigitating neuroendocrine cells. Whereas the potency of ACT production in epithelial cells almost always appeared to be suppressed under normal conditions, it was noted that a strong expression of ACT was apparent in the normal ducts or acini near a high grade carcinoma with a weak reaction to ACT. ACT expression is much more enhanced in high grade carcinomas and in the residual normal acini adjacent to carcinomas of low ACT expression, presumably representing scale down the elevated PSA. *(JKMS 1997; 12: 228~33)*

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Nam Hoon Cho, M.D., Chanil Park M.D., Dong Soo Park* M.D.

Departments of Pathology and Urology*, Yonsei University College of Medicine

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Address for correspondence
Nam Hoon Cho, M.D., Department of Pathology, Yonsei University College of Medicine, 146-92, Dogok-dong, Kangnam-ku, Seoul 135-270, Korea
Seoul, Yongdong P.O. Box 1217, Seoul, Korea

INTRODUCTION

Prostate specific antigen (PSA) is a tissue-specific serine protease similar in structure to the trypsin-like glandular kallikreins, but it is unique inasmuch as its enzyme activity is similar to that of chymotrypsin. Catalytically active PSA released in extracellular fluids may be inactivated by a complex formation with alpha-1-antichymotrypsin (ACT) and alpha-2 macroglobulin (MG). The active site remains unblocked and PSA fully retains its catalytic activity by being encased within the MG. Moreover, as PSA encased within MG is sterically protected from interacting with antibodies, the PSA molecule is undetectable by most immunohistochemical methods (1~3). But it is possible to detect encased PSA with ACT by immunohistochemical methods because there is an exposed epitope in its conformational structure. By means of this exposed epitope, the serological radioimmunoassay for the free-form and bound-form of PSA, or the ratio of the bound-form of PSA to total PSA, has been extensively studied (1~14). Hitherto, major claims have been made that the ratio of bound PSA to free PSA in serum can be utilized to discriminate easily and clearly between prostate carcinoma (PCa) and benign prostate hyperplasia (BPH). Recently, intensive studies about free/bound PSA on serum have been conducted to discriminate early PCa by observing higher level of a bound PSA ratio to total PSA in PCa than BPH, with elevation of serum total PSA (1~13). To our knowledge, however, there have been few histologic studies on PCa in relation to the ACT phenotype (15). Lilja previously stated that ACT, as well as PSA, can be produced in the epithelial cells of the normal prostate gland (16). But it is still unknown whether or not the differences in ACT expression predict the biologic behavior and correlate with tumor grade. This study is aimed to elucidate the validity of ACT in the prognostication of PCa.

MATERIALS AND METHODS

Tissue sampling

This study included 164 slides of 42 cases of prostate carcinoma demonstrating a variety of grades and stages, and 127 slides of 20 BPH, all of which were taken from the files of Severance hospital in the last 2 years. The carcinomas included 4 radical prostatectomies, 32 transurethral resections (TUR) and 6 needle biopsy specimens. All cases were reexamined by 2 urologic pathologists based on Gleason grade, 5 groups and 9 subgroups, and McNeal's classification, composed of 2 main types, tubular-scirrhotic and alveolar-medullary. High grade PIN, predominantly composed of micropapillary and cribriform pattern, were found in 5 cases of prostate carcinoma. The normal glands were separately examined according to the distance betweeen PCa and normal glands.

Immunohistochemistry

Tissue materials were fixed in 4% formaldehyde and processed to make paraffin block. Hematoxylin-eosin staining of tissue sections was used for histopathologic examination. Several unstained sections were prepared for immunohistochemical study on ACT, CK AE3 and chromogranin A. ACT was polyclonal rabbit anti-α 1-ACT IgG (Dako, Carpinteria, CA, USA), the clone of cytokeratin AE3 was 34βE12 (Dako, Carpinteria, CA, USA) and Chromogranin A was A0430 (Dako, Carpinteria, CA, USA). All of the antibodies were detected with the ABC technique using streptavidin-horseradish peroxidase (SA-HRP) with the chromophore of 3-amino-9-ethyl carbazole (AEC). ACT was diluted at 1:200, where the least background had been obtained. The incubation time was for 45 minutes at room temparature. CK AE3 specific for the basal cell layer of the prostate gland was diluted at 1:50. Neuroendocrine cells in the prostate gland were detected by chromogranin A with a working dilution of 1:75.

Interpretation methods

ACT expression was analyzed by 3-tier system according to a conventional semiquantitative method. When no cells were detectable, the grade was 0, with few positive cells +, moderate numbers of positive cells ++, and numerous cells, +++.

RESULTS

Normal prostate gland

The major portion of normal prostate glands remote from PCa seldom reacted to ACT, although nonspecific reactions such as diffusion into the luminal border were occasionally found. In contrast, a few prostate glands in the vicinity of PCa showed variable immunoreactions specific for ACT. ACT immunoreactive cells were detected in the scattered secretory epithelial cells of nor-

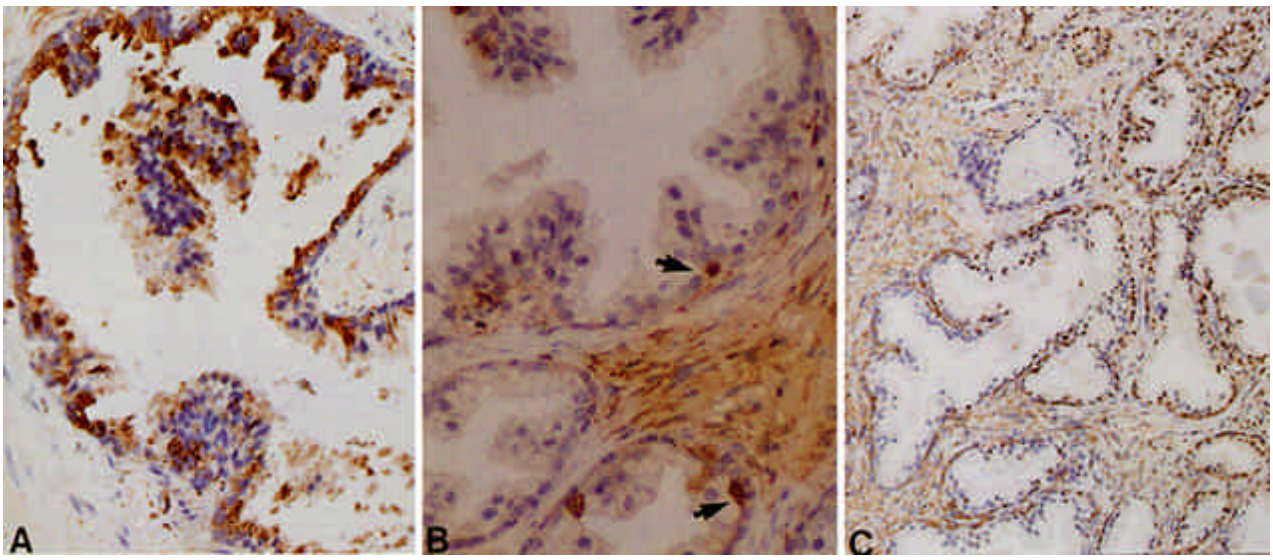

Fig. 1. Immnohistochemical demonstration of ACT in normal prostate gland. Immunoreactivity was observed mainly (A) in the secretory cells, or infrequently (B) in the basal cells or rarely (C) in the interdigitating epithelial cells (ABC method, AEC with hematoxylin counterstain; A, ×200, B, ×200, C, ×100).

mal ducts and acini (Fig. 1A). A few areas of normal prostate glands showed immunoreaction to ACT around the border of the basal layers (Fig. 1B), which were also verified as basal cells by CK AE3. Scattered interdigitating cells, not abutted to the luminal border, rarely reacted to ACT (Fig. 1C). These cells were also positive to chromogranin A. There was no immunoreaction to ACT in the transitional zone composed of urothelium. The central zone, including the seminal vesicle, contained weak diffuse immunoreaction predominantly along the luminal border.

Benign prostatic hyperplasia (BPH)

Both dilated and complex hyperplastic acini of BPH showed no reaction to ACT. Basal cell hyperplasia and transitional metaplasia were also negative. Atrophic glands which had shrunk showed a nonspecific diffuse reaction.

Prostate intraepithelial neoplasia (PIN)

Five cases of PIN, which were detected in the vicinity of PCa obtained from specimens of radical prostatectomies and one case from specimen of TUR, were all high grade and composed of the cribriform, or micropapillary type. These preneoplastic conditions were negative for the immunoreaction to ACT (Fig. 2A).

Prostate carcinoma (PCa)

In the analysis of 42 cases according to Gleason grade, 17 cases were grade 3, 4 cases were grade 4, and 7 cases were grade 5. All but 8 cases of PCa showed a variable positive reaction to ACT. Four cases below grade 3 showed hardly any reaction to ACT (Fig. 2B). Immunoreactivity to ACT was not so prominent until grade 4B, namely a hypernephroid pattern (Table 1). High grade PCa showed an intense reaction to ACT, and in some PCa, especially with deep infiltration into the periprostatic fat tissue, showed even more intense reaction to ACT (Fig. 2C). Almost all prostate ducts and acini remote from PCa repressed their potency of ACT expression. The normal ducts and acini in the midst of tumor nodules with an intense reaction to ACT showed a weak reaction to ACT. On the other hand, the normal ducts and acini adjacent to the carcinoma with weak reaction to ACT showed an intense expression of ACT. The prostate duct, partly filled with cribriform carcinoma with weak reaction to ACT, showed prominent immunoreaction to ACT (Fig. 3). There was a tendency for an inverse reaction between PCa and the normal prostate gland itself just in the vicinity of PCa.

According to McNeal's classification, we could integrate 9 subtypes of Gleason grade into only 2 groups. The first group, tubulo-scirrhotic, refers to the definite formation of tubules and infiltration within the sclerotic or desmoplastic background. This group included a constellation of high grade such as 3A, 3B, 4B and 5B. The second group, alveolar-medullary, refers to the formation of nodules with an expansile margin rather than infiltration, where we included some heterogeneous

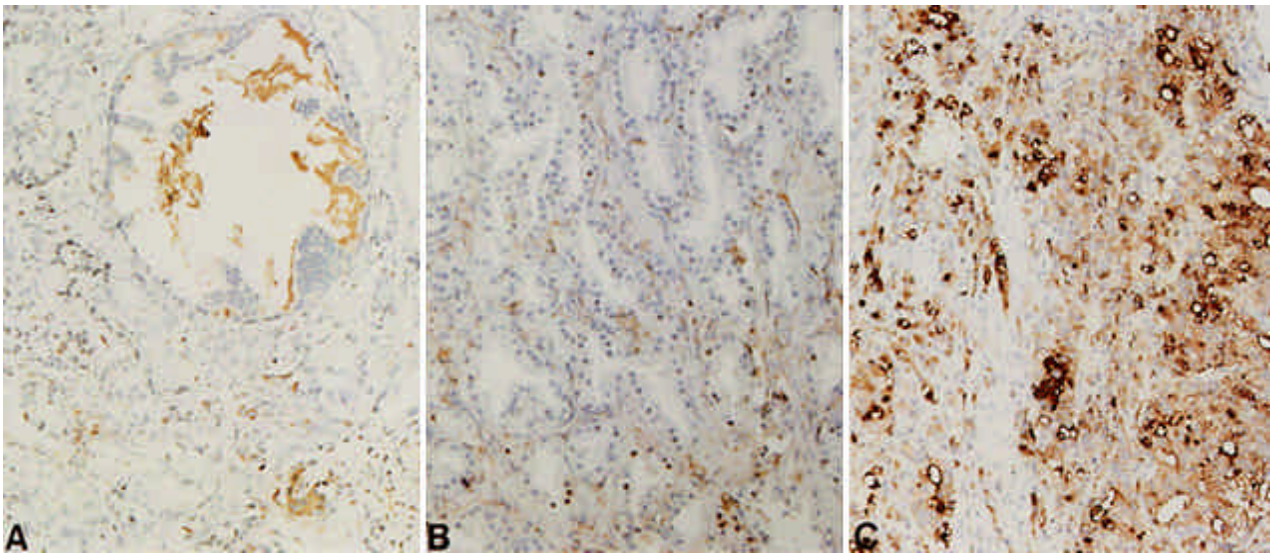

Fig. 2. Immunohistochemical demonstration of ACT in the preneoplastic lesion and carcinoma. (A) No positive cells were identified in high grade PIN; (B) Weak positivity was noted in low grade PCa; (C) Strong immunoreactivity was found in high grade PCa (ABC method, AEC with hematoxylin counterstain; A, B, C, ×100).

Table 1. Correlation between Gleason grade and alpha-1-antichymotrypsin expression in prostate carcinoma

Gleason grade	No.	−	+	++	+++
1	2	1	1	0	0
2	2	1	0	1	0
3	17	1	10	6	0
A	3	0	3	0	0
B	6	0	3	3	0
C	8	1	4	3	0
4	14	1	8	2	3
A	6	1	4	1	0
B	8	0	4	1	3
5	7	1	0	1	5
A	3	1	0	0	2
B	4	0	0	1	3
Total	42	5	19	10	8

No.: number of correspondent cases to the semiquantitative grade. −, no cells detectable; (+), few occasional cells; (++), moderate numbers of cells; (+++), numerous cells
The intensity of ACT expression was significantly correlated with the increase of grade (p-value: 0.003 by Spearman's correlation using spss v 6.0)

Table 2. Correlation between alveolar-medullary vs tubular-scirrhous pattern and alpha-1-antichymotrypsin expression in prostate carcinoma

	Weak (−, +)	Strong (++, ++)
Alveolar-Medullary	26	14
Tubular-Scirrhous	17	22

p-value: 0.041 by χ^2 test (spss v. 6.0)

grades composed of 1, 2, 3C, 4A, and 5A. On analysis by 2-tier semiquantitative comparison, the former showed a more intense and stronger reaction to ACT than the latter (Table 2).

DISCUSSION

PSA is the most prevalent glycoprotein originating from the prostate, and it is encoded in the human glandular kallikrein gene locus on chromosome 19 (12, 17). The gene locus harbors the gene for the pancreatic-renal tissue kallikrein (hK1) and the gene for human glandular kallikrein (12). PSA is a single chain form of 30 kd glycoprotein with the enzymatic activity of serine protease (3). An expression of the PSA gene is under complex control. A steady-state level of PSA mRNA is increased by androgen whereas it is decreased by epidermal growth factor-β and activation of protein kinase C (3,18~21). The majority of PSA in seminal fluid is present in the active single-chain form, whereas a minor part is devoid of enzymatic activity mainly due to

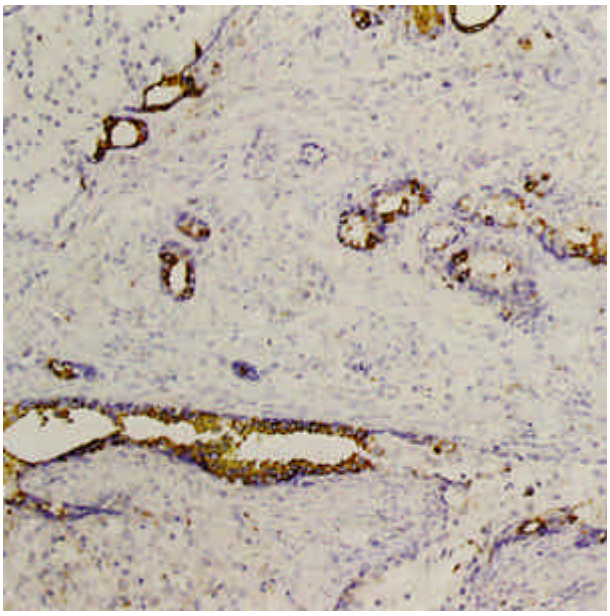

Fig. 3. A distinct inverse immunoreactivity between PCa and normal glands. Note strong reaction in normal duct in contrast to the carcinoma with weak expression of ACT (ABC method, AEC with hematoxylin counterstain, ×100).

an internal Lys-Lys cleavage resulting in an inactive two-chain form of PSA or complex formation with protein C inhibitor (PCI) similar to ACT (2~3,7,11~12). PCI and ACT are single chain glycoproteins and members of the superfamily of serine protease inhibitors showing similarities in size (~ 60 kd), overall structure and mechanisms of complex formation, except specificity of target proteases (2,22). The reaction between PSA, ACT and PCI results in the inactivation of enzymes due to the formation of covalently stabilized 1:1 molar ratio complexes of ~ 90 kd (2~3).

The complex formation with MG is believed to be irreversible and to regulate PSA activity by steric shielding; the bound protease is trapped by MG but remains catalytically active (2~3). Therefore, MG-reacted-PSA is sterically protected from competing protease inhibitors such as ACT. The active site remains unblocked and PSA fully retains the catalytic activity, being encased within the MG. Moreover, as PSA reacted with MG is sterically protected from interacting with antibodies, this molecule of PSA is undetectable by most immunohistochemical methods (1~3). It is possible to detect bound PSA with ACT by the conventional immunohistochemical method because there is an exposed epitope in its conformational structure. By means of this exposed epitope, the serological radioimmunoassay for free-form and bound-form of PSA, or the ratio of the bound-form of PSA to total PSA has been

extensively studied (1~14). Hitherto, major claims have been made that the ratio of the bound PSA to free PSA in serum can be utilized to discriminate easily and clearly between PCa and BPH. However, there have been several contradictory results which failed to confirm these claims, probably due to the different reactivities of the antisera or poor quality control. An immunohistochemical study for anti-PSA IgG (23~27) or anti ACT IgG (15) has been available in tissue sections so far. Recently, in a few immunohistochemical studies for PSA and ACT, the expression of both has been much more intense in PCa than in BPH, and more so in low grade PCa than in high grade PCa (15). Another study has shown that ACT was expressed in the epithelium of the prostate by in situ hybridization as well as by immunohistochemical stain, and then the prostate itself can produce ACT (16). So we intended to reevaluate ACT expression according to each Gleason grade, not Gleason score, by immunohistochemical stain for commercially available polyclonal anti-ACT antibodies.

In our study, we partly failed to duplicate the previous results. First, normal prostate glands scarcely revealed ACT expression. Lilja stated, however, that ACT production was never identified in the stroma, neuroendocrine cells or basal cells (16). Our study was too limited to determining conclusively whether basal and neuroendocrine cells can produce ACT. However, ACT expression was definitely noted in a few basal cells with interdigitating neuroendocrine cells, as well as in some luminar secretory epithelial cells. Those cells were also subsequently identified by specific immunostaining reaction for high molecular cytokeratin and chromogranin A, respectively.

Another contradictory point was that high grade PCa expressed ACT much more than low grade PCa. Previous surveys (15) have shown that the proportion of tumor cells producing both ACT and PSA was high in most PCa with a low Gleason score, but variably decreased in PCa with a high score. Generally speaking, the production of PSA per one cell in PCa is less than benign epithelium. But the greater number of malignant cells and more frequent stromal disruption in higher grade PCa account for the extremely elevated serum PSA level (28). Therefore, the greater amount of serum PSA, the higher expression of ACT to bind with PSA may occur (1~3). Therefore, it was very difficult to satisfactorily explain the previous result of higher ACT production in low grade PCa than in high grade PCa. Previous studies used Gleason score, not Gleason grade, in their assessment. Gleason score is frequently mixed with low grade and high grade. We examined the expression of ACT according to Gleason grade, not the concept of adding scores. We found very strong expression (+++) of ACT in high grade PCa more than 4B, even if it was highly variable from area to area. Though the cases of low Gleason grade PCa were very few, the expression of ACT was far less than that of high grade PCa. We failed to identify ACT expression in several foci of PIN and BPH, which was consistent with previous results (15).

McNeal reclassified the prostate carcinoma into tubular-scirrhous type and alveolar-medullary type according to morphogenesis (29). He insisted that the former appeared to arise from the atrophic duct, whereas the latter arose from an inactive involutional epithelium. On reanalysis according to McNeal's proposal, we found that PCa of tubular-scirrhotic type expressed ACT much more than that of alveolar-medullary type. We also found that tumor cells with extensive infiltration into the periprostatic fat tissue expressed ACT with very strong intensity. The more infiltrative and less differentiated was the prostate carcinoma, the more strongly ACT was expressed. This result correlates well with the previous hypothesis, in that PSA elevates in the infiltrative and less differentiated carcinoma by reason of the marked stromal disruption and large tumor volume (28).

Occasionally we found normal but enlarged prostate ducts partly filled with high grade PCa, where the residual epithelium of the prostate duct showed very strong expression of ACT in contrast to cancer cells with weak expression. McNeal et al. proposed that the cribriform carcinoma may be the intraductal component of grade 4 carcinoma, followed by intraluminal growth (30). Those lesions we found appeared to be compatible with intraductal components of grade 4 PCa. In cases where PCa showed weak expression of ACT, the overexpression of ACT in normal glands seemed to be the host defense mechanism for the inactivation of the spilled-out PSA by formation of a complex with ACT.

In conclusion, ACT is probably very prominent for the purpose of the inactivation mechanism of secreted PSA, particularly in the infiltrating margin of high grade PCa and in the remaining duct affected by a tumor with weak expression.

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