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Endopeptidase 24.11 activity in the human prostate cancer cell lines LNCaP and PPC-1

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Abstract Human endopeptidase 24.11 (EP) occurs in greatest abundance on terminally differentiated prostate cells; thus, loss of EP could mark dedifferentiation of prostate epithelium. To identify laboratory models that would permit continuous work on the biochemistry and hormonal regulation of EP, we examined the well-differentiated LNCaP and poorly differentiated PPC-1 human prostate cancer cell lines. Ultrastructural analysis revealed that LNCaP secretes electron-dense material that resembles the particulate matter of seminal plasma, which is associated with endopeptidase activity. LNCaP medium contained EP activity while PPC-1 medium did not. Whether the apparent deletion of EP from the PPC-1 cell line is characteristic of poorly differentiated prostate adenocarcinoma is not yet clear. However, it may be relevant to the carcinogenic process that EP can limit growth of lung small carcinomas by inactivating cell growth-promoting bombesin-like peptides. Because bombesin has been identified in aggressive human prostate cancers, loss of EP in PPC-1 could represent a necessary step in transformation to aggressive phenotype. The combination of LNCaP and PPC-1, which offers well-differentiated and poorly differentiated cancer phenotypes, appears well suited to studying the relevance of EP in prostate cancer biology.

Key words Prostate cancer · Endopeptidase 24.11 · Growth · LNCaP · PPC-1

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Human semen contains submicroscopic particles, ~130 nm in diameter, secreted by prostate acinar cells [9]. These particles, called prostasomes, present opportunities for direct and detailed analyses of prostate acinar gene expression and those disorders of gene expression that may be characteristic of prostate cancer. Prostasomes have a trilaminar outer membrane and an electron-dense amorphous core [2]. During ejaculation, prostasomes are released from acinar cells, primarily by exocytosis, in enormous numbers (~10^{14}/ml seminal plasma) [3]. Acinar cell apical inclusion bodies are now known to be intracellular stores of prostasomes [2]. As expected for a prostate secretion, prostasomes are present primarily in the second of six fractions of split ejaculates, along with acid phosphatase and prostate-specific antigen [9] and are undiminished by vasectomy [10]. Prostasomes remain suspended in seminal plasma under conditions in which spermatozoa are precipitated (centrifugation at 900 *g* for 10 min) [10].

Overall, development of a thorough understanding of prostasomes could provide dividends in terms of improved understanding of acinar cell (and prostate gland) function and dysfunction. Further, the methods required for obtaining prostasomes are noninvasive and acceptable to a large number of adult males who may have asymptomatic disease of the prostate but who are at risk. Thus, diagnostic studies for prostate diseases such as prostate cancer can be envisioned that, procedurally, are extensions of routine tests in male fertility clinics.

We have previously identified that endopeptidase 24.11 (EP) is among the proteins present in the prostasomal fraction of normal ejaculate (unpublished observations). However, because it is quite likely that prostasomes contain many functional proteins, a laboratory model that can be maintained and hormonally manipulated is essential for long-term biochemical and

molecular work; prostate epithelial cell lines could serve as initial models. With evidence that prostasomes contain specific endopeptidase activity, we set out to characterize a cell line(s) that could serve as a laboratory model of prostasome secretion and that would allow detailed biochemical analysis.

Materials and methods

The culture of well-differentiated, androgen-sensitive LNCaP and undifferentiated, androgen-insensitive PPC-1 human prostate cancer cell lines has been previously described [7]. For the purpose of the experiments, cells were grown in 10-cm dishes and harvested for enzymatic assays after 1 week, at 50% confluence. Culture media were collected by aspiration, and the cells were washed 3 times with serum-free phosphate-buffered saline. Cells were then scraped from the culture plate and collected by aspiration. Cell suspensions were sonified for 3 s.

Cell fractions were prepared as previously described (see introduction and references cited). In brief, centrifugation at 1000 *g* for 10 min provided supernatant 1 and precipitate 1. The supernatant was brought to a final concentration of 10 mM $MgCl_2$ and stored at 5°C; $MgCl_2$, a lipid membrane-aggregating agent, facilitates precipitation of prostasomes at relatively low *g*-forces (our unpublished data). The next day, the supernatant was centrifuged at 11 000 *g* for 120 min to obtain supernatant 2 and precipitate 2 (precipitate 2 of seminal plasma contains prostasomes and their enzymatic activity).

EP activity was measured by radiochemical methods as we have previously described [11, 12] , using glutaryl-Ala-Ala-Phe-[^{3}H]anilide (GAAFan), 25 nM, in 0.05 M MES buffer, pH 5.7 at 37°C [11]. GAAFan is hydrolyzed by EP to yield Phe-[^{3}H]anilide as the radioactive product. The reaction obeys first-order enzyme kinetics, which enabled direct measurement of the first-order rate constant, V_{max}/K_m. Protein was measured via the bicinchoninic acid

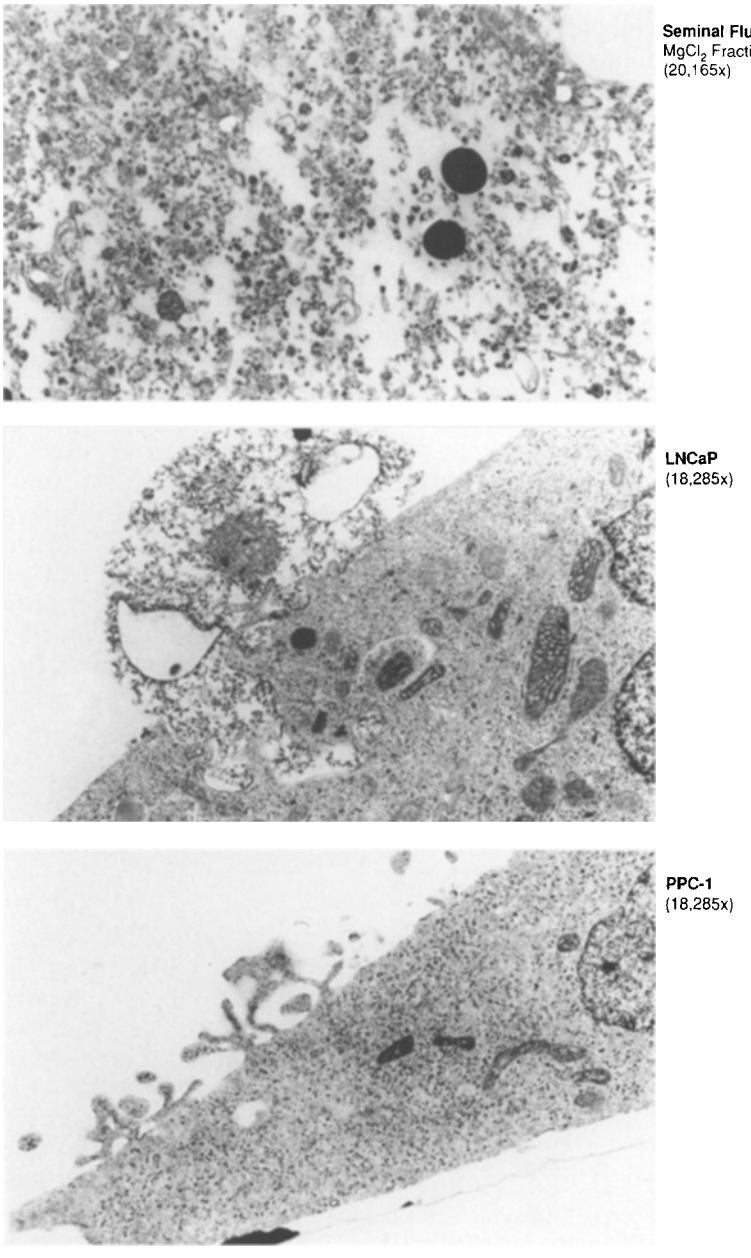

Fig. 1 Transmission electron micrographs of normal human seminal plasma ($MgCl_2$ fraction), LNCaP, and PPC-1 human prostate cancer cells

Table 1 Endopeptidase 24.11 specific activity in LNCaP and PPC-1

V_{max}/K_m (g prot./l)		
Conditioned medium	**LNCaP**	**PPC-1**
Whole medium	0.0018	0
Supernatant 1	0.0012	0
Precipitate 1	0.0496	0.0012
Supernatant 2	0.0083	0
Precipitate 2	0.0569	0.0015
Whole cells	**LNCaP**	**PPC-1**
Whole medium	1.0410	0.0126
Supernatant 1	0.9392	0.0184
Precipitate 1	0.7786	0.0030
Supernatant 2	0.1739	0.0164
Precipitate 2	14.0630	0.0310

procedure (Pierce Chemical Co.) and expressed in grams per liter. EP-specific activities were computed by dividing V_{max}/K_m per minute by protein concentration and expressed in liters per gram per minute [11, 12]. The latter units divided by the second-order constant, k_{cat}/K_m, yield specific activities in moles EP per gram protein.

Transmission electron microscopy was performed by methods we have previously described [13].

Results

The media of both cell lines contained particulate matter. To test the structural resemblance of this material to seminal plasma fractions associated with endopeptidase activity, we performed transmission electron microscopy (Fig. 1), which demonstrated that LNCaP, but not PPC-1, secretes material that strongly resembles the fibrillar and electron-dense matter found in the $MgCl_2$ fraction of seminal plasma.

Three separate experiments showed that medium with 10% fetal bovine serum (FBS), but without cells, had no measurable enzymatic activity (not shown). However, conditioned medium contained measurable EP activity (the results from one representative experiment are shown in Table 1). Of note, however, was the complete absence of EP activity in the undifferentiated cell line PPC-1.

Discussion

These results provide anatomic and biochemical support for the use of human prostate cancer cell lines as models for research into the structure and function of prostasomes and the associated EP activity. In this regard, the well-differentiated cell line LNCaP appears to possess more characteristics associated with human prostasomes. By contrast, the undifferentiated cell line PPC-1 does not secrete anatomic material resembling the $MgCl_2$ fraction (precipitate 2) of seminal plasma. Also, PPC-1 appears completely devoid of the EP activity associated with prostasomal fraction.

The last observation is potentially relevant to the highly aggressive behavior of PPC-1. It is known that EP antagonizes the growth-promoting actions of gastrin-release peptide/bombesin. In the lung, for example, EP hydrolyzes bombesin-like peptides, thereby slowing growth of small cell lung cancers [1, 14]. Because cigarette smoke inactivates EP and because small cell lung cancers tend to occur in smokers, the observations form part of a putative pathoetiologic model of small cell lung cancer. Similar models can be constructed for human prostate cancer. It is known that human semen contains bombesin-like activity [5]. It is also known that bombesin and vasoactive intestinal peptide increase the malignant behavior of human prostate cancer cells [6], including LNCaP and PC-3 (the less aggressive parent line of PPC-1); vasoactive intestinal peptide, like bombesin-like peptides, is metabolized by EP [4]. Because human prostate cancer cells can secrete bombesin and vasoactive intestinal peptide, one may reasonably hypothesize that the deletion of EP is a critical event in malignant transformation of human prostate cancers. This reasoning has been presented as an underlying rationale for new chemotherapeutic approaches to human prostate cancer [8].

Overall, the observations regarding the anatomic and functional endopeptidase characteristics of LNCaP indicate that it is a reasonable choice for initial studies of endopeptidase biology in human prostate epithelium. The usefulness of the PPC-1 cell line for the study of endopeptidase deletion in prostate carcinogenesis must await the availability of more direct analyses of endopeptidase activity in clinical samples. It is hoped that these observations and the derivative work will open the way to identification of new markers of prostate cancer.

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References

1. Aguayo SM, Schuyler WE, Murtagh JJJ, Roman J (1994) Regulation of lung branching morphogenesis by bombesin-like peptides and neutral endopeptidase. Am J Resp Cell Mol Biol 10:635
2. Brody L, Ronquist G, Gottfries A, Stegmayr B (1981) Ultrastructural localization of the prostasome: an organelle in human seminal plasma. Ups J Med Sci 88:63
3. Davis BK (1973) Occurrence of vesicles in rat seminal plasma. Experimentia 29:1484
4. Duggan KA, Jones DM, Ye VZC, Davis RE, MacDonald GJ (1995) Effects of endopeptidase 24.11 inhibition on plasma and tissue concentrations of vasoactive intestinal peptide. Clin Sci 89:267
5. Gnessi L, Ulisse S, Fabbri A, Jannini EA, Bonifacio V, Moretti C, Spera G, Isidori A (1989) Isolation of a human seminal plasma peptide with bombesin-like activity. Fertil Steril 51:1034
6. Hoosein NM, Logothetis CJ, Chung LWK (1993) Differential effects of peptide hormones bombesin, vasoactive intestinal polypeptide and somatostatin analog RC-160 on the

invasive capacity of human prostatic carcinoma cells. J Urol 149:1209
7. Krongrad A, Bai G (1994) c-*fos* promoter insensitivity of phorbol ester and possible role of protein kinase C in androgen-independent cancer cells. Cancer Res 54:6073
8. Logothetis C, Hoosein N (1992) The inhibition of the paracrine progression of prostate cancer as an approach to early therapy of prostatic carcinoma. J Cell Biochem 16:128
9. Ronquist G, Brody I (1985) The prostasome: its secretion and function in man. Biochem Biophys Acta 822:203
10. Ronquist G, Fritz GG, Jansson A (1988) Prostasome membrane associated enzyme activities and semen parameters in men attending an infertility clinic. Urol Int 43:133
11. Ryan JW, Chung AY, Nearing JA, Valido FA, Shun-Cun C, Berryer P (1993) A radiochemical assay for aminopeptidase N. Anal Biochem 210:27
12. Ryan JW, Chung AYK, Berryer P (1996) A radioassay for endopeptidase 24.11. FASEB J 10:A670
13. Ryan US, Hart MA (1986) Electron microscopy of endothelial cells in culture. J Tissue Cult Meth 10:31
14. Shipp MA, Tarr GE, Chen CY, Switzer SN, Hersh LB, Stein H, Sunday ME, Reinherz EL (1991) CD/neutral endopeptidase 24.11 hydrolyses bombesin-like peptides and regulates the growth of small cell carcinomas of the lung. Proc Natl Acad Sci USA 88:10662