Studies on Phenotypic Expression Patterns of Proteases and Protease Inhibitors in Benign Compared to Malignant Prostate Tissues

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The prostate gland produces several abundant proteolytic enzymes and protease inhibitors secreted into semen. Prostate-specific antigen (PSA) and human kallikrein-2 (hK2) are kallikrein-like serine proteases expressed both in the benign prostate and in prostate cancer (CAP). PSA manifests chymotrypsin-like activity, in contrast to the trypsin-like activity of hK2. Previously, it has been shown that the inactive PSA-zymogen (proPSA) is converted to active PSA by recombinant hK2 and by bovine trypsin *in vitro*. The PSA activity is inhibited extracellularly by complex binding to alpha-1-Antichymotrypsin (ACT), protein C inhibitor (PCI), alpha-1-Protease Inhibitor, and alpha-2-Macroglobulin. ACT is the predominant complexing ligand to PSA in blood whereas PCI is the most important regulator of hK2 activity in semen. Previously, immunohistochemistry (IHC), in situ-hybridisation (ISH) and time-resolved fluorescence imaging (TRFI) were used to show expression of ACT in prostatic secretory epithelium and in CAP. Now, we performed tissue studies of PSA, hK2, ACT, PCI and trypsinogen by conventional IHC, ISH, TRFI and at the ultrastructural level to improve our understanding of the protease-antiprotease interactions in benign compared to malignant prostatic tissue.

Methods

Paraffin sections from specimens obtained at urological operations were used for IHC and non-radioactive ISH. Double-immunogold histochemistry in transillumination electron microscopy (TEM) was used for co-localisation studies. Dual-label immunodetection of PSA and hK2 was accomplished by TRFI employing europium (Eu)-labeled anti-PSA and terbium (Tb)-labeled anti-hK2 Mabs, and a new workstation for TRFI (Signifer 1432 Microimager). This system was also used for evaluation of new labels for TRFI and for microarrays.

Results

PSA, ACT and PCI were detected in identical secretory granules using double-immunogold histochemistry in TEM.

The expression of PCI and trypsinogen was demonstrated in the secretory epithelium of the human prostate and in CAP by IHC and ISH. Trypsinogen was predominately distributed in ducts and in difference to PSA, infrequently in acini. PCI displayed an immunostaining pattern similar to that of PSA. Trypsinogen isoenzymes and PCI are also widely distributed in the secretory epithelium of the male genital tract.

TRFI

A dual-immunodetection method for quantitative IHC, revealed extensive variation in PSA/hK2 ratio in a small number of tissue specimens examined. A new Eu-chelate construct enabled excellent immunohistochemical localization of PSA expression in prostate tissues. Using the new reagent in a model microarray experiment with biotinylated mouse IgG as target, we demonstrate excellent spatial resolution of 5-10nL microspots. Preliminary results indicate that nanoparticles are useful as labels for improved sensitivity in TRFI.

Conclusions

- Double-immunogold histochemistry in TEM reveals that PSA and its inhibitors (ACT, PCI) are contained in secretory granules of the prostate epithelium.
- Trypsinogen isoenzymes produced locally in the prostate epithelium and in tumour cells, may be important for the activation of PSA and for tumour progression.
- Technical advances in TRFI may reflect quantitative immunohistochemistry for PSA, hK2, and ACT in tissue sections. Nanoparticles may be used to improve the sensitivity in direct quantification and for analysis of microarrays with a new microimager for TRFI.

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