Some Characteristics of Human Prostasomes and Their Relationship to Prostate Cancer

GÖRAN RONQUIST
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Abstract

Background: The secretory epithelial cells of the prostate gland use sophisticated vehicles named prostasomes to relay important information to sperm cells in semen. This prostasome-forming and secretory ability of the epithelial cells is also preserved in poorly differentiated prostate cancer cells.

Aim: The aim of this thesis was to examine different characteristics of prostasomes, especially those derived from malignant prostate cells, linked to their potential role in diagnosis and prognostication of prostate cancer.

Results: Serum samples of prostate cancer patients contained autoantibodies against seminal prostasomes in a higher concentration than did control sera. These autoantibodies were most frequently directed against 25 prostasome-associated proteins, but no one was prostate specific. Clusterin was one of the most frequently occurring prostasomal proteins. Elevated titers were however seen in both patients’ and control sera. Clusterin turned out to be a major antigen of seminal prostasomes. No prostate specific or prostate cancer specific protein was discovered upon proteomic analysis of prostasomes deriving from malignant cells of vertebral metastases of prostate cancer patients. Human chromosomal DNA was identified in both seminal prostasomes and PC-3 cell prostasomes and strong evidence existed that the DNA was localized inside the prostasomes. Four out of 13 DNA clones of seminal prostasomes featured gene sequences (31%). The corresponding figures for PC-3 cell prostasomes were 4 out of 16 clones (25%).

Conclusions: Prostasomes are immunogenic and give rise to serum autoantibodies. The most frequently occurring autoantibodies were directed against 25 prostasomal proteins but none of these was exclusively prostate specific. Thirty different proteins were identified in prostate cancer metastasis-derived prostasomes but none of these proteins was prostate cancer specific. Human chromosomal DNA was identified in prostasomes of both normal and malignant cell origin.

Keywords: Prostasomes, prostate, prostate cancer, clusterin, antibodies, metastasis, exosomes, PC-3 cells, DNA vehicles, gene therapy, immunoblotting, mass spectrometry, agarose gel electrophoresis, vertebral metastasis.

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Dedication
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Contents

Introduction ................................................................................................... 11
  The prostate gland .................................................................................... 11
  Prostate secretion ................................................................................... 12
  Prostasomes ............................................................................................ 12
  Prostasome biogenesis and secretion ..................................................... 14
  Prostasome interactions ......................................................................... 14
  Prostasome composition ........................................................................ 15
    Proteins ................................................................................................ 15
    Lipids ................................................................................................... 16
  Prostasome function ............................................................................... 16
    Sperm motility ..................................................................................... 17
    Immunosuppressive and complement inhibitory activity ...................... 17
    Antioxidant capacity ........................................................................... 18
    Antibacterial properties ..................................................................... 19
    Capacitation and acrosome reaction .................................................... 19
  Prostasomes in other species .................................................................. 20
  Prostate cancer ....................................................................................... 20
    Biochemical markers of prostate cancer .............................................. 21
    PC-3 cells ............................................................................................. 22
Aims ............................................................................................................. 24
  General aims ............................................................................................ 24
    Specific aims ........................................................................................ 24
Methods ........................................................................................................ 25
  Prostasome preparation (PAPERS I-IV) .................................................... 25
  Anti-clusterin enzyme-linked immunosorbent assay (ELISA) (PAPER II) ........................................................................................... 25
  2-D PAGE (Two-dimensional polyacrylamide gel electrophoresis) (PAPERS I, IV) ................................................................................... 26
  1-D and 2-D immunoblotting (PAPERS I, II) ............................................ 26
  Flow cytometry (PAPERS II, III) ............................................................ 26
  Clusterin purification (PAPER II) ............................................................ 27
  SELDI-TOF (surface-enhanced laser desorption ionization - time of flight) (PAPER II) ............................................................ 27
  Prostasomal DNA extraction and cloning (PAPER III) ............................. 28
  PC-3 cell culturing (PAPER III) .............................................................. 28
  Statistics (PAPERS II, III) ...................................................................... 28
Results .................................................................................................................. 29
PAPER I ........................................................................................................... 29
PAPER II ........................................................................................................... 30
PAPER III ........................................................................................................... 31
PAPER IV ........................................................................................................... 33
General discussion ............................................................................................ 35
Summary ............................................................................................................. 38
Svensk sammanfattning ...................................................................................... 39
Acknowledgements ............................................................................................. 42
References .......................................................................................................... 43
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AP</td>
<td>acid phosphatase</td>
</tr>
<tr>
<td>BLAST</td>
<td>basic local alignment search tool</td>
</tr>
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<td>BPH</td>
<td>benign prostatic hyperplasia</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>CD</td>
<td>cluster of differentiation</td>
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<td>CZ</td>
<td>central zone</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>GPI</td>
<td>glycosylphosphatidylinositol</td>
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<td>GTP</td>
<td>guanosine triphosphate</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>HRP</td>
<td>horse radish peroxidase</td>
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<td>HSP</td>
<td>heat shock proteins</td>
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<td>IMP</td>
<td>inosine monophosphate</td>
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<td>IP</td>
<td>isoelectric point</td>
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<tr>
<td>IPG</td>
<td>immobilized pH gradient</td>
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<tr>
<td>MAC</td>
<td>membrane attack complex</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MIRL</td>
<td>membrane inhibitor of reactive lysis</td>
</tr>
<tr>
<td>MVB</td>
<td>multivesicular bodies</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate, reduced form</td>
</tr>
<tr>
<td>PAP</td>
<td>prostatic acid phosphatase</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
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<td>PSA</td>
<td>prostate specific antigen</td>
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<tr>
<td>PZ</td>
<td>peripheral zone</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>SELDI-TOF</td>
<td>surface-enhanced laser desorption/ionization-time of flight</td>
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<tr>
<td>TZ</td>
<td>transition zone</td>
</tr>
<tr>
<td>VIP</td>
<td>vasoactive intestinal polypeptide</td>
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Introduction

The prostate gland

The prostate gland is located inferior to the urinary bladder neck, deep in the pelvis. It belongs to the male sex accessory glands together with the seminal vesicles, the ampullae of the vasa deferentes and the bulbourethral glands. The prostate is intimately associated with urethra and has a composite structure. It has the size of a walnut weighing about 18 g by the third decade, after which there is a gradual increase reaching a weight of about 31 g by the eighth decade (1). Not infrequently, both atrophy and hyperplasia are present in the prostate glands of elderly men. The prostate gland is difficult of access for examination. The posterior part of the peripheral zone (Fig 1) can be accessed by digital palpation via the rectum and here, most of the biopsies are taking place.

Fig 1. Left. Schematic view of the three different zones (except the periurethral zone) of the prostate. The central zone (CZ) and the transition zone (TZ) are embedded close to the urethra. Surrounding these zones is the peripheral zone (PZ). Right. The prostate gland in its anatomical location inferior to the urinary bladder neck. Lithograph plate from Gray’s anatomy originally published in 1918.

The prostate can be divided into three separate main zones (a fourth small zone of about 1% of the volume can be discerned in the periurethral zone): The peripheral zone (65-70%), the central zone (25%) and the transition zone (5-10%). The prostate represents a collection of 30-50 tubulo-alveolar glands secreting prostatic fluid into the prostatic urethra.
A fibroelastic capsule enriched in smooth muscle surrounds the prostate gland. The glandular compartment of the prostate (acinis and ducts) is built up by secretory, epithelial cells and basal cells. Neuroendocrine cells exist interfoliated among the secretory cells. The secretory cells are recognized in electron microscopy by: a high content of endoplasmic reticulum; a large Golgi-complex; manifold storage vesicles (multivesicular bodies, MVB) and lysosomes.

Prostate secretion

The prostate gland produces a protease-rich fluid that constitutes about 30% of the ejaculate. The pH of the secretion is slightly acid and the fluid is serous. There are a manifold of proteins in this fluid and some of them are generally considered specific for the organ, e.g. PSA (prostate specific antigen). PSA that is a member of the human kallikrein family, is a serine protease and degrades the gel forming proteins semenogelin I and II and fibronectin into multiple fragments, resulting in the liquefaction of semen (2). Other proteins with a high content in prostatic fluid are: acid phosphatase; albumin; other proteolytic enzymes and molecules involving mono- and divalent cations. In addition, the epithelial cells of the prostate also secrete small organelar structures, named prostasomes.

Prostasomes

Prostasomes were originally described in prostatic fluid and seminal plasma approximately 30 years ago (3-6). This observation was notable, since membrane-surrounded organellar structures could exist extracellularly \textit{in vivo} (4-6). The idea of an extracellular occurrence of organelles was subsequently corroborated by the existence of exosomes produced by other cell systems (7-9). Prostasomes are membrane-surrounded secretory granules produced by the luminal epithelial cells of the prostate gland (Fig 2).
At ejaculation, the prostasomes are expelled with the prostate secretion and mixed with other secretions of the accessory genital glands to constitute the seminal fluid. The prostasomes are encased usually by a lipid bilayered membrane with an exceptionally high cholesterol/phospholipid ratio (10). They have a globular appearance with a mean diameter of 150 nm, range 40-500 nm (Fig 3).
Prostasome biogenesis and secretion

Prostasomes are formed in the apical part of prostatic luminal epithelial cells. This locus, near the upper pole of the nucleus, is the area where the Golgi apparatus is most abundant (6, 11). Prostasome biogenesis presupposes the existence of so called "late endosomes" and involves the inward budding of what was originally called "storage vesicles" (6) (Fig 4). They should be understood as equivalent to multivesicular bodies (MVB), to produce the prostasome precursors reasonably originating from the Golgi membranes (6, 11). It should be noted that there were no ultrastructural indications that the prostasomes were formed in the cytoplasm and then incorporated in the storage vesicles (11). When fused with the plasma membrane of the prostatic luminal epithelial cell, the storage vesicles release the prostasomes into the extracellular environment to become constituents of the prostatic fluid and hence, the seminal fluid (6, 11), where they can play an important role in cell-to-cell signaling by the transfer of various molecules from their maternal prostatic epithelial cells to the spermatozoa.

![Fig 4. Invagination of the endosomal membrane to form multivesicular bodies or storage vesicles.](image)

Prostasome interactions

Prostasomes as real components of semen raised the justified question about their relevance to reproductive health. The release of prostasomes was early suspected to be a cellular mechanism of mediating one or more biological functions at a distance from one cell to another cell. The physiological relevance of prostasomes was highlighted by the finding that prostasomes were indeed able to interact with spermatozoa (12). This important extracellular joint action between a cell (spermatozoon) and an organelle (prostasome) was subsequently confirmed in different ways (13-15). The corollary of such an interaction is that in stead of a direct cell-to-cell interaction, *i.e.* an inte-
raction between the prostatic secretory epithelial cell and the sperm cell with an expected stoichiometric relationship of 1:1 (which for practical reasons is impossible), one prostatic luminal epithelial cell can reach several sperm cells via its messengers, i.e. the membrane-surrounded prostasomes. Here-with, a membrane-to-membrane contact can be established between prostasomes and sperm cells in an orderly fashion. Accordingly, one luminal epithelial cell of the prostate has the ability to mediate different competences to several sperm cells in favour of their survival in the female genital tract and their ultimate goal to reach and penetrate the zona pellucida for fertilization of the ovum. It is worthy of note that in this context, the number of prostasomes in an ejaculate on a stoichiometric basis exceeds by far that of spermatozoa (16).

Prostasome composition

Proteins
The protein composition of human prostasomes is varied and has been rather comprehensively investigated. The analysis of Utleg et al (17) comprised microcapillary high-performance liquid chromatography (HPLC)-electrospray ionization tandem mass spectrometry coupled with an iterative gas phase fractionation. In this way a minimum of 139 proteins were identified subdivided into 6 different categories: enzymes (35% of total); transport/structural proteins (19%); guanosine triphosphate (GTP) proteins (14%); chaperone proteins (6%); signal transduction proteins (17%); unnotated proteins (9%). A more recent investigation by Poliakov et al (18) using trypsin in-gel digestion and liquid chromatography/mass spectrometry led to the identification of 440 proteins. These latter authors also confirmed previous observations (4, 5, 11) of a certain degree of heterogeneity among the prostasomes.

Many of the prostosomal enzymes are involved in redox reactions. One enzyme, dipeptidyl peptidase IV (CD26) with an extremely high specific activity in prostasomes (19), was identified as the antigen of a monoclonal antibody to human prostasomes (20). The first membrane-bound enzyme (in a lipoprotein complex) identified in prostasomes was ATPase whose activity was divalent cation-dependent (3-5, 21, 22). Some other hydrolyzing enzymes are linked to the prostasome membrane via a glycosylphosphatidylinositol (GPI) anchor (23). In comparison with the surrounding seminal plasma, an unambiguous concentration of metals (magnesium, calcium, zinc) has been observed in prostasomes (24). The ATPase of the prostasome membrane (21, 22) may be the molecular basis for the vectorial transport of these divalent cations. Hence, prostasomes may exert a regulatory function on spermatozoa by modulating their microenvironment as regards divalent cations (25).
Among the transport/structural proteins of prostasomes, 6 members of the annexin family were identified (17). Annexins hold a central hydrophilic pore functioning as a calcium ion channel. Since prostasomes are rich in calcium and zinc, annexins may be involved in the recruitment of calcium to prostasomes as well. A zinc-binding ability of prostasomes was reported (26), well in line with the idea of prostasomes being regulators of the micro-environment of spermatozoa (25).

Lipids
The phospholipid composition of prostasomes reveals some extraordinary peculiarities. Sphingomyelin was the predominant phospholipid class representing almost half of the total phospholipid measured and the saturated fatty acids were quantitatively dominating (10). Cholesterol is known to be abundant (for physical-chemical reasons) in membranes that contain saturated phospholipid acyl chains and have a high content of sphingomyelin. This reasoning was in line with the finding of an ample representation of cholesterol in prostasomes resulting in a high cholesterol/phospholipid ratio close to 2 (10). These data have been confirmed by others (27). The discovery of sphingomyelin phosphodiesterase in prostasomes (18) is of interest, since this enzyme might be involved in the formation of secondary vesicles. Sphingomyelin phosphodiesterase cleaves off the ceramide portion of sphingomyelin releasing phosphocholine. The ceramide molecule has been implicated in the formation of secondary vesicles in giant unilamellar vesicles (28). The lipid composition of the plasma membrane of sperm cells is clearly different from that of the prostasome membrane although the sperm cells and prostasomes share the same external medium. Since the ordering of lipids in membranes is greatly influenced by the cholesterol and sphingomyelin content and by the degree of saturation of the phospholipid acyl chains (29), one would expect the lipids in the prostasome membrane to be highly ordered. Such was indeed the case and electron spin labelling experiments revealed very high order parameters for prostasomes and extracted prostasome lipids meaning that the prostasome membrane is a tight and highly ordered structure. This is also consonant with our experience of a firm resistance of prostasomes against mechanical treatment, including ultrasonication and changes in osmolarity in the surrounding fluid.

Prostasome function
Once released from the acinar prostate epithelial cells, and being constituents of semen, it is supposed that prostasomes interact and eventually fuse with the sperm cell membranes. Herewith prostasomes work as intercellular messengers between the prostate secretory cells and the sperm cells transferring molecules to the latter cells in favour of fertilization.
Sperm motility

Buffer washings of normozoospermatic spermatozoa terminate in a gradual loss of their forward motility (25). The perturbation that is the result of the buffer treatment is not of an irreversible nature since the sperm cells can be functionally restored fairly instantaneously by the addition of prostasomes (25). Furthermore, the sperm cells demonstrated a metabolic ability to exploit the energy potential of added hexoses manifested by the corroborative action by any of fructose, glucose or mannose on prostasome-promotive activity on sperm forward motility (25).

The mechanism by which prostasomes promote forward motility in spermatozoa is not obvious. Since prostasomes have the ability to interact with spermatozoa, several membrane properties may be affected including sperm membrane permeability to calcium ions (30) that might trigger contractile proteins. Another explanation could be an effect on sperm cells via cyclic adenosine monophosphate (cAMP) exerted by vasoactive intestinal polypeptide (VIP) which is a constituent of prostasomes (31). Previous research indicates that intracellular cAMP is related to induction of sperm motility (32, 33). A higher recovery of motile sperm cells was obtained after swim-up if albumin in the ordinary standard balanced salt medium was exchanged for prostasomes or if this solution containing albumin was supplemented with prostasomes (34). Prostasomes also have the ability to augment the number of hyper activated sperm cells (34), which is believed to be important for penetration of zona pellucida and fertilization (35). It might well be that swim-up media containing prostasomes contribute to a better recovery of hyperactivated motile sperm cells from semen samples with a reduced number of motile spermatozoa. A beneficial prostasomal effect was also observed on post-thaw spermatozoa after prostasome inclusion in swim-up medium (36).

Immunosuppressive and complement inhibitory activity

Sperm cells are conceived as foreign invaders in the female genital tract that is, accordingly, a potentially hostile environment. Prostasomes have shown to be able to assist the spermatozoa in evading potent female immune effectors and they have been identified as inhibitors in lymphoproliferation assays (37, 38). This activity accounts for a substantial proportion of the immunosuppressive activity of human seminal plasma (39). Since the prostasomes have the ability to adhere to spermatozoa (12, 15), there is the probability that the immunosuppressive effect associated with these organelles can be carried up the female genital tract with the spermatozoa (39). Prostasomes are capable of inhibiting mitogen-induced lymphoproliferation in a dose-dependent fashion with a concentration of prostasomes equivalent to 40% of that seen in seminal plasma giving 69% suppression of thymidine incorporation (39). In addition, prostasomes exert a direct effect on macrophage function (37).
Prostasomes bind rapidly to the plasma membrane of leukocytes leading to internalization of adsorbed material. Interaction of prostasomes with neutrophils and monocytes inhibits their ability to phagocytose latex particles (37). Similarly, endocytosis of prostasomes by the cells may suppress their ability to generate oxygen radicals (37). Accordingly, phagocytosing cells will become more or less invalidated due to ingestion of prostasomes. This in turn would favour the survival of sperm cells in the female genital tract.

Prostasomes contain in their membrane the membrane attack complex (MAC) inhibitory protein CD59, also known as membrane inhibitor of reactive lysis (MIRL) (40). There are reasons to believe that CD59 is carried on the surface of the prostasomes in a glycosylphosphatidylinositol (GPI) anchor and that sperm cells may acquire functionally active CD59 molecules as a result of interaction with prostasomes (40, 41). A direct transfer of functionally active CD59 molecules from prostasomes to CD59-deficient erythrocytes has been demonstrated (42). Accordingly, prostasomes may represent a pool of CD59 from which protein lost from sperm cells, possibly as a result of normal membrane turn-over or of low-level complement attack, may be replenished. Herewith, the sperm cells are decently guarded against the membrane attack complex when advancing in the female genital tract.

Antioxidant capacity
Reactive oxygen species (ROS) are a major cause of idiopathic male infertility. An abnormally high production of ROS has been demonstrated in 40% of semen samples from infertile patients, while very few were found in samples from fertile donors (43). Human sperm cells are sensitive to oxidative stress resulting in peroxidative damage. This sensitivity is due to the sperm cell membrane’s high content of unsaturated fatty acids combined with the small cytoplasmic volume that limit the scavenging potential of the sperm cell (44). Leukocytes infiltrating the semen seem to be a major source of ROS generation (45). As mentioned before, prostasomes have the ability to interact with neutrophil granulocytes and reduce their capacity to generate superoxide anion after stimulation (37). Later, it was revealed that the inhibitory effect of prostasomes on ROS production involved an interaction between prostasomes and the neutrophil granulocytes, since prostasomes lack the ability of directly acting as ROS scavengers (45). Subsequent studies established an inhibitory role of prostasomes on the NADPH (nicotinamide adenine dinucleotide phosphate, reduced) oxidase activity of these granulocytes as a consequence of lipid transfer from prostasomes to the plasma membrane of these cells (46).
Antibacterial properties

An antibacterial effect of prostasomes was previously reported from our laboratory (47). This effect was linked to deformation of the bacterial membrane, and in this process the creation of membrane cavities seemed to be essential, resulting in bacterial cell death (47). The bactericidal effect of prostasomes differed mechanistically from the one exerted by neutrophil granulocytes. The principle of action of these latter cells is based on ROS formation and action. As already mentioned, due to their paucity in cytoplasm and antioxidant mechanisms, sperm cells themselves have little defense against ROS that may damage their membrane and DNA (44, 48). Therefore, it is not incidental that prostasomes rather than neutrophil granulocytes serve as antibacterial agents in semen.

Capacitation and acrosome reaction

Spermatozoa must undergo capacitation and the acrosome reaction before being valid for fertilization. Capacitation means the removal of the glycoprotein coat and seminal plasma proteins overlying the acrosomal region of the spermatozoon. Capacitation is obligatory and must precede the acrosome reaction which in turn allows the sperm cell to fuse with the female egg. The influence of sperm-prostasome interaction and fusion on acrosome reaction has been studied to some extent but with somewhat controversial results. This may reflect the complex events involved in the whole process. During fusion with prostasomes, lipids, proteins, and ions can be delivered to sperm cells (30, 49, 50). It has been suggested that the interaction and fusion of prostasomes with sperm cells can delay the acrosome reaction (51) concomitantly with a cholesterol transfer from prostasomes to sperm cells. Contrary to this, it has been proposed that prostasome-sperm interaction and fusion can stimulate the acrosome reaction, making sperm cells more sensitive to the effect of progesterone (52). This latter report is consonant with findings of a stimulatory effect of porcine seminal prostasomes on the acrosome reaction (53). It should also be kept in mind that fusion of prostasomes with sperm cells will render these cells more sensitive to the effect of progesterone on acrosome reaction induction (54), and progesterone is believed to be one of the stimulatory factors of the acrosome reactions. Prostasomes seem to play a role in the complex reciprocal action between different factors leading to capacitation and acrosome reaction.
Prostasomes in other species

Prostasome-like vesicles have been observed in ram (55), dog (56), stallion (57), and boar (53). Additionally, bovine organelle production and secretion were described in seminal vesicles with no corresponding production in the prostate gland of the bull and these microvesicles were denoted vesiculosomes (58). Accordingly, the interplay between organelles (prostasomes) and sperm cells as a prerequisite for reproduction may not be confined to man but could well involve some other species as well.

Prostate cancer

Various pathological conditions can develop in the prostate. They are: benign prostatic hyperplasia (BPH); cancer; infection (prostatitis), both bacterial and non-bacterial, abscesses and atrophy. The most common abnormality in elderly males is BPH that showed a prevalence of 80% in men aged over 40 years (59).

Cancer in the prostate gland is the second most common disorder. BPH and cancer have in common that they increase rapidly in incidence and prevalence with age (1), and prostate cancer is one of the most common malignancies in the Western world (60).


Microscopic foci indicative of prostate cancer can be demonstrated in a majority of men above the age of 70 years (61). Still, only a subgroup of
these men will develop a clinically significant cancer (Fig 5). In its clinical presentation, prostate cancer is diagnosed as local or advanced, and treatments range from “watchful-waiting” to radical surgery, irradiation, or maximal androgen blockade. Androgen antagonism has been successful in 70-80% of cancer patients with advanced prostate cancer as regards to reduction of clinical symptoms, but most cancers relapse within two years to an androgen independent state (62). As a matter of fact, it has not been able to cure prostate cancer patients with metastatic disease. It should be added that the cellular and molecular mechanisms behind initiation and progression towards metastasis and androgen independence are not very well known. Also, the etiology of prostate cancer is not understood very well. Several factors, both genetic and environmental have been shown to correlate with the incidence of prostate cancer and this is reflected by the strikingly large international differences in the incidence of prostate cancer (63). No unambiguous causal relationships have been established, and apart from men with advanced age and familial aggregation, the identification of high risk patients is not possible at present. The risk of dying from prostate cancer is believed to be 2-3 times higher among first degree relatives of patients than among age-matched controls (64, 65).

Biochemical markers of prostate cancer

The most useful prostatic tumour markers in clinical practice have been prostatic acid phosphatase (PAP) and prostate specific antigen (PSA).

**PAP.** Prostate cancer was the first malignancy for which a biochemical marker in serum was used for diagnosis by testing serum acid phosphatase (AP) and subsequently prostatic acid phosphatase (66). PAP was the standard serum marker for prostate cancer (67) until tests for PSA took over due to higher specificity and sensitivity.

**PSA.** PSA is a serine protease of the kallikrein family produced by benign and malignant epithelial cells lining acini and ducts of the prostate gland (68, 69). Most of PSA is soluble in the prostate secretion but a smaller fraction is associated to prostasomes (17). Upon ejaculation, PSA is secreted into the seminal fluid, where it occurs in a concentration of about 1g/L (i.e. in a concentration of about 1 million times higher than that of blood plasma) (70). PSA was originally isolated from seminal plasma and some years later purified by Li and Beling (71). The same glycoprotein was isolated from human prostate tissue in 1979, and named “prostate specific antigen” (72). One year later PSA was identified in human serum and the identity with the molecule isolated from prostate tissue was verified (73). Shortly thereafter it was suggested to be a marker of prostate cancer (74, 75). It is important to point out
that PSA is organ-specific and not prostate cancer-specific. Therefore, increased serum concentrations of PSA are also typically found in patients with BPH. The relationship between BPH and PSA has been rather extensively investigated in repeated studies (76-79). Accordingly, the overlap in serum PSA concentrations between patients with BPH and those with prostate cancer diminishes the usefulness of serum PSA measurement in the early detection of curable prostate cancer. This overlap is most obvious at serum PSA concentrations between 3.0-10.0 μg/L. Therefore, as many as 45% of patients with prostate cancer will be missed if diagnosis is solely based on serum PSA value (80-82). In order to make the distinction between BPH and prostate cancer more stringent, estimation of the quota between free/total PSA has been used. A low quota indicates that prostate cancer is more probable while an increased quota is indicative of BPH. The details of the mechanism of PSA leakage to blood plasma from unharmed prostate tissue are not known. In prostate cancer, however, when the polarised structure of the secreting cells resolve, and the cancer invades surrounding tissues, a continuous leakage of PSA into interstitial fluid and blood plasma is to be expected.

PC-3 cells

PC-3 cells have been established as an epithelial cell line from a human prostatic adenocarcinoma metastatic to bone (83) and have been used as model cells for prostate cancer. Immunostaining of cytospins from monolayers of PC-3 cells demonstrated the content of a granular secretory component that displayed the same antigen epitope as did the secretion of the normal prostate epithelial cells when reacted with a monoclonal antibody against human prostasomes (84). Direct isolation of prostasomes and characterization of some prostasomal properties from PC-3 cells were subsequently made possible (15). PC-3 cells were used as a model to study differentiation therapy drugs for the treatment of prostate cancers that were refractory to standard chemotherapy (85). The idea was that cultured prostate tumour cells should be able to be induced to produce prostasomes or their building blocks coupled with replication arrest being implied that these organelles should represent a useful system to study potential differentiation therapy drugs (86). Inhibitors of IMP (inosine monophosphate) dehydrogenase, being a key enzyme in guanine nucleotide biosynthesis, have been found to effectively induce differentiation in a variety of distinct human tumour cell types (86, 87). Indeed, mycophenolic acid, an inhibitor, induced the expression of about half of the tested prostasomal proteins and a differentiation of PC-3 cells was achieved into cells that resemble normal luminal prostate cells (85). PC-3 cells were also used as model cells to acquire more detailed insights in the production and release of prostasomes. Llorente et al (88) ob-
served a high level of co-localization of caveolin-1 and raft-associated integral membrane proteins of the MAL family in an intracellular multivesicular compartment with resemblance to storage vesicles. What was more, these authors found again these proteins in the vesicular fraction (prostasome fraction) released from PC-3 cells into the culture medium (88). A subsequent study revealed that cholesterol can regulate the release of prostasomes from the PC-3 cells (89). This conclusion was reached after observing that PC-3 cells subjected to cholesterol-depleting drugs increased their secretion of prostasomes (89). Treatment of PC-3 cells with nanomolar concentrations of simvastatin, an inhibitor of biosynthesis of cholesterol, had a direct reducing effect on prostasome production and therewith prostasome release (90).
Aims

General aims
Cancers are in most cases a deceptive disease that in the early development slowly evolves by accumulating mutations into their genomes. The immune system has difficulties in finding the early stages of cancer growth since cancer cells originate from “self cells”. All nucleated cells have MHC I (major histocompatibility complex) receptors presenting peptides that represent newly synthesized proteins in the cells. MHC I is loaded with peptides in endoplasmic reticulum (ER) before this complex is transported to and exposed on the cell surface. Thereby, cells that have been hi-jacked by viruses or have become cancerous present foreign or altered proteins attached to their MHC I. This will signal to the immune cells that something is wrong with the MHC I-bearing host cell. This system hinders many cancers to evolve but, cancers evading detection will be a threat to the host.

Much research has been done for development of specific markers to detect evading cancers. Preferential properties of such markers should be: high sensitivity; high specificity; good reproducibility; and an easy way to carry out the test, e.g. a blood test. The prevailing opinion (based on experience) is that not a single antigen can possess all these abilities, instead future cancer markers are expected to represent a panel of antigens. It is so far unknown, whether prostasomes contain some protein(s) that is (are) unique for this organelle. However, there are data supporting the view that prostasomes deriving from prostate cancer cells differ from those deriving from normal cells.

Specific aims
- Identify prostasomal antigens giving rise to autoantibodies present in sera of patients with well characterized prostate cancer.
- Select an antigen of potential interest for monitoring prostate cancer patients and establish its association to prostasomes.
- Characterize the DNA presence in human prostasomes.
- Examine by 2D-electrophoresis and by ensuing mass spectrometry the identity of different spots derived from prostasomes from cells of vertebral metastases of prostate cancer.
Methods

Prostasome preparation (PAPERS I-IV)

Seminal prostasomes: Seminal plasma was centrifuged for 30 min at 10 000g to remove possible cell debris. The supernatant was subsequently subjected to ultracentrifugation for 2 h at 100 000g to pellet the prostasomes.

PC-3 cell prostasomes: Collected PC-3 cells stored in the freezer were thawed and mildly sonicated and the resulting suspension was centrifuged at 1500g for 30 min and then at 10 000g for 20 min to remove cell debris. The supernatant was subsequently subjected to ultracentrifugation for 2 h at 100 000g to pellet the prostasomes.

Prostasomes derived from metastatic cells: Tissues from metastases were homogenized using an Ultra-turrax homogenisator. The homogenates were centrifuged 3 times for 15 min at 3000g, then twice for 15 min at 10 000g. The supernatant was subsequently subjected to ultracentrifugation for 2 h at 100 000g to pellet the prostasomes.

All three types of prostasomes were resuspended, separately, in isotonic Tris-HCl (pH 7.6). The suspensions were further purified on a Sephadex G 200 column, equilibrated with the isotonic Tris-HCl buffer, to separate the prostasomes from an amorphous substance. Fractions with elevated A_{260} and A_{280} were collected and pooled for ultra-centrifugation at 100 000g for 2 h. The pellets representing the “purified prostasomes” were resuspended in the isotonic Tris-HCl buffer and adjusted to a protein concentration of 2 mg/mL using a Protein Assay ESL method. These protocols were used with only few exceptions.

Anti-clusterin enzyme-linked immunosorbent assay (ELISA) (PAPER II)

Microtitre plates were coated with clusterin and incubated with patients’ sera to investigate the possible presence of anti-clusterin autoantibodies in the sera. Secondary anti-human antibodies conjugated with HRP (horse radish peroxidase) enzyme were added. Finally, substrate for HRP was added and the enzymatic reaction was stopped with sulphuric acid and absorbances
were measured. The autoantibody titers of the patient sera were estimated. High and low standards were run in each plate.

\[
\text{Sample factor value} = \frac{(A_{450} \text{ of patient sample} - A_{450} \text{ of low standard})}{(A_{450} \text{ of high standard} - A_{450} \text{ of low standard})}
\]

2-D PAGE (Two-dimensional polyacrylamide gel electrophoresis) (PAPERS I, IV)

In paper I a large (17 cm strip) 2D-gel was used to separate seminal prostatic proteins and in paper IV the same sized 2D-gel was run with metastasizing cell prostatic proteins. In both cases and for first dimension electrophoresis, prostatic proteins were loaded onto the IPG (immobilized pH gradient, pH 4-10) strips for isoelectric focusing. Loaded proteins will migrate on the immobilized pH gradient towards the anode or the cathode in an electric field to the position of the pH gradient where the actual pH agrees with the isoelectric point (IP) of the protein, i.e. where the net charge of the protein is zero. The second dimension was carried out in a 4-12 % SDS gel and protein visualizations were performed by silver staining and by Coomassie blue for the mass spectrometry.

1-D and 2-D immunoblotting (PAPERS I, II)

After 1D and 2D electrophoresis, the proteins were electro-transferred to nitrocellulose membranes. The membranes were blocked to avoid unspecific binding.

In paper I, the membranes were incubated overnight with patients’ sera and next day, incubated first with biotinylated anti-human IgG antibodies and thereafter with streptavidin alkaline phosphatase. The visualization was made by NBT/BCIP (nitro-blue tetrazolium chloride/5-bromo-4-chloro-3’-indolyl phosphate p-toluidine) substrate. This was done for both 1D and 2D.

In paper II, murine anti-clusterin antibodies and secondary biotin-conjugated anti-murine antibodies identified the purified clusterin on 1D immunoblot. The visualization was performed by NBT/BCIP substrate.

Flow cytometry (PAPERS II, III)

Flow cytometry uses the reflection and refraction of light at boundaries between two media (buffer solution and cell membranes/cell interior) to measure size and complicity of membranous vesicles and cells. It can also be used to identify bound fluorescence molecules by selecting the emitted wavelength of those molecules.
In paper II, diluted seminal prostasomes were incubated with monoclonal murine anti-clusterin antibodies. Secondary FITC-conjugated anti-murine IgG antibodies were added. FITC conjugated CD46 antibodies incubated with seminal prostasomes were used as an irrelevant control.

In paper III, prostasomes were incubated with membrane-permeable and membrane-impermeable fluorescent DNA-staining in order to decide if DNA was attached to the outside of or packed inside prostasomes. The fluorescence was analyzed at appropriate emitting wavelengths. Unstained prostasomes were used as control.

Clusterin purification (PAPER II)
Blood was withdrawn from a volunteer and cooled at room temperature. The tubes were centrifuged and serum was collected. Two different concentrations of polyethylene glycol (PEG) were used to precipitate irrelevant material (lower concentration) and in a second step to precipitate the clusterin containing fraction (higher concentration) by centrifugation. The pellet was dissolved in 40 mL EDTA solution and filtered (0.2 μm nylon filter). The filtrate was applied to immunoaffinity chromatography where monoclonal clusterin antibodies had been coupled to the column. The clusterin-containing solution was applied and eluted. The eluate was further purified on a protein A column to remove contaminating immunoglobulins and the non-retained material was collected and desalted on Econo-Pac 10DG Desalting Column. The desalted solution was concentrated by freeze-drying and the resulting powder was dissolved in 1 mL MQ-water.

SELDI-TOF (surface-enhanced laser desorption ionization - time of flight) (PAPER II)
SELDI and other mass spectrometry applications provide the ability to accurately measure the mass of almost any molecule that can be ionized to the gas phase. In SELDI-TOF, an assisting laser energy absorbing matrix ionizes the sample to the gas phase and flying molecules are accelerated in an electric field. “Time of flight” is measured in a vacuum tube and (by assuming the law of preservation of energy) the kinetic energy equalizes the potential energy of the electric field, and the mass/charge ratio can be calculated.

Purified clusterin was applied onto active spots of the (NP20) array and the spots were covered with laser energy absorbing matrix. The SELDI spectrum was used for analysis of the purity of the applied clusterin-sample.
Prostasomal DNA extraction and cloning (PAPER III)

Prostasomal DNA was extracted by QIAmp DNA blood mini kit. This kit contains a lytic solution and a DNA-binding membrane. DNA from lysed prostasomes, attached to the membrane, was washed and eluted from the membrane. Enrichment of the DNA was performed by ethanol precipitation.

Extracted prostasomal DNA was sheared by pressurized helium gas (9-10 psi) resulting in fragments of about 1 000 base pairs. The fragments were blunted and cloned into blunt-ended plasmids. Transformation of TOP10 bacteria was performed by heat shocking at 42 °C for 45 s. Spread bacteria were grown at selective LB-plates over night. Growing colonies with successful ligation, detected by the Lac-operon blue white screening, were picked up and grown in LB-media under agitation over night.

The isolation of plasmid DNA was performed with a DNA-binding membrane-based kit.

PC-3 cell culturing (PAPER III)

The human prostatic carcinoma cell line PC-3 was obtained from the American Type Culture Collection. The cells were maintained in RPMI 1640 cell culture medium supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine. The cells were grown at 37°C in a humidified atmosphere of 5% CO₂ in air. When the cells reached 90% confluence, they were recovered by 0.25% trypsin-EDTA. Collected PC-3 cells were pooled and frozen for later purification of prostasomes.

Statistics (PAPERS II, III)

Spearman rank. In Spearman rank two sets of data are converted to rankings and correlation between ranks gives the correlation coefficient. The coefficient of correlation ranges between -1 to +1 whereas +1 is exact correlation and -1 is the exact inverse correlation. An example is the double run comparing clusterin coated ELISA plates with R=0.77.

BLAST. (Basic local alignment search tool) is an algorithm for comparing primary biological sequence information, such as the nucleotides of DNA sequences. A BLAST search enables comparisons between query sequences and target sequences in a library or database of sequences.
Results

PAPER I

The prostate acini are constituted by luminal epithelial cells on a layer of aligned basal cells. Surrounding these basal cells there is a basement membrane, which separates acini from stroma. The luminal epithelial cells are normally highly polarized both morphologically and functionally meaning that most products, e.g. PSA and prostasomes, are secreted mainly toward the glandular lumen. A destruction of the glandular structure could lead to leakage of prostasomes into the blood. A pilot study to find leaking prostasomes into blood of prostate cancer patients displayed too few prostasomes for reliable data. Therefore, instead of searching for prostasomes themselves in blood plasma, it was eligible to trace imprints of them and attention was changed towards the possible immune response against leaking prostasomes. Differences in the levels of serum antibodies against seminal prostasomes had been noted among patients with suspected prostate cancer by the ELISA-technique (500 sera investigated). The first paper had the ambition to identify antigens on prostasomes giving rise to (auto-) antibodies.

Out of the 500 sera, 44 sera with high prostasome auto-antibody titers were used as primary antibodies in 1D-immunoblots and 31 (70%) demonstrated reactivity against several prostasomal protein bands in the molecular weight range of 10-200 kDa. Thirteen sera with high reactivity were tested on small (11cm IEF-strips) 2D blots and 8 sera with strong 2D appearance were used for blotting on large (17cm IEF-strips) 2D immunoblots. Proteins stained on large immunoblots were retrieved on a corresponding large 2D PAGE-gel, punched out, and sent for identification by mass spectrometry (Fig 6).
Fig 6. Different analytical steps to identify prostasomal antigens. ELISA plates coated with seminal prostasomes sorted out 44 sera with high antibody titers against prostasomes. These sera were used in 1D-blotting to select 13 suitable sera for following 2D-blottings. Small (11cm) 2D-blottings displayed which sera (out of the 13) that should be used for the large (17cm) 2D-blottings. Finally, 8 sera were chosen for the large 2D-blottings.

Proteins in 25 localized positions were identified via MALDI and database searches. Heat shock 70 and 71 proteins (HSP70, HSP71) were present in all 8 of the large (17cm) Western blots. Clusterin and its isoforms were present in 6 out of 8 blots, ATPase and superoxide dismutase were present in 4 out of 8 blots, chromosome 17 open reading frame and peroxiredoxin 6 were present in 3 out of 8 blots, all other numbered positions were only appearing in one or at most two of the 8 blots (F-actin capping protein; alcohol dehydrogenase; syntenin 1; prostate specific antigen; HSP 27; glyoxalase I; semenogelin I; prolactin-inducible protein [PIP precursor]; DJ-1 protein).

PAPER II

Clusterin, one of the most frequently identified antigens in paper I, had at the time being, attracted considerable interest in the prostate cancer area. We purified clusterin from blood serum and coated wells in ELISA. We had at our disposal sera from 391 patients with suspected prostate cancer (150 benign prostate and 241 prostate cancers). These sera were used to track autoantibodies against clusterin and to correlate the antibody levels with different clinical variables of the patients (Fig 7).
Fig 7. Purification of clusterin and anti-clusterin serum titer determination. Blood withdrawn from a healthy volunteer underwent several steps to concentrate less pronounced serum proteins. Clusterin was enriched by immunoaffinity chromatography. The clusterin was recovered and used to coat ELISA wells for serum antibody titer determination in prostate cancer patients.

Anti-clusterin ELISA titers in sera of cancer patients did not differ significantly from those of a control group consisting of blood donors with low serum PSA. A significant inverse correlation (significance level 5%) existed between anti-clusterin ELISA titers and lymph node metastases ($p=0.047$), but only 11 out of 161 patients had metastases. Also, these titers correlated significantly with total prostate ($p=0.021$) and transition zone ($p=0.015$) volumes of the patients.

**PAPER III**

Purified seminal prostasomal DNA separated on 1% agarose gel displayed fragments from about 13 000 base pairs and below with two bands, one at 1 000 base pairs and the other at 2 000 base pairs (Fig 8).
Tests performed to investigate whether the DNA was attached to the prostasomal surface or located inside the prostasomes indicated an inside location; prostasomes treated with DNase did not display a lower quota of $A_{260}/A_{280}$ after treatment, and flow cytometric investigation with membrane-permeable and membrane-impermeable fluorescent DNA stains showed fluorescence only for the membrane-permeable stain.

Fig 8. Purified seminal prostasomal DNA separated on 1% agarose gel. DNA-fragments appeared from 13 000 base pairs and below. Two bands, one at 2 000 base pairs, and another at 1 000 base pairs were visible.

Fig 9. Prostasomal DNA identification. Sheared prostasomal DNA was ligated into plasmids. The plasmids were transformed into bacteria and spread onto LB-plates containing antibiotics. Plasmid DNA conferred antibiotic resistance and only transformed bacteria could grow on the plates. Formed colonies, descendent from a single transformed bacterium, were picked and grown in selective media over night. Plasmids were purified from each over night culture and the ligated DNA fragment was sequenced and aligned against BLAST.
Further investigations (by cloning, Fig 9) to trace the origin and identity of the DNA resulted in database alignments (BLAST-database) to human genomic sequences. Both seminal prostasomal (13 clones) and PC-3 cell prostasomal (16 clones) DNA-fragments were aligned and 4 clones (31%) of the seminal prostasomal fragments and 4 clones (25%) of the PC-3 cell fragments featured gene sequences, see Table.

<table>
<thead>
<tr>
<th>Seminal prostasomes (featured gene sequences)</th>
<th>PC-3 cell prostasomes (featured gene sequences)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q domain containing 1 isoform 1,2</td>
<td>breast cancer metastasis-suppressor like-1</td>
</tr>
<tr>
<td>ankyrin repeat domain 20 family, member A4</td>
<td>MAM domain containing 1</td>
</tr>
<tr>
<td>KH domain-containing, RNA-binding, signal transduction-associated 2</td>
<td>odd OZ/ten-m homolog 4</td>
</tr>
<tr>
<td>Down syndrome cell adhesion molecule</td>
<td>TSC22 domain family, member 1 isoform 1</td>
</tr>
</tbody>
</table>

**PAPER IV**

Proteins from prostasomes purified from prostate skeletal cancer metastases were separated on a 2D-gel and stained spots were punched out and identified by mass spectrometry. A total of 104 spots were punched out for identification. Twenty five unique protein spots had a MALDI-TOF score above 49 and another 5 proteins were determined by MS/MS. The remaining 74 spots were either identical to already determined proteins or had no reliable score. Annexins A1, A3, and A5 as well as dimethylarginine dimethylaminohydrolase 1 were among the identified proteins. The annexins (together with S100 calcium binding protein, that was also identified) and dimethylarginine dimethylaminohydrolase 1 found in metastatic cancer cell-derived prostasomes can act, among other things, as angiogenic factors and can increase the vascular development in the neighborhood of the tumour. Cancer-derived prostasomes may play an important role in the interaction between tumour cells and their environment. Other identified prostasomal proteins derived from prostate cancer metastases are given in Table.
<table>
<thead>
<tr>
<th>Protein name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione synthetase</td>
<td>stratifin</td>
</tr>
<tr>
<td>phosphoglycerate dehydrogenase</td>
<td>syndecan binding protein (syntenin)</td>
</tr>
<tr>
<td>leucine aminopeptidase 3</td>
<td>heat shock 27kDa protein 1</td>
</tr>
<tr>
<td>enolase 1, (alpha)</td>
<td>peroxiredoxin 6</td>
</tr>
<tr>
<td>keratin 10 (kontamine)</td>
<td>triosephosphate isomerase 1</td>
</tr>
<tr>
<td>creatine kinase B</td>
<td>phosphatidylethanolamine binding protein 1</td>
</tr>
<tr>
<td>actin beta</td>
<td>Semenogelin I</td>
</tr>
<tr>
<td>isocitrate dehydrogenase 1 (NADP+), soluble</td>
<td>SOD</td>
</tr>
<tr>
<td>aldo-keto reductase family 1</td>
<td>ubiquitin-conjugating enzyme E2N</td>
</tr>
<tr>
<td>sorbitol dehydrogenase</td>
<td>peptidylprolyl isomerase-like 5</td>
</tr>
<tr>
<td>F-actin-capping protein</td>
<td>prolactin-induced protein</td>
</tr>
<tr>
<td>dimethylarginine dimethylaminohydrolase 1</td>
<td>S100 calcium binding protein A9</td>
</tr>
<tr>
<td>annexin A1</td>
<td>histidine triad nucleotide binding protein 1</td>
</tr>
<tr>
<td>annexin A3</td>
<td>diazepam binding inhibitor</td>
</tr>
<tr>
<td>annexin A5</td>
<td>S100 calcium binding protein A11</td>
</tr>
</tbody>
</table>
General discussion

Homeostasis in normal tissue is strictly balanced by cell proliferation and apoptosis. One cell defeated by cell death must be replaced by exactly one new cell. An imbalance either at the level of cellular proliferation or at the level of cell death means a cancerous state under development.

Oncogenes, when activated, are a cluster of genes involved in this process, either by regulating cellular proliferation or by regulating cell death. One category of oncogenes encoding proteins involved in induction of cellular proliferation is, for example, growth factors, growth factor receptors, proteins in the signal-transduction pathways, and transcription factors. A second category of oncogenes inhibits excessive cell proliferation. A member of this group is the p53-gene which encodes a nuclear phosphoprotein that pauses the replication machinery for proofreading. A non-functional p53-protein increases the risk for mutations in the chromosome and cells transformed into cancer cells are in many cases deficient of functioning p53-protein. The p53-protein responds to various stress signals by initiating a program for transcription that restores cellular homeostasis and prevents the accumulation of errors in a cell (91). As part of this process, p53 regulates the transcription of a set of genes encoding proteins that populate the endosomal compartment and impact upon each of these endosomal functions including exosome production (91). A third category of oncogenes regulates programmed cell death (apoptosis).

It has been claimed that six essential alterations are necessary in the cancer cell pathophysiology, namely: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (92).

The incidence of prostate cancer is increasing world-wide, and is now the most common male malignancy in Sweden. Radical prostatectomy or radiation therapy are the current treatments for patients with early disease, i.e. the cancer is localized in the prostate gland. Androgen suppression therapy is the main measure for progressive prostate cancer. Androgen suppression results in apoptosis of the androgen-dependent tumour cells and the serum PSA-levels are concomitantly decreased. However, a majority of the patients will eventually fail this therapy and develop an androgen-independent disease. At present, there is no effective therapy for androgen-independent prostate cancer.
Early detection of prostate cancer improves prognosis. Limitations of the PSA test for early detection of prostate cancer (93), especially because of a high rate of false positive results (94), underline the need for other means of screening for this type of cancer. The observation that patients with cancer produce autoantibodies against antigens in their tumours (95-98) suggests that such antibodies could have diagnostic and prognostic value (96, 97, 99-101). To circumvent the well-known problems of PSA-screening for prostate cancer, we wanted to evaluate the use of autoantibody presence in serum to detect prostate cancer (102). Previous studies demonstrated that antibodies against prostasomes appeared in serum in association with prostate cancer (100, 102).

Among the most frequently occurring antigens in the present study were stress proteins (heat shock proteins and chaperons). This was not surprising since cancers induce a stressful condition resulting in high expression of such proteins (103). Over expression of different proteins can activate the immune system and antibody production (104). One of the frequently occurring antigens (clusterin) of the present work was highlighted in an immunohistological study of different stages of prostate cancer (105). Since clusterin appeared as big spots with several iso-forms in 2D-gels of prostasomes we proceeded with further examinations of auto-antibodies against clusterin. Purified clusterin, at the time being, could not be purchased why we purified it from serum. We hypothesized that clusterin in its prostasomal context would induce an immune response giving rise to an immune reaction in our assay. However, control sera displayed high antibody titers, too, against clusterin thwarting any discriminating power between prostate cancer patients and controls. Still, IgG titers in serum against prostasomes correlated well with corresponding titers against purified clusterin indicating clusterin being a major prostasomal antigen.

Defective sperm function is the largest single defined cause of human infertility affecting approximately one in 20 males of reproductive age (106, 107). The haploid genome of the sperm cell is condensed and therefore requires extensive remodeling of the chromatin. In the head of the sperm cells the histones have been replaced by other small basic proteins known as protamines. When fully protaminated, sperm DNA is extremely stable and difficult to damage. Deficiencies in the protamination process can however occur leaving portions of the DNA less compact and therefore vulnerable to injury (108). In the general population, an increased frequency of sperm DNA damage has been associated with early pregnancy loss (109). It has also been speculated whether environmental factors, e.g. cigarette smoke, may induce DNA damage in the sperm cells and therewith increase the risk of childhood cancer in the offspring (110). Accordingly, the integrity of the paternal genome appears important for embryonic and foetal development and also long-term health of the offspring. It has been claimed that oocytes may be able to repair DNA damage in sperm even if the exact mechanism for such
an interaction is unclear (111). Our finding of chromosomal DNA presence in prostasomes is unequivocal. The results of nuclease treatment and differential incubations with DNA fluorescent stains (membrane permeable and membrane impermeable) strongly favoured a location of the detected DNA within the prostasomes. Accordingly, a DNA packaging in prostasomes will protect DNA from enzymatic degradation in semen (112) and/or electrostatic interaction with seminal cationic proteins. Since prostasomes are able to interact with spermatozoa and since a transfer of proteins (even GPI-anchored proteins) can take place from prostasomes to sperm cells, there is a possibility of DNA delivery from prostasomes to sperm cells. A transfer of prostosomal DNA to sperm cells would tally with a previous finding regarding mast cell-derived exosomes containing mRNA that could be delivered to another cell being functional in this location (113). Also, artificial liposomes can transfer exogenous DNA into sperm cells and preferentially inside the sperm head (114) at which prostasomes cluster (15). Therefore, a potential role of prostasomes as DNA-delivery vehicles to spermatozoa is apparent. However, there are at present no solid grounds for a specified role of prostosomal DNA.
• Prostate cancer patients held serum antibodies against seminal prostasomes in a higher concentration than did control sera. These autoantibodies were most frequently directed against 25 prostasome-associated proteins among which none was prostate specific. Some of the proteins were stress proteins and such proteins are highly expressed in response to cancer.

• Investigation of serum antibody titers against one of the frequently occurring prostasomal proteins (clusterin) displayed elevated titers in both patient sera and control sera. Correlation with different clinical variables of the patients indicated a prostate-size relationship of clusterin autoantibodies. The serum antibody titers of the patients against purified clusterin correlated well with antibody (IgG) titers against prostasomes, meaning that patients with high anti-clusterin titers also possessed high anti-prostasome titers, corroborating the view that clusterin is a major antigen of seminal prostasomes.

• Prostasomes deriving from normal cells as well as from malignant cells contain chromosomal DNA and this DNA is not within reach of DNase breakdown. Most likely, the DNA is localized inside the prostasomes. The DNA appeared as fragments from 13,000 base pairs and below on agarose gel. Also, one distinct band at 1,000 base pairs and another at 2,000 base pairs were typically found in seminal prostasomes. Cloning of DNA from seminal prostasomes and from PC-3 cell prostasomes featured gene sequences in 31% of the seminal DNA clones and in 25% of the PC-3 cell DNA clones.

• Prostasomes deriving from malignant cells of vertebral metastases of prostate cancer patients were isolated and their protein content was subjected to 2-dimensional electrophoresis and mass spectrometry and 30 proteins were identified. None of these proteins was prostate specific or prostate cancer specific. Annexins A1, A3, and A5, dimethylarginine dimethylaminohydrolase 1, and S100 calcium binding protein were among the identified metastasis-derived prostasomal proteins. These proteins can act, among other things, as angiogenic factors thereby stimulating the vascular development in the neighborhood of the tumour.
Svensk sammanfattning


Prostata kan indelas i tre separata huvudzoner (en fjärde, mycket liten zon (1 %) kan dessutom urskiljas, den periurethrala körtelzonen): den perifera (65-70%), den centrala (25 %) och övergångszonen (5-10%). Prostata representerar en ansamling av 30-50 tubulo-alveolära körtlar som tömmer sitt sekret i prostatiska urethra. En fibroelastisk kapsel rik på glatt muskulatur omger prostatakörteln. Den glandulära delen av körteln (acini och utförsågar) byggs upp av sekretoriska, epitelia celler och s.k. basalceller. Bland de sekretoriska cellerna finns dessutom interfibrerat neuroendokrina celler. De sekretoriska cellerna igenkänns i mikroskopet på sitt rikliga innehåll av endoplasmatiskt retikel (ER), ett stort Golgi komplex, s.k. lagringsvesiklar (multivesiculära bodies, MVB) samt lysosomer. Prostatasekretet utgör c:a 30% av ejakulatvolymen. Det har ett pH kring 6,5 och är en serös vätska i vilken sekretionsprodukterna återfinns. I denna vätska kan höga koncentrationer av prostataspecifikt antigen (PSA) påvisas. PSA är egentligen ett proteolytiskt enzym, som anses ha stor betydelse för den s.k. likvelföringsprocessen i semen. Andra komponenter som utsöndras i stor mängd i prostatavätskan är srt fosfatias, albumin, proteolytiska enzym, mono- och divalenta katjoner samt citrat. Slutligen utsöndrar prostatakörtelns sekretoriska epitelceller små organellära strukturer i stor mängd vid namn prostasomer. Prostasomer bildas troligen genom invaginationer av det endosomala membranet. Den tidiga endosomen fungerar som en sorteringsvesikel och inkommande bidrag fås bl a från Golgi-ER vägen (syntetiserade proteiner och membran fetter) och endocytotisk transport från plasmamembranet. Sor-

I en proteomanalys från 2003 identifierades ett minimum av 139 prostasomala proteiner indelade i kategorierna: enzymer (34 %), transporter/strukturella proteiner (19 %), GTP-proteiner (14 %), chaperonproteiner (6 %), signalöverföringsproteiner (17 %) och hittills oidentifierade proteiner (10 %). Med andra metoder har ytterligare proteiner kunnat identifieras i prostasomer.

Prostasomer har visats kunna interagera och under vissa förhållanden också fusera med spermier och därigenom hjälpa till att överföra diverse prostasom-associerade molekyler till spermier. Således kan prostasomer fungera som budbärare mellan prostatas sekretoriska epitelceller och spermerna. Härigenom kan förmågor från prostateceller tillföras spermerna till gagn för deras överlevnad i en i princip fientlig miljö och migration till ägget för befruktning. Bland sådana funktioner kan nämnas: befördrande av framåtriktad rörlighet, immunsuppression, komplement hämning, anti-ROS (reactive oxygen species) aktivitet och antibakteriell aktivitet.

Undersökningar har visat att cancerceller från prostatacancer (prostatacancer startar i de allra flesta fall från de sekretoriska epitelcellerna i körteln) behåller förmågan till produktion och utsändring av prostasomer. Prostasomernas förmågor till gagn för reproduktionen synes kunna nyttjas av cancercellerna för deras överlevnad och reproduktion. Ett stort problem vid bekämpningen av prostatacancer är att upptäcka sjukdomen i tid och man letar


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Parents, family, relatives and friends.
References


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