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Regulatory Proteins in Progenitors and Differentiated Cells of the Human Prostate

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Doctoral dissertation to be publicly defended on Friday 14th of March 2008, at 13:00, in the CRC Aula, CRC, house 93, Plan 10, Malmö University Hospital, entrance 72, Malmö, Sweden, by due permission from the Faculty of Medicine, Lund University, Sweden.

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Till Elliot & Pommac

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List of papers

This thesis is based on studies reported in the following papers, which are referred to in the text by their Roman numerals (I-IV):

I. **Hansson*, J.A., Bjartell, A., Gadaleanu, V., Dizeyi, N., Abrahamsson, P.A.** Expression of Somatostatin Receptor Subtypes 2 and 4 in Human Benign Prostatic Hyperplasia and Prostatic Cancer. *Prostate 2002;53(1):50-59. Erratum in: Prostate 2002;53(4):330.*

II. **Ceder, J.A., Jansson, L., Ehrnström, R.A., Abrahamsson, P.A.** The Characterization of Epithelial and Stromal Subsets of Candidate Stem/Progenitor Cells in the Human Adult Prostate. *Eur Urol 2008;53(3):524-532.*

III. **Ceder, J.A., Jansson, L., Helczynski, L., Abrahamsson, P.A.** Delta-like 1 (Dlk-1), a novel marker of prostate basal and candidate epithelial stem cells, is down-regulated by Notch signalling in intermediate/transit amplifying cells of the human prostate. *Eur Urol, in press.*

IV. **Ceder, J.A., Jansson, L., Kjellström, K., Abrahamsson, P.A.** Somatostatin is expressed in the human prostate stem cell/progenitor niche and regulates expansion of intermediate luminal cells in primary cell cultures. *Manuscript.*

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*Surname changed to Ceder in 2007.

Abbreviations

AMACR	alpha-methylacyl-CoA racemase
AR	androgen receptor
bHLH	basic helix loop
BMP	bone morphogenic protein
BPH	benign prostatic hyperplasia
CgA	Chromogranin A
CK	cytokeratin
CSC	cancer stem cell
CSL/RBPJ-k	CBF1, Su(H), LAG-1
CZ	central zone
Dlk-1	Delta-like 1
DHT	dihydrotestosterone
DRE	Digital rectal examination
ECM	extracellular matrix
EGF	epidermal growth factor
EMT	epithelial to mesenchymal transition
ES cells	embryonic stem cells
FGF10	Fibroblast growth factor 10
FGFR	Fibroblast growth factor receptor
FRS2alpha	FGF receptor substrate 2alpha
GnRH/LHRH	gonadotropin releasing hormone
GSTP1	the *p*-class glutathione *S*-transferase
Hes	hairy/enhancer of split
HGF	hepatocyte growth factor
hK2	human glandular kallikrein 2
HMW-CKs	high-molecular weight cytokeratins
HSC	Hematopoietic stem cell
L-685,458	gamma-secretase inhibitor X, a Notch inhibitor
L-817818	SSTR5/1-specific agonist
LH	luteinising hormone
LMW-CKs	low-molecular weight ytokeratins
MET	mesenchymal to epithelial transition
MSC	mesenchymal stem cell
NE	neuroendocrine
NED	neuroendocrine differentiation
NICD	Notch intracellular domain

NSE	neuron specific enolase
PC	prostate cancer
PIA	proliferative inflammatory atrophy
PIN	prostatic intraepithelial neoplasia
PSA	prostate-specific antigen
PTEN	phosphatase and tensin homologue
PZ	peripheral zone
RT	room temperature
SC	stem cell
SCF	stem cell factor
SDF-1	stromal derived growth factor-1
Shh	Sonic hedgehog
ShoHu	Short Humour Canadian: an online repository of great Canadian humour writing
SRB	sulforhodamine B
SS	somatostatin
SSTR2	somatostatin receptor 2
SSTR4	somatostatin receptor 4
SYP	synaptophysin
TGF	transforming growth factor
TZ	transition zone
UGE	urogenital sinus epithelium
UGM	urogenital sinus mesenchyme
UGS	urogenital sinus

Background

The prostate and male reproduction

The human male reproductive system is a series of organs located in the scrotum (testes and epididymis), on the outside of the body (penis), and around the pelvic region (the accessory sex glands: prostate, seminal vesicles, and bulbourethral gland, connected to the epididymis through the vas deferens), that together contribute towards the reproductive process: the male gametes are produced through spermatogenesis in the testis, and later enter the epididymis, where they mature into motile spermatozoa (sperm) (Johnson and Everitt, 1988). When the male is sexually aroused, the male copulatory organ becomes erect, and the bulbourethral gland secretes a fluid that lubricates the urethra in order for sperm to pass more easily through the penis and to ejaculate into the female body, where fertilization of the female ovum (egg) takes place. During ejaculation, sperm leaves the penis in a fluid called seminal fluid. This fluid is produced by the prostate gland and the seminal vesicles. The ducts of the seminal vesicles merge with the vas deferens to form the ejaculatory ducts that empties into the prostatic urethra, where the prostatic ducts also empties its secretions. The prostate secretion contains prostate-specific antigen (PSA) that degrades the gelforming semenogelin-proteins secreted by the seminal vesicles. The prostate secretion also contains other proteins, including prostatic acid phosphatase and ß-microseminoprotein, with unknown functions, and human glandular kallikrein 2 (hK2), which is believed to become active in ejaculates, in order for PSA to become active (Lilja and Abrahamsson, 1988; Malm et al., 2000). The fertilized egg (zygote) then gradually develops into a foetus, which later is born as a child. Unfortunately, the development of benign and malignant tumours in the elderly prostate is very common, and may require that part of, or the complete prostate, is removed through surgery. Such interventions may reduce fertility, or lead to complete infertility. Sperm banking may be an option in such cases.

The normal adult prostate gland

The human adult prostate is a walnut shaped and sized (20g, 4x2.5 cm) exocrine organ. The organization of the prostate is commonly described in terms of three zones, a central zone (CZ: surrounding the ejaculatory ducts), a transition zone (TZ: surrounds the proximal urethra), and a peripheral zone (PZ: surrounds the distal urethra), reflecting three distinct sets of branched ducts present in the human prostate (McNeal, 1981; McNeal, 1988). The epithelial ducts are composed of a secretory luminal layer, that consists of tall columnar exocrine cells, and a basal cell layer with cuboidal cells, lined on a basement membrane, dividing the epithelial and the stromal compartments. Moreover, the presence of tight junctions between adjacent basal cells (Krajewska et al., 2007) suggests that these cells form a blood-luminal barrier, creating a ductal microenvironment.

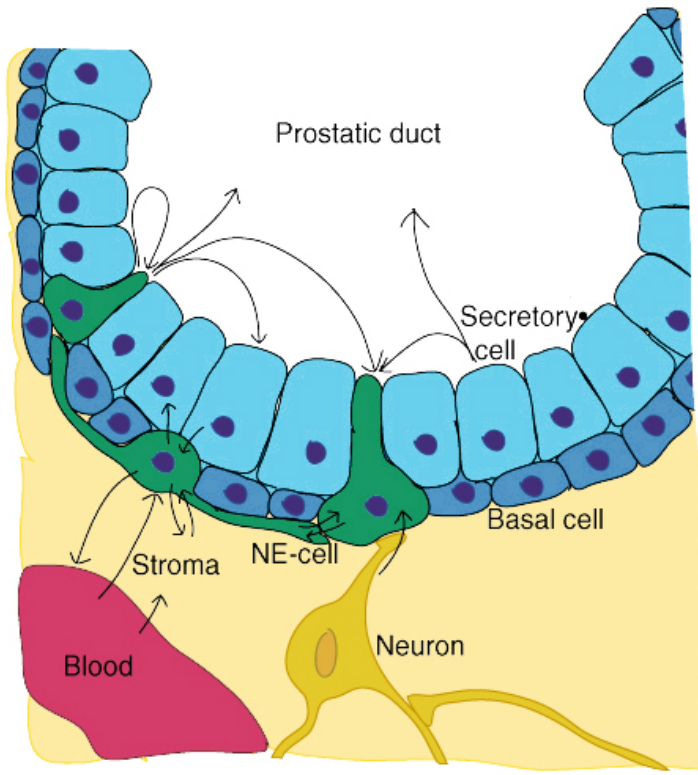

Figure 1. *Schematic drawing illustrating possible regulatory pathways of the prostatic neuroendocrine cell. Regulation may be endocrine, paracrine, autocrine, neurocrine or, in the open cell type, lumencrine.*

The basal layer consists of androgen-independent cells that express p63 and high-molecular weight cytokeratins (HMW-CKs), with CK5 and CK14 being the most common markers used to characterize them. The basal layer is believed to house prostate epithelial stem cells (SC), and the bulk of basal cells may have the capacity to differentiate into mature luminal cells. The luminal layer consists of androgen receptor (AR) expressing cells that secrete various AR-regulated proteins such as PSA, but is also characterized by CD57 and low-molecular weight CKs (LMW-CKs), including CK8 and CK18. The epithelium also contains a small population of isolated, seemingly randomly scattered cells, with dual properties of endocrine cells and neurons, i.e. the neuroendocrine (NE) cells, see figure 1. NE cells are androgen-independent, and are considered to be non-proliferating terminally differentiated cells (Bonkhoff, 2001). Morphologically, there are two types of NE cells; the open and closed cell types. The open cell type has extensions at their apex that connect with the lumen, and both types have dendritic processes that extend between adjacent cells. NE cells are characterized most commonly by the expression of the pan NE marker chromogranin A (CgA), but other markers such as synaptophysin (SYP) also indicate the NE lineage. The diversity of secretory products in the normal prostate suggests that NE cells consist of several subpopulations, where some may regulate luminal secretion (see further my reviews (Hansson and Abrahamsson, 2001; Hansson and Abrahamsson, 2003)). Once believed to only function as a passive support structure, the stroma is now considered to directly influence epithelial cell behaviour, in part by supplying androgen-regulated growth factors to the epithelium. Fibroblasts and smooth muscle cells are the principal stromal cell types in the human prostate gland. Structural and regulatory components of the extracellular matrix (ECM, e.g. collagen, fibronectin, and laminin etc), blood vessels, nerves, and immune cells are also integral part of the stromal compartment (Tuxhorn et al., 2002).

Prostate organogenesis

Pluripotent stem cells and embryogenesis

As described in the previous section, the prostate, as well as all other organs in the human body, contain multiple cell types that perform specialized tasks. Yet all cells in the human body contain the same genome, i.e. identical DNA. Cells in the adult body behave differently because they express the genes of the genome differentially: some genes are preferentially expressed, while others are restricted or silenced. In stark contrast, embryonic stem (ES) cells are pluripotent and defined by their potential to activate all gene-expression programmes found in embryonic and adult cell lineages. Directly after fusion of the sperm and egg and during the first divisions, epigenetic reprogramming of the parental genomes take place (imprinted genes are protected), and extensive DNA de-methylation occurs together with activating histone modifications (Fulka et al., 2008), and is necessary in order to achieve the pluripotent state. It is believed that the DNA chromatin now is generally accessible to transcription factors, and that this facilitates activation of genes (these cells are ES cells). Maternal factors originating from the oocyte (egg) cytoplasm are believed to regulate these early events. Indeed, somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997). Transcription of embryonic genes occurs at, or shortly before, the gastrulation stage, and cells loose their pluripotentiality and differentiate into the three germ layers of the embryo: ectoderm (derivates include skin and nerve cells), mesoderm (mesenchyme, circulatory system, bone etc), and endoderm (epithelial linings of most organs). At some time point, these germ layers engage in organogenesis: if the embryo is male, testes are formed and start to synthesise male hormones, including the androgen testosterone.

Prostate development

Androgens produced by the fetal testes are required to initiate prostate organogenesis: the androgen responsive urogenital sinus (UGS; situated immediately below the bladder) mesenchyme (UGM; mesodermally derived) induces the UGS epithelial layer (UGE; endodermally derived) to bud, and grow into the surrounding inductive fetal mesenchyme (stroma in adults) (Cunha and Donjacour, 1987). Subsequently, the epithelial buds elongate and bifurcate, shaping the arborised epithelial ducts of the human prostate. Postnatal development of the human prostate includes a period of growth during the first year, quiescence during childhood, and further growth and functionality with

the testosterone surge at puberty. Branching morphogenesis is a key feature of prostate organogenesis, and allows for an increased epithelial surface area, and greater storage capacity of prostatic fluid. Reciprocal cross-talk between epithelium of elongating ducts and mesenchyme regulate proliferation and cellular differentiation in both compartments: mesenchyme induces epithelial differentiation, and in turn, epithelium induces mesenchymal differentiation, including smooth muscle differentiation (Hayward et al., 1996; Taylor et al., 2006). Several studies have shown that it is mesenchymal AR, and not epithelial AR, that is critical for initiation of prostate organogenesis and epithelial budding (Cunha, 1994; Simanainen et al., 2007). However, knock-out of epithelial AR results in impaired terminal and functional differentiation of luminal cells (Simanainen et al., 2007). The epithelial cells that emerge in the budding tips show a very high proliferative index, which decrease in elongating ducts and with gestational age (Xue et al., 2001b). The budding tip is composed of cells positive for basal cell markers, including CK5 and CK14, whereas the majority of cells slightly more proximally/luminally located express markers associated with both basal and luminal cells, including the luminal cytokeratin CK18 (Xue et al., 1998) (Xue et al., 2001a). All fetal epithelial cells in acini and ducts express CK19, which becomes restricted to the basal cell layer in adults , whereas CK18, which only is expressed by the luminal cell layer in adults, diminishes its expression in the basal cell layer during prostate development (Letellier et al., 2007). This suggests a cellular hierarchy, and differentiation from a basal cell phenotype to luminal cells, with an intermediate (transit amplifying) phenotype between basal and luminal cells (Xue et al., 1998).
The primary determinants of cellular identity are the transcription factors that are expressed, defining the gene expression programme in the cell (by activating or silencing expression of target genes). Differentiation is likely a programming phenomenon that involves a tightly determined order of expression of transcription factors, regulated by external signals (growth factors and morphogens) at each developmental stage. Which are the factors that regulate prostate ductal morphogenesis and cytodifferentiation? Studies of gene knockout mice have elucidated some of the molecules that are involved in murine prostate development. Fibroblast growth factor 10 (FGF10) clearly play an important role, as FGF10 null mice develop an UGS, but fails to develop a prostate. Fibroblast growth factors constitute a large family of growth factors that have been found to be involved in cellular proliferation and differentiation. The FGF receptor family consists of four members, FGFR1-4, all receptor tyrosine kinases. However, alternate mRNA-splicing gives rise to multiple variants, of which the FGFR2iiib splice variant encode the canonical FGF10

receptor. FGF10 expression is spatially restricted to mesenchymal cells in the distal aspects of the murine prostate gland, where it is believed to function as a chemoattractant for elongating ducts. FGFR2iiib, on the other hand, is expressed exclusively in the distal tip epithelium of elongating ducts. Knockout of FGFR2iiib in mice results in smaller prostates with disorganized epithelial and stromal cells, and there is an emergence of an NE-like phenotype in the epithelial layer (Foster et al., 2002). The increased number of NE cells suggests that epithelial FGFRiiib directly or indirectly negatively regulates NE-differentiation. NE cells appear in the human prostate shortly after budding has initiated, and are negative for proliferative markers (Xue et al., 2001b), suggesting that maturation of NE cells precedes that of luminal cells, and the close association with developing epithelia suggests that NE cells may control developmental processes in the prostate (Aumuller et al., 2001). A key regulator of FGFR-signalling, FGF receptor substrate 2alpha (FRS2alpha), is an adaptor protein that links several signalling pathways to activated FGFRs, and has been shown to be uniformly expressed in the epithelial cells of developing murine prostate, suggesting that FGFs regulate several aspects of prostate development. In mature murine epithelium, FRS2alpha is expressed only in the basal cells. Ablation of FRS2alpha in developing mice prostates impairs intracellular MAP kinase activation, reduces cell proliferation and branching of elongating ducts (Zhang et al., 2008). That MAP kinase activity is crucial has been demonstrated through the use of specific chemical inhibitors, resulting in complete loss of epithelial budding (Kuslak and Marker, 2007). The expression of the Sonic hedgehog (Shh), an evolutionary conserved morphogen, and its receptor Patched, directly correlates with growth and branching morphogenesis of the murine prostate. Notably, Shh has been shown to down-regulate FGF10 and to up-regulate bone morphogenic protein (BMP) expression. Specifically, BMP4 has been found to restrict prostatic ductal branching (Lamm et al., 2001), whereas administration of the BMP antagonist Noggin can rescue branching in BMP4-exposed mice UGS-organ cultures (Cook et al., 2007).

One of the earliest known epithelium-specific transcription factors in the prostate is FOXA1, which together with AR has been found to regulate human PSA expression. The closely related FOXA2 has recently been found to be expressed in developing distal prostatic epithelium of the mouse (Mirosevich et al., 2005). It is noteworthy that in postnatal prostates, FOXA2 was associated with mouse NE-phenotype cells, suggesting that FOXA2 may be involved in several aspects of prostate development. The fact that Shh regulate the expression of FOXA2 in several human tissues suggests that FOXA2 may be under the direct or indirect control of Shh during prostate organogenesis, and

that a gradient of Shh may regulate FOXA2 and FGF10 expression and NE differentiation in the prostate. Notably, FOXA2 is required for the differentiation of endocrine pancreatic cells. It has recently been shown that the basic helix loop (bHLH) transcription factor Ash1 is expressed in foci of human NE-differentiated cells in prostate cancer tissues (Hu et al., 2002). The regulation of this transcription factor in the prostate is not known. However, BMP2 is a known inducer of Ash1 and neurogenesis in neural crest stem cells. A similar role for BMP2 (or any BMP) in the prostate gland has not been explored, although BMP2 mRNA has been detected in the developing prostate. A negative modulator of Asch1 expression in several human tissues is the bHLH transcription factor Hes1, at least partially regulated by Notch. Notch is one of the fundamental signalling pathways that regulate organogenesis throughout the animal kingdom. Humans have four Notch proteins that are single-pass transmembrane receptors. The Notch ligands, Jagged1 and 2, Delta1,3 and 4, are also transmembrane proteins; therefore signalling is restricted to neighbouring cells. The intracellular transduction of Notch seems archaic; no secondary messengers are required: ligand binding induces two proteolytic cleavages, and the Notch intracellular domain (NICD) is released and translocated to the nucleus were it co-operates with the DNA-binding protein CSL (CBF1, ShoHu, LAG-1), also known as RBPJ-k, and its co-activator Mastermind to promote transcription. To date, only a few target genes have been identified; the best known Notch target genes are members of the bHLH hairy/enhancer of split (Hes) family, including Hes1 and Hey1. Treatment of developing mice prostates with Notch-inhibitors, or knock-out of Notch-1, results in down-regulation of Hey1 expression, impaired branching and epithelial cytodifferentiation (Wang et al., 2006b). Specifically, luminal cells failed to differentiate in response to Notch-inhibition, and there was an increase in intermediate cells co-positive for basal and luminal cytokeratins in the adult mouse (Wang et al., 2006b), suggesting that Notch-1 is a key regulator of differentiation in the prostate gland.

Prostate cancer

Diagnosis and treatment

Prostate cancer (PC) is the most common malignancy in Sweden; More than 10 000 males are diagnosed annually (see further www.roc.se). Most patients presented symptoms and an advanced disease up to the 1990s. Although the incidence has continued to rise during the 21st century, the age at diagnosis has decreased during recent years (median now 70 years). Today most men are without symptoms, and have a treatable disease. This development is likely a cause of the introduction of prostate-specific antigen (PSA) testing in the early 1990s in Sweden. Of newly diagnosed patients in 2005, 28% reported "health control" as cause to diagnosis, suggesting increased testing. During a PSA test, a small amount of blood is analysed, and PSA levels below 4 ng/ml are considered normal. If the level is higher, there is a risk of PC. However, since PSA is expressed by both normal and the bulk of tumour cells, PSA is not a cancer specific marker. Consequently, prostatic inflammation or benign prostate tumours (benign prostatic hyperplasia, BPH) may influence PSA levels. Most men with BPH are intermediate in PSA levels, i.e. 4-10 ng/ml, but may also have levels above 10 ng/ml, and men with PC may have low PSA values (Thompson et al., 2004). PSA velocity, the absolute increase of PSA during time, may discriminate PC from BPH better than a single PSA test. (Another potential discriminator is PCA3: a molecular test that detects the presence of PCA3 mRNA in urine. PCA3 is not a replacement to PSA, but increased PCA3 is correlated with cancer, and may help to decide if a biopsy regimen (de la Taille, 2007). PCA3 testing is currently under investigation in several European countries, including Sweden). Digital rectal examination (DRE) remains a cornerstone in the diagnosis of PC, because of the ease, low risk, and contribution to detection of PC in men with lower PSA levels. Although the PSA and DRE tests cannot diagnose PC, they signal if there is a need for a biopsy: the needle-extraction of a small tissue sample to examine the prostate cells, and determine whether they are malignant or not. Malignant cells are assigned a Gleason grade (scale 1-5) by a pathologist, and is based on how closely cancer cells resemble normal glands. Grade 1-3 are less aggressive, whereas grade 4-5 are more aggressive. The two most prevalent grades are then added together to provide a Gleason score: a score between 2-4 is considered low grade, 5-7 intermediate grade, and 8-10 high grade. Staging then determines the extent of cancer, and together with Gleason score provides suggestions for how to treat the tumour. In the TNM system, T1 and T2 cancers

are confined to the prostate, whereas T3 and T4 tumours have spread elsewhere: T1 – cancer undetectable by DRE, usually discovered by PSA tests or biopsies; T2 – detectable by DRE, but confined to the prostate; T3 – tumour extends through the prostate capsule, and may have spread to the seminal vesicles, but has not spread to other organs; T4 – tumours have spread to organs near the prostate, e.g. bladder. Stage N+ tumour has spread to lymph nodes, and in stage M+ tumours there is systemic spread, e.g. to bone. Once the TNM categories have been determined, they are combined with the Gleason score, to give stage grouping:

Stage I: [T1, N0, M0, Gleason score 2-4]: the tumour is organ-confined, and is not likely to metastasise.

Stage II: [T1, N0, M0, Gleason score 5-10], or [T2, N0, M0, any Gleason score]: the tumour is organ-confined, but may metastasise.

Stage III: [T3, N0, M0, any Gleason score]: the tumour has begun to spread, but it has not invaded lymph nodes.

Stage IV: T4, or N1, or M1, any Gleason score: the tumour has spread to tissues next to the prostate, e.g. bladder, and/or lymph nodes, and/or more distant sites, e.g. bones.

Choosing the best treatment is then based on the grouped stage, age, general health, and the patients own evaluation of the risks and benefits of each therapy option. Stage I and II patients are usually offered radical prostatectomy, i.e. the surgical removal of the entire prostate gland, or radiotherapy (EAU guidelines; www.uroweb.org). However, since there is an increase of early detection, there is a risk of over treatment. For patients with organ-confined disease who are at low risk, waiting with radical treatment until early progression may increase quality of life. Less aggressive cancers may therefore be monitored through active surveillance, i.e. the cancer is carefully monitored for signs of progression by repeated PSA test, DRE, and biopsies. If the cancer has spread, treatment with hormone therapy to lower the androgen levels is typically initiated, although it is very unlikely to cure the cancer. Additionally, 20% of men undergoing radical prostatectomy due to localised PC experience PSA failure within five years (Pound et al., 1999), and may benefit from androgen ablation therapy. In 1941, Charles Huggins discovered that ablation of the androgen testosterone by removal of the testes (orchiectomy/castration) cause regression of prostate tumours (Huggins and Hodges, 1941). Later, the role of gonadotropin releasing hormone (GnRH/LHRH) in androgen metabolism was determined by Andrew Schally (Schally et al., 1971): the hypothalamus produce GnRH to stimulate the pituitary to release luteinising hormone (LH) into the blood stream. LH then stimulates the testes to produce

testosterone, which is converted into dihydrotestosterone (DHT) by 5-alpha reductase I and II in the prostate (reductase inhibitors are mostly given to BPH patients). The most successful hormonal treatments are orchiectomy and GnRH agonists, although today orchiectomy is rarely employed. GnRH agonists (e.g. Zoladex) actually suppress LH, albeit after an initial stimulatory effect. An alternative is to use anti-androgens (e.g. bicalutamide/Casodex), which block androgens from binding to the AR, either alone or in combination with GnRH analogues. Numerous studies have failed to demonstrate that total androgen blockade, i.e. drugs that in combination also blocks adrenal androgens, prolongs life more than simply blocking the production of androgens from the testes. Androgen ablation causes the majority of cancers cells to enter into apoptosis (die), and the PSA levels start to drop. However, hormone therapy may only work for a few years before the cancer starts to grow again, as indicated by a rising PSA or metastatic growth. Cancer that is no longer responding to any androgen ablation therapy is considered hormone-refractory, androgen-independent, and usually progress rapidly (Oh and Kantoff, 1998). Chemotherapy, e.g. docetaxel, may prolong life with a few months (Tannock et al., 2004), and bisphosphonates (e.g. Zometa) can help relieve bone pain and strengthen bone structures (Saad and Lipton, 2007). Radiation therapy can also help reduce bone pain, and lessen bone-related complications. However, these regimens are mostly palliative in nature. In Europe in 2004, PC was the third most common cause of male deaths (Boyle and Ferlay, 2005). Clearly, new treatment modalities in PC are urgently needed.

Carcinogenesis

The aetiology of PC is not known, but several risk factors have been identified or proposed. Currently, age, family history, race, inflammation, hormones, and dietary habits have all been suggested in the development of PC. An important risk factor seems to be heredity. The risk of developing prostate cancer is at least doubled if a first-line relative has the disease. A small subpopulation of individuals with PC (about 9%) has true hereditary disease, defined as three or more relatives affected, and often develop PC 6-7 years prior to spontaneous cases, but do not differ in other ways from spontaneous cases (Bratt, 2002). Genomewide analysis has identified variants in five chromosomal regions that are associated with an increased risk of PC. A very recent study identified that when four of these polymorphisms were present, the composite risk ratio for PC was at least 4-fold, or more than 9-fold if a first-line relative had the disease (Zheng et al., 2008). The loci of most of these polymorphisms do not reside within or near identifiable genes, but may exist in regions controlling gene

expression. Since the polymorphisms in the study appeared to be associated with a risk of PC in general, rather than with a more or less aggressive form, the results suggests that the genetic variants act at an early stage of carcinogenesis. PC develops from an androgen-dependent tissue that contains androgen receptors, and androgen ablation shrinks the tumour in the vast majority of PC patients. Thus, androgens are thought to be involved in the causation of PC. However, epidemiologic studies have failed to support this, and some recent studies have even suggested that high androgen levels might be protective, particularly against aggressive cancer (Severi et al., 2006). Controversial data has also been obtained from the famous PC prevention study; a 7-year intervention with a drug that inhibits 5-alpha reductase, the enzyme that converts testosterone to DHT, reduced PC risk in healthy men by about 25%. However, more high-grade cancers were reported in those men who did develop PC (Thompson et al., 2003), although the interpretation from this study is highly controversial (Andriole et al., 2007).

Somatic mutations have long been recognised in the development of PC. One prominent example is the gene for phosphatase and tensin homologue (PTEN), a tumour-suppressor gene encoding a phosphatase active against intracellular proliferative signals induced by upstream receptor tyrosine kinase growth factor receptors. PTEN is a common target for somatic alteration in PC (Yoshimoto et al., 2007). It has been shown that mutations to the PTEN locus leading to the loss of expression of the Pten protein in mice plays a central role in the initiation of PC (Wang et al., 2006a).

Chronic or recurrent inflammation probably has a role in the development of many types of cancers, including PC. De Marzo and colleagues has proposed that a prostatic lesion called proliferative inflammatory atrophy (PIA) is a precursor to PC. Focal areas of epithelial atrophy often contain proliferative epithelial cells that fail to differentiate into luminal cells, i.e. contain cells intermediate in phenotype, and tend to occur in the periphery of the prostate, where prostate cancers most commonly arise. The term PIA applies to focal atrophic lesions that are associated with chronic inflammation, and are most often found in the peripheral zone of the prostate (De Marzo et al., 2007). Additionally, PIA lesions are often found directly adjacent to lesions of prostatic intraepithelial neoplasia (PIN), PC, or both. Epithelial cells in lesions of PIA show many molecular signs of stress, such as high levels of the *p*-class glutathione *S*-transferase (GSTP1). GSTP1 belongs to a family of enzymes that play an important role in detoxification by catalyzing and reducing mutagenic free radicals and electrophilic compounds. Loss of GSTP1, as a result of epigenetic hypermethylation of the CpG island in the GSTP1 promoter, has

been hypothesised to result in increased mutations and to the transition of PIA to PIN and PC; loss of GSTP1 may serve as an initiating genome lesion for prostate carcinogenesis. As tumours progress to more advanced stages, they acquire an increasing number of genetic alterations and aberrant responses to regulatory stimuli and pathways. The evidence linking PIA to PC is however suggestive.

Environmental factors, such as diet, may contribute to the development of PC. Detoxification enzymes need different co-enzyme factors, such as some vitamins, including riboflavin, and certain minerals, e.g. selenium and zinc. Other factors, e.g. vitamin E, carotene and lycopene support detoxification through their anti-oxidant activities. Several studies suggest that intake of such factors may reduce the risk of developing tumours, including PC. In the Lady transgenic mouse, administration of vitamin E, selenium and lycopene dramatically inhibited PC development, and prostate tissue was shown to contain significantly lower numbers of NE cells (Palmer et al., 2008; Venkateswaran et al., 2004). It is also becoming clearer and clearer that bad fats, e.g. saturated and trans fats, increase the risk for certain diseases, while good fats such as monounsaturated and polyunsaturated fats, lower the risk. In a very recent study, total trans-fatty acid blood levels were positively associated with the development of non-aggressive PC, but unrelated to the risk of developing aggressive PC. This result suggest that trans fats may be involved in early stages of prostate carcinogenesis, but do not contribute importantly to disease progression (Chavarro et al., 2008). The molecular mechanisms underlining the effects of fat on cancer initiation and/or progression are largely unknown. However, malignant cells in PC show a strong increase in expression of alpha-methylacyl-CoA racemase (AMACR) (Luo et al., 2002), a protein that play an important role in breakdown of branched chain fatty acid molecules. Malignant NE cells are also positive for AMACR expression, whereas normal NE cells are not. In a recent study, it was shown that a high fat diet was correlated with elevated expression of NE markers and PC development in the Lady transgenic mouse model (Palmer et al., 2008). Although it is unclear if there is a link between increased expression of AMACR and diet, the up-regulation of AMACR in PC further supports a role for altered lipid metabolism as a factor in the process of PC carcinogenesis.

There is also emerging evidence to suggest that the stroma is affected in PC – either as a primary or a secondary phenomena. It has been hypothesised that prostate carcinogenesis requires functional stromal ARs, but not epithelial ARs, and that the deregulated stroma induces neoplasia in the epithelial compartment. Indeed, Cunha and co-workers have shown that cancer-

associated stroma can induce malignant transformation of pre-neoplastic BPH epithelia (Cunha et al., 2003). Clearly, investigating the contribution of the cellular microenvironment, including stromal-epithelial interactions, to the development of PC may be a key to generating a greater understanding of PC carcinogenesis.

In conclusion, genes and life-style factors contribute to the development of PC. Further research is needed to increase our understanding of the molecular changes involved in prostate cancer initiation, and most likely we will find that the pathways to the malignant phenotype are heterogeneous, as PC represent a diverse set of tumours.

Cancer initiating and maintaining cells

An overwhelming proportion (99%) of all PCs are adenocarcinomas, arising from the glandular compartment. Since the bulk of PC cells express luminal cell lineage markers, and since PC responds to androgen-ablation, it has been proposed that PC originates from mutated mature luminal cells (Nagle et al., 1987). However, although PSA levels increase with advancing disease, there is no direct relationship between PSA levels and stage (Partin et al., 1990). Moreover, the number of cells positive for the luminal marker PSA, and the intensity of PSA staining, is inversely correlated with Gleason grade (Aihara et al., 1994). It is also true that whereas most primary tumours predominantly consist of luminal cells, there seem to be an increased expression of basal cell genes in advanced disease (Liu et al., 2002). By using immunohistochemistry, it has been shown that immature intermediate luminal precursor cells are present in primary PC, and that these cells expand in number in androgen-independent disease (Lam et al., 2005; van Leenders et al., 2002). PSCA, a marker of normal late-intermediate prostate cells, is also often up-regulated in PC (Tran et al., 2002). Since advanced prostate malignancies become androgen-independent, it has been proposed that a more immature, androgen-independent cell type, is responsible for recurrent growth. As discussed above, PIA may represent lesions for the intermediate/transit amplifying cell phenotype, and intermediate cells have been proposed as a target of initial neoplastic transformation (van Leenders and Schalken, 2001). Recently, even more immature cells, cells with stem cell (SC) characteristics, were isolated from PC patients (Collins et al., 2005), and several candidate SC antigens have been reported to be up-regulated in PC (Gu et al., 2007; Kleeberger et al., 2007; McDonnell et al., 1992; Paronetto et al., 2004). Experiments have shown that malignant SC-like cells, when transplanted into nude mice, can reconstitute the tumour and the repertoire of epithelial cells, including neuroendocrine (NE) cells (Gu et al., 2007). This

suggests the existence of a common multipotent epithelial SC/progenitor in the human prostate, and a neoplastic process in very immature epithelia. If we consider PC as a differentiating system in which the initiating cell represents the most primitive cell, i.e. a SC/progenitor cell, this provides a new framework for viewing the cellular and molecular heterogeneity observed in PC; Experiments have shown that only a minor subpopulation of cells in human tumour samples have the capacity to self-renew and reconstitute the tumour upon transplantation (Hamburger and Salmon, 1977; Sabbath et al., 1985). To explain this phenomena, different theories have been put forward. The stochastic theory suggested that all cancer cells are equally malignant but only clones that randomly possess favourable biological properties will grow upon transplantation. Investigations by Bonnet and colleagues provided evidence for the hierarchy model, and showed that only a small subpopulation (0.1%–1.0%) of cells within human acute myelogenous leukaemia samples were capable of initiating disease when transplanted. These cells had SC-like capabilities and the same antigenic profile as normal human hematopoietic SCs (Bonnet and Dick, 1997), and since these tumour cells possess properties unique to normal adult SCs, they have been termed cancer stem cells (CSCs). Stem cells have a remarkable capacity for self-renewal, a property that enables their maintenance over the lifetime of the host, making them excellent candidates for the cells of origin of cancer. Although there is no single definition for a SC, there is general agreement that adult epithelial SCs should exhibit clonogenicity and, more importantly, the ability to regenerate and produce progeny that differentiate into a fully functional epithelium. The existence of SCs in the prostate was first suggested by androgen cycling experiments in the rodent. The prostate is an androgen-dependent organ that undergoes involution after castration, but the gland regenerate completely if androgen levels are restored. Isaacs and colleagues showed that involution–regeneration can be repeated many times, suggesting a population of androgen-independent SCs, (Isaacs, 1987), that in response to stromal AR-activation proliferate, giving rise to intermediate/transit amplifying cells that subsequently differentiate into mature luminal cells. Richardson et al recently identified AC133, which is also expressed by primitive haematopoietic, endothelial, and neuronal SCs, as an epithelial SC marker in the prostate (Richardson et al., 2004). Isolated prostate AC133 cells possess a high in vitro proliferative potential and can reconstitute prostatic-like acini in male nude mice with concomitant expression of differentiation markers, including CK18 and AR. Recent efforts to identify and characterize prostate cancer stem-like cells support the notion that CSC develops from normal SCs. Similar to their previous findings with normal prostate epithelial SCs, the rare

(approximately 0.1% of tumour cells) cancer SCs have a basal phenotype and proliferate extensively in vitro (Collins et al., 2005). This was not seen with the more differentiated tumour cells.
The lineage status of prostate CSCs has also been addressed in several other investigations. Using several human prostate xenograft tumours and cell lines, Patrawala and colleagues demonstrated that cells with a basal phenotype had increased tumour-initiating and metastatic activity in vivo, and expressed different putative SC genes (Patrawala et al., 2006). Several laboratories also investigate PC initiation in mouse models of the disease, and Wang and colleagues recently reported that in the PTEN-null murine model, there is an emergence of a proliferating Bcl-2–positive immature cell population with concomitant luminal differentiation and carcinogenesis (Wang et al., 2006a), suggesting that PTEN-deletion may initiate neoplasia in a stem/progenitor population.
However, it is important to note that acquisition of SC properties by other cells may enable them to support tumour initiation and maintenance. Recently, it was shown that by simply introducing four genes, Oct4, Sox2, c-Myc, and Klf4, into adult mouse fibroblasts, it was possible to induce pluripotent embryonic-like SC characteristics into these cells (Takahashi and Yamanaka, 2006). Since epigenetic changes are common in PC, I consider it plausible that key "stemness"-genes may be turned on by epigenetic changes, reprogramming and de-differentiating the target cell to behave as a SC, and to up-regulate SC antigens. Indeed, there is evidence to suggest that transfection of prostate basal/intermediate cells with the human telomerase catalytic subunit (hTERT) induces SC-characteristics in immature target cells, including expression of CD133 and the embryonic SC antigen Oct4 (Gu et al., 2007). (Interestingly, Oct4 has been reported to be restricted to embryonic SCs, not to be expressed in normal adult SCs, but to be up-regulated in several human malignant tumours, suggesting epigenetic re-activation). Never the less, if transformed cells behave, and express proteins, similar to normal SCs, we should learn more about normal SC biology, including its niche, in order to develop strategies to control or eradicate such cells.

Microenvironment, stromal growth factors and NE cells

Throughout the entire process of cancer aetiology, progression and metastasis, the surrounding microenvironment can be an active participant. Invasion occurs within a tumour–host microecology, where stroma and tumour cells exchange biomolecules that modify the local extracellular matrix, stimulate migration and metastatic processes, promote proliferation and survival, and

induce angiogenesis. It has been shown that human ES cells transplanted with UGM in nude male mice develops all epithelial cell lineages of the human prostate (Taylor et al., 2006), suggesting that mesenchyme instruct SCs to proliferate and generate tissue-specific cell types. Experimental evidence support the idea that signalling pathways essential for embryonic development also have a role in regulating self-renewal of tissues, and that these pathways are mutated or deregulated in human tumours. During prostate carcinogenesis, the stroma undergoes progressive loss of smooth muscle with the appearance of carcinoma-associated myofibroblasts. As described by Rowley's group, these peritumoral stromal cells have many features characteristic of wound repair (Tuxhorn et al., 2002). It has also been shown that carcinoma-associated stroma can induce preneoplastic epithelia to become malignant (Cunha et al., 2003). In this context, it has been hypothesized that repair and regeneration may provide opportunities for carcinogenesis, with chronic tissue injury resulting in a continuous need to replace damaged cells. The inability of the SC/SC-cell niche to control the return to a quiescent state may result in the progression to cancer. How prostate SCs/progenitor cells are regulated is not known, but it is plausible to assume that the stroma regulate SC biology to a greater or lesser extent, and is part of the SC-niche. As discussed previously, androgen administration to castrated mice regenerates the epithelium, suggesting that androgens act on the stroma to induce SC proliferation. Recently, it was shown that both normal and malignant stroma up-regulates AR expression in long-time castrated PC patients, suggesting that recurrent growth could be due to reactivation of stromal AR signalling (Wikstrom et al., 2007). Another potential regulator of prostate SC biology are the NE cells. Maturation of NE cells during prostate organogenesis precedes that of luminal cells, and NE cells are closely associated with the formation of the prostatic buds (Aumuller et al., 2001), suggesting that NE cells may control SC and/or developmental processes in the prostate, including differentiation. In PC it is well documented that the number of NE cells are increased and that they have a transformed phenotype (Huang et al., 2006). Normal NE cells are believed to be terminally differentiated, postmitotic cells, that do not express ARs. It has been suggested that NE cells support homeostasis and secretion in normal adult tissue, while de-regulation of the NE-system may promote carcinogenesis, progression and development of androgen-independent disease. NE differentiation (NED) of malignant cells appears to be a common phenomena in PC, especially in androgen-ablated cases (for a review, see (Hansson and Abrahamsson, 2001; Hansson and Abrahamsson, 2003)). Alterations in AR signalling causing receptor hypersensitivity, promiscuity, androgen-independent receptor transactivation,

or AR-independent growth by stromal growth factors have been suggested as mechanisms in androgen-independent disease. Similarly, NE cells may regulate the biology, including proliferation, survival and differentiation of neighbouring non-NE-phenotype cancer cells. It has been proposed that NE factors may transactivate the AR in androgen-independent disease, or stimulate proliferation and differentiation of immature cells. Notably, it has been shown that xenografts of NED-tumours can stimulate flanking non-NED PC xenografts to grow and express AR and PSA, even when the host is subjected to castration, while non-NED PC xenografts transplanted alone shrink and loose expression of AR and PSA (Jin et al, Cancer Res. 2004;64(15):5489-95), suggesting that NE-phenotype cells can support growth, survival, or differentiation of malignant cells in vivo. There are however conflicting data reported in the literature regarding the prognostic significance of NE tissue or serum markers in PC. Nevertheless, there may exist a real biological significance of NED even though controversial data has been reported regarding correlations between NED, tumour grade, stage, PSA levels and prognosis. Controversial data in terms of the prognostic value of NE markers may be explained by marker chosen, different patient cohorts, various methodological approaches, and other difficulties associated with the studies, e.g. limited amount of obtained tissue samples and unequal distribution of NE tumour cells etc.

Recently, Puccetti and colleagues reported that staining of the NE marker chromogranin A (CgA) in biopsies do not correlate to the CgA staining in whole mount sections of their homologous primary tumours (Puccetti et al., 2005). However, the authors found that staining of primary tumours showed a significantly greater degree of NED in high grade or high stage of disease than in low grade or low stage of disease, and that both CgA staining and PSA serum levels increased linearly. This highlights the methodological problems in evaluating NED, and suggests that we should re-evaluate past studies and conclusions.

Although it is widely accepted that CgA is an excellent marker of NE cell differentiation, different populations of NE cells produce different compounds. There is a lack of knowledge to which paracrine, and to what relative degree they influence PC biology. Yet, several studies have shown that regulation and function of malignant PC-cells in vitro is under the influence of several specific NE factors. For instance, serotonin and neuron specific enolase (NSE) are known to manifest tumour growth-promoting activity, and lately bombesin has been shown to stimulate androgen-independent proliferation (Lee et al., 2001), whereas somatostatin is thought to inhibit NE-secretion, proliferation and survival or proliferation of malignant cells (see my review (Hansson, 2005)).

However, several studies have reported that there is no correlation between the NE-markers CgA and NSE in serum samples from PC patients, suggesting that tissue or serum CgA may not necessarily reflect serum levels of potent mitogens or survival agents. Moreover, Roudier et al (Roudier et al., 2004) recently reported a case that lacked CgA staining in primary and metastatic lesions during the first six years of tumour progression, but that exhibited NED in metastatic lesions at time of death. This suggests that NED may occur late, and that absence of CgA staining in the primary tumour may not reflect inability to develop NED at later stages. We now know that metastatic lesions are heterogeneous, and that cancer cells evolve with time and microenvironment. It may be that cell populations and the profile of NE markers changes as the tumour progress. Malignant cells may also have different potential to respond to, or potential to induce release of various trophic factors from NE cells, depending on cell type and environment. Therefore, the prognostic significance of NED may increase if we can establish reliable methodologies and identify which critical NE factors to monitor, preferably in conjunction with markers that predict ability to respond to NE factors, and by identifying effects of NE factors on cells depending on their differentiation status. The neuropeptide-degrading protein NEP has been found to be down regulated in PC (Osman et al., 2004), but its expression in specific cell types and correlation with NED remains to be determined.

The identification of mechanisms and the source of NE cell differentiation could allow therapeutic intervention to inhibit the increase of the NE compartment, prevent production of growth factors and inhibit the progression to recurrent disease. Several studies have shown that NED increases in response to androgen-ablation, and in an elegant study by Sciarra et al (Sciarra et al., 2003) it was shown that intermittent administration of androgen deprivation significantly reduced increase of serum CgA levels. Such treatment may change the proportion of PC cells that have a NE-phenotype, and may affect tumour progression, as suggested by a study by Isshiki et al (Isshiki et al., 2002), where high serum CgA was associated with poor prognosis only in patients with a PSA value of 172 ng/ml or less, but not in those with higher PSA values.

In conclusion, the exact role of NE tumour cells, their neuropeptides and biogenic amines in disease progression is still not clear. Which specific NE-product that should be monitored is uncertain, or how we best should define the NE-phenotype, or potential to respond to NED. Refinement of the NE-concept may provide new opportunities in PC research.

Microenvironment and metastasis

Recent research suggests that cancer development and progression depends on the microenvironment, and is not exclusively regulated by genetic changes in cancer cells. There is considerable evidence that prostate tumour stroma is different than the stroma in normal tissue. The stroma is a dynamic environment that can perform tissue repair in response to injury, and the cancer-associated stroma has been described as "repair"-stroma by Rowley and colleagues (Tuxhorn et al., 2001), up-regulating growth factors, re-modelling the extracellular meshwork and modulating protease/protease-inhibitor expression, and inducing a stromal cell phenotype switch: there is a loss of smooth muscle cells concomitant with the emergence of undifferentiated myofibroblasts (an immature cell type that express a limited repertoire of both smooth muscle and fibroblast markers). It has been hypothesised that repair-stroma induces epithelial cells to proliferate and migrate through the wound tissue to re-establish the epithelial layer, and that a chronic wound response would lead to excessive free radicals from the immune system, deregulated stroma-epithelial interactions, and hyperplasia. Recently, GSTP1 inactivation through promoter methylation has been reported in the tumour-associated stroma (Hanson et al., 2006), suggesting epigenetic changes in the microenvironment. Recognition of epithelial to mesenchymal transition (EMT) is relatively new in PC research. EMT was first described in the mid 1980's as a central process in early embryonic morphogenesis; the primary mesenchyme is formed through EMT (Hay, 1995), resulting in the three-layers consisting of ectoderm, mesoderm (primary mesenchyme), and endoderm. The opposite, mesenchymal to epithelial transition (MET), also occur and is important in normal development. Recent research proposes that EMT is utilized as a mechanism by which solid epithelial cancers invade and metastasise. EMT is believed to allow cells to separate, lose their polarity, and gain a cell shape to facilitate cell movement, partly by changing the intermediate filaments from a cytokeratin phenotype to a mesenchymal phenotype (vimentin). However, evidence is still limited, and EMT in cancer progression is considered controversial. It has however been shown that EMT in adult epithelia can be induced by embryonic extracellular matrix. Matrix metalloproteases are frequently up-regulated in PC, putatively enabling cells to cleave E-cadherin, detach from each other and to penetrate the basement membrane. The classic cadherins (E-cadherin, N-cadherin, and P-cadherin) are transmembrane adhesion glycoproteins. EMT is characterised by reduced E-cadherin and increased N-cadherin expression, contributing to invasiveness. P-cadherin has been found to be expressed in the normal basal cell layer of the prostate. In a

study by Gravdal and colleagues (Gravdal et al., 2007), cadherin switching (high N- and low E-cadherin) showed significant associations with progression and cancer-specific death. Further, expression of P-cadherin in malignant cells was associated with poor differentiation and shorter time to skeletal metastasis, suggesting involvement of immature cells.

EMT probably is under strict control of growth factors and downstream transcription factors. For example, several growth factors, including FGFs and members of the transforming growth factor (TGF)-b family can induce up-regulation of members in the Snail family; members of transcription factors detected at EMT sites during embryonic development, recently also identified as key transcriptional repressors of E-cadherin expression in malignant tumours. Notch-1 signalling has also been shown to trigger EMT, in part by up-regulating Snail after TGF-b exposure in cardiac tissue, and as mentioned previously, Notch activity is critical for prostate branching morphogenesis in the mouse.

Interestingly, prostate carcinoma-associated stroma over express both TGF-b and stromal derived growth factor-1 (SDF-1). Whereas TGF-b inhibits proliferation of benign BPH1 cells in vitro, TGF-b also up-regulate membrane-expression of the SDF-1 receptor CXCR4. Administration of SDF-1 to BPH1 cells increased proliferation, and suppression of CXCR4 abrogated the tumorigenic response in tissue recombinants of BPH1 cells and carcinoma-associated stroma (Ao et al., 2007).

CXCR4 levels increase during PC progression, and has been hypothesised to guide PC cells to bone during the metastatic process. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) express CXCR4, and home to the SDF-1 abundant niche in the bone marrow, where MSCs are believed to regulate HSCs. SDF-1 is thought to function as a beacon for SC homing in development and injury. The use of MSCs for cell therapy relies on the capacity of these cells to home and engraft into the wound target tissue. Several papers have reported that MSCs have the ability for MET, although recent research suggests that rather than transforming to e.g. epithelial pancreatic insulin-producing cells, MSCs heal by regulating the stromal microenvironment. For example, injected MSCs that home to injured pancreas, results in improved healing and production of SDF-1. Application of SDF-1 alone to injured pancreatic tissue resulted in fully epitheliased wounds in a study by Badillo et al (Badillo et al., 2007). The involvement of MSCs in prostate development, wound healing, and cancer pathobiology is not known. However, in breast cancer, MSCs have recently been described to localize and integrate into the tumour-associated stroma. In a study by Karnoub and colleagues, when

otherwise weakly metastatic human breast carcinoma cells were mixed with MSCs, the tissue recombinant increased the metastatic potency greatly in tumour xenografts. This enhanced metastatic ability was shown to be reversible, and dependent on MSC secretion of the chemokine CCL5 (Karnoub et al., 2007).

The SC theory of cancer suggests that only CSCs may metastasise successfully (generate a large cell mass), but that the progeny may differentiate, to form heterogeneous lesions. Further, most of the daughter cells would not have a metastatic phenotype. However, heterogeneity in metastases may alternatively arise due to genetic instability of homogenous cells derived from the primary tumour, or to genetic instability of the primary tumour. In a recent study, the putative prostate SC/precursor protein nestin was identified to greatly induce migration, and was heavily up-regulated in metastatic PC lesions, but only after androgen-independent disease had developed. Since nestin was present in the primary tumour and in metastases only after androgen ablation, the authors suggested that the nestin-positive cells in metastases originated from within the prostate (Kleeberger et al., 2007). In conclusion, if we can develop treatments that target cells or processes in involved in metastasis, we could stop dissemination after carcinogenesis already has occurred.

The present investigation

Hypothesis and aims

Prostate cancer develops from an androgen-dependent tissue that contains androgen receptors in both the stromal and glandular compartment. Since the bulk of PC cells express mature glandular cell lineage markers, and since PC responds to androgen-ablation, it has been proposed that PC originates from mutated mature luminal epithelia. However, since advanced prostate malignancies become androgen-independent, it has also been proposed that a less mature, androgen-independent cell type, is responsible for recurrent growth. Recent research has shown that immature luminal intermediate/precursor cells and NE cells increase in numbers in advanced PC, and several lines of evidence suggests the existence of a CSC in PC.
We hypothesize that developmental mechanisms involved in organogenesis of the prostate have relevance to the genesis and biology of PC, and that the tightly controlled feedback loops that control self-renewal and differentiation are perturbed in PC. If we consider PC as a differentiating system, in which carcinogenesis occurs in primitive epithelial progenitor cells, this provides a framework for viewing the cellular and molecular heterogeneity observed in PC. We propose that the perturbed differentiation may have direct relevance to proliferation, invasiveness, apoptosis, and progression to the androgen-independent state. A detailed understanding of the hierarchy of PC cell populations is however hampered by the many uncertainties regarding the hierarchy of the early stages of normal differentiation processes in the human prostate. Here we aimed to investigate localisation, activity, and regulation of novel and key growth and differentiation modulating proteins in progenitors, precursors, and mature cell lineages of the adult human prostate gland, including benign and malignant tissue and cells.

Methods

Human prostate tissue collection and procurement

Paper I: Fresh tissue specimens of human prostates were obtained from patients undergoing transurethral resection of the prostate or transvesical prostatectomy because of benign enlargement of the prostate, or radical prostatectomy,

laminectomy, or pelvic lymph node dissection as treatment for PC. For positive control, pancreatic tissue was obtained at surgery from patients undergoing resection of pancreatic tumours. The tissue specimens were dissected into 3 to $4mm^3$ pieces and either placed on a small cork square, drenched in Tissue-Tek, and submerged in 2-methylbutene (isopentane) cooled by liquid nitrogen, and stored at -70°C until analyzed; or fixed in Bouin's fixative (for 4 to 18 hr), with a subsequent paraffin-embedding standard procedure.
Paper II – IV: All tissue samples were surgically obtained from patients who had undergone radical prostatectomy for organ-confined PC (preoperative staging). Prostates were put in ice-cold physiological saline media immediately after surgery. Two tissue cores representing normal/benign tissue were excised from the transition zone, cut into 2–4-mm cubes and immediately fixed in 4% paraformaldehyde (1 h/mm at 4°C). Explants were then serially passed through a sucrose gradient in PBS at 4°C, until sunk. Subsequently, tissue was frozen. To validate that no cancer was present in obtained tissue cores, sections were stained with haematoxylin–eosin and evaluated together with original prostates by three uro-pathologists according to the Gleason scoring system.

Explant cultures

Immediately after resection, excised prostate tissue cubes from patients were placed on collagen-I–coated dishes in RPMI 1640 media, supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 2mM L-glutamine, and 10% fetal bovine serum (RPMI-FBS media), and maintained at 37°C (5% CO_2), until processed for immunofluorescence, after three, seven, or fourteen days of tissue culture.

Primary cell cultures

Prostate tissue cubes were placed on collagen-I–coated dishes, and primary epithelial cell cultures were initiated in RPMI-FBS (37°C, 5% CO_2) media without, or with treatment factors as described in the primary cell treatment sections. Tissue was removed after three days, and epithelial cells were allowed to reach 80% confluency before passaging or fixation. Media was changed every third day. For paper II, primary cell cultures were further initiated from additional prostates on un-coated, on collagen-I-coated, and on laminin-coated dishes in the above media, with or without 10% FBS, supplemented with or without 1nM of the synthetic androgen R188.

Primary cell treatment

Wound-healing assay

Primary passage null cell cultures were scratched when 80% confluent and immediately treated with or without 10 ng/ml hepatocyte growth factor (HGF), and monitored until closed/healed. Mean closure time (h) +/- average absolute deviation from the median was reported.

Notch inhibition

To inhibit cleavage and activation of the Notch-pathway, 10 μM of gamma-secretase inhibitor X (L-685,458; DMSO solubilized) was administered at initiation of primary cell cultures, or to 50% confluent passage null cultures, or to passage one cultures 12 hours after plating, and at media change. In addition, some L-685,458-initiated cultures were administrated 500 nM human Growth Hormone at initiation, and at media changes, omitting L-685,458 from the third and subsequent final media change. Control cultures received DMSO at levels paralleling DMSO content in L-685,458-treated cultures.

Somatostatin (SS) analogue treatment

Twelve hours after plating, media in passage one cultures was changed to FBS-free RPMI 1640 media after washing cells with PBS. Then 1μM of the non-selective SSTR-subtype agonist SS-14 (solubilized in DMSO), or 1μM of the SSTR5/1-specific agonist L-817818 (solubilized in DMSO), or DMSO was administrated to cells. Five minutes later, 10ng/ml epidermal growth factor (EGF) was administrated to half of the DMSO-treated cultures, and to the SS-14 and L-817818 treated cultures, and cultures were incubated. Identical media was replenished after four days. All cells were treated with DMSO at levels paralleling DMSO-content in SS-agonist treated cultures.

Cell density assay

Triplicate control and treated passage one cultures were assayed for cell density using the sulforhodamine B (SRB) method as described [19], after seven days of culture. In addition, at media change and at day seven, apoptotic floating cells were collected from the media and bürker-chamber counted for paper III. Student's t-test was performed on collected data.

Riboprobe synthesis

To synthesize human somatostatin receptor 2 (SSTR2) and somatostatin receptor 4 (SSTR4) cRNA probes of appropriate lengths and specificity, riboprobe templates were generated by PCR from purified plasmid DNA of cloned SS-receptors. The primers were directed against SSTR2 or SSTR4, with extensions containing sequences for T7 and T3 RNA polymerase promoters. In vitro transcription with T7 and T3 RNA polymerase of the PCR-derived riboprobe templates were then used to synthesize fluorescein-labeled SSTR2 and SSTR4 antisense and sense cRNA probes. The specificity of the synthesized SSTR2 and SSTR4 antisense riboprobes were evaluated by hybridization against synthesized SSTR1-5 RNA spotted on membranes in slot blots, to verify that the hybridization signals obtained for the different forms of SSTRs were specific, and not a consequence of cross-hybridization between related members of the SSTR-family.

In situ hybridization

Paraffin-embedded specimens were sectioned, mounted on SuperFrost Plus slides and deparaffinized according to standard procedures. Fresh-frozen tissue specimens were sectioned and mounted on SuperFrost Plus slides. All slides were subsequently subjected to the same hybridization conditions as the membranes in the slot-blot protocol, and SSTR2 and SSTR4 signals were detected by adding the chromogenic substrates nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) after incubation with an anti-fluorescein alkaline phosphatase conjugated antibody. Due to the nature of RNA, which is easily and quickly degraded, signal intensity was only determined in adjacent cells on the same tissue section. The specificity of the in situ hybridization experiments were ensured by using the same buffers and temperatures as in the slot blot control experiments, and the specificity of the antisense signals was further corroborated by the occurrence of positive in situ hybridization signals in the islets of Langerhans and negative signals in the surrounding cells of pancreatic tissue. Moreover, the negative control sense riboprobes generated no signals in prostatic tissue.

Immunofluorescence

Frozen sections 8 μm thick were cut from fixed fresh and cultured explants onto SuperFrost Ultra Plus slides. Sections were permeabilized with 0.1% Triton X-100 in PBS for 15 min at room temperature (RT), and then rinsed in

PBS. Sections were subsequently blocked in 2% bovine serum albumin fraction V in PBS (A-PBS) for 1 h at RT, and then incubated at RT with primary antibodies (directed against putative stem cell-, intermediate cell-, cell lineages-, proliferative-, and apoptotic antigens) diluted in A-PBS. Slides were then washed in PBS and incubated with secondary donkey Alexa 488/594 fluor-conjugated antibodies and counterstained with DAPI, according to the manufacturer's instructions, mounted in ProLong Gold mounting media, and coverslipped. Negative control reactions omitting primary antibody were performed. In addition, pre-absorption using immunizing CD133 peptide, Notch-1 peptide, and SSTR5 peptide was performed. Signals were detected with an Olympus AX70 microscope, equipped with appropriate filters from Semrock, and fitted with a Nikon DS-U1 digital colour camera. Quantification of Ki-67 cells in paper II: Sections stained with anti-Ki-67 and anti-KIT or with anti-Ki-67 and anti-CD133 antibodies, were allocated into five groups: snap-frozen fresh tissue (glandular, group 1); cultured for 3 days (glandular, group 2; surface, group 3); cultured for 7 days (glandular, group 4; surface, group 5). Twelve sections per group were randomly photographed at 20X magnification: the nearest random gland or surface in view was centred and photographed, and the number of Ki-67–positive nuclei was reported as a percentage of epithelial DAPI nuclei. Statistical analysis between groups was done using Fisher's exact test.

Results

Paper I

In this paper, we investigated tissue localization of SSTR2 and SSTR4 mRNA in BPH and malignant cells of the human prostate. Hybridization signals for SSTR4 mRNA transcripts were confined to the prostatic epithelium (12 of 16 BPH cases, and in 12 of 13 carcinoma cases), whereas SSTR2 transcripts were predominantly localized in the stromal compartment, but were also detectable in epithelial cells in a significant number of specimens (11 of 17 BPH cases, and in 12 of 14 carcinoma cases). Importantly, the staining intensity for SSTR2 and SSTR4 transcripts was stronger in malignant cells when compared to adjacent BPH epithelium, suggesting that SSTR2 and SSTR4 receptors are up-regulated in PC.

Paper II

Investigation of dynamic expression patterns in an in situ prostate explant renewal model of the human normal/benign adult prostate showed that cells with a basal phenotype proliferated significantly in prostate tissue cultures, with a peak at three days, whereas luminal cells went into apoptosis. Results further showed down-regulation in tissue cultures of the basal and hypothetical SC marker Bcl-2 in the majority of cells, except in rare candidate epithelial SCs. Investigation of established (AC133) and novel candidate prostate SC/progenitor markers, including the cell surface receptor tyrosine kinase KIT and its ligand stem cell factor (SCF), showed that these rare epithelial cells are positive for AC133, CD133, Bcl-2, cytokeratin, KIT, and SCF, but negative for vimentin. In addition, we found a stromal population that expressed the mesenchymal marker vimentin and that are positive for CD133, KIT, and SCF, but negative for AC133, Bcl-2, and cytokeratins, suggesting a mesenchymal/stromal progenitor phenotype. We further observed that basal cytokeratin phenotype cells migrated in an invasive manner towards the surface of the explants, where they typically formed an epithelial lining. In a few cases, CD133 positive putative epithelial SCs were found in the epithelial layer lining the surface of the explants, suggesting that SCs actively migrate in prostate tissue cultures. In primary cell cultures, epithelial cells responded to HGF, expressed lower levels of basal cell cytokeratins, and were negative for luminal cell cytokeratins even in the presence of androgens, suggesting an early intermediate phenotype, and incomplete differentiation towards the luminal cell lineage; other, androgen-additional factors, are needed for terminal luminal differentiation.

Paper III

Mature luminal cells in the adult human prostate were found to co-express the cytodifferentiation-modulatory protein Notch-1 and its ligand Jagged1, whereas the Notch-ligand Delta1 was expressed in endothelial cells. Active Notch-signalling was confined to blood vessels of normal/benign prostate tissue. The basal cell layer, rare candidate epithelial SCs, and a subpopulation of NE cells were found to express the progenitor and differentiation marker Dlk-1. In prostate explant cultures, luminal cells and Jagged1 expression were lost, whereas intermediate cells expanded and up-regulated and activated Notch-1, concomitant with Dlk-1 down-regulation. Administration of a Notch-inhibitor to primary epithelial cell cultures led to lower cell proliferation and suppressed down-regulation of Dlk-1, i.e. Notch-signalling down-regulates the novel prostate SC and basal cell marker Dlk-1 in differentiating intermediate cells.

Paper IV

Characterization of somatostatin, SS, expression in relation to candidate epithelial SCs in non-malignant adult prostate tissue showed that these two very rare cell types sometimes localized adjacent to each other. In addition, SS-positive cells were detected in the stroma, and somatonergic processes innervated stromal progenitor phenotype cells. Populations of both candidate epithelial SCs and putative stromal progenitor cells expressed SSTR1, but only stromal progenitor phenotype cells expressed SSTR5. SSTR5 was further expressed by terminally differentiated luminal cells, and found to be up-regulated in emerging intermediate cells. Administration of an unspecific SS-receptor agonist (SS-14) or the SSTR5/1-specific agonist L-817818 inhibited epidermal growth factor (EGF)-driven androgen-independent growth in prostate primary epithelial cell cultures. Although intermediate cells did not express SSTR1, we cannot exclude the possibility that contaminating SSTR1 positive epithelial candidate stem cells were present in the primary cell cultures, i.e. contributing to the growth of such cultures, and hence to diminished growth in response to SSTR-agonists. Our results suggest that SS analogues should be evaluated for treatment of PC; non-specific agonists may target both progenitors and mature malignant cells.

Discussion

In European countries, the incidence of PC has increased more than any other cancer over the past two decades, and despite the widespread use of prostate specific antigen testing, the number of deaths from PC is still increasing (Ferlay et al., 2007; Jemal et al., 2007). Current treatment options with curative intent for patients with early disease are radical prostatectomy and/or radiation therapy. Androgen suppression therapy is the golden standard for progressive prostate cancer. However, most men will eventually fail this therapy and develop recurrent androgen-independent disease. At present, there is no effective therapy for this patient group.

Recently, the concept of CSCs has changed the perspective on PC. Only a minority of cancer cells within most human tumours are endowed with tumorigenic potential when transplanted, suggesting the existence of CSCs. Importantly, CSCs have the ability to reproduce the phenotypic diversity and differentiation pattern of the parent tumour when xenografted. In a recent landmark finding from Collins and colleagues (Collins et al., 2005), it was

shown that there are only minor populations of cells in each PC malignancy that have CSC-characteristics; These cells are AC133-positive and appear to be capable of asymmetric division and self renewal, and constitute only a minor fraction among the bulk of more differentiated cells in the tumour, and can reconstitute the tumour when transplanted into nude mice. It is possible that under the influence of androgens, malignant SCs generate the bulk of differentiated androgen-dependent daughter tumour cells, and that androgen suppression leads to apoptosis of these more mature cells, leaving less differentiated cells to survive. If we can target these androgen-independent immature cells with SC-characteristics, it is possible that we could eradicate the cells that makes the tumour recur. It is, however, unclear whether tumour SCs arise exclusively from normal tissue SCs, or from progenitors that have differentiated from the SC itself, or from more mature cells that have de-differentiated. Normal SCs are however the most likely candidate targets for the initial carcinogenic insult, as they have the long life necessary for accumulating the multiple genetic or epigenetic changes required for malignant transformation. The limited knowledge of the biology of normal prostate SCs and their differentiation programs and hierarchy of cells currently limits our understanding of PC pathobiology. Characterization of normal prostate SCs, SC-niche, differentiation processes, and biology of intermediate cells, could provide knowledge and open up for development of novel treatment modalities in PC.

In this thesis, I have investigated dynamic expression patterns and activity of regulatory proteins in prostate candidate SCs, SC-niche, intermediate/transit-amplifying cells, and cell lineages, in an in situ explant renewal model of the human non-malignant adult prostate. The regulation of proteins and effects of growth factors and inhibitors were further investigated in primary prostate epithelial cell cultures. In addition, SSTR2 and SSTR4 mRNA expression was investigated in both benign and malignant prostate glands.

Our investigation provides evidence for epithelial renewal in adult human prostate explants in response to tissue culture and for basal and epithelial stem/progenitor cell recruitment leading to an expansion of an intermediate luminal precursor phenotype. Data further showed that immature epithelia migrated in an invasive manner towards the surface of the explants, and that cells up-regulated Notch-1. In the future, we propose to investigate if intermediate/immature prostate epithelia exhibit features of (presumably transient) EMT in explant cultures. EMT implies an acquisition of a migratory phenotype, and has been proposed to occur not only during embryogenesis, but also during tumour progression, when cells disseminate and form metastases.

It is possible that only certain cell populations, depending on differentiation status, have the ability for EMT. In paper I, we identified a novel putative stromal progenitor phenotype, positive for CD133, KIT, SCF, and vimentin. Notably, the stroma is subverted in prostate malignant lesions, and such lesions have been hypothesized to support carcinogenesis, and we suggest that involvement of this cell type, or possible daughter cells, should be investigated, including possible role in EMT. In paper IV, we noted that the stromal progenitor phenotype differentially expressed SS-receptors, with only a minor population expressing SSTR5.

The nature and regulation of the stromal SSTR1- and SSTR5-positive SCF/KIT-populations reported here needs to be further investigated, including possibility to differentiate into different cell types. Recently it was shown that stromal primary cell cultures obtained from BPH tissue contained SCF and cells with SC-characteristics (Lin et al., 2007). The notion that stromal progenitor cells may be involved in BPH has been raised (Ceder et al., 2007), and we suggest investigating a possible SS-SCF-KIT axis and aberrant stromal differentiation in this disease.

Whereas stromal putative progenitor cells did not express the Notch-homologous protein Dlk-1, candidate prostate epithelial SCs, basal cells, and a subpopulation of NE cells were found positive for this cytodifferentiation-regulatory protein. The identification of Dlk-1 expressing candidate stem and rare NE cells suggests a hierarchical relationship. Here we further found that candidate SCs retain Bcl-2 expression in tissue culture, but was lost in intermediate cells. Interestingly, it has been demonstrated previously that most PCs express SC-related genes, especially so in the advanced stages of the disease. Notably, Wang and colleagues recently reported that in the PTEN-null murine model, there is an emergence of a proliferating Bcl-2–positive immature cell population with concomitant luminal differentiation and carcinogenesis, suggesting that PTEN deletion may initiate neoplasia in a stem/progenitor population. In androgen-independent PC, it has been found that Bcl-2 is up-regulated, and that malignant NE-phenotype cells often are found in close proximity to Bcl-2 expressing cells. In conjunction with our results, previous findings suggest that emerging NE cells in PC may arise from Bcl-2 positive SCs. The identification of the source and mechanisms of NE differentiation in PC could allow therapeutic intervention to inhibit the increase of NE-characteristics, prevent production of growth factors, putatively inhibiting the progression of the disease. NE cells were negative for Notch-1 expression in our study, whereas differentiating epithelia up-regulated and activated Notch-1 to down-regulate the expression of Dlk-1. Importantly, administration of a Notch-

inhibitor to primary epithelial cell cultures decreased growth significantly. It will be interesting to investigate whether ectopic Dlk-1 expression inhibits engagement or terminal differentiation of luminal precursor cells. Modulation of Notch-related family members in PC may possibly provide a method for controlling differentiation status of malignant cells and growth characteristics. The microenvironment, including stroma and NE cells, is thought to maintain growth quiescence and differentiation status in the adult prostate. During repair and regeneration, it is believed that SCs proliferate and give rise to differentiating daughter cells, and that the microenvironment in the SC-niche controls SC behaviour. The inability of the stem cell/SC-cell niche to control the return to a quiescent state may result in the progression to cancer. Modulating the SC-niche may prevent or diminish malignant features of putative precancerous SCs. We have provided evidence to suggest that SS is part of the prostate SC/progenitor niche, and that SS acts through specific SS-receptors in both stromal and epithelial stem/progenitor phenotypes cells. Additionally, terminally differentiated luminal cells were found to express SSTR5, and SSTR5 was up-regulated in emerging intermediate epithelial precursor cells. Importantly, administration of SS-agonists inhibited expansion of immature luminal cells in prostate primary epithelial cell cultures. Further, SSTR2 and SSTR4 were found to be up-regulated in malignant cells when compared to benign cells in tissue from PC patients, and to be expressed in disseminated cells. Together, our results suggest that SS regulates several aspects of prostate biology. Of note, KIT kinase activity has been shown to be down-regulated via the cytosolic phosphatase Shp-1, a phosphatase known to be activated by SS signalling, suggesting that SS may directly abrogate KIT activity. In combination with previously described findings that SS inhibits SCF synthesis in the human testis and that SCF stimulates self-renewal in hematopoietic SCs, we suggest that a loss of prostatic SSTR1 activation may lead to increased levels of SCF/KIT activity, causing a slight change in SC cycling and an expansion of the epithelial progenitor population, leaving the opportunity for prostate carcinogenesis.

In conclusion, androgen ablation does an effective job killing the majority of cells within the tumour, but as suggested by the CSC theory and recent PC research, today we may be targeting the wrong cell type, missing the cells that have the capacity to make the tumour recur. Our limited knowledge of normal and malignant SCs is in part due to the overall paucity of SC markers. In the current investigation, we have reported on several novel candidate SC-antigens in the human prostate. It is safe to assume that ex vivo purification of prostate

SCs based on here described novel SC antigens coupled with in vitro functional assays will provide one of the most effective probes for the study of prostate SC biology in the near future.

The characterization of normal prostate SCs, SC-niche, intermediate cells, and cell lineages, could provide knowledge to the understanding of self-renewal, transit amplifying/intermediate cell proliferation, and cell differentiation processes to supply information regarding possible key regulators, opening up for development of novel treatment modalities in PC. Novel treatment modalities of PC may be based on inhibitors/agonists of critical SC/SC-niche signalling molecules or of their receptors, or of cytodifferentiating modulatory molecules. Current results may set the stage for the development of new therapeutics in the treatment of prostate stem/progenitor cell derived diseases. SS analogues make logical drug candidates, as SS analogues may target both prostate SCs, intermediate cells, and the bulk of more differentiated cells (Halmos et al., 2000; Hansson et al., 2002; Reubi et al., 1995) simultaneously.

Svensk sammanfattning

Antalet prostatacancerfall i Sverige har ökat dramatiskt sedan PSA (prostata-specifikt antigen) testet infördes. PSA utsöndras av körtelcellerna i prostatakörteln, och PSA är en viktig komponent i mannens sädesvätska. Vid tumörsjukdomar i prostatakörteln läcker en del PSA till blodet, som kan detekteras med ett enkelt blodprov. De flesta patienter har dock en godartad tumör, eller en mild form av PC, som inte, eller endast långsamt, ger upphov till dottersvulstrar/metastaser. Avlägsnar man tumören genom ett kirurgiskt ingrepp kan patienten bli frisk.
En del patienter drabbas dock av en mer allvarlig form av PC, som mer ofta ger upphov till metastaser. Metastaserad PC behandlas oftast genom att strypa tillgången på androgener, och merparten av cancercellerna dör. Dock överlever en del celler, och sjukdomen blir efter ett tag hormon-oberoende, dvs den tillväxer trots avsaknad av androgener. I dagsläget finns ingen bra behandlingsmetod mot hormon-oberoende PC.
Mycket forskning inom PC området går ut på att hitta nya mediciner mot denna form av PC, hormon-oberoende PC. Vissa forskare tror att cancercellerna är muterade och sjuka fullt mogna körtelceller, dvs de celler som utsöndrar PSA, och att man bör utveckla mediciner som bättre slår ut dessa celler än vad dagens mediciner förmår. Andra forskare tror att mer primitiva, stamcells-liknande celler ger upphov till PC, och är anledningen till att sjukdomen blir hormon-oberoende, eftersom sådana celler ej behöver androgener för att överleva eller tillväxa.
Tyvärr vet man ej så mycket om vilka celler som finns i prostatan, eller hur de mognar och blir funktionella körtelceller som utsöndrar PSA. Vi vill ta reda på vilka celler som finns i prostatan, hur de mognar, och vilka proteiner cellerna uttrycker under tiden de mognar, och vad som reglerar deras tillväxt och funktionella

mognad. Genom att titta på och behandla celler från PC patienter, har vi sett att stamcells-liknande celler som ger upphov till körtelcellerna i prostatan uttrycker ett protein/markör som kallas CD133, och ett specifikt protein som kallas KIT, och som tros reglera om cellerna skall ge upphov till dotterceller. Vidare uttrycker de stamcells-liknande primitiva körtelcellerna ett protein som kallas Dlk-1, som tros hämma mognad av celler. Ett annat protein, som kallas Notch-1, stänger dock av Dlk-1 i utmognande körtelceller, dvs i celler som påbörjat mognaden mot funktionella körtelceller. Hämmar vi aktiviteten av Notch-1, avtar tillväxten av omogna celler. Vi har även hittat en ny celltyp i prostatans bindväv, som uttrycker stamcells markören CD133. Vid godartad/benign prostatatumör, är det framförallt bindvävnaden som tillväxer ohämmat. I framtiden vill vi undersöka om dessa 'nya' celler bidrar till godartad tillväxt i prostatan.
Vi har även sett att stamcells-liknande bindvävsceller, stamcells-liknande körtelceller, utmognande körtelceller, och fullt mogna körtelceller uttrycker receptorer för det hämmande proteinet somatostatin, och att receptorer för somatostatin även uttrycks av cancerceller i metastaser. Dessa celler kan alltså känna av och reagera på somatostatin-liknande substanser. Vi föreslår att man skall undersöka om somatostatin-liknande mediciner kan hämma tillväxt i såväl godartade som elekartade tumörer i prostata, och om Notch-1 samt Dlk-1 modulerande mediciner kan påverka mognadsgraden av cellerna, och därmed sjukdomsförloppet. Förhoppningsvis kan sådana framtida studier leda till bättre behandlingsmetoder av tumörsjukdomar som uppstår i prostatakörteln, inklusive hormon-oberoende metastaserad PC.

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Appendix: papers I – IV