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HIGH-THROUGHPUT SCREENING FOR NOVEL PROSTATE CANCER DRUG TARGETS

Getting personal

by

Paula Vainio

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To my dear family

"Some see a hopeless end, while others see an endless hope."
- Author Unknown

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High-Throughput Screening for Novel Prostate Cancer Drug Targets – Getting Personal
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ABSTRACT

Prostate cancers form a heterogeneous group of diseases and there is a need for novel biomarkers, and for more efficient and targeted methods of treatment. In this thesis, the potential of microarray data, RNA interference (RNAi) and compound screens were utilized in order to identify novel biomarkers, drug targets and drugs for future personalized prostate cancer therapeutics. First, a bioinformatic mRNA expression analysis covering 9873 human tissue and cell samples, including 349 prostate cancer and 147 normal prostate samples, was used to distinguish *in silico* prevalidated putative prostate cancer biomarkers and drug targets. Second, RNAi based high-throughput (HT) functional profiling of 295 prostate and prostate cancer tissue specific genes was performed in cultured prostate cancer cells. Third, a HT compound screen approach using a library of 4910 drugs and drug-like molecules was exploited to identify potential drugs inhibiting prostate cancer cell growth. Nine candidate drug targets, with biomarker potential, and one cancer selective compound were validated *in vitro* and *in vivo*. In addition to androgen receptor (AR) signaling, endoplasmic reticulum (ER) function, arachidonic acid (AA) pathway, redox homeostasis and mitosis were identified as vital processes in prostate cancer cells. ERG oncogene positive cancer cells exhibited sensitivity to induction of oxidative and ER stress, whereas advanced and castrate-resistant prostate cancer (CRPC) could be potentially targeted through AR signaling and mitosis. In conclusion, this thesis illustrates the power of systems biological data analysis in the discovery of potential vulnerabilities present in prostate cancer cells, as well as novel options for personalized cancer management.

Keywords: Prostate cancer, high-throughput screening, gene expression, RNA interference, drug target, biomarker, drug

Paula Vainio
Uusien eturauhassyövän hoitokohteiden identifiointi tehoseulonta-menetelmiä hyväksi käyttäen – kohti täsmähoitoa
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TIIVISTELMÄ

Eturauhassyöpä on monimuotoinen ja epäyhtenäinen joukko sairauksia, joiden hoitamiseksi tarvitaan uusia tehokkaampia merkkiaineita, sekä kohdennettuja hoitovaihtoehtoja. Tässä väitöstutkimuksessa yhdistettiin tieto geenien ilmentymisestä geenin hiljentämisen mahdollistavaan RNA-interferenssi (RNAi) -tekniikkaan sekä lääketehoseulontoihin uusien merkkiaineiden, lääkehoidon kohteiden sekä lääkeaineiden löytämiseksi, ja kohdennettujen eturauhassyöpähoitojen mahdollistamiseksi. Aluksi hyödynsimme tietoja geenien ilmentymisestä 9873:ssa ihmiskudos- ja solunäytteessä erityisesti eturauhas- (n = 147) ja eturauhassyöpäkudoksessa (n = 349) ilmentyvien geenien havaitsemiseen. Seuraavaksi 295:n eturauhassyöpäkudokselle ominaisen geenin vaikutusta viljeltyjen eturauhassyöpäsolujen kasvuun tutkittiin RNAi–tehoseulontatekniikkaa hyödyntäen. Samanaikaisesti 4910:n eri lääkeaineen tehoa eturauhassyöpäsolujen kasvun estossa tutkittiin lääketehoseulontoja hyväksi käyttäen. Yhdeksän uuden lupaavan lääkehoidon kohteen sekä yhden syöpäsolujen kasvua estävän lääkeaineen toiminta varmennettiin jatkotutkimuksissa. Tulokset osoittivat, että androgeenireseptorin (AR) signaloinnin lisäksi solulimakalvoston toiminta, arakidonihappo-aineenvaihdunta, hapetus-pelkistys –tasapainotila ja tuman jakautuminen, mitoosi, ovat tärkeitä eturauhassyöpäsolujen kasvulle. Uudet lääkehoidon kohdegeenit ilmentyivät eri eturauhassyövissä ja osoittivat, että ERG syöpägeeniä ilmentävät syöpäsolut olivat herkkiä oksidatiiviselle stressille ja solulimakalvoston toiminnan häiriölle, kun taas mitoosin estoa voitaisiin mahdollisesti hyödyntää pitkälle edenneiden ja hormonihoidoille vastustuskykyisten eturauhassyöpien hoidossa. Yhteenvetona voidaan todeta, että tämän väitöstutkimuksen tulokset havainnollistavat systeemibiologisen tutkimuksen mahdollisuudet uusien syöpähoitojen kehityksessä.

Avainsanat: Eturauhassyöpä, tehoseulonta, RNA interferenssi, geenin ilmentyminen, lääkehoidon kohde, merkkiaine, lääke

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ABBREVIATIONS

12,13-EODE	12,13-cis epoxide of linoleic acid (12(13)epoxy-9Z-octadecenoic acid)
AA	arachidonic acid
ABC	ATP-binding cassette
aCGH	array-based comparative genomic hybridization
ACTR3	ARP3 actin-related protein 3 homolog (yeast)
AD	androgen dependent
AI	androgen independent
AIM1	absent in melanoma 1
AKT	v-akt murine thymoma viral oncogene homolog 1
ALDH1A1	aldehyde dehydrogenase 1A1
ALOX15B	15-lipoxygenase 2
AMACR	alpha-methylacyl coenzyme A racemase
APC	antigen-presenting cells
AR	androgen receptor
AS	androgen sensitive
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
B2M	beta-2-microglobulin
BCL2	B-cell CLL / lymphoma 2
BCL2L1	Bcl-2-like protein 1
BPH	benign prostatic hyperplasia
CASP8	caspase 8, apoptosis-related cysteine peptidase
CDC42	cell division cycle 42 (GTP binding protein)
CDKN1A	cyclin-dependent kinase inhibitor 1A, p21
CDKN1B	cyclin-dependent kinase inhibitor 1B, p27
cDNA	complementary deoxyribonucleic acid
COX	cycloxygenase
cPARP	cleaved poly(ADP-ribose) polymerase
CRPC	castrate-resistant prostate cancer
CTNNB1	catenin (cadherin-associated protein), beta 1
CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1
CYP4F8	cytochrome P450, family 4, subfamily F, polypeptide 8
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DSCAM	Down syndrome cell adhesion molecule
DSF	disulfiram
ECL	enhanced chemiluminescence reagent
EGF	epidermal growth factor
EPHX2	epoxide hydrolase 2, cytoplasmic
ER	endoplasmic reticulum
ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)
ERGIC1	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1
ETV	ets variant
EZH2	enhancer of zeste homolog 2
FAAH	fatty acid amide hydrolase

FAK	focal adhesion kinase
FAM110B	family with sequence similarity 110, member B
FDA	U.S. Food and Drug Administration
FGF	fibroblast growth factor
GSTP1	glutathione S-transferase pi 1
HDAC	histone deacetylase
HIF1A	hypoxia-inducible factor 1-alpha
HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD) / prostaglandin dehydrogenase 1
HPV	human papillome virus
HSP90	heat shock protein 90
HT	high-troughput
hTERT	telomerase reverse transcriptase
HTS	high-troughput screening
IGF1	insulin-like growth factor 1
IHC	immunohistochemistry
IL6	interleukin 6
ITGB1	integrin, beta 1
IRF7	interferon regulatory factor 7
JAK	Janus kinase
KGF	keratinocyte growth factor
KIF11	kinesin family member 11
KLK	kallikrein
LAS1L	LAS1-like (S. cerevisiae)
LDL	low-density lipoprotein
LHRH	luteinising hormone-releasing hormone
LIMK1	LIM domain kinase 1
LPC	lysophosphatidyl choline (1-hexadecanoyl-sn-glycerol-3-phosphorylcholine)
MAPK	mitogen-activated protein kinase
MCM	minichromosome maintenance complex component
mRNA	messenger ribonucleic acid
MT	metallothionein
mTOR	mechanistic target of rapamycin
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
NCAM1	neural cell adhesion molecule 1
NKX3.1	prostate specific NK3 homeoprotein 1
PAF	platelet-activating factor
PAK	p21 protein (Cdc42/Rac)-activated kinase
PAP	prostatic acid phosphatase (ACPP)
PCA3	prostate cancer antigen 3
PDGFR	platelet-derived growth factor receptor
PGE_2	prostaglandin E2
PIN	prostatic intraepithelial neoplasia
PI3K	phosphatidylinositol 3-kinase
PLA2G2A	phospholipase A2, group IIA
PLA2G7	phospholipase A2, group VII

PLK1	polo-like kinase 1
pPAK	phosphorylated p21 protein (Cdc42/Rac)-activated kinase
PSA	prostate specific antigen
pSTAT3	phosphorylated signal transducer and activator of transcription 3
qRT-PCR	quantitative real-time polymerase chain reaction
PTEN	phosphatase and tensin homolog
RB	retinoblastoma
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
siRNA	small interfering ribonucleic acid
SPINK1	serine protease inhibitor Kazal type 1
SRC	v-src sarcoma viral oncogene homolog, avian
STAT1/3	signal transducer and activator of transcription 1/3
TGF-β	transforming growth factor beta
TMED3	transmembrane emp24 protein transport domain containing 3
TMPRSS2	transmembrane protease, serine 2
TP53	tumor protein p53
TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)
TSA	trichostatin A
uPA	urokinase plasminogen activator
UPLC-MS	Ultra Performance Liquid Chromatography - Mass Spectrometry
VEGF	vascular endothelial growth factor
YIPF6	Yip1 domain family, member 6

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-V:

I. **Vainio P**, Mpindi JP, Kohonen P, Fey V, Mirtti T, Alanen KA, Perälä M, Kallioniemi O, Iljin K. High-throughput transcriptomic and RNAi analysis identifies AIM1, ERGIC1, TMED3 and TPX2 as potential drug targets in prostate cancer. Submitted.

II. **Vainio P**, Gupta S, Ketola K, Mirtti T, Mpindi JP, Kohonen P, Fey V, Perälä M, Smit F, Verhaegh G, Schalken J, Alanen KA, Kallioniemi O, Iljin K. Arachidonic acid pathway members PLA2G7, HPGD, EPHX2, and CYP4F8 identified as putative novel therapeutic targets in prostate cancer. Am J Pathol. 2011 178(2):525-36.

III. **Vainio P**, Lehtinen L, Mirtti T, Hilvo M, Seppänen-Laakso T, Virtanen J, Sankila A, Nordling S, Lundin J, Rannikko A, Orešič M, Kallioniemi O, Iljin K. Phospholipase PLA2G7 is a drug target and a potent biomarker in prostate cancer. Submitted.

IV. **Vainio P**, Wolf M, Edgren H, He T, Kohonen P, Mpindi JP, Smit F, Verhaegh G, Schalken J, Perälä M, Iljin K, Kallioniemi O. Integrative genomic, transcriptomic and RNAi analysis indicates a potential oncogenic role for FAM110B in castration-resistant prostate cancer. The Prostate. 2011 Sept 14 [Epub ahead of print].

V. Iljin K, Ketola K, **Vainio P**, Halonen P, Kohonen P, Fey V, Grafström RC, Perälä M, Kallioniemi O. High-throughput cell-based screening of 4910 known drugs and drug-like small molecules identifies disulfiram as an inhibitor of prostate cancer cell growth. Clin Cancer Res. 2009 15(19):6070-8.

 In addition, some unpublished data are presented in this thesis.

1. INTRODUCTION

Prostate cancer is the most commonly diagnosed malignancy and the third most common cause of cancer mortality in the Western male population (Jemal et al. 2011). Despite the high frequency of the disease, the opinions on the usefulness of prostate cancer screening and diagnostics with the currently existing methods, the prognostic significance of the screenings, as well as valid treatment options remain controversial (Crosswell et al. 2011). The main treatment options in prostate cancer are "watchful waiting", active surveillance, prostatectomy, radiation therapy, and androgen-deprivation therapy. Chemotherapy is used in hormone-refractory and metastatic prostate cancer, but survival benefits have been modest (Tannock et al. 2004, de Bono et al. 2010). There is a lack of efficient targeted treatments and rationally designed therapeutic approaches are needed.

In recent years microarray technique and high-throughput DNA sequencing have offered novel efficient means to examine tumour gene expression profiles. This provides important information for biomarker discovery, and for the identification of novel drug targets and therapeutics for cancer (Gimba et al. 2003, Golias et al. 2007). Furthermore, the discovery of RNA interference (RNAi) technique now enables the exploration of the role of individual genes on cancer cell characteristics, such as growth and survival (Bauer et al. 2010, Cole et al. 2011, Meacham et al. 2009). These techniques enhance the development of novel targeted, personalized and efficient therapeutic option for cancer. In this thesis, the potential of microarray and RNAi techniques, as well as compound screens was combined in order to identify novel potential biomarkers, drug targets and drugs for prostate cancer.

We carried out bioinformatic mRNA expression analysis based on 9873 human tissue and cell samples, and performed a high-throughput (HT) functional profiling of 295 statistically and bioinformatically selected *in silico* prevalidated prostate and prostate cancer tissue specific genes in prostate cancer cell lines. The potential drug targets or target pathways highly expressed in clinical prostate cancers and regulating prostate cancer cell growth were validated *in vitro* and *vivo*. In addition, a parallel unbiased approach to identify compounds against prostate cancer was taken and the responses to 4910 compounds were studied in cultured prostate cells. This combinatorial approach enabled us to identify potential vulnerabilities in prostate cancer cells, which could be exploited to inhibit tumour cell proliferation and survival, and help us to advance the development of targeted treatments for prostate cancer.

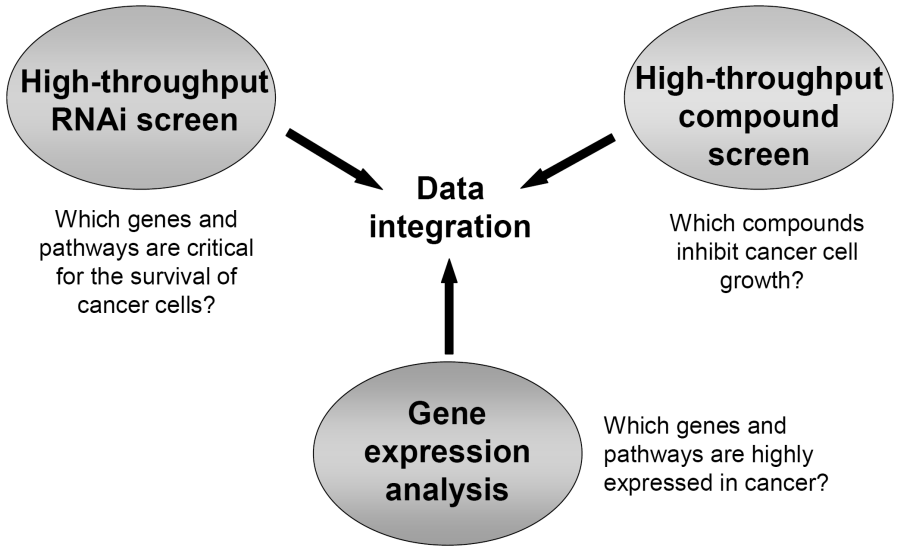

Figure 1.
An overview of the combinatorial usage of gene expression data, RNAi technique and compound screens in order to identify potential vulnerabilities present in prostate cancers which could be exploited to develop targeted and personalized approaches to prostate cancer treatment.

2. REVIEW OF THE LITERATURE

2.1. Prostate Cancer

2.1.1. Etiology

Carcinogenesis is a gradually progressing process, where genetic changes alter normal control mechanisms enabling cells to proliferate and survive limitlessly and eventually develop into cancer. Accordingly, prostate cancer is a slowly developing disease arising from prostate cancer stem / progenitor cells and differentiating prostate epithelial cells due to activation of oncogenes and loss of tumour suppressor genes (Gu et al. 2007, Kasper 2008, van Leenders and Schalken 2003). Prostate cancer cells are known to contain wide range of somatic mutations, gene deletions and amplifications as well as gene expression pattern altering changes in DNA methylation (Nelson et al. 2003).

The most consistent risk factor for developing cancer is advancing age. In addition, inflammatory diseases are known to increase the risk of prostate cancer (De Marzo et al. 2007). Especially chronic inflammation induces epithelial cell proliferation and causes tissue damage and prostate malignancy (Naber and Weidner 2000). Regions of inflammation are known to generate free radicals, and as a part of the response to oxidative stress cells produce arachidonic acid (AA) from cell membranes (Sciarrra et al. 2008). The AA pathway is a key inflammatory pathway involved in cellular signaling and has been implicated in prostate carcinogenesis (Patel et al. 2008).

A variety of environmental factors have also an impact on prostate carcinogenesis. Numerous physical, chemical or biological agents are known to mutate and activate oncogenes, or inactivate tumour suppressor genes. Especially a diet rich in fats, obesity and smoking have been associated with a higher incidence of prostate cancer (Rohrmann et al. 2007, Venkateswaran and Klotz 2010). However, the putative role of vitamin D is under debate (Gilbert et al. 2011, Swami et al. 2011).

Only 5-10 % percent of prostate cancers are hereditary (defined by Mendelian inheritance of a susceptibility gene), but approximately 20 % of prostate cancers are familial (Bratt et al. 2002, Hemminki et al. 2008). Familial passage of prostate cancer reflects both shared genetic background, as well as shared environment and common behaviors.

2.1.2. Epidemiology

Prostate cancer is the most commonly diagnosed malignancy and the third most common cause of cancer mortality in the Western male population (Jemal et al. 2011). However, ethnicity has an important effect on the occurrence of prostate cancer and there are large regional differences in the incidence rates (Ferlay et al. 2010). Currently approximately 4600 new prostate cancer cases are diagnosed and 800 patients die of prostate cancer every year in Finland (http://www.cancer.fi/syoparekisteri/).

Prostate cancer is a heterogeneous group of cancers and although some men are still diagnosed with high-grade disease and ultimately fail treatment, approximately 92 % of new cases of prostate cancer are diagnosed at localized or regional stage with as high as 100 % 5-year relative (adjusted for normal life expectancy) survival (Jemal et al. 2011).

2.1.3. Histology and grading

Most malignancies of the prostate are adenocarcinomas, tumours of glandular epithelium, and they originate in the posteriorly locating peripheral zone of the prostate. The histological diagnosis is based on the architectural and cytological features of the tissue. Malignant acini are small or medium, have irregular architecture and are randomly scattered in the stroma. In poorly differentiated prostate cancers the outline of the glands is lost and cancer cells form irregular masses and sheets of cells. The cytologic features include nuclear and nucleolar enlargement, and there is a variable amount of cellular and nucleolar pleomorphism. Most prostate cancers are multifocal and the malignant acini invade the stroma, lymphatics and perineural spaces.

Gleason prostate cancer grading system estimating the glandular epithelial architecture of the tumour tissue was introduced in 1966 (Gleason 1966). This system grades the tumours based on the degree of loss of the normal glandular tissue architecture from well-differentiated and closely resembling the normal prostate tissue (1) to poorly differentiated with no recognizable glands (5). The sum of the most prevalent pattern in a tumour (primary grade) and the second most prevalent pattern (secondary grade) is called the Gleason score, ranging from 2 to 10. Gleason score is an important prognostic factor in prostate cancer, and it strongly influences treatment decisions. However, treated (radiotherapy or androgen ablation) prostate cancers can show atrophy, shrinkage of nuclei and nucleoli, or glandular epithelial architecture collapse, and grading after treatment is thus controversial (Bostwick et al. 2004, Epstein 2004, Epstein et al. 2005).

2.1.4. Molecular pathology

2.1.4.1. Primary prostate cancer

Prostate cancer is a slowly developing heterogeneous disease arising from normal prostate epithelial cells or prostate cancer stem / progenitor cells due to accumulation of somatic genetic and epigenetic changes, and resulting in activation of oncogenes and inactivation of tumour suppressor genes (Gu et al. 2007, Kasper 2008, van Leenders and Schalken 2003). Besides regulating the development and maintenance of the prostate (Roy et al. 1999), androgens support the development and growth of most primary prostate cancers, and androgen receptor (AR) acts as an oncogene in prostate cancer (Berger et al. 2004, Heinlein and Chang 2004, Hååg et al. 2005).

Numerous studies have utilized gene expression profiling to identify AR dependent genes contributing to prostate cancer development and progression (DePrimo et al. 2002, Ngan et al. 2009, Nelson et al. 2002, Segawa et al. 2002, Velasco et al. 2004). Androgens and AR signaling have been reported to regulate prostate cell apoptosis and cell cycle progression (Kimura et al. 2001). Androgen-deprived prostate cancer cells arrest in G1 phase due to AR dependent regulation of cyclin D1, CDKN1A (cyclin-dependent kinase inhibitor 1A, p21) and CDKN1B (cyclin-dependent kinase inhibitor 1B, p27) (Comstock and Knudsen 2007, Knudsen et al. 1998). Furthermore, increased levels of growth factors associate with prostate cancer, and androgens are known to regulate IGF1 (insulin-like growth factor 1), EGF (epidermal growth factor) and VEGF (vascular endothelial growth factor) signaling, as well as FGF (fibroblast growth factor) expression (Byrne et al. 1996, Kaaks et al. 2000, Kwabi-Addo et al. 2004, Zhu and Kyprianou 2008).

In addition to inducing autocrine activation, cancer cells are known to harbour mutated or overexpressed growth factor receptors producing continuous mitogenic signals. Sequential activation of cellular signal transduction requires activation and / or inactivation of protein kinases, phosphatases and GTPases, as well as regulation of the concentrations and localization of intracellular signaling molecules. In cancer cells these signaling pathways are often altered, and especially tyrosine kinases show promise as cancer drug targets (Pytel et al. 2009). Among others, emerging evidence support the role of non-receptor tyrosine kinase SRC (v-src sarcoma viral oncogene homolog, avian) in multiple prostate cancer promoting cellular processes interacting with multiple signaling pathways. SRC transduces signals from numerous upstream receptors to downstream molecules such as FAK (focal adhesion kinase), JAK1/2 (Janus kinase 1/2), STAT3/5 (signal transducer and activator of transcription 3/5), Ras oncogene, MAPK1/3 (mitogen-activated protein kinase 1/3), AKT (v-akt murine thymoma viral oncogene homolog 1), HIF1A (hypoxia-inducible factor 1-alpha), as well as AR (Amorino et al. 2007, Chang et al. 2007, Gray et al. 2005, Gu et al. 2010).

Recently perturbations in Ras/Raf (v-raf murine leukemia viral oncogene homolog), phosphatidylinositol 3-kinase (PI3K) as well as retinoblastoma (RB) signaling pathways were proposed as additional prostate tumourigenesis driving alterations

(Taylor et al. 2010). Accordingly, tumour suppressor RB is frequently deleted in early prostate tumourigenesis (Phillips et al. 1994), whereas loss of PI3K inhibitor PTEN (phosphatase and tensin homolog) expression is more easily detected in advanced stage and high-grade prostate tumours (McMenamin et al. 1999). Other common somatic genetic and epigenetic changes in primary prostate cancers include deletions of CDKN1B and NKX3.1 (prostate specific NK3 homeoprotein 1) tumour suppressor genes, overexpression of MYC (v-myc myelocytomatosis viral oncogene homolog, avian) oncogene, chromosomal rearrangements of ERG (v-ets erythroblastosis virus E26 oncogene homolog, avian) and other ETS-like transcription factors, activation of telomerase enzymatic activity, hypermethylation and silencing of GSTP1 (glutathione S-transferase pi 1), a gene protecting cells from oxidative damage, as well as telomere and centrosome abnormalities (Gonzalgo et al. 2003, Shand et al. 2006).

2.1.4.2. TMPRSS2-ERG fusion oncogene

Although the prevalence varies by the race and ethnicity of patients, approximately 40-70 % of all prostate cancer samples harbour an oncogenic gene fusion combining androgen regulated transmembrane serine protease (TMPRSS2) with oncogenic ETS transcription factors (Magi-Galluzzi et al. 2011, Tomlins et al. 2005). Most frequently, the fusion partner is ERG, followed by ETV1 (ets variant 1), ETV4, and ETV5 (Helgeson et al. 2008, Tomlins et al. 2006, 2007). The fusions occur early in carcinogenesis and recent evidence suggest that, in addition to inducing the translation of the fusion genes, AR has a role also in the formation of the fusions via binding to the promoter of TMPRSS2 and bringing the genes closer to each other (Haffner et al. 2010). Furthermore, different areas in primary prostate cancer can have differing gene fusion status, whereas different sites of prostate cancer metastasis (in the same patient) are all either fusion positive or fusion negative (Perner et al. 2010).

ERG mRNA is not expressed in healthy prostate tissues, but as a result of the TMPRSS2-ERG gene fusion, a significant increase in ERG transcript levels can be detected in prostate cancers. Ectopic ERG oncogene expression promotes multiple signaling pathways associated with cancer formation and progression, including plasminogen, MYC and EZH2 (enhancer of zeste homolog 2) activation, PI3K and Wnt signaling as well as epigenetic programming (Gupta et al. 2010, Iljin et al. 2006, Kunderfranco et al. 2010, Sun et al. 2008, Tomlins et al. 2008a, Zong et al. 2009). Although ERG oncogene expression is not enough to induce prostate carcinogenesis in transgenic mouse model, it is able to induce prostatic intraepithelial neoplasia (PIN), a precursor lesion of prostate cancer (Tomlins et al. 2008a). Furthermore, in combination with inactivated PTEN tumour suppressor, ERG enhances tumourigenesis (Carver et al. 2009). Accordingly, copy-number loss of PTEN and TP53 (tumor protein p53) have been associated with ERG oncogene expression and suggested as possible cooperating genomic events (Taylor et al. 2010).

ETS gene fusions are associated with a specific molecular signature in prostate cancer (Iljin et al. 2006), but reports on the possible prognostic effects of activated ERG

oncogene expression have been contradictory. Although multiple studies have supported the association of ERG oncogene expression with aggressive prostate cancer (high risk of recurrence, poor survival, poor differentiation and high pathological stage, as well as invasion and presence of metastatic disease involving pelvic lymph nodes), opposing (better overall and recurrence-free survival, normal and moderate differentiation, lower pathological stage and grade, as well as negative surgical margins) and insignificant effects have also been published (Boormans et al. 2011, Gopalan et al. 2009, Kumar-Sinha et al. 2008, Leinonen et al. 2010, Reid et al. 2010, Saramäki et al. 2008, Yoshimoto et al. 2008). The exact reason for the discordant findings is unknown, but it may reflect the differences in cohort race and ethnicity, fusion detection technique, TMPRSS2-ERG fusion isoform expression and genetic rearrangement mechanism, as well as in the primary end point of the studies (Barwick et al. 2010, Kumar-Sinha et al. 2008, Wang J et al. 2008).

Although ERG activation mediated oncogenic processes may be bypassed in advanced prostate cancer (Hermans et al. 2006), hormone-regulated expression of *ERG* has been described to persist also in castrate-resistant prostate cancer (CRPC) (Attard et al. 2009a, Iljin et al. 2006), supporting the importance of this rearrangement also in advanced disease.

In conclusion, emerging evidence suggests that ETS fusions are key molecular alterations driving the development and progression of a distinct class of prostate cancers, and providing opportunities for targeted therapy. However, due to their transcriptional role, ETS gene fusions are a challenge to target and novel therapeutic approaches for this patient group are needed.

2.1.4.3. Castrate-resistant and metastatic prostate cancer

Initially prostate cancer cells are highly androgen dependent and androgen withdrawal results in tumour regression. However, castrate-resistant cancer cells typically start to appear during therapy, eventually leading to recurrent, hormone-refractory disease. The median survival time for men with CRPC is only around two years (Tannock et al. 2004, de Bono et al. 2010).

It is known that androgen signaling pathways are re-activated and re-directed during the progression of CRPC (Amler et al. 2000, Mousses et al. 2001). Tumour cells may use multiple mechanisms to become castrate-resistant, but in most cases it happens by increased AR expression. AR gene is amplified in 30% of CRPCs (Visakorpi et al. 1995), and prostate cancers with AR gene amplification have been suggested to be androgen hypersensitive instead of independent, as well as dependent on the remaining androgens. Accordingly, AR gene amplification has been suggested to prognosticate better response to maximal combined androgen deprivation than AR without amplification (Palmberg et al. 2000).

In addition to AR amplification and overexpression, mechanisms underlying the failure of hormonal therapy in prostate cancer have been attributed to outlaw activation of the AR, increased local synthesis of androgens, as well as to other mechanisms, such as blockage of apoptosis (Feldman and Feldman 2001; Schröder 2008). AR mutations are rare in untreated prostate cancers as well as in tumours treated with castration alone (Culig et al. 2001, Wallén et al. 1999). However, AR mutations have been detected in about 20–25% of tumours treated with anti-androgens (Haapala et al. 2001, Taplin et al. 1995). Activation of AR can be achieved by mutation induced ligand diversification or in a ligand-independent manner. In addition to other steroids, and even anti-androgens, AR has also been shown to be activated by IL6 (interleukin 6), IGF1, KGF (keratinocyte growth factor) and EGF (Culig et al. 1994, Ueda et al. 2002, Zhu and Kyprianou 2008). Furthermore, other important signaling pathways, such as Wnt signaling pathway, have been reported to activate AR when androgen levels are low (Yang et al. 2006). It has also been suggested, that castrate-resistant cells express constitutively active AR splice variants, and that the altered expression of AR coregulators could have a role in prostate tumourigenesis (Hu et al. 2009, Linja et al. 2004).

Despite the very low levels of androgen in the blood circulation of castration treated patients, prostate cancer cells have been reported to maintain sufficient androgen levels to activate AR, likely through *de novo* androgen synthesis (Feldman and Feldman 2001, Locke et al. 2008, Mohler et al. 2004, Schröder 2008). Testosterone levels within castrate-resistant metastases have been reported to be three times higher than the levels within untreated primary prostate cancers (Montgomery et al. 2008).

Among others, AR was recently suggested to upregulate the expression of M phase genes in order to enhance CRPC growth (Wang et al. 2009). However, although AR has an important role in advanced prostate cancer, parallel survival pathways regulated by other oncogenes and tumour suppressor genes have also been implicated. In contrast to MYC overexpression in primary prostate tumours, MYC oncogene is commonly amplified in aggressive disease (Nupponen et al. 1998, Cher et al. 1996). Important tumour suppressors influencing development of advanced prostate cancer include NKX3.1, PTEN and TP53. NKX3.1 is commonly lost in prostate cancer and the loss of NKX3.1 expression has been shown to associate with hormone-refractory disease and advanced tumour stage (Abdulkadir et al. 2002). Similarly, PTEN is also known to be highly mutated in metastatic lesions of prostate cancer, whereas it is infrequently deleted and mutated in primary prostate tumours (Vlietstra et al. 1998). Furthermore, the same applies to mutations in TP53 gene. They are rare in localized prostate cancer, whereas in advanced prostate cancers TP53 mutations are found in 20-40% of tumours (Bookstein et al. 1993, Navone et al. 1993, Visakorpi et al. 1992). Also the expression of anti-apoptotic BCL2 (B-cell CLL / lymphoma 2) is significantly increased in CRPC (McDonnell et al. 1992), supporting thus the importance of inhibition of programmed cell death in CRPC growth. Furthermore, recently phosphorylation of AKT was reported to be upregulated in response to long-term androgen ablation, and further activated by docetaxel, highlighting the unfortunate capability of cancer cells to acquire resistance to cancer therapeutics (Kosako et al. 2011).

Prostate cancer metastasis occurs most commonly in bone and induces high level of morbidity. The molecular mechanisms of prostate cancer metastasis are complex and involve a number of sequential steps and interrelated mechanisms. Among others, many of the androgen-regulated signaling pathways discussed earlier are also important for prostate cancer metastasis. Androgens have been suggested to control E-cadherin, N-cadherin and cadherin-11 expression (Jennbacken et al. 2010, Lee et al. 2010, Patriarca et al. 2003).

2.1.5. Clinical management

2.1.5.1. Prevention

Due to the high prevalence, prostate cancer and cancer as a whole presents a challenging task for public health care and causes considerable financial costs. However, due to the well-known risk factors, such as physical inactivity, diet, obesity, use of alcohol and tobacco, it has been estimated, that at least 30 % of all cancers could be prevented (Ott et al. 2011).

Multiple non-pharmaceutical cancer preventive strategies have been introduced to improve diets, increase the level of physical activity, reduce tobacco and alcohol consumption, and to prevent exposure to infectious and environmental carcinogenic agents. In addition, chemopreventive interventions designed to delay or prevent cancer have also been intensively studied and reported. The agents with most proven effects include cyclooxygenase 2 (COX-2) enzyme inhibitors and aspirin (Cuzick et al. 2009). Published evidence suggests that COX-2 inhibitors prevent prostate cancer (Jacobs et al. 2005, Mahmud et al. 2008), but the occurrence of cardiovascular side effects may exclude their use as general cancer preventive agents (Kearney et al. 2006). In addition, some hormonal approaches have also provided positive results in cancer prevention. Finasteride and dutasteride, inhibitors of dihydrotestosterone (DHT) forming 5-alpha-reductase, have been shown to reduce the incidence of prostate cancer (Thompson et al. 2003, Andriole et al. 2010). However, the discovery of higher Gleason score tumours in the patients treated with 5-alpha-reductase inhibitors raises concerns (Thompson et al. 2003).

Although vitamins, antioxidants and other dietary supplements have long had a strong reputation as cancer preventive agents, the evidence is controversial. For the moment, there is no single dietary factor reported to conclusively reduce the risk of developing prostate cancer. However, it is evident that diet plays a major role in prostate carcinogenesis (Venkateswaran and Klotz 2010). Studies on green tea containing antioxidant polyphenolic compounds (Bettuzzi et al. 2006, Kurahashi et al. 2008) and on soy phytooestrogens (Hwang et al. 2009, Yan and Spitznagel 2005) have yielded promising results. Similarly, high tomato (lycopene) consumption has been reported to potentially prevent prostate cancer development (Etminan et al. 2004).

2.1.5.2. Screening and diagnosis

PSA (prostate specific antigen; kallikrein 3, KLK3), introduced already almost 30 years ago (Stamey et al. 1987, Wang et al. 1979), is the only prostate cancer serum biomarker nowadays widely used in clinics, both in screening, detection and prognostication. It is more prostate tissue than prostate cancer specific and has many limitations. Numerous non-malignant processes, including benign prostatic hyperplasia (BPH) and prostatitis, frequently cause elevated PSA levels (Nadler et al. 1995). Thus, despite the high frequency of prostate cancer, the use of PSA in prostate cancer screening and diagnostics remains controversial (Heidenreich et al. 2011). PSA screening has been reported to decrease mortality, but PSA tests also result in a large number of false positives and overdiagnosis, as well as to unnecessary and repeated biopsies (Schröder et al. 2009). Accordingly, major urologic societies have concluded that at present widespread mass screening for prostate cancer is not appropriate (Heidenreich et al. 2011). Although several modifications of serum PSA value have been described to improve the specificity of PSA in prostate cancer diagnosis, developing additional serum biomarkers for early detection would be invaluable for improving early detection, while reducing the number of unnecessary biopsies.

Prostate cancer is detected and diagnosed using digital rectal examination, serum concentration of PSA, and transrectal ultrasound guided biopsies. The clinical suspicion for prostate cancer is confirmed using histological analysis of biopsy specimen, and the Gleason score strongly influences following treatment decisions.

2.1.5.3. Management of primary prostate cancer

The treatment of choice for a patient with prostate cancer depends on several considerations, including but not limited to disease stage, age and physical condition of the patient, as well as co-morbidities. As prostate cancer is a slowly developing and heterogeneous disease, the therapeutic options vary from "watchful waiting", prostatectomy, radiation therapy and androgen deprivation to chemotherapy (Heidenreich et al. 2011, Mottet et al. 2011).

"Watchful waiting" and the more intensive, active surveillance are options for men having low risk prostate cancer with good prognosis, especially at high age or alongside with severe co-morbidities. Watchful waiting is based on a delayed symptomatic treatment of patients who are not candidates for aggressive therapy, whereas patients treated with active surveillance may be offered curative approach if necessary. The purpose of these non-invasive therapeutic options is to optimize the quality of life by avoiding aggressive treatment for as long as possible. However, if necessary, intervention can be initiated later in order to escape from the potential mortality associated with prostate cancer. Currently there are no definite ways to predict the outcome or behaviour of individual cancer in the early phases of the disease.

In patients with organ confined prostate cancer, main treatment options are radical prostatectomy and radiation therapy. Prostatectomy is recommended especially for patients with low- and intermediate-risk localised prostate cancer and with over 10 year life expectancy (Heidenreich et al. 2011). In addition, extended pelvic lymphadenectomy is recommended in intermediate- and high-risk prostate cancers (Briganti et al. 2006). Furthermore, adjuvant radiation therapy immediately after prostatectomy significantly improves clinical or biologic survival in high-risk cancers as well as in patients with positive surgical margins (Bolla et al. 2005, Swanson et al. 2008, Wiegel et al. 2009). Transperineal brachytherapy is a safe and effective technique for low-risk prostate cancer. In addition, three-dimensional conformal radiotherapy or intensity-modulated radiotherapy present an option for prostatectomy in low- and intermediate-risk localised prostate cancer. In high-risk prostate cancer, radiation therapy is combined with adjuvant androgen deprivation therapy (Heidenreich et al. 2011).

In most cases prostatectomy and radiation therapy are curative, but both are associated with local adverse effects and reduce the quality of life of the patient. Furthermore, about one third of these patients eventually experience a relapse (Amling et al. 2000).

2.1.5.4. Hormonal treatment

If the cancer has progressed and invaded adjacent or remote tissues at the time of diagnosis, or relapsed after the local treatment, local radical therapies are not sufficient to eliminate cancer. Hormonal therapy aiming at inhibiting the production of prostate cancer growth promoting androgens or their effect on AR signaling has remained a valid treatment approach for decades and LHRH (luteinising hormone-releasing hormone) agonists are the standard method of treatment in metastatic prostate cancer (Mottet et al. 2011). Chemical or surgical castration inhibits the production of testicular androgens, whereas anti-androgens block AR from binding testosterone and 5-α-DHT. Reduction of DHT levels in androgen dependent tumour tissue leads to apoptosis of cancer cells and eventually reduces cancer volume (Knudsen et al. 1998). Adjuvant androgen deprivation therapy is used especially in high-risk advanced and metastatic cancers to improve symptom- and cancer-free survival (Mottet et al. 2011).

However, like other treatment options, hormonal treatment also has limitations, the most critical being the emergence of CRPC. Treatment of CRPC includes second-line hormonal therapy as well as chemotherapy (Mottet et al. 2011).

2.1.5.5. Chemotherapy

Chemotherapy is used in castrate-resistant and metastatic prostate cancer, but survival benefits have been modest (Tannock et al. 2004). Furthermore, chemotherapy is toxic and associated with severe adverse effects. Although mitoxantrone achieved only

palliative benefits, it maintained the position as the established treatment for CRPC for years (Tannock et al. 1996). At 2004 docetaxel was demonstrated as the first compound with at least minor survival effects in CRPC, and became thus the treatment of choice in the management of advanced CRPC (Tannock et al. 2004, Petrylak et al. 2004). However, most patients treated with docetaxel still relapse within the first year of treatment and the median survival time is only around two years (Tannock et al. 2004). Cabazitaxel is a novel taxane drug which has been shown to be effective in docetaxel resistant prostate cancer cells. It induced significantly more efficient overall survival than mitoxantrone in advanced disease following docetaxel treatment (de Bono et al. 2010) and was recently approved to be used in the treatment of docetaxel resistant CRPC. Despite novel therapeutic options improving survival, most CRPC patients receiving chemotherapy eventually relapse and die of prostate cancer.

In conclusion, there is an evident lack of efficient targeted treatments, and rationally designed therapeutic approaches are needed. The main problems to be solved in prostate cancer management still remain the discovery of reliable biomarkers for distinguishing between the "well-behaving" and aggressive prostate cancers as well as development of efficient therapeutic options for castrate-resistant and advanced disease. The ultimate aim is to target and efficiently treat cancers with poor prognosis already at early stages of the disease.

2.1.6. Emerging prostate cancer biomarkers

Despite intensive prevention strategies, cancer remains a major cause of death world-wide. Novel efficient methods are needed to improve early diagnosis and screening of cancer. In recent years molecular genetic techniques have opened efficient ways to examine tumour gene expression profiles. Analysis of a selected set of genes has potential for clinical use and allows better, more individual, diagnosis and prognosis of each disease. In addition, gene expression profiling provides information on how individual patients may respond to various treatments and allows identification of novel drug targets, therapeutics and therapeutic biomarkers (Gimba et al. 2003, Golias et al. 2007). Gene signatures and biomarkers also offer important knowledge of the genes and pathways influencing prostate carcinogenesis and progression as well as the aggressiveness of the disease.

2.1.6.1. Diagnostic biomarkers

A promising new prostate cancer marker, PCA3 (prostate cancer antigen 3), is a highly prostate specific non-coding mRNA overexpressed especially in prostate cancer tissues. PCA3 expression in urine has been reported to correlate with tumour volume (Whitman et al. 2008). In addition, PCA3 expression has been suggested to associate with tumour aggressiveness (Whitman et al. 2008). Various assays have been developed for PCA3

detection. A commercially available urine assay amplifies PCA3 together with PSA and the result is calculated as the PCA3 / PSA mRNA ratio (Groskopf et al. 2006).

Due to the high incidence of TMPRSS2-ERG fusion gene in prostate cancer, ERG expression and fusion transcripts have also been suggested to be candidate biomarkers for early detection of prostate cancer. Evaluation of TMPRSS-ERG transcripts in urine, alone or in combination with other prostate cancer biomarkers, like PCA3, have been suggested to be useful for prostate cancer screening (Laxman et al. 2008).

In addition to PCA3 and TMPRSS2-ERG fusion gene, various other biomarkers have been presented as possible diagnostic biomarkers for detecting prostate cancer (Madu and Lu 2010). Notably, the potential of KLK2, another member of the kallikrein family (Becker et al. 2000, Nam et al. 2000, Steubler et al. 2006), hypermethylated GSTP1 (glutathione S-transferase pi) (Goessl et al. 2000), AMACR (alpha-methylacyl coenzyme A racemase) (Luo et al. 2002), as well as uPA (urokinase plasminogen activator) (Gupta et al. 2009, Steuber et al. 2007) have been studied. In addition to numerous protein, RNA and DNA biomarkers, also sarcosine, a glycine metabolite, was recently proposed as a urine marker of prostate cancer (Sreekumar et al. 2009). Although the potential of sarcosine as a single biomarker has been disproved, recent study indicates that sarcosine in combination with PCA3, TMPRSS2-ERG and Annexin A3 could be a potential diagnostic biomarker panel (Cao et al. 2011, Jentzmik et al. 2010, Struys et al. 2010).

Although these markers show early promise, they require additional investigation and further validation to fully understand their potential clinical utility in prostate cancer diagnostics. So far, none of the biomarkers can be utilized to determine if an individual needs a prostate biopsy to exclude prostate cancer, or to determine whether a patient has prostate cancer or not.

2.1.6.2. Prognostic biomarkers

Presently, most prostate cancers are diagnosed at early stage and most cases have a good prognosis (Jemal et al. 2011). However, prostate cancer is a heterogeneous group of cancers and some are still diagnosed with high-grade disease or fail to respond to initial treatment and eventually develop metastatic or CRPC. So far, the only indicators generally accepted to be relevant for clinical management of prostate cancer are serum PSA and Gleason score in biopsy samples (Cuzick et al. 2006). However, neither of these provides accurate predictive information for the individual prostate cancer patient. It is thus crucial to identify prognostic biomarkers able to distinguish between indolent and aggressive cancers at early phases of carcinogenesis.

The first serum marker used for management of prostate cancer was prostatic acid phosphatase (PAP). It was used as a biomarker for progression and for response of metastatic disease to castration therapy (Huggins and Hodges 1941). After the introduction of PSA PAP was largely forgotten in the clinics, but has since been

reintroduced as an interesting prognostic marker for patients with aggressive disease undergoing local therapy and at high risk for relapse (Taira et al. 2007). Recently also some chromosomal aberrations, including amplification of 5p as well as deletions in 5q, 13q and 18q, have been shown to predict high risk for relapse after prostatectomy (Taylor et al. 2010). In addition, COX-2, TGF-β (transforming growth factor beta) and Ki67 have been shown to be highly expressed in metastatic prostate cancer, and COX-2 expression has been found to associate with increased risk of death (Richardsen et al. 2010). SPINK1 (serine peptidase inhibitor, Kazal type 1) is a secreted serine protease inhibitor expressed in approximately 10 % of prostate cancers. High expression of SPINK1, detectable also in urine, has been associated with aggressive disease characteristics among ETS fusion negative prostate cancers in radical prostatectomy samples (Tomlins et al. 2008b). Moreover, further highlighting the potential of SPINK1 as a biomarker, SPINK1 has been shown to regulate prostate cancer invasiveness and tumour growth *in vivo*, and has thus been proposed also as a promising therapeutic target (Ateeq et al. 2011).

Although novel biomarkers for prognostication have been identified, it is questionable whether single markers are sufficient to distinguish the presence of cancer, disease stage, metastasis or the need for targeted systemic therapy. Sets of biomarkers or signatures might be needed for potent management of the heterogeneous disease.

2.1.6.3. Therapeutic biomarkers

A therapeutic biomarker can be utilized to detect a specific group of cancer patients benefiting from selected therapeutic approaches, whereas theranostic markers can be utilized to predict and indicate the presence and nature of drug response to a specific treatment. Furthermore, therapeutic biomarkers can also have potential as therapeutic targets. Therefore, identifying potential biomarkers and understanding their role in carcinogenesis can lead to promising novel treatments for prostate cancer (Gann et al. 2001).

For anti-tumour vaccines and immunoconjugate antibodies, the most important feature of the therapeutic target is disease specific expression. The target itself is not required to be involved in cancer cell growth. However, other biomarker targeted therapeutic approaches, including epigenetic therapy, pro-apoptotic agents, and anti-angiogenesis approaches rely on targets with a role in carcinogenesis and cancer progression. Potential biomarkers utilized in clinical trials as therapeutic targets include histone deacetylases (HDAC), anti-apoptotic BCL2 and survivin, VEGF and PDGFR (platelet-derived growth factor receptor), as well as cell growth and motility promoting protein kinase mTOR (mechanistic target of rapamycin) (Detchokul and Frauman 2011).

2.1.7. Novel prostate cancer therapeutics

2.1.7.1. Antiandrogens and inhibitors of de novo steroid synthesis

Improved understanding of the mechanisms leading to CRPC has facilitated the development of novel therapeutic agents for this incurable disease. Novel highly efficient antiandrogens (MDV3100, ARN-509) as well as drugs targeting *de novo* intratumoural steroid synthesis (Abiraterone acetate, ketoconazole and TAK700) are evaluated for their ability to increase the efficacy of hormonal treatments (Attard et al. 2009b, Attard et al. 2011, Scher et al. 2010). Abiraterone acetate has yielded promising results especially in the treatment of patients with advanced treatment resistant CRPC (de Bono et al. 2011) and was approved by the U.S. Food and Drug Administration (FDA) in April 2011.

2.1.7.2. Novel antineoplastic agents

One of the most promising novel therapeutic options for prostate cancer are the antineoplastic HDAC inhibitors that have been shown to specifically reduce the cell proliferation of ERG positive prostate cancer cells (Iljin et al. 2006, Björkman et al. 2008, Iljin et al. 2009). Although the results with HDAC inhibitors as single agents in preclinical and clinical cancer studies have been modest, combined treatment strategies have yielded more promising results (Ellis and Pili 2010). In clinical trials for new prostate cancer treatments, HDAC inhibitors are currently being studied alone and in combination with various drugs, including bicalutamide or docetaxel (www.clinicaltrials.gov).

Heat shock proteins are essential for the post-translational stabilization of proteins. AR is stabilized by interacting with heat shock protein 90 (HSP90) enabling it to interact with androgens (Solit et al. 2003). In preclinical models, HSP90 inhibitors have been shown to inhibit AR signaling independently of serum testosterone levels. However, in clinical trials the results have been modest (Heath et al. 2008, Pacey et al. 2009).

Results from studies investigating intracellular molecular pathway inhibitors targeting IGF1 receptor, PTEN and PI3K / AKT / mTOR suggest modest anti-tumour activity which could potentially be enhanced by combining them with chemotherapy (Bianchini et al. 2010). Pro-apoptotic therapeutic approaches targeting BCL2 and survivin have given promising results, and their use alone or in combination with docetaxel is being further investigated (Detchokul and Frauman 2011). VEGF inhibition has proven to be challenging, but multiple VEGF receptor and small molecule multi-tyrosine kinase inhibitor studies are ongoing (Bianchini et al. 2010).

Other potential therapeutic options especially for advanced disease include EGF receptor, PDGFR and SRC family kinase inhibitors, endothelin receptor antagonists,

immunomodulatory agents as well as immunotherapy. Most of these have been studied in combination with docetaxel (Nabhan et al. 2011).

2.1.7.3. Therapeutic prostate cancer vaccines

Sipuleucel-T (Provenge) is a prostate cancer vaccine used to activate the patient's own immune system to attack cancer cells expressing PAP. Sipuleucel-T is prepared individually for each patient from blood sample derived antigen-presenting cells (APCs). Although significant overall survival benefits have been obtained, the production of the vaccine is time consuming and expensive (Chambers and Neumann 2011, Higano et al. 2009, Kantoff et al. 2010, Small et al. 2006). Another vaccine approach, poxviral vectors using PROSTVAC, has also demonstrated beneficial effects (DiPaola 2009). However, GVAX, composed of two allogenic inactivated prostate cell lysates, has not been as successful (Higano et al. 2008, Small et al. 2007). Sipuleucel-T was approved by the FDA in April 2010 for the treatment of asymptomatic or minimally symptomatic metastatic CRPC.

2.1.7.4. Targeted and personalized therapeutics

In conclusion, although three novel treatment approaches (Cabazitaxel, Abiraterone and Sipuleucel-T) have recently been introduced as novel therapeutic options for advanced prostate cancer, their impact on survival has been relatively modest, and there is still a lack of efficient targeted and personalized therapeutic approaches.

The introduction of microarray techniques has enabled efficient analysis of tumour gene expression profiles. This facilitates diagnosis and staging of the disease, provides information on how individual patients may respond to various treatments, leads to reduced drug toxicity and allows the identification of novel drug targets and therapeutics (Gimba et al. 2003, Golias et al. 2007). Furthermore, RNAi technique now enables the exploration of the role of individual genes on cancer cell phenotype such as growth and survival (Bauer et al. 2010, Cole et al. 2011, Meacham et al. 2009). Combination of these technologies enables the detection of potential biomarkers and drug targets specifically expressed in cancer tissues, as well as functional profiling of these targets. Possible noteworthy benefits include targeted, personalized and efficient therapy without unwanted side effects. However, as a clinical treatment option the efficacy of siRNA (small interfering RNA) based therapeutics is dependent on achieving successful delivery to cancer tissue. In localized disease siRNAs can be given locally, but for the metastatic disease systemic delivery is essential. Extensive research is ongoing in order to develop efficient siRNA delivery technologies to treat cancer (Guo et al. 2011). In some cancers, targeted therapies based on small molecules or monoclonal antibodies, such as HER-2 targeted Herceptin treatment in breast cancer, have given promising results.

In advanced prostate cancer, metastases arising from the same primary tumour, have been shown to be surprisingly similar genetically (Liu et al. 2009), supporting the hypothesis that targeted therapeutics could have potential also in metastatic prostate cancer. However, given the heterogeneous nature, complexity and crosstalk of molecular pathways in prostate cancer and the emergence of drug resistance, combining different therapies may be necessary to yield significant therapeutic progress.

3. AIMS OF THE STUDY

The general motivation for this study was the need to understand better the genes and pathways critical for prostate oncogenesis and progression, and to identify novel drug targets and biomarkers in order to advance the development of efficient diagnosis, prognosis and personalized treatment options for prostate cancer.

The first aim was to identify genes and pathways that play critical roles in cancer cell growth and survival by combining the data from gene expression profiles in primary tumours with data from functional high-throughput gene silencing screening in prostate cancer cell lines.

The second aim was to identify potential drugs inhibiting prostate cancer cell growth and survival with high-throughput screening using a library of 4910 compounds.

Taken together, these studies contribute to the identification of genes, biomarkers, drug targets as well as potential future therapeutic strategies in human prostate cancer.

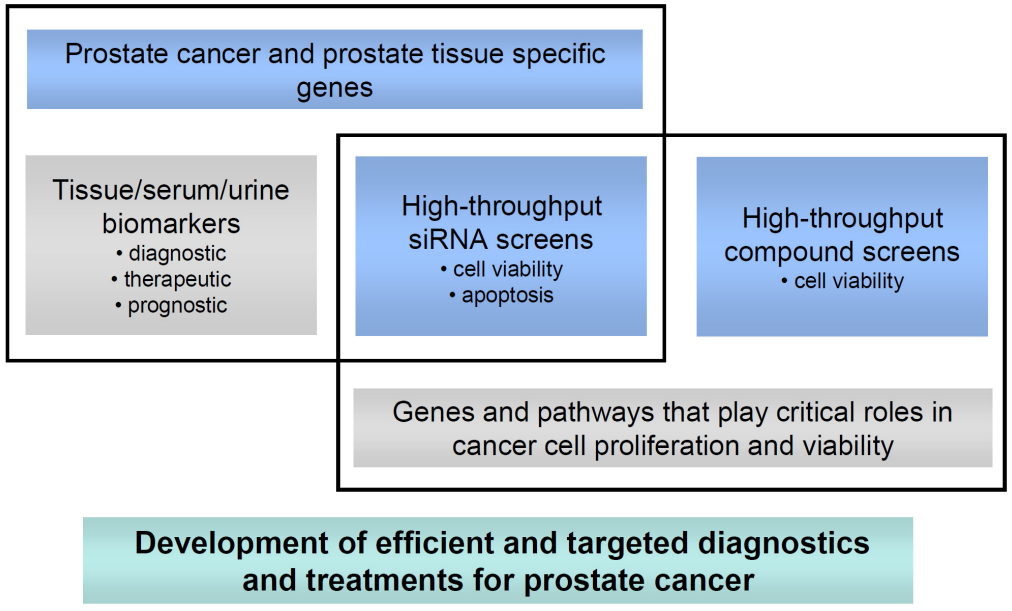

Figure 2. The aims and potential outcome of this study. Blue color indicates the material and methods utilized, grey color the putative results, and green color the main aim and outcome.

4. MATERIALS AND METHODS

More detailed information on materials and methods is available in the original publications (I-V).

4.1. *In silico* gene expression analysis

GeneSapiens database (www.genesapiens.org) (Kilpinen et al. 2008) was utilized in the *in silico* gene expression analyses. GeneSapiens website is a collection of Affymetrix microarray experiments. All data is re-annotated and normalized with a custom algorithm. The data is collected from various publicly available sources such as Gene Expression Omnibus and ArrayExpress.

4.2. Clinical tissue samples

Tissue sample histology	n	Methodology	Used in
Normal	14	IHC	II
Non-malignant	3	qRT-PCR	I, II
Adjacent non-malignant	409	IHC	III
Benign prostate hyperplasia	5	IHC	II
Primary prostate cancer	33	qRT-PCR, IHC	I, II
Primary prostate cancer	1137	IHC	III
Advanced prostate cancer	19	Microarray, aCGH, qRT-PCR	II, IV
Metastatic prostate cancer	103	IHC	II

4.3. Cell lines

Cell line	Tissue of origin	Used in
AI LNCaP	Prostate adenocarcinoma, lymph node metastasis; AI	IV
DU-145	Prostate adenocarcinoma, brain metastasis	I, V
EP-156T	Primary prostate cell line; hTERT immortalized	I, III, V
LNCaP	Prostate adenocarcinoma, lymph node metastasis	I-V
LNCaP C4-2	Prostate adenocarcinoma, lymph node metastasis, AI xenograft	IV
MDA-PCa-2b	Prostate adenocarcinoma, AI bone metastasis	I, IV
PC-3	Prostate adenocarcinoma, AI bone metastasis	I, III, V
PrEc	Primary prostate epithelial cell	I, III
RWPE-1	Histologically normal prostate; HPV-18 immortalized	I, III-V
VCaP	Prostate adenocarcinoma, AI vertebral metastasis	I-V
22Rv1	Prostate adenocarcinoma; AI CWR22 xenograft	I, III

4.4. High-throughput screening (HTS)

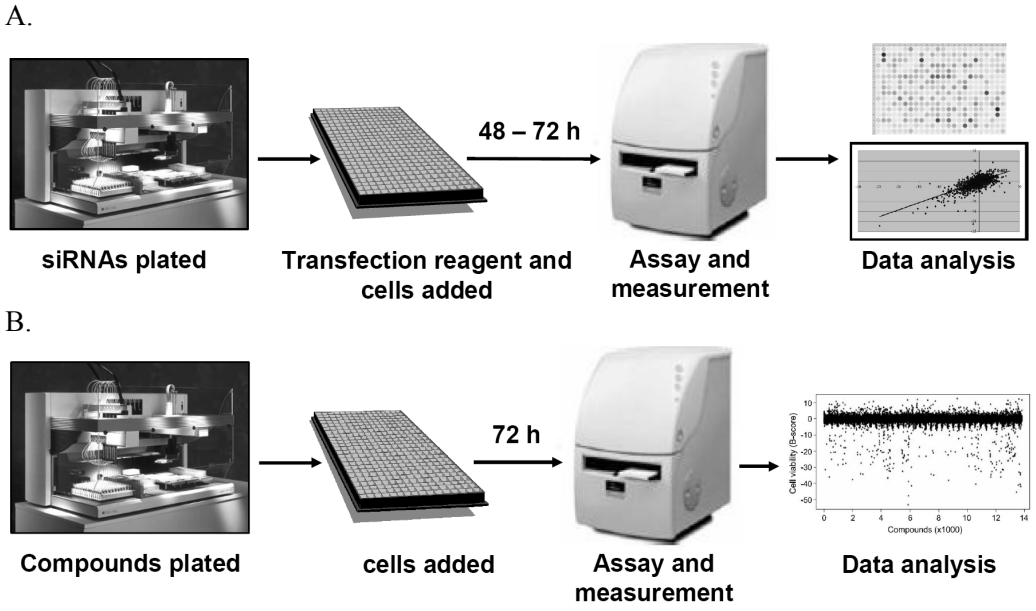

Figure 3. A schematic presentation of the HTS protocols (I, V). A. In the HT siRNA screen siRNAs are plated on 384-well plate followed by addition of transfection reagent and cells. B. In the compound screen compounds are plated followed by addition of cells. The end-point assays are performed after 48-72 h incubation, followed by data normalization and analysis.

4.5. siRNAs

Target Gene		siRNA ID	Used in
AIM1	absent in melanoma 1	SI03126704	I
		SI03212846	I
ALOX15B	15-lipoxygenase 2	SI03089877	II
AR	androgen receptor	SI02757258	I, IV
		SI02757265	IV
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	SI00306558	IV
		SI02660756	IV
CYP4F8	CYP450, family 4F, polypeptide 8	SI03058923	II
EPHX2	epoxide hydrolase 2, cytoplasmic	SI00380520	II
ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)	SI03089443	I, II
ERGIC1	endoplasmic reticulum-golgi intermediate compartment 1	SI03164763	I
		SI04302872	I
FAAH	fatty acid amide hydrolase	SI02626302	II

Target Gene		siRNA ID	Used in
FAM110B	family with sequence similarity 110, member B	SI00640507	IV
		SI00640514	IV
		SI00640521	IV
		SI00640528	IV
HPGD	prostaglandin dehydrogenase 1	SI00017171	II
LAS1L	LAS1-like (S. cerevisiae)	SI00392273	IV
		SI00392280	IV
MCM5	minichromosome maintenance complex component 5	SI04156712	V
MT1A	metallothionein 1A	SI04372914	V
MT1B	metallothionein 1B	SI04348470	V
MT1F	metallothionein 1F	SI04154731	V
MT1G	metallothionein 1G	SI03162775	V
MT1X	metallothionein 1X	SI04305994	V
MT2A	metallothionein 2A	SI00650720	V
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	SI00300902	IV
		SI02662611	IV
PLA2G2A	phospholipase A2, group IIA	SI03027689	II
PLA2G7	phospholipase A2, group VII	SI00072177	II, III
		SI00072184	II, III
TMED3	transmembrane emp24 protein transport domain containing 3	SI00746711	I
		SI00746718	I
TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	SI00097188	I
		SI00097195	I
YIPF6	Yip1 domain family, member 6	SI00635159	IV
		SI00635166	IV
Positive control (KIF11; kinesin family member 11)		SI02653770	I-IV
Positive control (PLK1; polo-like kinase 1)		SI02223844	I, V
AllStars negative control			I-V

In addition to the siRNAs mentioned above, an siRNA library consisting of 1207 siRNA molecules was used in the HT siRNA screen. For detailes see Supplementary Table S2 (I).

4.6. Constructs

FAM110B cDNA (TC127806, OriGene Technologies Inc., Rockville, MD) was cloned into two vectors, one with (pEGFP-C1, BD Biosciences Clontech, Mountain View, CA) and one without a GFP tag (pcDNA-3.1+, Invitrogen). The amplified *Hind*III-*Apa*I FAM110B fragment was ligated into the respective cloning sites in the expression vectors. Sequence was verified with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies Corporation**,** Foster City, CA, USA) according to manufacturer's instructions, and analyzed with ABI 3100 genetic Analyzer (Applied Biosystems).

4.7. qRT-PCR primers and probes

Gene	Forward	Reverse	#	Used in
ACTB	ccaaccgcgagaagatga	ccagaggcgtacagggatag	64	I-V
ACTR3	gaaaggtgttgatgacctagacttc	actataccatggcggattgg	9	III
AIM1	ctggaatgtcattatcagacacaa	tcagagacgtcgggttcact	85	I
ALDH1A1	gcaactgaggaggagctctg	gtcttgcggccttcactg	88	III
ALOX15B	tgaggtcttcaccctggcta	ttgatgtgcagggtgtatcg	43	II
AR	gccttgctctctagcctcaa	gtcgtccacgtgtaagttgc	14	I, IV
BCL2L1	gctgagttaccggcatcc	ttctgaagggagagaaagagattc	10	III
CASP8	gtctgtgcccaaatcaacaa	caaggctgctgcttctctct	40	III
CDC42	catcggaatatgtaccgactgtt	tgcagtatcaaaaagtccaagagta	22	III
CYP11A1	aggaggggtggacacgac	ttgcgtgccatctcataca	59	IV
CYP4F8	catcttcagctttgacagcaa	tgagctccatgatcgcagta	2	II
DSCAM	aacctcatggacggagagc	agctccagtgaaggctgtgt	9	III
EPHX2	ttctgctggacaccctgaa	ttcagattagccccgatgtc	45	II
ERG	caggtgaatggctcaagga	agttcatcccaacggtgtct	44	I, II
ERGIC1	agtacacggtggccaacaa	aaccagattgcagggatgat	5	I
FAAH	ctctgctgccaaggctgt	tgcagttcccagagttttcc	73	II
FAM110B	gaggacggccacatcaatag	tgctgtcctttcttaaaatgctc	18	IV
	ggcaggagactgctggag	cggatgtccgaagagctg	69	IV
HPGD	tggtcaataatgctggagtga	ggttccactgataacagaaacca	48	II
IRF7	caaggtgtacgcgctcag	gcctctgcctcagtctggt	24	IV
ITGB1	cgatgccatcatgcaagt	acaccagcagccgtgtaac	65	III
KIF11	catccaggtggtggtgagat	tattgaatgggcgctagctt	53	I-IV
LIMK1	ggggcatcatcaagagca	tgtccttggcaaagctcact	36	III
MCM5	ccttgtccggtaccctgtc	gatgcggctcagcatctc	20	V
MT1A	tgggatctccaacctcacc	atttgcaggagccagtgc	68	V
MT1B	gaactccaggcttgtcttgg	catttgcactctttgcacttg	68	V
MT1F	ccactgcttcttcgcttctc	caggtgcaggagacacca	68	V
MT1G	ctagtctcgcctcgggttg	gcatttgcactctttgcact	68	V
MT1X	cttctccttgcctcgaaatg	acaggcacaggagccaac	15	II, V
MT2A	ctagccgcctcttcagca	gcaggtgcaggagtcacc	68	II, V
NCAM1	taccgcggcaagaacatc	ccacctgcagagaaactgc	20	III
PLA2G2A	acctgccctgtctccaaac	tttgttctgcactcctgctc	32	II
PLA2G7	tggctctaccttagaaccctga	ttttgctctttgccgtacct	63	II, III
PLK1	cacagtgtcaatgcctcca	ttgctgacccagaagatgg	30	I, IV
STAT1	ttggcacctaacgtgctg	ttcgtaccactgagacatcctg	68	IV
STAT3	cccttggattgagagtcaaga	aagcggctatactgctggtc	14	III
TMED3	gggttctctgtacctgaggaaa	caccgagggtgagcagat	81	I
TPX2	acatctgaactacgaaagcatcc	ggcttaacaatggtacatccctta	51	I

4.8. Antibodies

Antigen	Supplier / Antibody ID	Species	Used in
AR	NeoMarkers, Thermo Fisher Scientific Inc.	mouse	I, II, IV, V
β-actin	Sigma-Aldrich	mouse	I-V
B2M	Sigma-Aldrich, HPA006361	rabbit	IV
cPARP	Abcam	rabbit	V
CTNNB1	Abcam, ab32572	rabbit	IV
FAM110B	Sigma-Aldrich, HPA008318	rabbit	IV
HPGD	Sigma- Aldrich	rabbit	II
PAK	Santa Cruz Biotechnology, sc-881	rabbit	III
PLA2G7	Cayman Chemical	rabbit	II, III
pPAK	Cell signaling technology, 2606S	rabbit	III
PSA	DakoCytomation, A0562	rabbit	I, II, IV
pSTAT3	Santa Cruz Biotechnology, sc-7993	goat	III

4.9. Reagents and chemicals

Reagent	Supplier	Used in
Aldefluor	Stemcell Technologies	III
Alexa conjugated Phalloidin	Molecular Probes, Invitrogen	III, IV
Alexa Fluor antibodies	Molecular Probes, Invitrogen	I, III, IV
ApoONE	Promega	I, II, IV
BigDye Terminator v3.1 Cycle Sequencing Kit	Applied Biosystems	IV
Calcein AM live cell dye	Invitrogen	III
CellTitre Blue	Promega	I, IV, V
CellTitre Glo	Promega	I-III
$CuCl_2$	Sigma-Aldrich	V
Cy5-dUTP and Cy3-dUTP	PerkinElmer	IV
Disulfiram (DSF)	Sigma-Aldrich	V
ECL IgG HRP-linked antibodies	Amersham Life Sciences	II-V
ECL reagent	Amersham Biosciences	II-V
Fibronectin	CalbioChem	III
Flutamide	Sigma-Aldrich	II
Fluvastatin	Tocris Bioscience	III
Fugene HD	F. Hoffmann-La Roche Ltd	IV
Hydrogen peroxide	Sigma-Aldrich	II
Lovastatin	Tocris Bioscience	III
LPC	Cayman Chemical	III
Male genomic DNA	Promega	IV
Matrigel	BD Biosciences	III

Reagent	Supplier	Used in
Monensin	Sigma-Aldrich	V
Paraformaldehyde	Sigma-Aldrich	III, IV
PowerVision+ IHC detection kit	ImmunoVision Technologies	II
Pravastatin	Tocris Bioscience	III
Propidium Iodide	Biofellows	III, IV
R1881	PerkinElmer	I, IV
siLentFect	Bio-Rad Laboratories	I-V
Simvastatin	Tocris Bioscience	III
Soybean trypsin inhibitor	Sigma-Aldrich	III
Thiram	Sigma-Aldrich	V
Triton X-100	Sigma-Aldrich	III, IV
TRIzol	Invitrogen	IV
Vectashield	Vector Laboratories	III, IV
Vectastain	Vector Laboratories	II
$ZnCl_2$	Sigma-Aldrich	V

In addition, five compound libraries were used in HTS. The libraries, and summary of the compounds, were the following: Biomol (80 known kinase and phosphatase inhibitors), LOPAC (1280 existing Food and Drug Administration approved drugs and other compounds with pharmacologically relevant structures), IBIS (1473 compounds derived from natural sources), Microsource Spectrum (2000 compounds including most of the known drugs and other bioactive compounds and natural products), and an inhouse library (77 experimental compounds).

4.10. Equipment

Equipment and software	Supplier	Used in
ABI 3100 genetic Analyzer	Applied Biosystems	IV
Acumen Assay Explorer	TTP LabTech Ltd	III
Automated liquid handling robot	Hamilton	I
Automated liquid dispenser	ThermoFisher	I
BD FACSarray Flow cytometer	BD Biosciences	IV
BeadArray Reader	Illumina	III, IV
Bioanalyzer 2100	Agilent Technologies	III, IV
Bioprime aCGH Genomic Labeling Module	Invitrogen	IV
CGH Analytics software	Agilent Technologies	IV
Feature Extraction software	Agilent Technologies	IV
GeneTools software	SynGene, Synoptics Ltd	III
GraphPadPrism 4 software	GraphPad Software, Inc.	V
EnVision Multilabel platereader	PerkinElmer / Wallac	I-V
Incucyte Live-Cell Imaging System and software	Essen Instruments	III

Equipment and software	Supplier	Used in
Ingenuity Pathway Analysis Software	Ingenuity Systems Inc.	III, IV
Lab Vision autostainer	Thermo Fisher Scientific	II
Laser confocal scanner	Agilent Technologies	IV
Multicycle software	Phoenix FlowSystems	IV
Odyssey Infrared Imaging System	LI-COR Biosciences	I-IV
Odyssey v2 analysis software	LI-COR Biosciences	IV
Olympus IX81-ZDC microscope	Olympus Europa GmbH	IV
RQ manager 1.2 software	Applied Biosystems	I-V
ScanR scanning microscope and software	Olympus Biosystems	IV
Universal ProbeLibrary Assay Design Center	Roche Diagnostics	I-V
VTT Acca software	VTT	III
Wound Maker 96 Tool	Essen Instruments	III
Zeiss Axiovert 200M fluorescence microscope	Carl Zeiss AG	III, IV
7900HT Fast Real-Time PCR System	Applied Biosystems	I-V

4.11. Methodology

Method	Used in
aCGH	IV
Aldehyde dehydrogenase activity assay	III
Apoptosis assay	I, II, IV
Cell adhesion assay	III
Cell culture	I-V
Cell viability assay	I-V
Compound treatments	II-V
Flow cytometric analysis	IV
Gene expression analysis	III-V
HTS	I, V
IHC	II, III
Immunofluorescence staining	III, IV
In silico data mining	I, II, IV
Lipidomic analysis	III
Oxidative Stress Response Analysis	II
PLA2G7 activity assay	III
qRT-PCR	I-V
RNA interference	I-V
Statistical analysis	I-V
Transfection of overexpression construct	IV
Western blot analysis	I-V
Wound healing assay	III
Xenograft experiment	V
3D cell culture	III

5. RESULTS

5.1. RNAi screen (I)

5.1.1. Prostate and prostate cancer specific genes

To identify potential vulnerabilities present in prostate cancer, a bioinformatic mRNA expression analysis was first carried out based on 9 873 human tissue and cell samples, including 349 prostate cancer and 147 non-malignant prostate samples, available in GeneSapiens database (Kilpinen et al. 2008) to distinguish the most potential *in vivo* prevalidated prostate cancer drug targets and biomarkers for further studies in cultured prostate cancer cells. In total, 295 prostate and/or prostate cancer specific genes were selected based on their high mRNA expression levels in prostate tissue, prostate cancer tissue or in metastatic prostate cancer tissue samples (I: Figure 1), and an siRNA library was constructed targeting these genes for further functional studies.

5.1.2. Functional RNAi screen

For the RNAi studies 4 siRNAs per gene were purchased and plate based HT siRNA screens were performed in VCaP (a model for TMPRSS2-ERG positive prostate cancer, AI/AS, wild type AR) and LNCaP (AD/AS, AR mutant) prostate cancer cells to identify therapeutically relevant genes and pathways in prostate oncogenesis. Changes in cell viability and induction of apoptosis (caspase -3 and 7 activation) were studied as end-point measurements, and the cell viability screen was performed in three replicates and the apoptosis assay once in both cell lines. The siRNA screens resulted in 94 potential proliferation promoting (hits in at least two of the cell viability screens) and 97 anti-apoptotic genes in LNCaP cells. A total of 45 (47.9 %) of the reproduced proliferation hit genes were also apoptosis hits. In VCaP cells the final hit rate was 35 reproduced proliferation promoting and 34 anti-apoptotic hit genes, resulting in 9 (25.7 %) cell viability promoting and apoptosis inhibiting hit genes. Silencing of 17 genes resulted in an anti-proliferative response in both LNCaP and VCaP cells. (I: Figure 2B-C).

The *in silico* co-expression analysis of proliferation hit genes suggested that with these targets three major prostate cancer sub groups with different mechanisms for cell growth regulation could be targeted. The largest set of genes had a role in prostate gland development, endoplasmic reticulum (ER) and Golgi apparatus function as well as in oxidation reduction. Other subgroups were associated with in actin cytoskeleton activity and mitosis (I: Figure 2D).

5.2. Putative novel prostate cancer drug targets (I-IV)

5.2.1. Targets related to endoplasmic reticulum function (I)

The dysfunction of proteostasis and ER function, inducing a stress response (unfolded protein response) and leading to apoptosis, has been suggested as an opportunity for targeted cancer therapy (Liu et Ye 2011; McLaughlin and Vanderbroeck 2011). Moreover, in prostate cancer cells the expression of ER stress response genes is known to be induced by androgens (Segawa et al. 2002).

Based on the *in silico* correlation analysis of the 112 prostate cancer cell proliferation promoting genes in clinical prostate cancer samples, possible novel prostate cancer drug targets AIM1, ERGIC1, and TMED3 were expressed in the same samples as genes involved in protein synthesis and transport at ER and Golgi apparatus as well as in prostate gland development and redox homeostasis. In the HT RNAi screen AIM1 and TMED3 siRNAs induced antiproliferative effects in both of the cell lines studied, and were also able to induce apoptosis in LNCaP cells. ERGIC1 was among the few genes, whose silencing induced antiproliferative effect only in the ERG oncogene positive VCaP cells (I: Supporting Table S2).

5.2.1.1. AIM1

AIM1 (absent in melanoma 1) protein is a member of the βγ-crystalline superfamily. Unlike other β- and γ-crystallines, known to be specifically expressed in elongating lens fiber cells that are undergoing large changes in cytoskeletal architecture and composition, AIM1 has a non-lens role. However, AIM1 protein sequence has a weak similarity with filament or actin-binding proteins, supporting a possible role in the maintenance of cell morphology and shape (Ray et al. 1997). Previous studies have suggested AIM1 as a tumour suppressor in melanoma (Ray et al. 1996). In addition, AIM1 methylation has been associated with nasopharyngeal carcinoma and primary tumour invasion of bladder cancer (Brait et al. 2008, Loyo et al. 2011). On the other hand, AIM1 expression has been shown to be high in TRAIL resistant cancer cell lines (Araki et al. 2010).

The results from our study support the potential oncogenic role of AIM1 in a subset of prostate cancers. Among six prostate cancer and three non-malignant prostate epithelial cell lines studied, AIM1 mRNA was most highly expressed in VCaP cells (I: Figure 3A). Furthermore, the results from secondary cell viability and apoptosis assay confirmed the antiproliferative effect of AIM1 silencing specifically in VCaP cell line (I: Figure 3C). *In vivo* validation of AIM1 mRNA expression in clinical prostate cancer samples (n = 33) and non-malignant prostate tissues (n = 3) confirmed high expression of AIM1 especially in the cancer samples. Importantly, all cancer samples expressed AIM1 more highly than any of the non-malignant samples studied (I: Figure 4A).

Furthermore, high AIM1 expression correlated significantly ($p = 0.03$) with young age (< 60 years) (I: Figure 4E).

Although AIM1 expression was not found to be significantly correlating with ERG expression in primary prostate cancer samples, further *in vitro* validation indicated AIM1 to be regulated by ERG oncogene expression, as ERG silencing in VCaP cells significantly decreased AIM1 mRNA expression (I: Figure 5A). In addition, AR silencing and androgen deprivation decreased, and the synthetic androgen R1881 induced the mRNA expression of AIM1 in LNCaP cells (I: Figure 5B-C). However, AIM1 silencing did not downregulate AR signaling (I: Supporting Figure S2)

Based on the *in silico* correlation analysis of the 112 prostate cancer cell proliferation promoting genes in clinical prostate cancer samples, AIM1 clustered in the same samples as genes involved in redox homeostasis and protein synthesis and transport at ER and Golgi apparatus. When analyzing all genes co-expressed ($R > 0.5$, $p < 0.001$) with *AIM1* in prostate cancer samples, the genes show enrichment in the ribosomal and mitochondrial location, and have a role in the regulation of cell morphology. In addition, high *AIM1* expression associates with genes involved in lipid metabolism (I: Table 1).

5.2.1.2. ERGIC1

ERGIC1 (endoplasmic reticulum-Golgi intermediate compartment protein 1) is a cycling membrane protein contributing to the membrane traffic and selective transport of cargo between the ER, the intermediate compartment and the Golgi apparatus (Breuza et al. 2004).

In our study, ERGIC1 was shown to be highly expressed in the cancer but not in the non-malignant prostate cell lines. Highest expression was detected in VCaP cells (I: Figure 3A). The results from secondary cell viability and apoptosis assay confirmed the antiproliferative effect of ERGIC1 silencing specifically in VCaP cell line (I: Figure 3C). However, ERGIC1 silencing was able to induce apoptosis also in LNCaP cells (I: Figure 3D). *In vivo* validation of mRNA expression levels in clinical prostate cancer samples ($n = 33$) and non-malignant prostate tissues ($n = 3$) confirmed high expression of ERGIC1 especially in the cancer samples. Based on the results 94 % of the cancer samples expressed ERGIC1 more highly than any of the non-malignant samples (I: Figure 4A). Furthermore, the mRNA expression of ERGIC1 correlated significantly ($R = 0.51$) with AR expression in the cancer samples (I: Figure 4C), as well as with ERG expression levels in the ERG positive cancer samples ($p = 0.002$) (I: Figure 4D).

Further *in vitro* validation showed that ERG silencing in VCaP cells significantly decreased ERGIC1 mRNA expression (I: Figure 5A). In addition, although no major changes were observed in the expression of ERGIC1 in response to AR silencing (I: Figure 5B), androgen deprivation decreased and the synthetic androgen R1881 induced

the expression of ERGIC1 in LNCaP cells in comparison to the expression levels detected in androgen deprived conditions (I: Figure 5C). Although no consistent effect was seen on AR or PSA protein expression (Supporting Figure S2), ERGIC1 silencing was able to systematically downregulate ERG mRNA expression (I: Figure 5F).

In silico co-expression patterns in clinical prostate cancer samples confirmed, that ERGIC1 is expressed in the same tumours as genes involved in protein transport at ER and Golgi apparatus. In addition, cancer was among the top disease processes associated with the ERGIC1 co-expressed genes. Furthermore, high ERGIC1 expression associated with genes involved in cellular redox homeostasis. (I: Table 1).

5.2.1.3. TMED3

TMED3 (transmembrane emp24 protein transport domain containing 3) is a constituent of the coated vesicles that are involved in the transportation of cargo molecules from the ER to the Golgi complex and function as receptors for specific secretory cargo (Ananthraman and Aravind 2002). In our study, TMED3 was shown to be highly expressed in the cancer but not in the non-malignant prostate cell lines. Highest expression was detected in VCaP cells (I: Figure 3A). The results from cell viability and apoptosis assay confirmed the antiproliferative effect of TMED3 silencing in VCaP and LNCaP cell lines, as expected based on the screening results (I: Figure 3C). A pro-apoptotic effect was observed especially in LNCaP cells (I: Figure 3D).

Despite the promising results of TMED3 expression patterns in prostate cell lines, TMED3 was equally expressed in the non-malignant ($n = 3$) and cancer ($n = 33$) prostate tissues (I: Figure 4A). However, the mRNA expression of TMED3 correlated significantly ($R = 0.69$) with AR (I: Figure 4C), as well as with ERG expression levels in ERG positive samples ($p = 0.007$) (I: Figure 4D). Further *in vitro* validation indicated TMED3 to be regulated by ERG oncogene expression (I: Figure 5A). However, although androgen deprivation decreased and the synthetic androgen R1881 induced the expression of TMED3 (I: Figure 5C), AR silencing was shown to increase the mRNA expression of TMED3 in both VCaP and LNCaP cells (I: Figure 5B).

In silico co-expression patterns in clinical prostate cancer samples confirmed that TMED3 is expressed in the same tumours with other genes involved in protein transport in the ER and Golgi apparatus. In addition, high TMED3 expression associated with genes involved in lipid metabolism and redox homeostasis (I: Table 1). In conclusion, although TMED3 was equally expressed in both non-malignant and cancer prostate tissues, our results suggest TMED3 to be a candidate prostate cancer drug target for further studies.

5.2.2. Anti-mitotic target (I)

5.2.2.1. TPX2

TPX2 (targeting protein for Xklp2) is exclusively expressed in proliferating cells from the G1/S transition until the end of cytokinesis. TPX2 is known to be highly expressed in various cancer tissues, and it has been suggested as a biomarker for poor prognosis (Kadara et al. 2009, Li et al. 2010, Stuart et al. 2011). As an important regulator of cell cycle and a binding partner for Aurora A kinase, TPX2 has been suggested also as a drug target in multiple malignancies (Ramakrishna et al. 2010, Satow et al. 2010, Warner et al. 2009). However, the role of TPX2 in prostate cancer has not been studied previously.

In accordance with the earlier reports, our results show high TPX2 expression in the cancer but not in the non-malignant prostate cell lines. Highest expression was detected in LNCaP cells (I: Figure 3A). In addition, *in vivo* validation of TPX2 mRNA expression in clinical prostate cancer samples (n = 33) and non-malignant prostate tissues (n = 3) confirmed high expression of TPX2 especially in the cancer samples. In total, 64 % of the cancer samples expressed TPX2 more highly than any of the non-malignant samples (I: Figure 4A). Furthermore, high TPX2 expression significantly correlated with PSA failure ($p = 0.02$), and associated with high WHO grade and young age (I: Figure 4F).

The results from cell viability and apoptosis assay confirmed the antiproliferative and pro-apoptotic effect of TPX2 silencing in both VCaP and LNCaP cell lines (I: Figure 3C-D). Further *in vitro* validation indicated TPX2 to be regulated by ERG oncogene expression, as ERG silencing in VCaP cells significantly decreased TPX2 mRNA expression (I: Figure 5A). In addition, AR silencing decreased the mRNA expression of TPX2 in VCaP and LNCaP cells (I: Figure 4B), and androgen deprivation decreased and the synthetic androgen R1881 induced the expression of TPX2 in LNCaP cells in comparison to the expression levels detected in androgen deprived conditions (I: Figure 5C). Interestingly, TPX2 silencing was able to significantly reduce PSA protein expression in both VCaP and LNCaP cell lines, as well as to decrease AR protein expression in LNCaP cells (I: Figure 5D). Furthermore, qRT-PCR results confirmed that *TPX2* regulates the expression of AR and PSA already at mRNA level (Figure 5E).

In silico co-expression patterns in clinical prostate cancer samples confirmed that high TPX2 expression correlated with the expression of genes involved in the M phase of mitotic cell cycle. In addition, cancer was again among the top five disease processes associated with the co-expressed genes (I: Table 1). Taken together, the results indicate TPX2 as an attractive candidate drug target also in prostate cancer. Furthermore, in addition to strongly affecting prostate cancer cell proliferation and apoptosis, TPX2 inhibition has the ability to inhibit AR signaling.

5.2.3. Arachidonic acid pathway enzymes (II-III)

The AA pathway has been implicated in prostate carcinogenesis (Patel et al. 2008), and the rate of AA turnover is 10-fold enhanced in prostate cancer cells, in comparison to normal prostate epithelial cells (Chaudry at al. 1991). Furthermore, AA, as well as many eicosanoids, induce prostate cancer proliferation *in vitro* (Ghosh et al. 1997, Patel et al. 2008, Wang et al. 1995). Recently AA synthesis was also shown to induce androgen production in androgen deprived prostate cancer cells, suggesting a contribution to the activation of AR in CRPC progression (Locke et al. 2010). Widely used COX-2 inhibitors suppress the growth of prostate cancer cells *in vitro* and tumourigenesis *in vivo* (Hsu et al. 2000, Narayanan et al. 2000, Patel et al. 2005). However, because of cardiovascular adverse effects, the use of COX-2 inhibitors as cancer drugs raises safety concerns (Kearney et al. 2006). Understanding the roles of different downstream pathways and individual enzymes in AA metabolism may provide more effective therapeutic opportunities with fewer adverse effects (Wang D et al. 2010).

We applied bioinformatics to systematically explore the expression patterns of 36 key AA pathway members *in vivo*. The results highlighted ALOX15B, CYP4F8, EPHX2, FAAH, PLA2G2A, and PLA2G7 to be highly expressed in prostate cancer samples, compared with expression levels in the normal tissues studied (II: Figure 1A). ALOX15B, CYP4F8, EPHX2, FAAH, and PLA2G2A showed more prostate specific than prostate cancer specific expression, whereas PLA2G7 mRNA levels were clearly elevated in prostate cancer, compared with normal prostate. In addition, HPGD mRNA expression was significantly elevated in a subset of prostate cancer samples, compared with normal prostate (II: Figure 1A). Next, targeted clinical validation and functional siRNA knockdown studies were performed with the seven most prostate cancer-specific AA pathway genes.

To evaluate the expression patterns of the novel candidate drug targets in prostate tissues their mRNA expression was first analyzed in 3 non-malignant prostate and 33 primary prostate cancer samples (II: Supplemental Table S1), and for PLA2G7 and HPGD also in 19 advanced prostate cancer samples. Second, the protein expression of HPGD and PLA2G7 was evaluated in 14 histologically normal and 5 hyperplastic non-malignant prostate samples as well as in 103 metastatic prostate cancer tissue samples.

RNAi mediated gene silencing in VCaP and LNCaP prostate cancer cells revealed a significant role for AA pathway in prostate cancer cell growth. In addition to the known regulators of prostate cancer growth, PLA2G7, HPGD, EPHX2 and CYP4F8 were identified as potential novel therapeutic targets for prostate cancer (Figure 4).

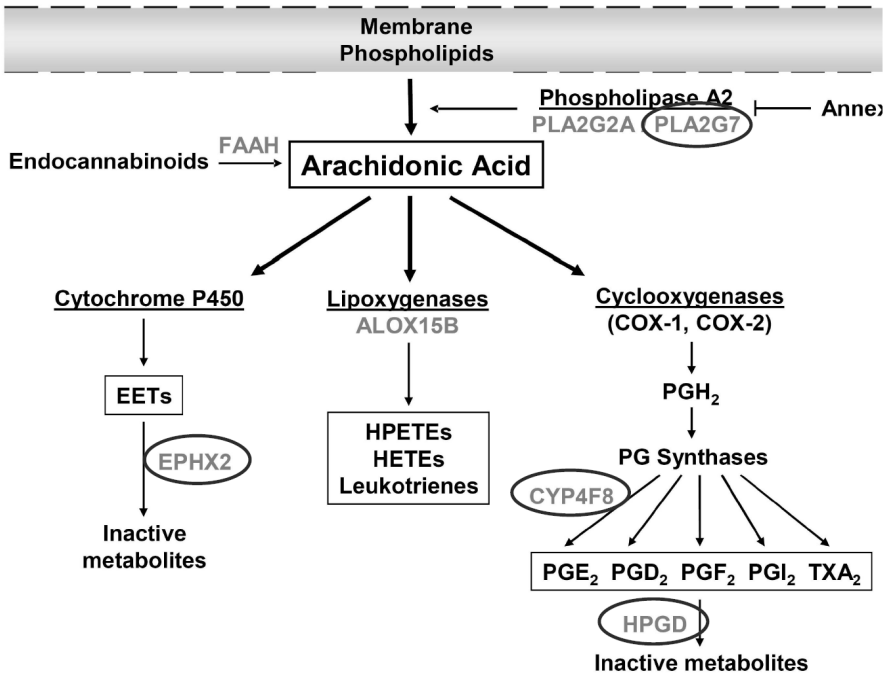

Figure 4. A schematic presentation of the AA pathway. Genes included in the functional experiments are indicated with gray text and identified novel drug targets with a circle. For detailed information see figure legend in manuscript II (II: Figure 1).

5.2.3.1. CYP4F8

Based on enzymatic assays, CYP4F8 (cytochrome P450 4F8) has been proposed to oxygenate and hydroxylate COX-derived products to 19-hydroxy-prostaglandin E2 (19-hydroxy-PGE_2) (Bylund et al. 2000). In contrast to PGE_2, which may stimulate a variety of prostanoid receptor subtypes, 19-hydroxy-PGE_2 has been found to exhibit selectivity for the PGE_2 receptor EP2 subtype (Woodward et al. 1993). In prostate cancer, the EP2 receptor activation has been shown to induce VEGF secretion, cell motility, growth, and angiogenesis (Jain et al. 2008, Wang et al. 2007), suggesting CYP4F8 inhibition as an attractive therapeutic alternative to COX-2 inhibition. However, the role of CYP4F8 had not been previously studied in prostate cancer.

The results from *in vivo* validation of the expression profile in prostate showed CYP4F8 expression to be elevated in 10 of the 33 (30 %) primary cancer samples (II: Figure 2), but no correlation with AR or ERG mRNA expression was observed. However, silencing of CYP4F8 reduced cell viability in both VCaP and LNCaP prostate cancer cell lines (II: Figure 5A), indicating possible therapeutic potential in a subset of prostate cancers.

5.2.3.2. EPHX2

The EPHX2 protein is a bifunctional enzyme harbouring epoxide hydrolase and phosphatase domains, both with different biological functions (Newman et al. 2003). EPHX2 (epoxide hydrolase 2) has the ability to degrade AA-derived and CYP-produced bioactive epoxy fatty acids, but EPHX2 has also been associated with androgen signaling (Pinot et al. 1995) and was recently suggested to regulate testosterone levels in mice (Luria et al. 2009).

Although earlier reports have suggested EPHX2 as a potential metastasis suppressor gene in breast cancer (Thomassen et al. 2009), the results from our study show that in clinical prostate samples EPHX2 mRNA is expressed at the same level in both primary cancer and non-malignant prostate samples (II: Figure 2). Furthermore, EPHX2 expression showed positive correlation (R = 0.43) with AR mRNA expression in primary prostate cancer samples (Figure 5), supporting the association between EPHX2 and androgen signaling.

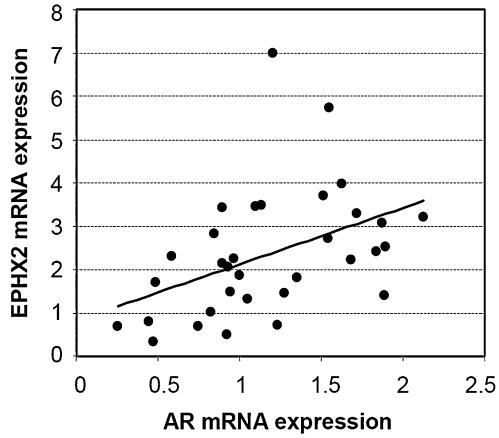

Figure 5. EPHX2 and AR mRNA expression levels correlate in primary prostate cancers. The relative mRNA expression of EPHX2 (y-axis) and AR (x-axis) in 33 primary prostate cancer samples.

In functional assays, EPHX2 silencing was able to reduce cell viability in LNCaP cells (II: Figure 5A). In addition, the results also indicated EPHX2 silencing to induce a moderate but significant pro-apoptotic effect (II: Figure 5B). In further functional validation EPHX2 silencing was shown to reduce AR signaling and to potentiate the growth inhibitory effect of flutamide in LNCaP prostate cancer cells (II: Figure 7), confirming a regulatory role of EPHX2 in androgen receptor signaling.

Interestingly, EPHX2 has been recently suggested also as a novel drug target for cardiovascular diseases (Imig and Hammock 2009, Ni et al. 2011). EPHX2-null mice are reported to be fertile and healthy (Luria et al. 2009), further suggesting that EPHX2 is a safe and attractive candidate drug target.

5.2.3.3. HPGD

HPGD (15-hydroxyprostaglandin dehydrogenase or 15-PGDH) has an important role in the inactivation of eicosanoids, and it has been suggested to be a tumour suppressor in several cancers (Wolf et al. 2006, Huang et al. 2008, Thiel et al. 2009, Tseng-Rogenski et al. 2010). In our data set HPGD mRNA was highly expressed in 15% of the primary cancer samples (II: Figure 2) and in 25 % of the advanced prostate cancer samples, compared with normal prostate (II: Figure 4B). Furthermore, the mRNA expression of HPGD associated with AR mRNA expression in the primary prostate cancer samples (II: Figure 4B), supporting the earlier findings suggesting that HPGD expression is induced by androgens (Tong et al. 2000).

Immunohistochemical staining of histologically normal and hyperplastic samples confirmed low HPGD expression in non-malignant prostate, and staining of metastatic samples (n = 77) highlighted the enhanced expression of HPGD in more than half (52 %) of the advanced metastatic samples (II: Figures 4C-D). Furthermore, in the functional assay HPGD silencing was shown to decrease LNCaP prostate cancer cell viability (II: Figure 5A).

In conclusion, high HPGD expression was associated with advanced and metastatic disease, as well as high AR expression, indicating a possible therapeutic opportunity for HPGD inhibition especially in the aggressive advanced prostate cancers, and warranting further studies to fully understand the clinical relevance of this promising candidate biomarker and drug target.

5.2.3.4. PLA2G7

PLA2G7 (platelet-activating factor acetylhydrolase, or PAF-acetylhydrolase; also known as LDL-associated phospholipase 2) is an enzyme degrading PAF and truncated membrane phospholipids generated by oxidative stress (Stafforini 2009). PLA2G7 is secreted mainly by leukocytes and macrophages and associated with circulating low-density lipoprotein (LDL) (Elstad et al. 1989, Stafforini et al. 1987). Although PLA2G7 has been shown to exert anti-inflammatory effects in a variety of experimental models, it also degrades apoptosis inducing oxidized phospholipids, including oxidized LDL, and simultaneously generates inflammatory products which have broad atherogenic effects (Stafforini et al. 2009, Wilensky et al. 2008, Zalewski and Macphee 2005). The expression of PLA2G7 is regulated by differentiation state, oxidized phospholipids and inflammatory cytokines, as well as steroid hormones, such as estrogen, progesterone

and glucocorticoid dexamethasone (Cao et al. 1998, Yasuda et al. 1992, Yoshimura et al. 1999).

Similarly to EPHX2, also PLA2G7 has been recently under intensive research in the area of cardiovascular diseases. PLA2G7 mass and activity have been associated with an increased risk of acute coronary syndrome, myocardial infarction, cardiac death, as well as ischemic stroke (May et al. 2006, O'Donoghue et al. 2006, Oei et al. 2005, Packard et al. 2000). Interestingly, early results with PLA2G7 inhibitor, darapladib, have been promising in the prevention and treatment of coronary heart disease (Serruys et al. 2008, Wilensky et al. 2008). In addition, lipid-lowering statin treatment is known to inhibit PLA2G7 in both plasma and atherosclerotic plaques, and darapladib has been suggested to offer substantial benefit especially when added to lipid-lowering therapy (O'Donoghue et al. 2006, Racherla and Arora 2010, Schaefer et al. 2005).

In our preliminary data set PLA2G7 mRNA was highly expressed in 24 of 33 (73%) primary tumours and in 74 % of advanced prostate tumours (Figure 6). In addition, PLA2G7 mRNA expression correlated positively with ERG expression in primary prostate cancers (II: Figure 3A).

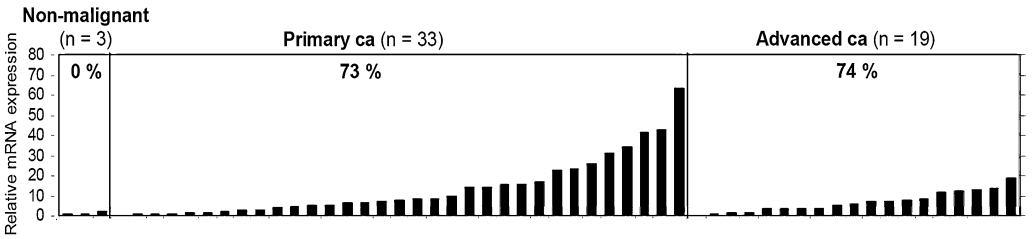

Figure 6. PLA2G7 is expressed in a cancer-specific manner. The mRNA expression of PLA2G7 in 3 non malignant prostate, 33 primary prostate cancer and 19 advanced prostate cancer samples.

Preliminary immunohistochemical staining results of histologically normal (n = 14) and hyperplastic (n = 5) samples supported low PLA2G7 expression in non-malignant prostate (II: Figures 3C), and further validation using 1137 primary prostate cancer samples and 409 adjacent non-malignant prostate samples from 453 patients confirmed PLA2G7 to be expressed in a cancer-specific manner. In total, 50 % of the cancer samples were PLA2G7 positive, whereas only 2.7 % of the non-malignant samples expressed PLA2G7 (III: Figure 1B-C). Importantly, the positive staining of PLA2G7 in primary prostate cancer samples significantly correlated with high (≥ 7) Gleason score (III: Figure 1D). In accordance to the association of PLA2G7 expression and higher Gleason score, the results from Kaplan-Meier analysis suggested that PLA2G7 positivity associates with poor survival and more aggressive disease (III: Figure 1E). Supporting the association of PLA2G7 expression with aggressive disease, staining

results of 80 metastatic prostate cancer samples from 47 patients showed PLA2G7 expression in 70% of the samples (II: Figure 3D).

The PLA2G7 silencing induced anti-proliferative and pro-apoptotic effect was seen specifically in the ERG oncogene-positive VCaP cells (II: Figure 5A-B). Furthermore, PLA2G7 silencing was shown to sensitize VCaP cells to oxidative stress induced damage (II: Figure 6A). Due to the expression of PLA2G7 especially in the ERG positive prostate tumours and the selective anti-proliferative effect observed in ERG positive prostate cancer cells, the effect of ERG on PLA2G7 expression, and vice versa, was studied. The results indicated that ERG induces PLA2G7 expression, whereas PLA2G7 expression does not influence ERG expression (II: Figure 6D).

In order to reveal the molecular alterations induced by PLA2G7 expression, lipidomic and gene expression profiling was analyzed in response to PLA2G7 silencing in cultured prostate cancer cells. In agreement with the earlier publications (Stafforini 2009, Wilensky et al. 2008), Ultra Performance Liquid Chromatography - Mass Spectrometry (UPLC-MS) results indicate that the most prominent change in response to PLA2G7 silencing also in prostate cancer cells was a decrease in the cellular lysophosphatidylcholine (LPC, PC(16:0/0:0), 1-hexadecanoyl-*sn*-glycero-3-phosphocholine) level (III: Figure 2A).

Results from the gene expression analysis (III: Table 1) showed that PLA2G7 silencing induced the mRNA expression of pro-apoptotic CASP8 and decreased the mRNA expression of anti-apoptotic BCL2L1 (III: Figure 2B), indicating that PLA2G7 silencing activates both intrinsic and extrinsic apoptotic pathways. The results from the genome-wide gene expression profiling and validation experiments indicated also that PLA2G7 silencing has the potential to regulate cell adhesion, motility and invasion. To validate the phenotype, cell attachment on fibronectin as well as cell motility and invasion in 2D and 3D matrix was monitored. The results indicated a significant increase in the amount of adherent cells (III: Figure 4A), and a decrease in the cellular motility and invasion potential (III: Figure 4B) in response to PLA2G7 silencing. Furthermore, PLA2G7 silencing was shown to reduce tumourigenesis and metastasis inducing aldehyde dehydrogenase mRNA expression and activity, supporting also the association of PLA2G7 expression with aggressive disease (III: Figure 2C).

As combinatorial therapeutic approaches may be required for adequate and efficient prostate cancer management, the ability of statins to inhibit PLA2G7 and to potentiate the anti-proliferative effect of PLA2G7 impairment in VCaP cells was investigated. The results indicated that the enzymatic activity of PLA2G7 was reduced by all four statins studied (III: Figure 5B). In addition, simvastatin, fluvastatin and lovastatin were able to inhibit PLA2G7 enzymatic activity synergistically with PLA2G7 siRNA. The results from cell viability assay proved that statins synergistically reinforced the anti-proliferative effect of PLA2G7 silencing in prostate cancer cells (III: Figure 5C).

To conclude, the expression levels of AIM1, CYP4F8, EPHX2, ERGIC1, HPGD, PLA2G7, TMED3 and TPX2, as well as the key prostate cancer oncogenes AR and

ERG have been presented as a heatmap in Figure 7, to illustrate the differences in their expression patterns.

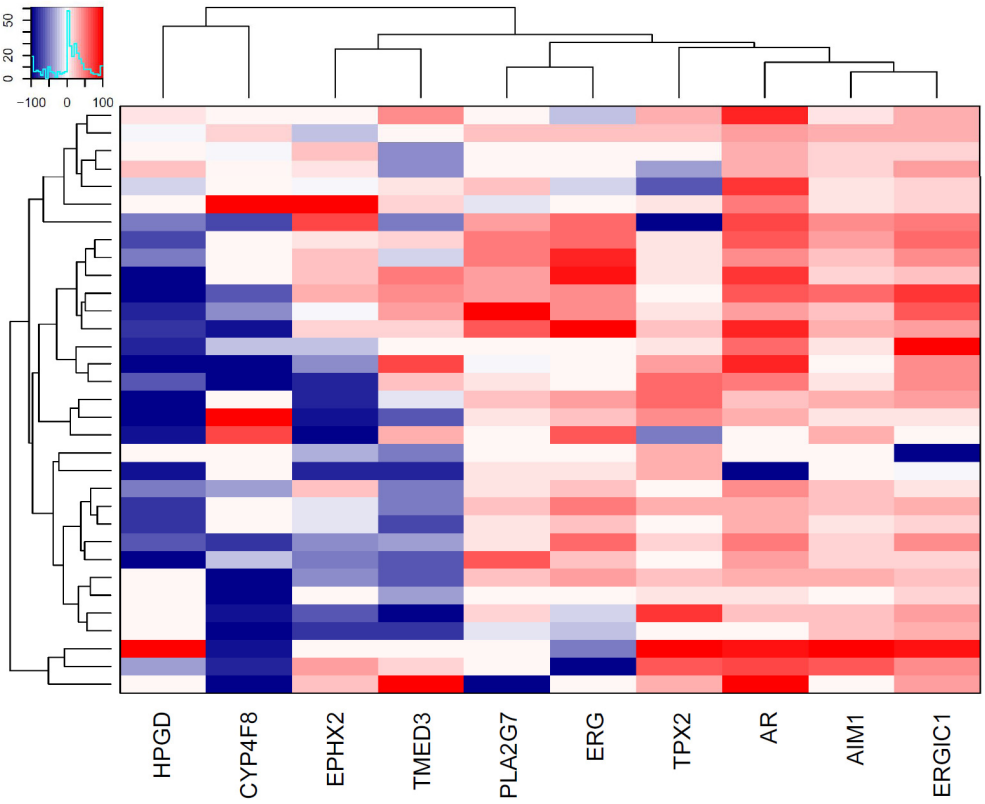

Figure 7. The mRNA expression of the novel putative drug targets in primary prostate cancer samples. Heatmap visualization of the gene-wise scaled relative mRNA expression values for AIM1, CYP4F8, EPHX2, ERGIC1, HPGD, PLA2G7, TMED3, TPX2, ERG, and AR in 33 primary prostate cancer tissues. The heatmap is drawn based on unsupervised hierarchical clustering of the expression values. Relative mean expression level in normal control samples was set as 0.

5.2.4. CRPC genomic targets (IV)

Rationally designed novel therapeutic approaches are needed, especially for treating castrate-resistant tumours. Thus, gaining a better understanding of the mechanisms leading to the emergence and progression of CRPC may facilitate more effective means to prevent and treat this currently fatal disease.

In order to investigate the molecular pathogenesis of advanced and CRPC, genome-wide DNA and RNA data from 13 castrate-resistant, advanced and metastatic prostate

cancers were integrated to distinguish genes whose overexpression was driven by their amplification. In total, 18 genes were found to be overexpressed and amplified in at least two of the samples (IV: Supplemental Table SII). Out of these, only six genes (AR, ATP1B1, FAM110B, LAS1L, MYC and YIPF6) were detected as recurrent genomic targets in CRPC samples (IV: Table II and Figure 1A-B). Functional and bioinformatic studies were then applied to explore the roles and mechanisms of six key candidate genes, with FAM110B appearing as a previously undescribed gene that may have a critical role in CRPC (IV: Figure 1C-D and Figure 2A).

5.2.4.1. FAM110B

The FAM110 (family with sequence similarity 110) gene family was originally identified in a search for centrosome and spindle pole associated proteins by yeast two-hybrid approaches (Hauge et al. 2007). FAM110B was shown to be highly expressed in testis, spleen, and thyroid tissues, and to have a role in cell cycle progression.

In this study, the siRNA experiments showed that FAM110B silencing had a significant anti-proliferative effect in LNCaP, LNCaP C4-2 and MDA-PCa-2b prostate cancer cells (IV: Figure 2A-B). Further support for the role of FAM110B in regulating prostate cancer cell growth was obtained from apoptosis assay, indicating that FAM110B knock-down slightly induces caspase-3 and 7 activity in LNCaP cells (IV: Figure 2C). In addition, FAM110B silencing was shown to reduce cell viability more efficiently in the androgen independent (AI) LNCaP cell line, than in the parental cell line cultured in normal media (IV: Figure 3E).

In silico data mining indicated a clear positive correlation ($R = 0.5$, $p < 0.001$) between FAM110B and AR mRNA expression in prostate cancer samples (IV: Figures 1C and 3A) and the overexpression of FAM110B in a subset of CRPC samples supported a link between FAM110B and AR signaling. Interestingly, FAM110B siRNA was able to reduce AR and PSA protein levels, and AR silencing reduced FAM110B mRNA and protein levels in LNCaP cell (IV: Figure 3B-C). Furthermore, FAM110B mRNA and protein levels were decreased by androgen deprivation and increased by stimulation with synthetic androgen R1881 (IV: Figure 3D). Interestingly, CYP11A1 expression was upregulated as a consequence of forced FAM110B overexpression in prostate cancer cells (IV: Supplemental Figure S1C). This steroid hormone synthesis enzyme has previously been associated with activated de novo androgen synthesis in hormone-refractory prostate cancer cells (Dillard et al. 2008). On the other hand, FAM110B silencing decreased the expression of beta-catenin in prostate cancer cells (IV: Supplemental Figure S1D). Wnt / beta-catenin signaling pathway has previously been associated with aberrant activation of the AR during progression of prostate cancer to the terminal castrate-resistant stage (Wang G et al. 2008). Therefore, these results suggest FAM110B, a gene overexpressed and amplified in a subset of CRPCs, as an effector in the reprogramming and maintenance of androgen signaling as well as androgen independent (AI) growth. In conclusion, FAM110B is an androgen

responsive gene with the ability to regulate AR signaling in cultured prostate cancer cells.

To further assess the possible functional role of FAM110B in prostate cell growth and carcinogenesis, the effect of ectopic FAM110B expression on the overall amount of living non-malignant prostate epithelial cells was studied. FAM110B overexpression significantly increased the proliferation of non-malignant RWPE-1 cells (IV: Figure 4A), supporting the growth promoting role of FAM110B in prostate cancer. Furthermore, FAM110B overexpression induced aneuploid, multinuclear phenotype in these cells (IV: Figure 4D), adding thus more evidence to the possible oncogenic role of FAM110B in prostate carcinogenesis. In addition, the gene expression analysis in cultured prostate cells also indicated a potential oncogenic role for FAM110B (IV: Supplemental Table SIII). As an example, the ectopic FAM110B expression in RWPE-1 normal prostate epithelial cells was confirmed to downregulate interferon response and antigen presentation (IV: Figure 5), essential to anti-cancer immune surveillance and host immune response.

In summary, the results highlighted the role of FAM110B in the promotion of prostate cancer cell growth, and further suggested FAM110B to have a possible role in regulating distinct molecular mechanisms of cancer and CRPC.

5.3. Prostate cancer cell specific anti-proliferative compounds (V)

Information on putative drug targets, based on the data from gene expression analyses and siRNA experiments, has been utilized to identify existing drugs for these individual targets or target pathways. In addition, a parallel unbiased approach to identify drugs (and drug targets) against prostate cancers by HT compound screens was taken and responses to 4910 compounds in multiple prostate cancer cells studied. Cell lines screened with HT proliferation assay included prostate cancer cell lines VCaP, LNCaP, PC-3 and DU-145, as well as normal prostate epithelial cell lines RWPE-1 and EP-156T. The results highlighted four novel prostate cancer-selective growth inhibitory compounds; disulfiram (DSF), thiram, trichostatin A and monensin, among marketed drugs (V: Figure 2). We validated DSF as a potential prostate cancer therapeutic agent and suggest a possible advantage by promoting oxidative stress in prostate cancer management.

5.3.1. Disulfiram

Due to its excellent safety profile and long-term use as an alcohol deterrent in the clinic, DSF is an attractive therapeutic option for cancer. In addition to acting as an aldehyde dehydrogenase inhibitor, DSF has been shown to inhibit DNA topoisomerases, matrix metalloproteinases, and ABC (ATP-binding cassette) drug

transport proteins, and thereby to have antitumour and chemosensitizing activities (Yakisich et al. 2001, Sauna et al. 2005). Previous studies with melanoma and breast cancer cells have shown that the growth inhibitory potential of DSF was potentiated with copper or zinc cotreatment (Brar et al. 2004, Chen et al. 2006).

To study the growth inhibitory mechanism of DSF in prostate cancer cells, the effect of DSF exposure on ERG and AR expression was first studied. The results indicated that DSF decreases ERG mRNA expression, whereas AR expression is not consistently affected (V: Figure 3A). Second, a genome-wide gene expression analysis was performed and the results indicated metal-binding activities to be altered. Further validation confirmed the induction of metallothionein (MT) MT1B, MT1G, MT1F, MT1X, and MT2A mRNA expression in response to 6 h DSF exposure (V: Figure 3B). Whereas, at later time point (24 h) DNA replication related genes were downregulated (V: Figure 3C). To find out whether MTs and minichromosome maintenance complex (MCM) genes affect prostate cancer cell proliferation, the effect of MT and MCM gene expression on prostate cancer cell proliferation was studied. The results indicated that silencing of MCM5, MT1F, or MT1G, is alone sufficient to reduce the proliferation of both VCaP and LNCaP cells (V: Figure 4).

The differentially expressed genes in DSF exposed VCaP cells were compared with the expression profiles representing drug responses to over 1300 compounds using Connectivity Map. The results indicated the highest enrichment with oxidative stress inducing 12,13-EODE (12,13-cis epoxide of linoleic acid) (V: Supplementary Table S3). Also irinotecan, a topoisomerase 1 inhibitor was among the most enriched drugs. These correlations support the gene expression results indicating oxidative stress and inhibition of DNA replication as the DSF induced biological processes.

To study the inhibitory potential of DSF *in vivo* on prostate cancer growth, VCaP cell xenograft experiments were done in immunocompromized mice. The results showed that DSF reduced tumour growth up to 40% but was not able to block it, indicating the need for a combinatorial treatment (V: Figure 5A). Combinatorial effects of DSF and copper or zinc were thus studied in VCaP cells. Cell viability assay results indicated a significant reduction in cell viability in response to combinatorial treatment with DSF and copper chloride ($CuCl_2$) (V: Figure 5B). In addition, $CuCl_2$ and DSF cotreatment reduced AR protein levels and induced poly ADP ribose polymerase cleavage, whereas neither of the agents had an effect alone (V: Supplementary Figure S4). Because MTs are known to regulate the intracellular copper levels and DSF induces MT mRNA expression, we studied MT expression in VCaP cells in response to DSF, $CuCl_2$, and DSF-$CuCl_2$ cotreatment. The results indicated that in response to DSF-$CuCl_2$ cotreatment, the expression of MTs is more highly induced, whereas MCM expression is even more repressed, than in response to either one of the agents alone (V: Figure 5C-D).

6. DISCUSSION

6.1. HT RNAi screen

In carcinogenesis genetic changes alter normal control mechanisms and enable cancer cells to proliferate and survive limitlessly. Accordingly, prostate cancer cells are known to contain wide range of somatic mutations, gene deletions and amplifications, as well as gene expression pattern altering changes in DNA methylation leading to increased expression of oncogenes and loss of tumour suppressor genes (Nelson et al. 2003). Accumulating gene expression data from normal and cancer cell lines and human tissues provide important information for biomarker discovery, as well as for the identification of potential novel drug targets and therapeutics for personalized medicine. Furthermore, RNAi-based loss of function screening has proven powerful for the identification of new and interesting cancer drug targets (Bauer et al. 2010, Cole et al. 2011, Meacham et al. 2009). These techniques enable the development of novel targeted and personalized therapeutic options for cancer. In this thesis, the potential of microarray and RNAi techniques was combined to identify novel potential drug targets for prostate cancer.

To identify genes that may be essential for prostate cell proliferation and survival, and suitable for targeted and personalized therapeutics, a bioinformatic *in silico* mRNA expression analysis was performed to select a set of prevalidated prostate and prostate cancer tissue specific genes for further functional assays in cultured prostate cancer cells. The GeneSapiens database (Kilpinen et al. 2008) was applied to bioinformatically explore the gene expression levels across 9783 human tissue samples from 175 different tissue types. Briefly, GeneSapiens (http://www.genesapiens.org/) is a collection of Affymetrix microarray experiments. The data are collected from various publicly available sources, including Gene Expression Omnibus and Array-Express. All samples have been reannotated (with detailed information on sample collection procedures, anatomic location, disease type, and clinicopathological details) and normalized with a custom algorithm to enable direct comparison of the observed gene expression patterns across five different array platforms. However, although each sample was systematically manually annotated, the differences in experimental setting, conditions and sample handling might cause minor bias in the gene expression patterns observed. However, this database is the world's largest fully integrated and annotated human gene expression data source and provides unique data analysis options for identification of potential candidate biomarkers and drug targets for the development of personalized medicine.

Since siRNAs are known to induce off-target effects (Jackson et al. 2003), four siRNAs per gene were used in the screening. In addition, to confirm the validity of the results, positive and negative controls were utilized, and the cell proliferation siRNA screen was performed in triplicates in two different clinically relevant prostate cancer cell lines. In addition, apoptosis screen was performed in both of the cell lines. Post-screen

validation included expression profile analysis of the cancer cell proliferation and survival influencing drug targets in prostate cell lines, as well as in clinical prostate samples. Furthermore, the results from the functional assays were validated *in vitro*. Since most HT RNAi studies, as the one described in this thesis, evaluate only a single parameter at a time, the molecular mechanisms of the most potent targets were further investigated.

By utilizing a preselection approach for the genes included in the RNAi analysis, instead of a commercial siRNA library, the aim was to maximize the discovery rate of prostate cancer cell viability affecting genes, and to enable the discovery of personalized and efficient prostate cancer therapy without unwanted side effects. However, the gene expression based approach excludes most activated or inactivated protein kinase signaling pathways from the study, since their activation is not dependent only on changes in gene expression but rather depend on alterations in phosphorylation status. Hence, although promising candidate drug targets were discovered, the results presented here illustrate the potential of combining gene expression analysis and RNAi technique in the discovery of potential novel drug targets, but do not give a comprehensive view of the complex processes associated with prostate carcinogenesis and cancer progression. However, as evidenced by the high rate of anti-proliferative hit siRNAs especially in LNCaP cells, the focused approach was successful in maximizing the amount of potential prostate cancer relevant drug targets identified, and the results provide several starting points for preclinical and eventually clinical efforts to treat prostate cancer.

6.2. Novel prostate cancer drug targets

In total 9 novel drug targets were validated for different subsets of prostate cancer (Table I). The clinical validation showed that the putative drug targets selected for further validation were expressed in clinical prostate cancer samples, thereby confirming the results of the bioinformatic surveys. Furthermore, most of the targets were clearly upregulated at least in a subset of prostate cancers compared to the non-malignant prostate tissues analyzed. The expression of ERGIC1, PLA2G7 and TMED3 was associated with ERG oncogene expression, whereas FAM110B, HPGD and TPX2 expression was associated with advanced, metastatic and castrate-resistant tumours. Furthermore, FAM110B, EPHX2 and TPX2 were able to decrease AR and PSA expression. Further functional studies supported the important differences in the involvement of the target genes in different types of tumours.

Although several potential novel drug targets for prostate cancer were identified from the targeted siRNA screen, the future of siRNA based therapeutics is dependent on successful siRNA delivery to the target tissue (Guo et al. 2011). Intensive research is ongoing to develop efficient delivery technologies to enable siRNA based therapeutics for cancer. However, a few of the targets identified already possess potent inhibitors used for other indications, providing a significant opportunity for repositioning of drugs already in clinical development to new indications.

Table I. **Characteristics of the 9 novel prostate cancer drug targets identified using RNAi.** The effect of each target gene on AR and ERG expression, the possible co-expression with AR or ERG in primary prostate cancer samples, and the effect on prostate cancer cell proliferation, as well as the potential target prostate cancer patient group is indicated.

Target gene	Effect on expression		Co-expression		Effect on proliferation		Target cancer patient group
	AR	ERG	AR	ERG	VCaP	LNCaP	
AIM1	No	No	No	No	**Yes**	No	Primary
CYP4F8	No	N/A	No	No	**Yes**	**Yes**	Primary
FAM110B	**Yes**	N/A	**Yes**	No	No	**Yes**	CRPC
EPHX2	**Yes**	N/A	**Yes**	No	No	**Yes**	Primary
ERGIC1	No	**Yes**	**Yes**	**Yes**	**Yes**	No	ERG positive
HPGD	No	N/A	**Yes**	No	No	**Yes**	Advanced
PLA2G7	No	No	No	**Yes**	**Yes**	No	ERG positive
TMED3	No	No	**Yes**	**Yes**	**Yes**	**Yes**	Primary
TPX2	**Yes**	No	No	No	**Yes**	**Yes**	Primary, CRPC

6.2.1. Targets related to endoplasmic reticulum function

Three of the potential drug target genes (AIM1, ERGIC1, TMED3) were associated with redox homeostasis and ER and Golgi apparatus function, suggested as an promising opportunity for targeted cancer therapy (Liu et Ye 2011; McLaughlin et Vanderbroeck 2011). Since the reports of the exact role of AIM in different cancers are controversial (Araki et al. 2010, Brait et al. 2008, Loyo et al. 2011, Ray et al. 1996), further studies are needed to evaluate its potential in cancer management. However, the evidence suggest AIM1 to be highly expressed in most primary prostate cancers, as well as support the potential role of AIM1 in the regulation of prostate cancer cell growth and morphology. ERGIC1 and TMED3 have not been previously associated with cancer. Results from this study associate their expression with ERG oncogene expression, and support their potential as prostate cancer drug targets. Since ERGIC1 was highly expressed in most primary prostate tumours, and ERGIC1 silencing was able to downregulate ERG expression, it is an intriguing candidate drug target especially for the ERG oncogene expressing tumours. Furthermore, all of the ER related genes were upregulated by androgens, supporting earlier reports suggesting, that the expression of ER stress response genes is regulated by androgen in prostate cancer cells (Segawa et al. 2002). Increase in the transcription of genes involved in the adaptive mechanism of melanoma cells to ER stress has been shown be mediated by the transcription factor Ets-1 (Dong et al. 2011), thus supporting also the potential role of ERG in the general regulation of ER function related genes in prostate cancer. Importantly, this would also suggest the sensitivity of ERG positive prostate tumours to pharmacological ER stress inducers.

6.2.2. TPX2

TPX2 has been proposed as a potential drug target in multiple cancers (Ramakrishna et al. 2010, Satow et al. 2010, Warner et al. 2009), and our results suggest TPX2 to be a potent drug target also in prostate cancer. Similarly to AIM1 and ERGIC1, TPX2 was highly expressed in most prostate tumour samples analyzed. TPX2 was shown to be regulated by AR and androgens, but more importantly, TPX2 silencing was able to downregulate AR signaling. Furthermore, supporting recent reports associating TPX2 expression with poor prognosis in cancer (Kadara et al. 2009, Li et al. 2010, Stuart et al. 2011), in our data set high TPX2 expression was associated with PSA failure. In conclusion, the results indicate potential therapeutic relevance for TPX2 in majority of prostate cancers, possibly also in advanced and castrate-resistant disease.

6.2.3. Arachidonic acid pathway enzymes

The AA pathway is a promising area for translational research, because many targets along this pathway have been already intensively investigated in other indications, such as cardiovascular diseases and pain, providing an opportunity for repositioning of drugs already in clinical development to new indications. Furthermore, understanding the roles of different downstream pathways and individual enzymes in AA metabolism may provide more effective therapeutic opportunities with fewer adverse effects. Accordingly, our results suggest the potential of CYP4F8, EPHX2, HPGD and PLA2G7 in the management of prostate cancer.

Although COX-2 inhibitors have been reported to be efficient in both prostate cancer prevention and treatment, their use has been restricted due to the unexpected cardiovascular side effects (Hsu et al. 2000, Jacobs et al. 2005, Kearney et al. 2006, Mahmud et al. 2008, Narayanan et al. 2000, Patel et al. 2005). CYP4F8 is known to produce 19-hydroxy-PGE_2 that, unlike COX-2 produced PGE_2, specifically activates the EP2 receptor associated with prostate carcinogenesis and cancer progression (Jain et al. 2008, Wang et al. 2007). In addition, EPHX2 has been recently suggested as a novel drug target for cardiovascular diseases (Imig and Hammock 2009, Ni et al. 2011), and EPHX2-null mice are reported to be fertile and healthy (Luria et al. 2009). Similarly to EPHX2, also PLA2G7 is intensively studied as a potential drug target in cardiovascular diseases (Serruys et al. 2008, Wilensky et al. 2008). Thus, the inhibition of CYP4F8, EPHX2 or PLA2G7 could be an attractive therapeutic alternative to COX-2 inhibition in prostate cancer prevention and treatment.

EPHX2 has previously been associated with androgen signaling (Luria et al. 2009, Pinot et al. 1995), and in the present study, EPHX2 expression was shown to correlate with AR mRNA in clinical primary prostate tumour samples. In addition, EPHX2 silencing reduced AR signaling and potentiated the antiproliferative effect of antiandrogen flutamide, confirming a regulatory role of EPHX2 in AR signaling and indicating a putative combinatorial therapeutic approach in prostate cancer treatment.

Although HPGD has been suggested to function as a tumour suppressor (Wolf et al. 2006, Huang et al. 2008, Thiel et al. 2009, Tseng-Rogenski et al. 2010), our results showed a clear dependency of LNCaP cell growth and survival on HPGD expression. In addition, HPGD was highly expressed in a subset of androgen receptor overexpressing advanced and metastatic prostate tumours, indicating potential therapeutic relevance in this subset of typically incurable prostate cancer.

In contrast to cancer, the role and therapeutic potential of PLA2G7 has been under intensive research in the area of cardiovascular diseases (Serruys et al. 2008, Wilensky et al. 2008). Results from our study indicate that PLA2G7 is a potent biomarker distinguishing prostate cancer from non-malignant prostate tissues. Furthermore, PLA2G7 positivity was associated with high Gleason score and poor prognosis. In functional experiments, PLA2G7 impairment reduced aldehyde dehydrogenase activity, considered as a marker of prostate cancer stem cells as well as tumour- and metastasis-initiating prostate cancer cells (Li et al. 2010, van den Hoogen et al. 2010, Yu et al. 2011), supporting the possibility that PLA2G7 expression may have prognostic significance. This hypothesis was further supported by our results demonstrating PLA2G7 protein expression in 70 % of metastatic prostate tumours compared to the 50 % positivity observed in the primary tumours. Interestingly, the results also suggested PLA2G7 mRNA expression to correlate with ERG expression, and silencing of ERG reduced PLA2G7 mRNA expression in ERG-positive prostate cancer cells, supporting a functional link between these two genes. Furthermore, knock-down of PLA2G7 significantly reduced the growth of ERG positive, but not ERG negative, prostate cancer cells *in vitro*, indicating potential as a biomarker and personalized drug target especially in ERG positive prostate cancers. Further functional validation suggested PLA2G7 to regulate cell adhesion, mimicking the previously described ERG knock-down phenotype (Gupta et al. 2010), and supporting the possibility that PLA2G7 is an important mediator of ERG oncogene in prostate cancer.

One of the main functions of PLA2G7 is to hydrolyze truncated phospholipids generated by oxidative attack and to participate in the maintenance of membrane integrity (Stafforini 2009). Furthermore, a yeast PLA2G7 ortholog has been shown to suppress oxidative death (Foulks et al. 2008). In the current study, PLA2G7 silencing was shown to reduce the expression of the protective MTs and to sensitize ERG-positive prostate cancer cells to oxidative stress. Moreover, ERG oncogene-positive prostate cancer samples were found to express low levels of MTs, known to protect cells against oxidative stress, suggesting that ERG-positive prostate cancers are vulnerable to oxidative stress.

In addition to regulating cell viability, the results from this thesis suggest PLA2G7 to have a role in prostate cancer cell migration and invasion. LPC, found to be decreased in response to PLA2G7 silencing in prostate cancer cells, has been linked to cancer cell migration and metastasis via promoting invadopodia formation in multiple cancer cell lines as well as migration of PC-3 prostate cancer cells (Harper et al. 2010, Monet et al. 2009). Furthermore, PLA2G7 silencing also reduced cell migration and invasion in

prostate cancer cell culture models. Since the anti-migratory effect was not restricted to ERG positive prostate cancer cells, PLA2G7 inhibition is potential therapeutic option also in the prevention and treatment of aggressive and metastatic tumours.

As combinatorial therapeutic approaches may be required for efficient prostate cancer management, the ability of statins to potentiate the anti-proliferative effect of PLA2G7 silencing in prostate cancer cells was studied. Epidemiologic evidence supports the possible chemopreventive potential of statins in prostate cancer (Murtola et al. 2010, Platz et al. 2006). In addition, statins suppress tumour growth in prostate cancer mice xenografts (Wang C et al. 2010). The results from this study showed that statins reduce the enzymatic activity of PLA2G7 and potentiate the anti-proliferative effect of PLA2G7 silencing in cultured prostate cancer cells.

Taken together, the present results highlight the significance of the AA pathway in prostate cancer cell growth regulation. Although the mechanisms inducing the changes observed after target gene silencing are most likely diverse, inhibition of this metabolic signaling cascade, or the balance between different branches of the pathway, appears to affect the growth and survival of prostate cancer cells. Finally, inhibition of EPHX2 and PLA2G7 may reduce prostate cancer cell viability even more effectively when combined with other treatments, such as androgen deprivation, induction of oxidative stress or lipid-lowering statins.

6.2.4. FAM110B

Unlike the other potential novel drug targets, FAM110B was selected for further functional validation based on high expression and amplification in a subset of CRPC samples. FAM110B, identified here as a novel genomic target in CRPC, also significantly affected prostate cancer cell growth and survival *in vitro*. Further studies indicated that in cultured prostate cancer cells FAM110B is regulated by AR, and FAM110B silencing decreases AR and PSA protein levels. Furthermore, FAM110B silencing specifically potentiated the inhibition of LNCaP prostate cancer cell growth in androgen-deficient, CRPC tissue environment mimicking conditions. These results support an important dual role for FAM110B in androgen signaling as well as prostate carcinogenesis.

In addition to the potential role as an effector in the reprogramming and maintenance of androgen signaling as well as androgen independent growth, ectopic FAM110B expression moderately promoted aneuploidy. Genomic instability is a hallmark in cancer and it induces a large number of cancer progression promoting genetic alterations in cancer cells (Negrini et al. 2010). Furthermore, ectopic FAM110B expression decreased the expression of genes involved in immune surveillance and antigen presentation allowing the tumour to escape killing by immune cells and thus promote cancer progression and metastasis (Smyth et al. 2001).

In conclusion, our results suggest FAM110B to have a role in regulating distinct molecular mechanisms of cancer as well as those of CRPC. Thus, inhibition of FAM110B could have therapeutic potential in CRPC.

6.3. HT compound screen

In this project, together with the siRNA screening results, cell-based HT compound screen was utilized to identify potential vulnerabilities present in prostate cancers, which could be exploited to inhibit tumour cell proliferation and survival *in vivo*. To identify cancer specific antineoplastic compounds, four prostate cancer and two normal prostate cell lines were screened with HT proliferation assay. A library consisting of 4910 drug-like small molecule compounds, including most currently marketed drugs, was screened, and the results highlighted four novel prostate cancer-selective growth inhibitory compounds. Interestingly, vast majority of the anticancer drugs identified in the screen were equally effective in cancer and control cells. These nonselective growth inhibitory compounds included also docetaxel, which is currently used in the clinic to treat patients with hormone-refractory prostate cancer.

DSF was one of the most promising compounds identified. Earlier studies on cultured cells have indicated that DSF inhibits myeloma, leukaemia, lymphoma, small cell lung cancer, cervical adenocarcinoma, melanoma, neuroblastoma, and colorectal cancer cell survival as well as osteosarcoma invasion (Wang et al. 2003, Wickström et al. 2007). Due to the excellent safety profile and long-term use as an alcohol deterrent, DSF was selected for more detailed mechanistic studies. We validated DSF as a potential prostate cancer therapeutic agent and suggested a possible advantage by promoting oxidative stress in prostate cancer management.

Gene expression profiling results linked decreased prostate cancer cell growth to inhibition of DNA replication and indicated that DSF induces metallothionein expression in VCaP cells. The results suggest induction of oxidative stress as a DSF-induced biological process, supporting the sensitivity of VCaP cells to oxidative stress inducers.

In vivo studies using VCaP cell xenografts showed reduced tumour growth in response to DSF exposure. However, DSF alone was not able to completely block tumour growth, indicating need for combinatorial approaches. Further *in vitro* studies showed that the growth inhibitory potential of DSF was potentiated with copper. Interestingly, recent results associate DSF-copper complexes with ER stress and massive vacuolization in the absence of apoptotic features. When combined with DSF, copper acts simultaneously as an ER stress inducer and a caspase-3 inhibitor, forcing the cell into caspase-independent cell death (Tardito et al. 2011).

In agreement with previous data (Björkman et al. 2008), the HDAC inhibitor trichostatin A (TSA) was among the most selective antiproliferative compounds especially for ERG oncogene expressing VCaP cells. Interestingly, according to the

Connectivity Map results HDAC inhibitors were among the drugs altering gene expression in an opposite direction than DSF. This finding indicates that even in a defined subset of prostate cancers, such as ERG positive tumours, the mechanisms of action for different potent growth inhibitory compounds and siRNAs may be completely different.

7. SUMMARY AND CONCLUSIONS

The primary aim of this study was to identify possible novel drug targets, genes and pathways critical for prostate oncogenesis and progression, and to advance the development of personalized therapeutic options for prostate cancer. In this thesis, the potential of microarray data, RNAi technique and compound screens were combined in order to identify potential novel biomarkers, drug targets and drugs for future personalized prostate cancer therapeutics.

The bioinformatic mRNA expression analysis covering 9873 human tissue and cell samples was used to identify the most promising *in vivo* prevalidated prostate cancer drug targets and biomarkers for further studies in cultured prostate cancer cells. Second, RNAi based HT functional profiling of 295 *in silico* prevalidated prostate and prostate cancer tissue specific genes was performed in prostate cancer cell lines. Potential drug targets or target pathways highly expressed in clinical prostate cancers and regulating prostate cancer cell growth were validated *in vitro* and *vivo*. In addition, a parallel unbiased HT compound screen approach was used to identify cancer selective compounds among 4910 currently marketed drugs and drug-like molecules in cultured prostate cells. In addition to identifying novel potential therapeutic options for prostate cancer, this combinatorial approach enabled us to identify vulnerabilities in prostate cancer cells, which could be utilized in the inhibition of tumour cell proliferation and survival.

Nine novel drug targets, with biomarker potential, as well as one compound were validated *in vitro* and *in vivo*, and the results highlight ER function, lipid metabolism and arachidonic acid pathway, redox homeostasis, AR signaling as well as mitosis as potential therapeutic processes critical for prostate oncogenesis and progression. Moreover, ERG oncogene positive cancer cells especially exhibited sensitivity to induction of oxidative and ER stress, whereas advanced and castrate-resistant tumours could be potentially targeted through androgen signaling and mitosis. Based on the experimental results and information available so far, PLA2G7 and TPX2 appear as the most potential candidate drug targets discovered with the combinatorial gene expression analysis and RNAi based approach. PLA2G7 shows strong prognostic and therapeutic biomarker potential as well as is an attractive drug target affecting multiple processes involved in prostate carcinogenesis and progression. Furthermore, PLA2G7 is inhibited by the widely used statins and associates with the ERG positive cancers currently lacking targeted therapeutic options. TPX2 strongly affects prostate cancer cell viability and AR signaling, as well as associates with poor prognosis in our data set. In addition, results from other malignancies support the potential of TPX2 as an effective cancer drug target. Since combinatorial therapeutic approaches may be required for efficient prostate cancer management, DSF or inhibition of the putative novel drug targets may reduce prostate cancer cell viability even more effectively when combined with other treatments, such as androgen deprivation, inducers of oxidative stress or lipid-lowering statins. However, further studies are needed to confirm the

expression pattern of these targets in prostate cancer, as well as to investigate their molecular mechanisms of action *in vitro* and effectiveness *in vivo* in suitable animal models.

In conclusion, this thesis illustrates the power of systems biological data analysis in the exploration of potential new target genes and lead compounds for prostate cancer management. The results from the combinatorial usage of gene expression, RNAi and compound screens provide several novel starting points for preclinical and eventually clinical efforts to treat prostate cancer. Furthermore, the combinatorial approach enabled the identification of potential vulnerabilities present in prostate cancers, which can be exploited to develop efficient, targeted and personalized treatments for prostate cancer.

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