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ANALYSIS OF STROMA-DERIVED FACTORS WITH POTENTIAL PROGNOSTIC AND THERAPEUTIC SIGNIFICANCE IN PROSTATE CANCER

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ABSTRACT

Prostate cancer is the most common malignancy in Sweden with about 9000 new cases diagnosed every year. New markers are needed to improve diagnostic accuracy. The most commonly used tissue-biomarkers are basal cell markers and AMACR, often used in combination. We identified three potential tissue biomarkers, CYCS, ICK and IKBKB, by using the Human Protein Atlas database and investigated their diagnostic accuracy. The potential of these biomarkers was also compared with AMACR. Immunohistochemical analysis of the markers was performed on a tissue microarray (TMA) consisting of tissue from 40 prostate specimens, including benign prostatic tissue, atrophy, high-grade prostatic intraepithelial neoplasia (HGPIN) and prostate cancer. In addition, qRT-PCR analysis of malignant and benign frozen tissue samples from 32 radical prostatectomy specimens was performed. All four biomarkers showed a higher protein expression in prostate cancer and HGPIN than in benign tissue. The prognostic accuracy was highest for AMACR, but the results indicate that in some cases CYCS, ICK and IKBKB may serve as additional diagnostic markers.

It is known that prognostic information can also be derived from tumor stroma. The prognostic value of stromal expression of PDGFRβ was therefore evaluated by immunohistochemical analysis of PDGFRβ on a TMA containing cancer and non-malignant tissue from more than 300 prostate cancer patients. The association between stromal staining intensity and a number of clinical characteristics were then analyzed. Expression of PDGFRβ in non-malignant and tumor stroma was associated with high Gleason grade and reduced cancer specific survival.

Cancer associated fibroblasts (CAFs) are found in many solid tumors and promote tumor growth and progression. Identification and inhibition of molecules mediating these interactions constitute an attractive strategy for development of new cancer therapies. By comparative analyses of CAFs and normal fibroblasts from prostate tissue we have identified a number of genes upregulated in prostate CAFs. CXCL14, an orphan chemokine, was the most upregulated transcript. Overexpression of CXCL14 in fibroblasts increased their proliferation and migratory capacity. Also over-expression in fibroblasts of CAF led to increased ability of these cells to stimulate proliferation and migration of prostate cancer cells. Furthermore, fibroblasts overexpressing CXCL14 enhanced tumor growth, vascularisation and macrophage infiltration in a stroma-dependent prostate cancer model.

Another transcript identified to be upregulated in prostate CAFs was GDF15, a member of the TGFβ superfamily. GDF15 was shown to stimulate fibroblast proliferation and enhanced growth, migration and invasion of prostate cancer cells. Fibroblasts over-expresssing GDF15 was also able to stimulate prostate xenograft growth when co-injected with prostate cancer cells. Interestingly, these fibroblasts also increased the ability of tumor xenograft to promote growth at a distant site suggesting direct or in-direct systemic pro-tumoral effects of fibroblast-derived GDF15.

These studies thus identify a set of new diagnostic and prognostic markers for prostate cancer and stroma-derived potential therapeutic targets.

LIST OF PUBLICATIONS

I. Lars Häggarth, **Christina Hägglöf**, Sara Jonmarker Jaraj, Kenneth Wester, Fredrik Pontén, Arne Östman and Lars Egevad
Diagnostic biomarkers of prostate cancer
Manuscript

II. **Christina Hägglöf**, Peter Hammarsten, Andreas Josefsson, Pär Stattin, Janna Paulsson, Anders Bergh and Arne Östman
Stromal PDGFRβ expression in prostate tumors and non-malignant prostate tissue predicts prostate cancer survival
PLoS One 2010 May 20;5(5):e10747

III. Martin Augsten*, **Christina Hägglöf** *, Eleonor Olsson, Claudia Stolz, Panagiotis Tsagozis, Tetyana Levchenko, Mitchell J. Frederick, Åke Borg, Patrick Micke, Lars Egevad and Arne Östman
CXCL14 is an autocrine growth factor for fibroblasts and acts as a multi-modal stimulator of prostate tumor growth
Proc Natl Acad Sci. 2009 Mar 3;106(9):3414-9

IV. Francesca Bruzzese, **Christina Hägglöf**, Alessandra Leone, Arne Östman, Alfredo Budillon and Martin Augsten
Local and systemic pro-tumorigenic effects of fibroblast-derived GDF15
Manuscript

* Authors contributed equally

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AMACR	Alpha-Methylacyl-CoA Racemase
AP-1	Activating Protein-1
AR	Androgen Receptor
BMP	Bone Morphogenetic Protein
BRAK	Breast- and kidney-Expressed Chemokine
Cav-1	Caveolin-1
CCL5	Chemochine(CC-motif) Ligand 5
CMML	Chronic Myelomonocytic Leukemia
COL1A1	Collagen type 1a1
CXCL14	CXC Chemokine Ligand 14
CYCS	Somatic Cytochrome C
DFSP	Dermatofibrosarcoma Protuberans
EGF	Epidermal Growth Factor
EGF-R	Epidermal Growth Factor-Receptor
EGR-1	Early Growth Response Gene Product 1
ELR	Glutamic acid-leucine-arginine
EMT	Epithelial-Mesenchymal Transition
EndMT	Endothelial-to-Mesenchymal Transition
EPCA	Early Prostate Cancer Antigen
ERK	Extracellular Signal-Regulated Kinase
FAK	Focal Adhesion Kinase
FAP	Fibroblast Activation Protein
FGF-2	Fibroblast Growth Factor 2
FSP	Fibroblast Specific Protein
GDF15	Growth-Differentiation Factor 15
GIST	Gastrointestinal Stromal Tumor
HGF	Hepatocyte Growth Factor
HU	Hydroxyurea
ICK	Intestinal Cell Kinase
IKBKB	Inhibitor of nuclear factor kappa-B kinase subunit beta
IL-1β	Interleukin-1 Beta
MAPK	Mitogen-Activated Protein Kinase
MIC-1	Macrophage Inhibitory Cytokine 1
MIP-2γ	Macrophage Inflammatory Protein 2 Gamma
MMPs	Matrix Metalloproteinases

NAG-1	Nonsteroidal Anti-inflammatory Drug-activated Gene 1
NFκB	Nuclear Factor Kappa B
NG-2	Nerve/Glial Antigen 2
NK	Natural Killer
NO	Nitrous Oxide
PAP	Prostatic acid phosphatase
p53	Protein 53
p63	Protein 63
PDF	Prostate-Derived Factor
PDGF	Platelet Derived Growthfactor
PDGFRα	Platelet Derived Growthfactor Receptor Alpha
PDGFRβ	Platelet Derived Growthfactor Receptor Beta
PI3K	Phosphatidyl-Inositol-3-Kinase
PLAB	Placental Bone Morphogenetic Protein
PLCγ	Phospholipase C-Gamma
PSA	Prostate-Specifik Antigen
PTEN	Phosphatase and Tensin Homolog
PTGF	Placental Transforming Growth Factor
RhoA	RAS Homolog gene family A
ROS	Reactive Oxygen Species
SDF1α	Stromal Derived Growth Factor Alpha
SH-2	Src Homology 2
SHP-2	(SH)2-Containing Phosphotyrosine Phosphatase
shRNA	Short hairpin Ribonucleic Acid
SMAD	Mothers Against Decapentaplegic, Drosophila
SNPs	Single Nucleotide Promoter Polymorphism
SPARC	Secreted Protein Acidic and Rich in Cysteine
Src	Rous Sarcoma Oncogene
TGFβ	Transforming Growth Factor Beta
VEGF	Vascular Endothelial Growth Factor
TMPRSS2	Transmembrane Protease Serine 2
α-SMA	Alpha Smoothmuscle Actin

1 INTRODUCTION

Around 50 000 persons are diagnosed with cancer and approximately 20 000 persons die from cancer in Sweden every year. One out of three persons in Sweden will develop cancer during their lifetime and cancer is the second most common cause of death, following cardiovascular disease. Breast and prostate cancer are the most common cancers in Sweden, making up one third of all cases.

The incidence of both breast and prostate cancer is increasing, as well as skin cancer and lung cancer among women. Some of the increase might be due to an older population, and other explanations are increased smoking among women and higher exposure to the sun. Cervix and gastric cancer are decreasing, likely due to screening for cervix cancer, improved diet and less *Helicobacter pylori* infections. There are a number of known risk factors for cancer. Smoking is the most important risk factor and it is estimated that 20% of all cancer cases can be connected to smoking. Examples of other known risk factors are alcohol, obesity, foodstuff, exposure to chemical or physical carcinogens and virus infections. Also, the risk of developing some cancers can be increased due to genetic predispositions [1].

Cancer is a consequence of genetic alterations, including point mutations, deletions, amplifications, translocations and epigenetic changes. These alterations in turn provide the cells with new capacities which make them able to circumvent their normally strictly controlled homeostasis. Hanahan and Weinberg identified six attributes necessary for tumor development that are shared by most tumors: limitless replicative potential, evasion of apoptosis, self sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis and capacity to invade tissue and metastasize [2].

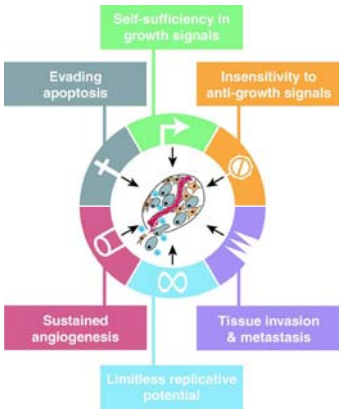

Figure 1. The hallmarks of cancer.
Figure adapted from [2].

Lately, additional hallmarks of cancer have been discussed as being equally important for cancer. The tumor stroma is being increasingly recognized as an important factor for tumor development, progression and metastasis [3].

1.1 PROSTATE CANCER

Prostate cancer is the most common form of cancer in Sweden. Around 9 000 new cases are diagnosed yearly. Approximately 50 percent of the patients are over 70 years of age when they are diagnosed, and the disease is extremely rare before 40 years of age [4].

1.1.1 The prostate gland

The prostate is a gland located under the urinary bladder, surrounding the upper part of the urethra. The prostate produces a secretion containing citrate, acid phosphatase and several proteolytic enzymes (e.g. PSA). This secretion contributes to seminal fluid and raises the pH of semen and improves motility and fertility of sperm.

The prostate gland is small before puberty, but under the influence of testosterone it grows to the size of a chestnut [5].

The prostate gland is composed of glands surrounded by a fibromuscular stroma. The prostate is divided into three anatomical zones: the peripheral zone which contains the main prostatic glands and where most of the glandular tissue is located in the young man, the central zone containing about 25 percent glandular tissue and the transition zone which surrounds the urethra.

Benign prostatic hyperplasia, a condition that leads to obstruction of the urinary tract, occurs mainly in the transition zone, whereas prostate cancer most commonly develops in the peripheral zone [6].

1.1.2 Prostate tumors

Prostate carcinoma is usually an adenocarcinoma that develops from the prostatic glands [7]. Among clinically detected cancers, 70 - 80% arise in the peripheral zone and 15 - 25% are of transition zone origin while only 5 - 10% are assumed to be of central zone origin [8].

Prostate cancer can spread directly to the bladder and adjacent tissues or via the lymphatic or blood vessels to lymph nodes and distant organs [7]. The most common site for prostate cancer metastasis is bone [4].

1.1.3 Epidemiology and genetics

The risk of developing prostate cancer increases with age. Most patients are diagnosed after 65 years of age and the mean age of diagnosis is 72-74 years [9].

Many different environmental factors and their effect on the risk of developing prostate cancer have been studied. Epidemiological studies have shown that tomatoes, selenium, vitamin E and green tea decrease the risk of developing prostate cancer, whereas a high intake of red and processed meat and dietary fat is associated with increased risk [10].

Race can also influence the risk of developing prostate cancer. When analyzing the American population, African-American men were found to have a 2-fold higher risk of prostate cancer as compared to Caucasians, while the risk for Japanese men was 2/3 of the risk of Caucasian men [11].

Hereditary factors also influence the risk to develop prostate cancer. An affected first-grade relative will increase the risk 2-3-fold. The genetic component of hereditary prostate cancer is quite complex, and several different chromosomal regions and loci are known to be involved [9,12]. No single gene can therefore be used to predict risk of developing prostate cancer.

A number of SNPs associated with the risk of developing prostate cancer have been identified. Most variants indicate only a small risk increase, but combining data from several SNPs might provide important information in predicting prostate cancer risk [13].

Somatic genetic aberrations found in prostate cancer include fusions of ETS transcription factors members, the most common variant being TMPRSS2-ERG. ETS fusion genes are found in approximately 60-70 percent of prostate cancers [14].

1.1.4 Detection and grading

The most widely used biomarker for detection of prostate cancer is the serine protease PSA. The expression of PSA is controlled by androgens, and the expression is restricted to the prostate. During normal conditions, PSA is secreted into the seminal fluid and only a very small amount will leak out to the blood stream. Prostate cancer or other diseases of the prostate might cause a release of PSA to the blood. Detection of elevated levels of PSA in serum is therefore used as a tool for diagnosing prostate cancer. Since elevation of PSA is not specific for cancer but can also be caused by other disesases of the prostate, histopathological examination of biopsies are needed for the final diagnosis [15]. In addition to the actual PSA level, also other factors such as PSA density (PSA/prostate volume), PSA velocity (yearly change of PSA level) and PSA doubling time can be used as prognostic markers [16]. The use of PSA screening has been shown to reduce mortality in prostate cancer but the method also generates problems with overdetection and overtreatment [17]. Examples of other biomarkers in the serum are PAP, EPCA and EPCA-2 [16].

Cancer is diagnosed based on morphological analysis of biopsies using light microscopy. If morphological changes are insufficient for a conclusive diagnosis the

biopsies can be further analyzed with immunohistochemistry. Since the basal layer is lost in prostate cancer, detection of basal cells with high-molecular weight keratin or p63 is commonly used as a diagnostic tool. AMACR, which is an enzyme involved in fatty acid metabolism, is sometimes also used as a biomarker for cancer. However, not all cancers are positive for AMACR and the protein is also expressed in some benign lesions [18].

The Gleason grading method is the most established prognostic indicator for prostate cancer. The system is based on histology, where the growth patterns of prostate cancer is analyzed with light microscopy of hematoxylin and eosin stained tissue sections. The growth pattern is graded from 1 to 5 according to glandular achitecture. A Gleason score (from 2 to 10) is obtained by adding the two predominant Gleason grades [19]. Increased Gleason score is associated with a number of prognostic features, such as lymph node invasion, tumor size and stage, with biochemical recurrence after radical prostatectomy and with disease-specific survival in patients on deferred treatment [19,20,21].

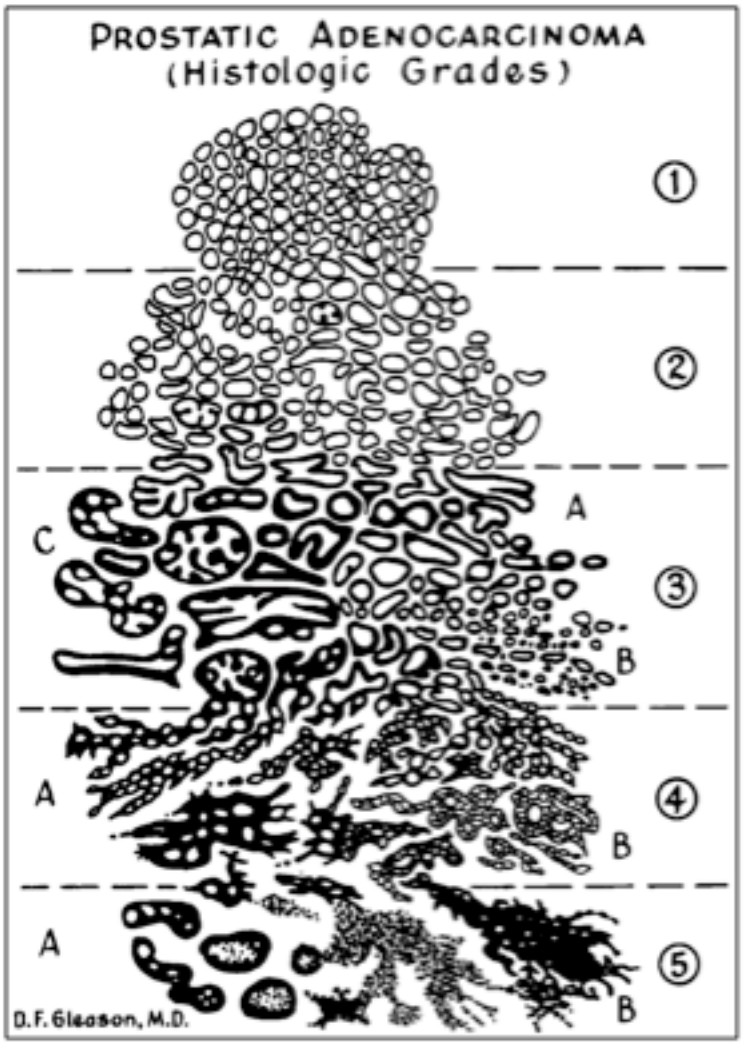

Figure 2. The Gleason grading system.
Figure adapted from [19]

1.1.5 Treatment

In Sweden, older patients with an expected remaining lifetime of less than 10 years, prostate cancer without symptoms is not always treated. Active surveillance, i.e. careful monitoring of disease progress and deferred treatment may be an alternative to immediate therapy in selected cases [18].

Radical prostatectomy, where the prostate is surgically removed, is an efficient way of treating localized disease. Radiotherapy is an alternative treatment, where the prostate is kept intact. In both cases, side effects such as impotence can occur.

Since prostate cancer cells normally are androgen dependent, deprivation of androgen can be used in combination with other treatments in all stages of the disease. This can be achieved, either by reducing the production of testosteron through chemical or surgical castration or by treatment with anti-androgens or estrogen. Most prostate tumors initially respond to this treatment, but eventually advanced cancers usually become androgen independent.

When tumors no longer respond to hormonal therapy, chemotherapy or radiation is commonly used to relieve symptoms [4].

1.2 TUMOR MICROENVIRONMENT

For many years, cancer research has mainly focused on the cancer cells. However, other components of tumors are now becoming established as important factors for cancer growth and progress. These components consist of e.g. blood vessels and pericytes, lymph vessels, cancer associated fibroblasts (CAFs), immune cells, adipocytes and extracellular matrix (ECM) [22,23]. Below follows a short introduction to blood vessels, immune cells and ECM in tumors, and a more thorough description of CAFs.

1.2.1 Tumor vessels

As other tissues, tumors are dependent on vessels to deliver oxygen and nutrients and for removal of waste products. Hence, the ability to recruit vessels is an important and growth limiting step for tumors. The angiogenic process is normally tightly regulated. However, the hypoxic conditions and dysregulated production of angiogenic factors that are common in tumors stimulate angiogenesis [24].

Tumor vessels differ from normal vessels in several ways. They have a chaotic and irregular shape and are often twisted and leaky. New vessels are also constantly formed [24,25]. Experimental data have shown that endothelial progenitor cells are recruited

from the bone marrow and contribute to tumor vessels and affects tumor growth [26,27]. Besides endothelial cells, tumor vessels have been reported to contain also cancer cells, giving rise to what is referred to as mosaic vessels [28]. There are also reports of genetic instability and altered gene expression in tumor vessels [29,30].

Apart from being a requisite for tumor progression, vessels are also a common route for tumors to metastazise.

Targeting of tumor angiogenesis by the use of VEGF-inhibitors are in clinical use for treatment of renal, breast, colorectal and lung cancers [31,32,33,34].

Pericytes are perivascular cells, known to be involved in for example vessel maturation and remodelling [35]. Tumor pericytes are different from their normal counterparts. Tumor vessels are commonly covered by fewer pericytes, which are more loosely attached. The expression of markers also differs from that of pericytes in normal tissues [36].

The significance of pericytes in cancer is not clear. Some studies have demonstrated that reduced pericyte coverage in tumor vessels is associated with an increased risk of tumor metastasis [37,38]. Other studies show that increased coverage of pericytes on tumor vessels enhances tumor growth [39,40].

1.2.2 Immune cells

The association between cancer and inflammation is substantiated in many different tumor types. Inflammation leads to accumulation of various immune cells, contributing to cancer in various ways. How different immune cells influence tumors is not completely sorted out. A polarization of immune cells in tumors, leading to a more pro-tumorigenic phenotype, is commonly observed [41]. The literature concerning immune cells and cancer is vast and complicated and below follows a few examples of the involvement of immune cells in cancer.

Some immune cells, such as cytotoxic T-cells and NK cells are usually associated with targeting and suppression of malignant cells, whereas others such as mast cells, myeloid cells, granulocytes and macrophages can enhance tumor growth [42]. Macrophages have also been shown to be involved in tumor metastasis [41].

Presence of macrophages in tumor tissue is correlated with bad prognosis in many cancers such as breast, skin and cervix cancers [43,44,45]. However, in colon cancer, a high infiltration of macrophages is associated with a better outcome [46]. It is possible that markers with the ability to distinguish between different macrophage subsets would sort out these contradictory results.

1.2.3 The ECM

The ECM is mainly composed of proteoglycans, collagens, hyaluronan, fibronectin, elastin and laminin. The role of the ECM is to provide e.g. support, structure and anchorage for cells and to sequester proteins such as growth factors. In the normal tissue, degradation and production of ECM components are tightly regulated. When tumors grow and invade into tissues, the ECM is degraded by proteases produced by cancer cells and stromal cells, leading to release of proteins that can influence tumor growth and progression [47].

The basement membrane (BM) is a form of ECM, surrounding the epithelium of tissues and thereby organizes the epithelial layer and forms a barrier to the neighboring tissue. A tumor is defined as being invasive when the BM is degraded and the cancer cells infiltrate the stroma. The cleaved fragments from the BM are known to regulate angiogenesis [48,49].

1.2.4 CAFs

Stromal changes occurring during the development of many solid tumors is a well known phenomenon. These changes often consist of an increased fibroblast proliferation and production of ECM constituents. The fibroblasts in a tumor, often termed CAFs, have a specific phenotype including the expression of stress fibers and a number of markers such as FAP, FSP, α-SMA, PDGFRα, PDGFRβ and NG-2 [50,51]. The CAF population is heterogenous and it is likely that a number of different subsets of CAFs exist [52].

The specific phenotype of CAFs is very similar to fibroblasts observed in wound healing and fibrosis. The prominent function of fibroblasts in wound healing is to generate ECM components and contracting the wound. In response to wounding, fibroblasts are recruited and acquire the activated phenotype. After the wound is healed, the normal phenotype is restored. However, during tumorigenesis, they stay constantly active. This has led to the concept of tumors as "wounds that do not heal" [53].

1.2.4.1 Recruitment of CAFs

The origin of CAFs is not completely clear. One suggestion is that fibroblasts already present in the tissue are converted to the activated phenotype by signals from other tumor compartments [54]. Different growth factors, such as TGFβ, PDGF, FGF-2 and hedgehog are thought to be involved in the activation of fibroblasts [55]. The effects of TGFβ and PDGF are thought to have the greatest influence, and their effect on tumor-stroma interactions have been extensively studied.

Some experimental studies, where transplanted bone marrow genetically different from the host was used, also indicate that a proportion of the CAF population is originating from the bone marrow [56]. Additional support for this hypothesis is provided by the

finding that human bone marrow derived mesenchymal stem cells obtain a CAF-like phenotype and CAF-properties when exposed to conditioned medium from cancer cell lines [57].

EMT and EndMT are other suggested sources of CAFs. Evidence for the contribution

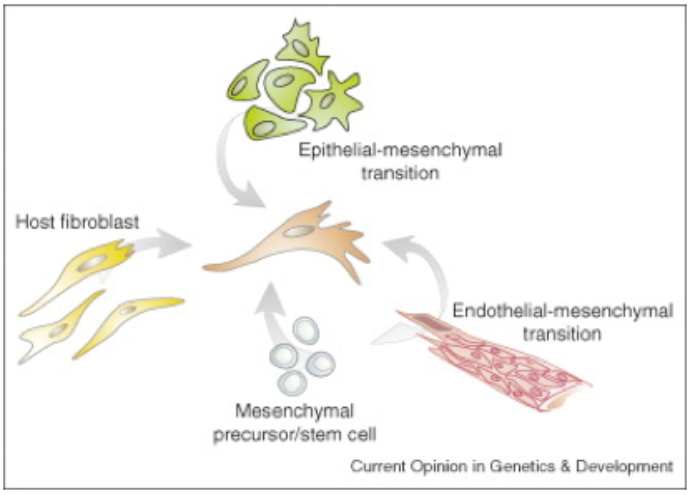

Figure 3. Suggested origins of CAFs. Figure adapted from [51].

of EMT to the fibroblast population have been shown in renal fibrosis models and support for EndMT has been found both *in vitro* and in animal tumor models [58,59,60].

1.2.4.2 Genetic and epigenetic characterization of CAFs

The non-malignant cells of the tumor have generally been considered to be genetically stable. However, the observation that the CAF phenotype is maintained in the absence of cancer cells motivated analysis of the genetic and epigenetic profile of CAFs from different tumors [61].

Some studies indicate that fibroblasts in tumors might carry genetic alterations. Stromal cells in breast cancer were shown to have p53 mutations associated with allelic imbalance, loss of heterozygosity and lymph node metastasis. Genetic alterations in breast cancer were also shown to have stronger associations with clinical characteristics than alterations in the cancer cells, suggesting that genetic analysis of stromal cells might have clinical relevance in breast cancer [62,63]. Also, analysis of stroma from head- and neck tumors from smokers revealed genetic alterations correlating with clinical parameters [64].

In contrast, Allinen et al report genetic alterations exclusively in the epithelial cells of breast cancer tissue [65]. Analysis of a set of breast and ovarian cancer tissues could not detect any genetic aberrations in stromal cells and similar results were found when pancreatic CAFs were analyzed [66].

Epigenetic changes in CAFs could be another explanation for the stable CAF-phenotype. Indeed, epigenetic changes in stromal cells from breast and prostate cancer have been demonstrated [67,68].

1.2.4.3 Experimental support for various roles of CAFs in tumor growth

Several different functional studies have shown the importance of CAFs in initiation, promotion and metastasis of cancer. CAFs have also been shown to influence the sensitivity of tumors to anti-cancer therapies and stem cell properties of cancer cells. Below follows a short description of a number of selected studies.

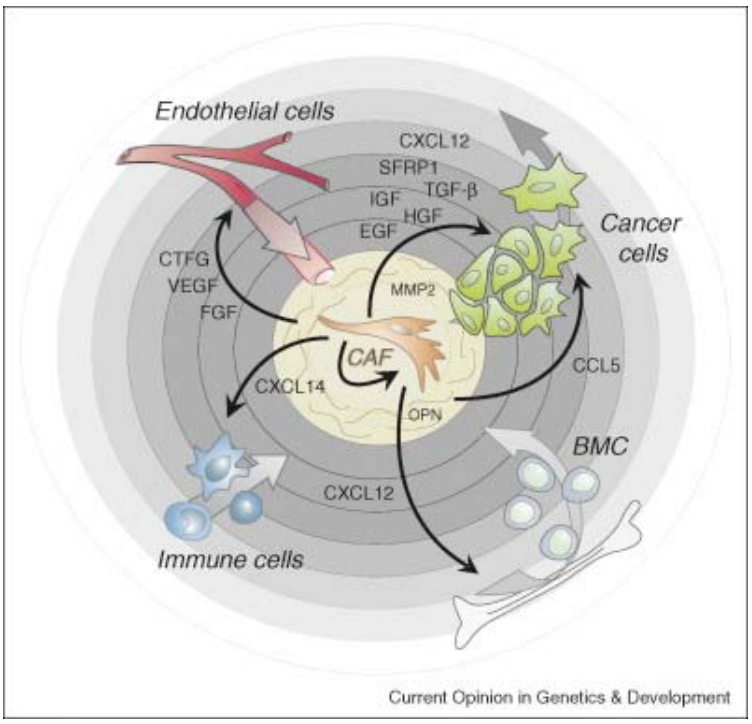

Figure 4. Effects of CAFs on the tumor microenvironment. Figure adapted from [51]

Fibroblasts and tumor initiation

In chicken infected with the Rous sarcoma virus, a retrovirus carrying the *src* oncogene, tumors could only be observed at the sites of injection. The oncogene itself was not sufficient for tumor formation, but wounding and activation of the fibroblasts was necessary. This provides evidence that the tumor microenvironment supply the epithelial cells with factors essential for tumor formation [69].

In another study, the mammary fat pads of mice were cleared from epithelial cells in an early stage of development. The mice were then irradiated before the epithelial cells were reintroduced. Compared to the non-irradiated group, the irradiated mice developed a significantly higher number of tumors, indicating that radiation-induced damage of stromal cells influence tumor formation [70].

There are also various different co-injection models where cancer cell-lines are combined with fibroblasts before injection into immunosuppressed mice. In one study, five different human low-malignant cancer cell lines were injected alone, or combined with mouse fibroblasts before injection. The addition of fibroblasts reduced the latency and increased the frequency of tumors [71]. Subsequent experiments analyzed the effect of combining human cancer cells with fibroblasts of different origin. The conclusion was that different fibroblasts vary in their potential to promote tumor growth [72]. In another study, the properties of human primary prostate cultures of CAFs and normal fibroblasts were evaluated. The combination of CAFs and initiated prostate epithelial cells displayed a dramatic tumor growth as compared to when combined with normal fibroblasts [73]. A similar experiment was performed with CAFs and normal fibroblasts isolated from human breast tissue, also indicating a cancer promoting effect of CAFs [74].

Genetic approaches have also been used to investigate the importance of stromal factors in tumorigenesis. Knocking out the TGFβ receptor II specifically in fibroblasts gave mice that developed spontaneous tumors in the prostate and forestomach after a few weeks, indicating a disturbed paracrine signaling between the fibroblasts and the epithelium [75]. Another study report decreased tumor latency and increased tumor incidence in p53 knock-out mice as compared to p53 wild type mice after injection of mammary cancer cells, indicating that the genetic status of stromal cells affect tumor formation [76]. A recent study show that fibroblast-specific deletion of PTEN leads to remodeling of ECM, increased angiogenesis and immune cell infiltration and increased tumor growth in a mouse mammary carcinoma model. This effect was associated with upregulation of the transcription factor Ets2 following PTEN loss in fibroblasts [77].

Effects of CAFs on epithelial cells

The role of CAFs in tumor metastasis

CAFs have also been suggested to contribute to tumor metastasis. Bone marrow derived mesenchymal cells were able to enhance metastatic potential of breast cancer cells [78]. This effect was mediated by CCL5, a chemokine produced by the mesenchymal cells in response to cancer cell stimulation. Further evidence for the importance of stromal factors comes from experiments with mice lacking the gene encoding FSP1, a protein known to be upregulated in CAFs. This study demonstrates that FSP1 knock-out mice display a lower tumor frequency than wild type mice when inoculated with cancer cells and the tumors do not metastasize. Moreover, if cancer cells are co-injected with FSP1 wild type fibroblasts, the tumor formation is enhanced and the ability to metastasize is restored [79]. Also, pancreatic stellate cells increased the tumor forming and metastatic potential of cancer cells in a co-injection model [80].

CAF-regulation of stem cell traits in cancer cells

Stromal cells were recently described to be capable of affecting stem cell features of cancer cells. In this study, colon cancer stem cells were characterized by high activity of the Wnt-pathway. CAFs enhanced the cancer cell Wnt-signaling and CAF-produced factors were able to re-install a stem cell phenotype in differentiated cancer cells. The finding that nuclear β-catenin could be detected in cancer cells surrounded by fibroblasts in human colorectal cancer suggests that this might occur also in human cancer [81].

Effects of CAFs on drug sensitivity

Pancreatic stellate cells have been shown to be able to reduce the cancer cell sensitivity to drug and radiation therapy [80]. Another study supporting this data shows that coculture with fibroblasts reduced drug sensitivity of pancreatic cancer cells in an IL-1β and NO dependent manner [82]. In breast cancer, tumor fibroblasts have been shown to influence the tamoxifen sensitivity of cancer cells [83]. A recent study demonstrated the involvement of fibroblasts in inducing tyrosine kinase-inhibitor resistance in lung cancer cells with activating EGF-R mutations. The fibroblast-induced resistance could be abolished by inhibiting HGF-signaling [84].

Effects of CAF produced factors on other tumor compartments

CAFs can influence tumor development and progression in many different ways. Besides being able of stimulating cancer cells, they also exert effects on tumor vessels, immune cells and the ECM [23,51].

Concerning the effect of CAFs on angiogenesis, fibroblasts and inflammatory cells are a major source of VEGF, which is a crucial factor in inducing tumor angiogenesis [85,86]. Another important component, known to be involved in the tumor-stroma interactions is SDF1α. SDF1α is produced by the CAFs and can promote mammary epithelial proliferation, migration and invasion [65]. This factor was further investigated in a study where CAF-produced SDF1α was blocked with a neutralizing antibody. This resulted in a greatly attenuated tumor growth, caused by reduced angiogenesis. The proposed mechanism for this effect was that SDF-1 assists in recruiting endothelial progenitor cells to the tumor [74]. Moreover, CAFs have recently been shown to have the capacity to induce resistance to anti-angiogenic treatment by producing PDGF-CC [87].

It has also been demonstrated that CAFs in different mouse and human tumors express a pro-inflammatory expression profile. The study also shows that CAFs are involved in the recruitment of inflammatory cells and tumor vessels in an NFκB dependent manner, and that this effect is an early event in tumor development [88]. CAFs have also been shown to interact with NK cells in tumors and to inhibit their cytotoxic ability [89].

Another study suggest that depletion of stroma-derived S100A4 affects the number of infiltrating T-cells in tumors which in turn influence the metastatic potential of cancer cells [90].

CAFs are also known to produce various ECM-components and different proteolytic enzymes like MMPs, serine proteases and cathepsins. Proteases are able to modify the tumor microenvironment and thereby facilitate migration and invasion of cancer cells [91,92,93]. An *in vitro* study shows how fibroblasts promote cancer cell invasion in the ECM by creating tracks in which the cancer cells can follow [94].

1.2.4.4 Prognostic and response-predicative information in tumor stroma

The possibility of deriving prognostic information from tumor stroma has been evaluated with e.g. genetic profiling, gene expression profiling and immunohistochemstry.

As mentioned before, genetic alterations in stromal cells of head and neck and breast cancers have been shown to associate with clinical characteristics [63,64].

Gene expression analysis of microdissected stroma from a large set of breast tumors identified a set of stroma-genes which could predict patient outcome, independently of known prognostic factors [95]. Also, a wound healing-signature derived from serum-stimulated fibroblasts was shown to associate with increased risk of metastasis in breast cancer patients [96]. Furthermore, a recent study demonstrates response-predicative capacities of stroma-associated genes. High expression of stromal genes was associated with resistance to chemotherapy in breast cancer patients [97].

Immunohistochemical analysis of breast cancer tissue shows that high stromal expression of PDGFRβ correlates with worse patient outcome [98]. Another prognostic marker in breast cancer stroma is Cav-1. Absence of Cav-1 in breast tumor stroma was recently shown to associate with poor clinical outcome [99]. Analysis of AR expression in prostate stroma demonstrated that low expression of the receptor in tumor and non-malignant tissue was associated with bad prognosis [100]. Moreover, a high abundance of fibroblasts, identified by αSMA immunoreactivity, correlates with disease recurrence in colon cancer patients [101]. Similarly, immunohistochemical analysis of stromal FAP expression in colon and pancreatic cancers revealed that high expression of FAP was associated with bad prognosis [102,103]. For pancreatic cancers, stromal expression of SPARC is also correlated with worse outcome [104].

Besides from tissue based analysis of prognostic information, detection of cleaved basement membrane fragments, such as endostatin, in serum has also been suggested as possible stroma-derived biomarkers [49].

Moreover, in a study where the migratory behavior of skin fibroblasts from patients with breast cancer was analyzed, an association between abnormal migratory behavior

and family history of breast cancer was found [105]. This finding suggests that a certain mesenchymal genotype might predispose for development of cancer. To further explore this finding might provide information about risk of developing cancer.

1.2.4.5 CAFs as potential drug targets

Since CAFs are important for many aspects of cancer it would be interesting to explore the possibility of therapeutic targeting of CAFs. CAFs are also likely to be more genetically stable than cancer cells, and might therefore not be as prone to develop resistance.

Targeting of CAFs could be accomplished either by interrupting CAF-derived, tumor promoting signals or by direct inhibition of the cells.

Immuno-therapeutic approaches of CAF-targeting have been rather efficient in experimental models. By inducing an anti-FAP immune response, tumor growth and metastasis of breast and colon cancer cells could be inhibited and tumor drug uptake was increased [106]. Also, treatment of xenograft tumors with a therapeutic immunoconjugate targeting fibroblasts inhibited tumor growth [107]. In addition, inhibition of FAP reduced tumor growth in mouse models of lung and colon cancer and the tumors demonstrated a decreased number of CAFs and vessels [108]. A FAP antibody has also been tested in patients with colon cancer, demonstrating tumor-specific location and almost no side effects [109].

PDGF is an important factor for fibroblast growth and recruitment. Effects of PDGF inhibition on tumor growth have been studied in several experimental settings. Treatment with PDGF inhibitors reduces the interstitial fluid pressure (IFP) and increase tumor drug uptake. Also, in a stroma-rich cervical cancer model, imatinib treatment reduced tumor growth and tumors demonstrated impaired angiogenesis and less pericyte coverage of vessels [110]. Moreover, imatinib combined with cytostatic treatment reduced growth and metastasis of colon cancer cells [111]. However, since imatinib is not only targeting PDGF-signaling, contributing effects by inhibition of other pathways cannot be excluded. Nevertheless, the finding that PDGFRβ expression in stroma of human breast tumors is associated with bad prognosis motivates further studies of inhibition of PDGF-signalling and stromal cells in human cancers.

In addition to PDGF, TGFβ is another growth factor that is important for fibroblast recruitment and activation. Fibroblast-specific knock-out of TGFβ receptor II resulted in the development of tumors in the prostate and forestomach, demonstrating that disturbance of stromal signals is sufficient to cause spontaneous cancer growth [75]. TGFβ is known to have both tumor inhibiting and promoting traits, depending on e.g. tumor stage and context, hence targeting of TGFβ in cancer might have undesired effects [112].

A recent study shows that inhibition of hedgehog signaling specifically in the stroma lead to reduced xenograft growth, proposing the hedgehog signaling pathway as a potential stroma drug target [113]. Moreover, targeting of stromal cells by inhibiting hedgehog signaling improves drug uptake in a pancreatic cancer model [114].

It is likely that CAF targeting drugs would not be used as a single treatment, but rather in combination with conventional treatments. As mentioned earlier, CAFs are known to interfere with cancer cell drug sensitivity and to integrate CAFs in the process of developing new cancer therapies might be very useful. An interesting recent study show how CAFs can be included in drug screening and the study also identifies a drug with increased activity in the presence of stromal cells [115].

1.3 INTRODUCTION TO PDGF, CXCL14 AND GDF15

1.3.1 PDGF

There are five different isoforms of PDGFs (PDGF-AA, -AB, -BB, -CC, and –DD), and they signal via PDGF α- and β-receptors. They are secreted as disulfide-bonded dimers and contain a conserved motif of eight cysteine residues. The PDGF α- and β-receptors consists of an extracellular domain controlling ligand binding and receptor dimerization, a transmembrane domain and an intracellular kinase domain. *In vitro* data show ligand-receptor interactions between PDGFRα and PDGF-AA, PDGF-BB, PDGF-AB and PDGF-CC. PDGFRβ interact with PDGF-BB and PDGF-DD and the heterodimeric receptor binds to PDGF-AB and PDGF-BB [116]. Upon ligand binding, the receptor will dimerize and autophosphorylate. This in turn leads to recruitment and activation of SH-2 domain containing proteins such as PI3K, Src, SHP-2 and PLCγ, finally resulting in cellular responses such as proliferation, migration and survival [117].

PDGFs are potent growth factors, stimulating primarily cells of mesenchymal origin such as pericytes, fibroblasts, smooth muscle cells and macrophages [118]. Besides important regulatory functions during embryogenesis, PGDFs are also involved in wound healing processes and regulation of IFP in tissues [119,120,121].

As with many other important growth factors, dysregulation of PDGFs are found in different malignancies.

Mutations and translocations affecting PDGF receptors and ligands are not commonly found in cancer, but some examples are known. DFSP, a rare sarcoma affecting the skin, is caused by fusion of the PDGFB and COL1A1 genes [122,123,124]. GISTs are mesenchymal, gastrointestinal tumors, most commonly found in the stomach. In approximately 10% of GISTs, activation mutations of PDGFRα are found [125]. Fusion of the PDGFRβ gene and the TEL gene is found in approximately 30% of all

CMML cases [126]. Also other fusions of PDGF receptors with various genes have been identified [127]. In glioblastoma, amplifications of the PDGFRα gene are found in 5-25% of all cases [128,129,130].

PDGF receptors and ligands are known to be involved in tumor angiogenesis, mainly by mediating the recruitment of pericytes to the vessels. Pericytes are important for vessel stability and PDGF-mediated vessel recruitment has been shown to accelerate tumor growth [39,40]. Expression of PDGFRβ is a common feature of pericytes in most solid tumors [98]. PDGF receptor expression has also been shown in endothelial cells from experimental tumors and metastasis [80]. It can however be considered that the endothelial expression might rather reflect a perivascular cell expression.

PDGF is also a strong chemo-attractant and mitogen for fibroblasts [131]. The ligand is produced by the cancer cells but acts mainly in a paracrine fashion to stimulate cells in the tumor stroma. Cancer cells are considered not to respond to PDGF stimulation. PDGFRβ is commonly expressed in tumor stroma and receptor expression has been shown to associate with prognosis in human breast cancer [98]. There is a great deal of experimental data supporting the role of PDGF in stroma formation. Mice injected with melanoma cells overexpressing PDGF-B obtain tumors with a higher content of stroma as compared to control mice, and similar results have been shown with keratinocytes [132,133]. PDGF has also been shown to be important for the development of desmoplasia in a xenograft model [134].

Another feature of solid tumors where PDGF is known to be involved is in the regulation of IFP [135]. Treatment with PDGF antagonists in tumor models results in a reduction of IFP and enhanced drug uptake [136,137,138,139].

Imatinib, sunitinib and sorafinib are FDA approved drugs inhibiting PDGF signaling. They are not specific for PDGF receptors, but target also a number of other tyrosine kinases. Treatment of GIST, CMML and IHES with imatinib has shown good results. In addition, promising results from treatment of glioblastoma with HU and imatinib have been reported [129].

1.3.2 CXCL14

CXCL14, also referred to as BRAK, MIC-1, MIP-2γ or KS1, is a member of the CXC chemokine family. CXCL14 however lacks the N-terminal ELR motif and would therefore be predicted to inhibit angiogenesis. The CXCL14 receptor remains to be identified [140].

CXCL14 is expressed in most normal tissues, including heart, placenta, lung, brain, liver, pancreas, skeletal muscle and kidney. The expression of CXCL14 is however absent in many cancer cell lines[141]. *In situ* hybridization of tumor and adjacent non-malignant tissue from several different organs show expression of CXCL14 primarily

in the epithelium of normal tissues and in the stroma of cancer tissues. Hence, the epithelial expression of CXCL14 is commonly lost during cancer progression, whereas the expression is induced in stromal cells [140,142]

Biological activities of CXCL14 include chemotactic activity on activated monocytes, immature dendritic cells and activated NK cells [142,143].

Concerning the effects of CXCL14 on cancer growth, present information is contradictory. Some studies indicate a growth-inhibitory function of CXCL14, whereas others point to a tumor promoting effect.

Data supporting a tumor inhibitory role of CXCL14 includes the absence of CXCL14 expression in cancer cells and a number of studies also show decreased tumor growth as an effect of CXCL14 stimulation in different xenograft models. For example, CXCL14 overexpression in prostate cancer cells has been shown to reduce tumor growth [143]. Also, expression of CXCL14 in oral cancer cells inhibited tumor growth [144]. Moreover, treatment of oral cancer cells with EGF has been shown to reduce CXCL14 expression and the same group also demonstrated that treatment of head and neck cancer cell lines with the EGF-inhibitor gefitinib increase the levels of CXCL14 and inhibit xenograft growth [145,146]. Furthermore, epigenetic silencing of CXCL14 is frequently found in human lung cancer samples, and restoration of CXCL14 expression reduces growth of lung cancer xenografts [147].

As mentioned before, CXCL14 is lackning an ELR motif, and is therefore expected to inhibit angiogenesis. Some experimental data support this hypothesis and demonstrate that CXCL14 has anti-angiogenic effects by inhibiting endothelial cell migration [142].

Since CXCL14 is a potent chemoattractant for immune cells, loss of CXCL14 has also been suggested as a way for tumors to escape immune surveillance.

Other studies instead suggest CXCL14 to stimulate tumor growth. Many of these studies demonstrate an increased mobility and proliferation of cancer cells upon CXCL14 stimulation. CXCL14 is upregulated in myoepithelial cells of breast tumors and stimulate proliferation, migration and invasion of breast cancer cells [65]. A recent paper shows that reactive oxygen species induce CXCL14 expression in breast cancer cells via AP-1 and promote their migratory and invasive properties [148]. In pancreatic cancer tissue, upregulation of CXCL14 is commonly found and CXCL14 stimulation increased the invasive capacity of pancreatic cancer cells *in vitro*. Interestingly, the CXCL14 expression was frequently located at the invasive front of tumors, which might suggest a role for CXCL14 in metastasis mechanisms [149]. In fact, CXCL14 is included in a set of genes that can predict time for metastasis of breast cancer [150]. Moreover, upregulation of CXCL14 in papillary thyroid carcinoma is associated with mutational activation of BRAF and lymph node metastasis [151].

The conflicting literature of CXCL14 might be explained by the different settings and models that are used in the different studies. Receptor identification and subsequent analysis of receptor expression might help in resolving this. The role of CXCL14 in cancer is likely context dependent, and microenvironment and cancer cell type are likely to influence the effect of CXCL14 on tumor growth.

1.3.3 GDF15

GDF15, also designated MIC-1, PTGF, PLAB, PDF and NAG-1, is an orphan member of the BMP family of proteins that is included in the TGFβ superfamily [152]. GDF15 was originally cloned from myelomonocytic cells with the aim of finding genes associated with macrophage activation [153]. GDF15 is first produced as an inactive pro-peptide, but after disulfide-linked dimerization of the precursor the pro-peptide is cleaved by a furin- like pro-protein convertase to release the C-terminal mature GDF15 protein (112 aa) [152].

GDF15 expression is induced in response to the MAPK signaling pathway [154]. GDF15 is also induced and secreted as a response to p53, which might suggest GDF15 to be a paracrine mediator of p53 signaling [155]. Also other transcription factors, for example EGR-1, have been shown to stimulate GDF15 expression [156]. The downstream signaling of GDF15 is not completely sorted out, but there are reports of SMAD, AKT and ERK activation [152].

Under normal physiological conditions, GDF15 is only expressed at high levels in the placenta [157]. However, in response to stress such as injury, inflammation and cancer, GDF15 expression is increased [157,158,159].

Like with other members of the TGFβ superfamily, the role of GDF15 in cancer is not completely clear. Some studies suggest an antitumorigenic capacity of GDF15, whereas others report tumor-supporting effects.

The anti-tumor effects of GDF15 often consist of p53 dependent or independent induction of apoptosis and growth arrest. Several *in vitro* studies and tumor xenograft models report decreased growth of cancer cell lines and xenografts when the cancer cells overexpress GDF15 [152]. GDF15 has also been shown to inhibit angiogenesis [160].

Support for a tumor-stimulatory role of GDF15 can be found in a number of studies. GDF15 is expressed in many different cancer cell lines such as breast, pancreas, colon and prostate [159]. Upregulation of GDF15 is also found in many melanoma cell lines and GDF15 shRNA can reduce the tumorigenicity of melanoma cells [154]. Increased levels of GDF15 in cerebrospinal fluid have been shown to be a marker for glioblastoma, and are also correlated with worse outcome of the disease [161].

Furthermore, overexpression of GDF15 can be found in metastatic prostate, breast and colon tumors, which in turn leads to increased levels of GDF15 in the serum [159]. Serum levels of GDF15 have also been shown to correlate with prostate cancer stage, prognosis and presence of bone metastasis [162,163]. Reports of increased serum levels of GDF15 in response to cancer progression have also been shown in colon cancer and pancreatic cancer [164,165].

Another recent study report an increased actin reorganization and phosphorylation of FAK and Rho A as an effect of GDF15 overexpression in cancer cells, which in turn leads to an increased motility and metastatic capacity of the cancer cells [166].

GDF15 has also been shown to be upregulated in human prostate cancers in response to chemotherapy. Overexpression of GDF15 in prostate cancer cell lines or treatment with recombinant GDF15 induced resistance to chemotherapy [167]. Additional *in vitro* data report that GDF15 protect cells against chemotherapy in a p53-dependent manner, by inducing the PI3K/Akt signaling pathway [168].

SNP analysis of the GDF15 gene revealed a polymorphism consisting of a variation from a histidine (H) to an aspartic acid (D) at position 6 in the GDF15 protein. The homozygous histidine variant is most common (54%), thereafter the heterozygots (39%) and least common is the homozygous aspartic acid variant (7%) [169]. These different variants seem to influence the risk of developing cancer. Whereas presence of the D allele is associated with better survival in an analysis of 200 colon cancer patients, the H allele was found to be associated with an increased risk for prostate cancer in a study including 1383 prostate cancer patients and 700 controls [164,170].

2 PRESENT INVESTIGATION

Aims:

- To identifiy new diagnostic biomarkers for prostate cancer
- To find potential prognostic markers and therapeutic targets in prostate cancer stroma

Results

Paper I

Diagnostic biomarkers of prostate cancer

Since the sensitivity and specificity of currently used biomarkers for diagnosing prostate cancer is not optimal, identification of additional biomarkers would facilitate diagnosis in difficult cases.

With the aim of finding markers of diagnostic relevance in prostate cancer, a screening was performed in the HPA atlas. The screening resulted in a number of candidates from which three novel proteins proteins, CYCS, ICK, IKBKB, were selected for further analysis. In addition, AMACR that is an already known diagnostic marker was also analyzed.

Expression of these proteins was analyzed with immunochistochemical staining of a TMA containing 40 prostate specimens with benign tissues, precursor lesions and invasive carcinoma represented from the same cases. Staining intensity and extent was then scored. In addition, expression of all four genes was evaluated with qRT-PCR on material isolated from fresh frozen tumor and benign prostate tissue from 32 independent patients.

The immunohistochemical analysis demonstrated that all four biomarkers had an increased expression in prostate cancer as compared to non-malignant tissue. The best prognostic performance was achieved with IKC and AMACR.

The qRT-PCR analysis could confirm upregulation in cancer of AMACR. However, upregulation of CYCS, ICK and IKBKB could only be detected in a minority of cases.

In summary this study shows that AMACR is a useful diagnostic tissue marker for prostate cancer, but CYCS, ICK and IKBKB might be useful as additional diagnostic markers.

Paper II

Stromal PDGFRβ expression in prostate tumors and non-malignant prostate tissue predicts prostate cancer survival

The possibility to acquire prognostic information from tumor stroma is being increasingly explored and many stroma-derived tissue-biomarkers have been identified. High PDGFRβ expression in breast cancer stroma has been shown to associate with poor outcome of patients and to further explore this finding in other tumors might provide new prognostic tools.

In this study, we analyzed the expression of PDGFRβ with immunohistochemistry on a TMA containing tumor and non-malignant tissue from more than 300 prostate cancer patients. The staining intensity was scored independently in stroma and around the vessels and the results were associated with histopathological data

Expression of PDGFRβ was primarily found in the fibromuscular stroma and in perivascular cells.

The perivascular PDGFRβ expression in tumor tissue was found to correlate with advanced stage, increased tumor vessel density and high Gleason score. Moreover, the perivascular PDGFRβ expression in non-malignant tissue correlated with increased epithelial cell proliferation.

PDGFRβ expression in non-malignant and tumor fibromuscular-stroma was associated with large tumor size, advanced stage, high Gleason score and reduced cancer-specific surivial. In addition, the PDGFRβ expression in non-malignant stroma was also associated with increased proliferation of epithelial cells whereas PDGFRβ expression in tumor stroma was associated with high vessel density.

Overall, this study identifies a number of previously unidentified associations between stromal PDGFRβ expression and clinical parameters, including Gleason score and survival.

Paper III and IV

CXCL14 is an autocrine growth factor for fibroblasts and acts as a multi-modal stimulator of prostate tumor growth and **Local and systemic pro-tumorigenic effects of fibroblast-derived GDF15**

CAFs and other cells in the tumor microenvironment are known to interact with the malignant cells and affect tumor growth, progression and metastasis. A number of studies have also shown that CAFs influence drug sensitivity of cancer cells and tumor drug uptake. Further characterization of CAFs and identification of proteins mediating these effects might provide new ways of inhibiting tumors.

To determine gene-expression differences between normal fibroblasts and CAFs, we performed microarray analysis comparing normal fibroblasts and CAFs isolated from prostate cancer tissue.

CXCL14, an orphan chemokine, was found to be 40-fold upregulated in prostate CAFs. Another transcript, GDF15 was up-regulated 8-fold. Expression of CXCL14 and GDF15 was analyzed with qRT-PCR in additional prostate cancer tissues and upregulation of both genes were confirmed in six of eight cases.

To evaluate the functional importance of these genes, we generated fibroblasts overexpressing CXCL14 or GDF15. Overexpression of CXCL14 in fibroblasts increased their proliferation and migration and co-culture with fibroblasts overexpressing CXCL14 stimulated growth and migration of prostate cancer cells. The effects on cancer cells could however not be recapitulated by stimulation with recombinant CXCL14, indicating that the effects on cancer cells is not triggered by CXCL14 but rather some factor/s produced by fibroblasts in response to CXCL14 treatment. Furthermore, co-injection of fibroblasts overexpressing CXCL14 with prostate cancer cells in mice increased xenograft growth as compared to when cancer cells were combined with control fibroblasts. The xenograft tumors were characterized by increased vessel density and infiltration of macrophages. These results were supported by experiments showing that CXCL14 increased migration of monocytes and that fibroblasts overexpressing CXCL14 enhanced endothelial cell recruitment.

Similarly, GDF15 overexpression in fibroblasts increased growth of fibroblasts and enhanced proliferation, migration and invasion of prostate cancer cells. Also, GDF15 overexpression in fibroblasts promoted xenograft tumor growth and stimulated tumor growth at a distant site.

These studies identify stroma-derived CXCL14 and GDF15 as novel tumor promoting factors.

Conclusions and future perspectives

Paper I

The conclusion from the first paper is that AMACR is a good tool for detection of prostate cancer, but the other markers might help in cases where AMACR fails.

The discrepancy between the results obtained with immunohistochemistry and qRT-PCR might depend on different things. Either the two sets of patients differ with regard to their expression of the markers or the protein levels are not corresponding to mRNA levels. A very local increase of expression might be diluted in the qRT-PCR analysis and marker expression in the benign stroma could further obscure the data. For this purpose, immunohistochemical analysis is probably superior to qRT-PCR analysis since information about protein location and distribution can be obtained.

Since all four proteins were found to have increased expression in prostate cancer, further functional studies might be of interest.

Also, to further explore the potential of CYCS, ICK and IKBKB in cases that are very challenging to diagnose would be interesting. One of the analyzed patients was negative for AMACR but positive for the other markers, indicating that the CYCS, ICK and IKBKB might be used as a complements to AMACR.

Paper II

Expression of PDGFRβ in tumor and non-malignant stroma of prostate tissue was associated with a number of clinical charactersitics and shorter cancer specific survival.

In the present study we have analyzed PDGFRβ expression. To instead analyze receptor activation might further improve the prognostic capacity. This could be achieved by using a recently developed technique, *in situ*-PLA [171,172].

The finding that PDGFRβ expression also in non-malignant prostate tissue was interesting and might be useful since biopsies sometimes captures non-malignant tissue.

It would also be interesting to understand if PDGFRβ is expressed in normal tissue also in other organs. If this is the case, it is possible that these patients carry a specific mesencymal phenotype and that this phenotype might predispose for prostate cancer and possibly also for other cancers. This topic merits further investigation.

Paper III

CXCL14 was found to stimulate proliferation and migration of fibroblasts. Fibroblasts overexpressing CXCL14 was able to enhance growth and migration of prostate cancer cells and increase growth of prostate cancer xenografts.

It is possible that macrophages contribute to some of the effects seen in the xenograft experiment and also the matrigel plug assay, such as stimulation of angiogenesis. *In vitro* analysis of the effect of conditioned medium from CXCL14 overexpressing fibroblasts on endothelial cells might provide more information about this.

Our data indicate that the effects of CXCL14 on cancer cells and angiogenesis is not caused by the CXCL14 producing fibroblasts directly but rather by factors produced by fibroblasts as a response to CXCL14 stimulation. In this model, the tumor and angiogenesis promoting effects of CXCL14 are likely to be fibroblast-dependent. Less stroma-dependent models, including other call lines might provide different results.

Identification of the CXCL14 receptor would help to further investigate the context dependence of CXCL14 effects. A known receptor would also provide new strategies for further analysis of CXCL14 and would also present an additional way of targeting CXCL14 signaling. Developing neutralizing antibodies would be another possible strategy.

To further validate the expression of CXCL14 in human prostate tissue and to analyze potential associations between expression and clinical parameters would be very interesting.

Paper IV

GDF15 increased fibroblast growth and stimulated proliferation, migration and invasion of prostate cancer cells. GDF15 overexpression in fibroblasts enhanced prostate xenograft growth and also tumor growth at another site in the mouse.

As for CXCL14, receptor identification and neutralizing antibodies would offer more tools for analysis and inhibition of GDF15 function.

Since the cancer cells produce GDF15 themselves, it is not apparent why they are stimulated by the fibroblast-derived GDF15. To analyze the differences between cancer cell and fibroblast produced GDF15 and investigate the dose-dependency might provide more information about this observation.

The assumed systemic, tumor-promoting effect of fibroblast-derived GDF15 needs to be further substantiated. Also, the underlying mechanisms should be further explored, including analysis of effects of GDF15 on EMT and on recruitment of bone marrow derived cells.

To examine the expression of GDF15 in human prostate tissue might provide data on possible correlations with histopathological findings.

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4 POPULÄRVETENSKAPLIG SAMMANFATTNING

Cancer uppstår som en konsekvens av genetiska förändringar som leder till att celler frångår sitt normalt kontrollerade beteende. Orsaker till dessa genetiska förändringar kan sällan förklaras enkelt. Man känner dock till en rad olika faktorer som ökar risken för att utveckla cancer, som till exempel rökning, solexponering, alkohol, övervikt och vissa typer av infektioner.

Prostatacancer är den vanligaste cancerformen i Sverige, med ungefär 9 000 nya fall varje år. Sjukdomen drabbar framförallt äldre män. Cirka hälften av patienterna är över 70 år gamla vid tiden för diagnos och det är mycket ovanligt att drabbas av prostatacancer före 40 års ålder.

Diagnosticering av prostatacancer kan ibland vara komplicerat och att hitta nya markörer i tumörer för att underlätta detta arbete har varit syftet med ett av projekten. Vi har identifierat tre nya markörer som eventuellt kan vara användbara som tillägg till de markörer som redan används rutinmässigt. Vidare utvärdering av tillförlitligheten av dessa markörer är dock nödvändig.

Tumörer består inte av enbart cancerceller utan även av omgivande stödjevävnad som består av till exempel kärl, en sorts stödjeceller som kallas fibroblaster och olika typer av immunceller. Denna stödjevävnad kallas ofta för tumörstroma. Cancerforskningen har länge koncentrerats på de maligna cellerna, men på senare tid har man förstått att även de celler som ingår i tumörstromat är viktiga för bildning och spridning av tumörer.

En målsättning har varit att identifiera faktorer i stromala celler i prostatavävnad som kan användas för bedömning av prognos och även att hitta nya stroma-producerade proteiner som eventuellt kan fungera som mål-proteiner för nya läkemedel.

I våra studier har vi identifierat två proteiner, CXCL14 och GDF15, som produceras av tumörfibroblaster i prostatacancer. Experiment i cell- och djur-modeller har visat att dessa proteiner kan stimulera tumörväxt. Fortsatta studier kommer att ytterligare undersöka betydelsen av dessa proteiner för tumörväxt. Vi har även funnit att patienter som uttrycker ett specifikt protein, PGDFRB, i prostatastroma har kortare överlevnad. Eventuellt kan detta protein användas för bedömning av en patients prognos.

Sammanfattningsvis har studierna identifierat nya potentiella diagnostiska markörer för prostata cancer och tre olika stroma-producerade proteiner (PDGFRB, CXCL14 och GDF15) som förhoppningsvis kan utvecklas till prognostiska markörer och mål-proteiner för nya läkemedel.

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