Tumour-stroma interactions between metastatic prostate cancer cells and fibroblasts

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Abstract. Previous work has shown the importance of tumour-stroma interactions for prostate cancer development at the primary site. The aim of the present study was to find out whether evidence can be found for a tumour-stroma cross-talk also between metastatic prostate cancer cell lines and non-prostatic stromal fibroblasts which are encountered by metastatic cells at most sites. We addressed this issue in cell culture systems using 3 metastatic human prostate cancer cell lines (LnCaP, PC-3 and DU-145) on the one hand, and a human fibroblast line (HFF, human foreskin fibroblasts) on the other. We incubated fibroblasts with tumour cell- and tumour cells with fibroblast-conditioned media and evaluated several parameters important for the establishment of metastases such as cell proliferation, migration and expression of matrix degrading proteases. We also determined in the conditioned media the concentrations of several growth factors and cytokines which might be responsible for the observed effects. We found that media conditioned by all 3 metastatic prostate cancer cell lines stimulated fibroblast proliferation which corresponds to fibrous stroma induction *in vivo*. DU-145 cell conditioned media induced in fibroblasts expression of mmp-1 mRNA known to be important for tumour invasion. ELISA assays revealed that tumour cells secrete bFGF, PDGF and TNFα known to stimulate fibroblast proliferation and/or MMP-1 expression. Cultivation of DU-145 carcinoma cells in fibroblast conditioned medium resulted in an enhanced proliferation and anchorage-independent growth of this cell line in soft agar. Fibroblast conditioned medium also increased migration of PC-3 cells in the wound assay and slightly augmented *mmp-1* expression. KGF (able to stimulate proliferation of normal and neoplastic prostate epithelial cells) was secreted by fibroblasts at higher concentrations than by all 3 tumour cell lines. In addition, fibroblasts secreted TNFα, bFGF, PDGF, HGF and also VEGF, the most important factor for tumour vascularization. Our results provide evidence that tumour-stroma interactions do not only exist at the primary site but also between metastatic prostate cancer cell lines and their fibroblastic microenvironment. These interactions, which are mediated through secreted factors, affect several steps of the metastatic cascade including proliferation, anchorage-independent growth, migration and the secretion of matrix-degrading proteases.

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Introduction

Prostate cancer is among the most frequent malignancies in industrialized nations and continuous research efforts are undertaken in order to better understand its development and progression. Androgen dependence of this tumour is long known (1,2) (reviewed in ref. 3) and epidemiological studies suggest factors in Western life style to contribute to its development (4,5). Linkage analyses point to hereditary prostate cancer genes (6,7) and polymorphisms of so-called modifier genes (related to hormone response, cell protection or DNA repair) may increase the risk of prostate carcinoma (7-9). Several proto-oncogenes such as *myc* (10), *EIF3S3* (11), *bcl2* (12) or growth factor encoding genes (13) have been identified. TP53 (14) and PTEN (15) are among tumour suppressor genes shown to be inactivated in prostate cancer. Comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analyses suggest further oncogenes and tumour suppressor genes (16-20) (reviewed in ref. 21). DNA methylation and histone acetylation may modify the activity of prostate cancer relevant genes (22-24) and signalling pathways (such as Wnt signalling) have been shown to be deregulated (25). Alterations of the proteasome pathway may finally change the degradation of proteins involved in growth or apoptosis (26).

Epithelial stromal interactions are also considered important for prostate cancer development and progression (reviewed in refs. 27,28). Carcinomas in general are composed of two interdependent components: the neoplastic epithelial cells and the supporting tumour stroma, which plays decisive roles in pivotal processes such as tumour proliferation, vascularization and invasion (29-31). An

epithelial-stromal cross-talk is already necessary for normal prostate development during which the mesenchyme of the urogenital sinus determines growth and differentiation of the prostate glands (27,32-34). Epithelial-mesenchymal interactions are maintained during life (35) and also play important roles in prostate cancer development (36) (reviewed in refs. 27,37). Prostate fibroblasts can influence prostate carcinoma cell growth by modifying proliferation or apoptosis via paracrine mechanisms (38-41). Using SELDI-TOF mass spectrometry we found a number of differences in gene expression at the proteome level not only between normal and neoplastic epithelial cells but also between normal and peritumoural stromal cells (42).

Only early localized stages of prostate cancer can be cured by therapeutic measures such as radical prostatectomy. However, a significant proportion of tumours metastasizes via the lymph or blood stream (43,44). Hematogenous metastases (most frequentl in the skeleton) occur in ~35% of patients and lead to uncontrollable disease (45-47). The metastatic process comprises many steps such as tumour cell detachment, migration, matrix degradation, vascular transportation and attachment to metastatic sites by tissue-specific adhesion mechanisms (48,49). Proliferation in the new microenvironment and induction of angiogenesis finally lead to continuous growth of metastases (50). During this cascade tumour-stroma interactions are as important as they are for the development of the primary tumour. However, little is known about this cross-talk at metastatic sites.

Tumour-stroma communications consist of multiple dynamic interactions between neoplastic and stromal cells. The complexity of this cross-talk cannot be directly studied *in vivo* except in transgenic models able to focus at one given factor such as the role of VEGF-C in lymphangiogenesis (51,52). Appropriate *in vitro* models are cell culture systems which we used in the present work to study tumour-stroma interactions between metastatic prostate cancer cells and fibroblasts.

We used three established metastatic prostate cancer cell lines from both lymphatic and hematogenous metastatic sites [LNCaP, DU-145 and PC-3 (53-55)] on the one hand, and a human fibroblast line (56) on the other, in order to investigate how a tumour-stroma cross-talk may affect important cellular functions during the establishment of metastases such as proliferation, migration and the secretion of matrix degrading proteases.

Materials and methods

Cell lines. Cancer cells were received from ATCC (American Type Culture Collection). We used the following three human prostate cancer cell lines derived from different metastases: LNCaP from a lymph node metastasis (53), PC-3 from a bone metastasis (55) and DU-145 from a brain metastasis (54). A short description of cell characteristics is given in Table I. Fibroblasts were human foreskin fibroblasts which we had already used and characterized in previous studies (56).

Culture conditions and production of conditioned media. Cells were cultured at 37˚C in a humidified atmosphere containing 5% CO_2. They were maintained either in Dulbecco's modified Eagle's medium (DMEM, Invitrogen NV, Leek, The Netherlands, DU-145, PC-3 and HFF) or in RPMI-1640 (Invitrogen NV) medium (LNCaP) supplemented with 10% heat-inactivated fetal calf serum (FCS, Invitrogen NV), 100 U/ml penicillin/streptomycin and 2 mM L-glutamine (Invitrogen NV). Culture media were completely eliminated by washing with phosphate-buffered saline (PBS, Invitrogen NV) and cells were starved in medium with 0.5% FCS prior to the production of conditioned media. Conditioned media with 0.5% FCS were prepared by incubating subconfluent cells ($2x10^5$) in 24-well plates for 24 h. Media were separately harvested, clarified by centrifugation (2.000 x g at 4˚C for 15 min) and stored frozen at -20˚C until further use.

Table I. Characteristics of the used prostate carcinoma cell lines.

Cell line	Origin	Androgen responsiveness	Tumourigenicity
LNCaP	Human lymph node metastasis	Androgen sensitive	Low
PC-3	Human bone metastasis	Androgen independent	High
DU-145	Human brain metastasis	Androgen independent	High

Enzyme-linked immunosorbent assay (ELISA) for determination of growth factor and cytokine concentrations. Separately, harvested conditioned media as well as unconditioned control media were examined for the presence of the following growth factors and cytokines by ELISA: EGF (epidermal growth factor), KGF (keratinocyte growth factor), bFGF (basic fibroblast growth factor), PDGF (platlet derived growth factor), TNFα (tumour necrosis factor α), and VEGF (vascular endothelial growth factor). Media stored at -20˚C were thawed on ice for analysis. Concentrations of growth factors and cytokines were examined by ELISA kits (R&D Systems, Minneapolis, MN, USA). All determinations were performed in triplicates according to the manufacturer's instructions. Concentrations were determined by comparing the optical density in the samples to standard curves.

Incubation of prostate cancer cell lines and fibroblasts in fibroblast (FCM) and tumour cell (TCM) conditioned media. In order to examine the effects of tumour cell and fibroblast conditioned media on different properties of cancer cells and fibroblasts we incubated cells with the conditioned media for 24 h. All experiments were performed at least in triplicate. Cells cultivated in starvation medium were used as controls.

Proliferation assay. Proliferation of different prostate carcinoma and HFF cells, which had been cultured in conditioned or control media, was determined by a modified MTT test system for living cells (EZ_4U, Biomedica, Vienna, Austria). Cells ($2x10^3$) were cultured in 96-well plates in

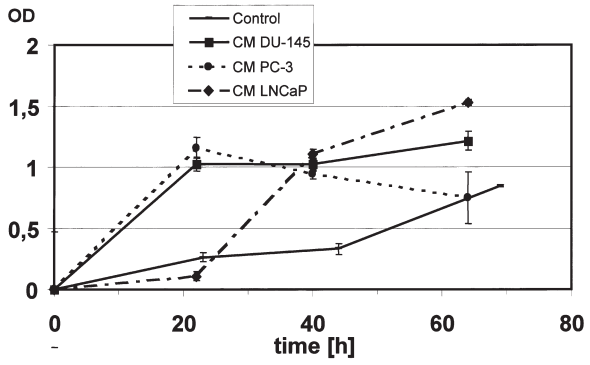

Figure 1. Proliferation of human foreskin fibroblasts (HFF) in tumour cell conditioned media. Proliferation of HFF was determined in prostate carcinoma cell conditioned media (CM) by a modified MTT-test. Cells/well ($2x10^3$) were cultured in 96-well plates in 200 μl of conditioned or control media for 24 h. The mean values of five independent determinations are shown.

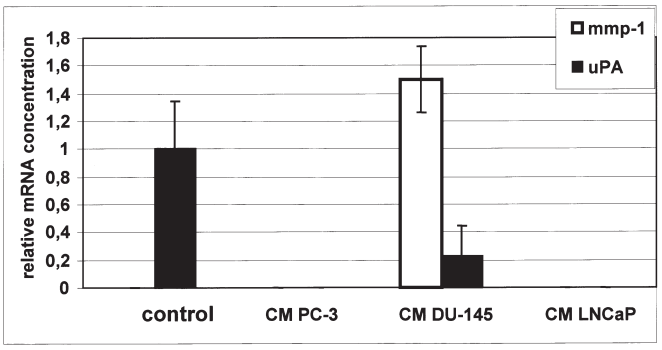

Figure 2. Quantitative RT-PCR analysis of mmp-1 and uPA expression in HFF cells. Expression of mmp-1 and of uPA was determined in HFF cells without stimulation (control) and after stimulation by media conditioned by the three carcinoma cell lines (CM). The mean value of three independent measurements is shown.

200 μl of conditioned or control medium. The proliferation rates of cells were determined at the time points indicated in Figs. 1 and 4. Each measurement was performed 5 times according to the manufacturer's instructions.

Soft agar assay. For soft agar assays $1x10^5$ tumour cells in DMEM and 20% FCS were mixed with an equal volume of 0.8% agarose (FMC BioProducts, Rockland, USA). The mixture was poured onto a bed of 1.4% agarose. The plates were supplied every 3 days with 2 ml of either fibroblast-conditioned or starvation medium with 0.5% FCS and observed for colony formation.

Migration assay. The migratory and motility behavior of the three tumour cell lines was analyzed by the wound assay. Monolayers of confluent cultures were lightly scratched with a pipette tip. After washing to remove detached cells, the cultures were incubated with either fibroblast-conditioned or control media and observed at distinct time intervals.

Invasion assay. Invasion assays were performed in Matrigel Chambers (Becton Dickinson) containing polyethylene membranes with 8-μm pore size coated with basement membrane (Matrigel, Becton Dickinson). Cells were harvested by trypsinization, resuspended in conditioned or control media at a density of $5x10^4$ cells/ml and $2.5x10^4$ cells were plated in the upper compartment of the chamber. After incubation at 37˚C for 22 h filters were removed. Cells adhering to the lower surface were fixed, stained with Hemacolor® (Merck) and counted.

RNA isolation and reverse transcription. Total cellular RNA was extracted from cells by the RNeasy kit (Qiagen, Hilden, Germany). The integrity of RNA preparations was controlled visually in 1% agarose/formaldehyde gels. Generation of cDNAs by reverse transcription was performed in 20 μl reaction volume containing 2 μg of total cellular RNA, 4 μl of 5X first-strand buffer (Invitrogen NV), 2 μl of 0.1 M DTT, 1 μl of dT8TP primer (10 mM), 1 μl of dNTPs (10 mM) and RNAse-free water. After incubation at 70˚C for 10 min, 1 μl of Superscript II reverse transcriptase (Invitrogen NV) was added for the reverse-transcription at 42˚C for 1 h. The enzyme was inactivated by heating at 70˚C for 10 min. cDNA quantities were evaluated by comparison to the constitutively expressed ribosomal protein L13A (*rp*L13A)-gene.

Quantitative RT-PCR analysis of MMP-1 and uPA expression. Quantitative real-time RT-PCR was performed on a Light-Cycler™ (Roche, Mannheim, Germany) using SybrGreen in combination with RNA-FastStart master kit (Roche). The mixture contained 1 μl of cDNA template, 2.4 μl $MgCl_2$ (25 mM), 0.75 μl of each oligonucleotide (10 mM) and 2 μl SybrGreen mix in a final volume of 20 μl. After 5 min of denaturation, 34 cycles were performed with a denaturation step at 95˚C for 5 sec, 20 sec annealing at 56˚C and 35 sec extension at 72˚C. Oligonucleotides had been synthesized by TIB-Molbiol (Berlin, Germany): RPL13f (5'-TAC GCT GTG AAG GCA TCA AC-3'), RPL13r (5'-CAC CAT CCG CTT TTT CTT GT-3'), MMP1f (5'-GGA CCG ACA ACA ATG AGG AT-3'), MMP1r (5'-TCA AAG GTT CCT GGC AGA AG-3'), uPAf (5'-AGT GCA TGGTGC ATG ACT GC-3'); uPAr (5'-CCA AAG CCA GTG ATC TCA CA-3').

Results

We stimulated the three prostate cancer cell lines with media which had been conditioned by fibroblasts and the latter with those which had been conditioned by the tumour cells. We then evaluated stimulated and non-stimulated cells for basic cellular functions such as proliferation, invasion and production of proteases involved in matrix degradation. Results are presented first for effects of tumour cell conditioned media on fibroblasts and then for effects of fibroblast conditioned media on tumour cells.

LNCaP, PC-3 and DU-145 cell conditioned media stimulate HFF proliferation. According to current models, the first step of tumour-stroma-interactions at primary or metastatic sites is stroma induction by tumour cell-derived factors. Therefore, we evaluated first the effects of tumour cell conditioned media on fibroblast proliferation. We found HFF proliferation to be

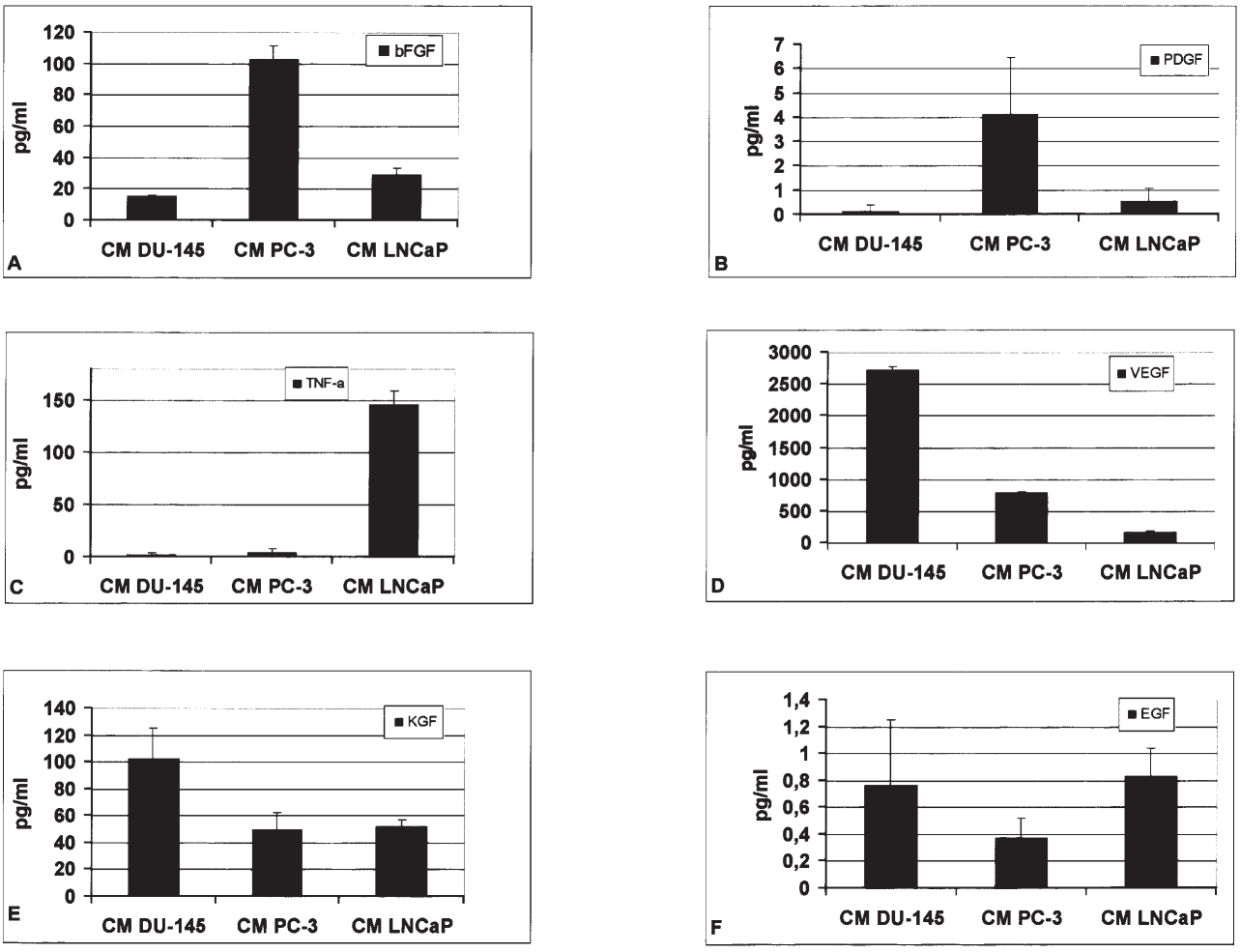

Figure 3. Concentrations of different growth-factors and cytokines in tumour cell conditioned media. Concentrations of bFGF (A), PDGF (B), TNFα (C), VEGF (D), KGF (E) and EGF (F) were determined by ELISA in media conditioned by the three carcinoma cell lines. Cells ($2x10^5$) were cultured in 24-well plates in 2 ml medium. After 24 h, medium was harvested and concentrations were determined by commercially available ELISAs. The mean value of four independent measurements is shown.

stimulated by media which had been conditioned by all three metastatic prostate carcinoma cell lines (Fig. 1). Stimulation by LNCaP conditioned media augmented proliferation after a lag phase of 24 h (Fig. 1).

Expression of proteases by HFF. Invasion of tumour cells is another important step during the establishment of metastases. Stroma fibroblasts are suggested to significantly contribute to tumour invasion through a secretion of different matrix-degrading metalloproteinases (MMPs) as well as their activators, such as urokinase-type of plasminogen activator (uPA), which initiates the proteolytic cascade leading to MMP activation. We therefore assessed fibroblasts for expression of MMP1 (an important MMP for invasion) as well as of uPA after stimulation by tumour cell conditioned media.

In quantitative light cycler analyses we found that fibroblasts held in control medium expressed uPA but not mmp-1 transcripts (Fig. 2). Media conditioned by DU-145 cells induced expression of mmp-1 mRNA in contrast to LNCaP and PC-3 conditioned media. Media which had been conditioned by all 3 cell lines down-regulated uPA mRNA in fibroblasts (Fig. 2).

Determination of growth factors and cytokines secreted by tumour cells. We next determined by ELISA in tumour cell conditioned media the concentrations of several important stroma-inducing or activating growth factors and cytokines known to induce fibroblasts to proliferate and/or to express matrix-degrading proteases such as MMP-1 and uPA according to our previous findings (56). Results are shown in Fig. 3.

We found that bFGF, PDGF and TNFα which stimulate fibroblast proliferation and/or induce MMP-1 and uPA in HFF are actually secreted at different concentrations by the metastatic prostate cancer cell lines. Both bFGF and PDGF were secreted at highest amounts by PC-3 cells followed by LNCaP and DU-145 cells. TNFα concentration was highest in LNCaP conditioned media. In contrast TNFα was barely detectable in media conditioned by PC-3 and DU-145 cells.

VEGF is considered the most important angiogenic factor for tumour vascularization which is the prerequisite for continuous tumour growth at primary or metastatic sites. This factor was secreted at the highest amounts by DU-145 cells followed by PC-3 and LNCaP cells (Fig. 3D).

All three tumour cell lines also secreted KGF and EGF known to stimulate proliferation of prostatic epithelial cells

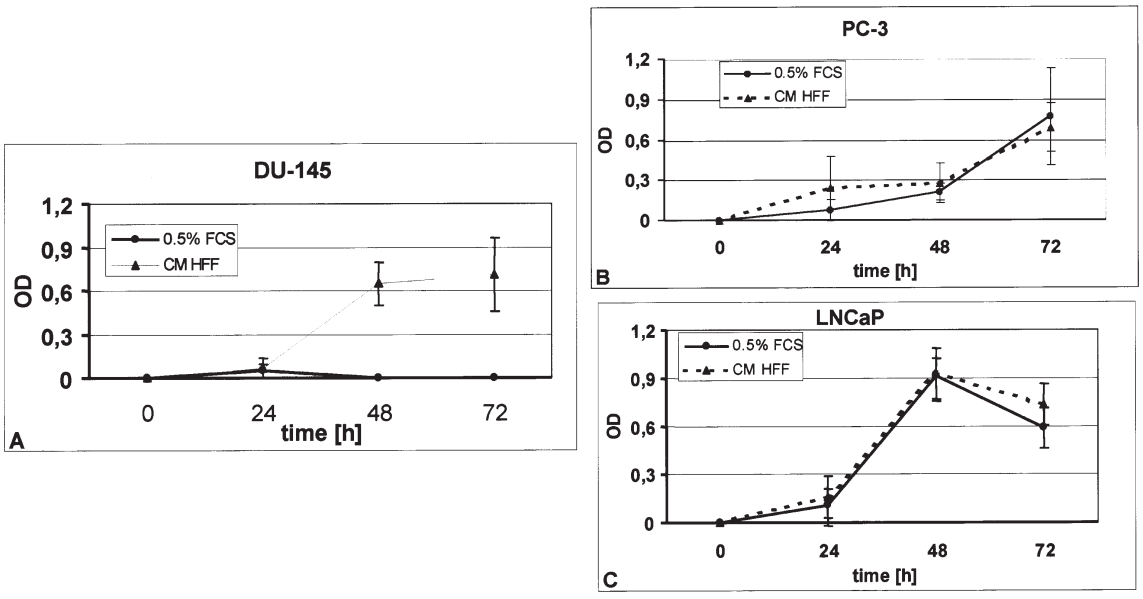

Figure 4. Proliferation of prostate carcinoma cell lines in fibroblast conditioned or starvation media. Proliferation of DU-145 (A), PC-3 (B) and LNCaP cells (C) was determined in starvation medium (0.5% FCS) and HFF conditioned medium (CM HFF) by a modified MTT-test. Cells ($2x10^3$) were cultured in 96-well plates in 200 μl medium. Stimulation by fibroblast conditioned media was performed for 24 h. The mean value of five independent measurements is shown.

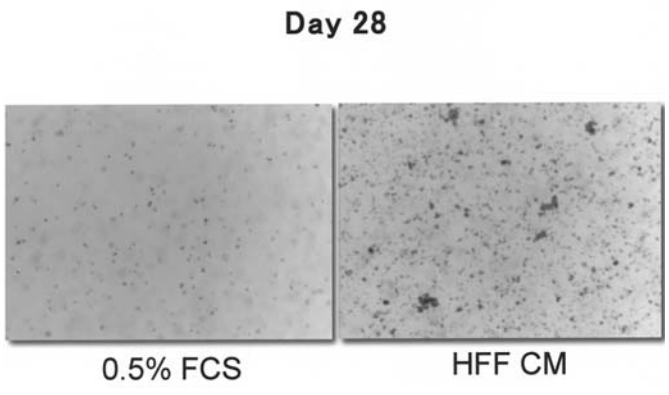

Figure 5. Fibroblast conditioned medium (FCM) increases soft agar growth of DU-145 cells. When anchorage-independent growth of the three prostate carcinoma cell lines was analysed in soft-agar, Du-145 cells showed an increased growth after treatment with fibroblast conditioned medium compared to the starvation medium. Results are shown for day 28. In contrast, fibroblast conditioned medium had no influence on anchorage-independent growth of LNCaP and PC-3 cells.

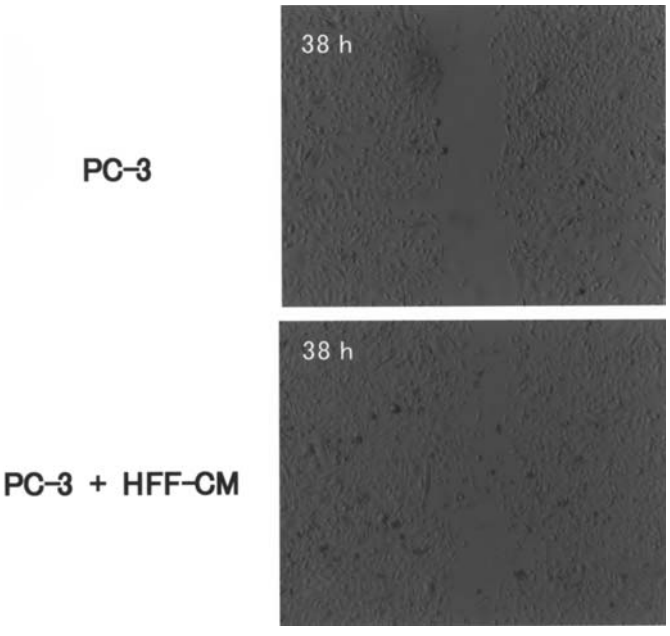

Figure 6. Fibroblast conditioned medium (FCM) increases migration of PC-3 prostate carcinoma cells in the wound assay. Monolayers of confluent cultures of all three tumour cell lines were lightly scratched with a pipet tip. Detached cells were removed by washing and the cultures were incubated with either fibroblast conditioned or control media. Fibroblast conditioned media increased migration of PC-3 cells after 28 and 38 h of incubation. Migration of the two other tumour cell lines was not changed (data not shown).

(57). We next assessed diverse effects of fibroblast conditioned media on the three metastatic prostate cancer cell lines.

Effects of fibroblast conditioned media on proliferation of prostate carcinoma cell lines. We cultivated the 3 carcinoma cell lines in media which had been conditioned by fibroblasts for 24 h and used tumour cell lines starved in medium with 0.5% FCS as negative controls. Results are shown in Fig. 4.

Cultivation of DU-145 carcinoma cells in fibroblast conditioned medium resulted in an enhanced proliferation at all investigated time points compared to the control (Fig. 4A). In the case of PC-3 cells measurements after 24 and 48 h demonstrated a slightly increased proliferation in fibroblast conditioned media (Fig. 4B). However, these differences were not significant (p=0.242). LNCaP cells had a comparable proliferation rate in control medium and in HFF conditioned medium (Fig. 4C).

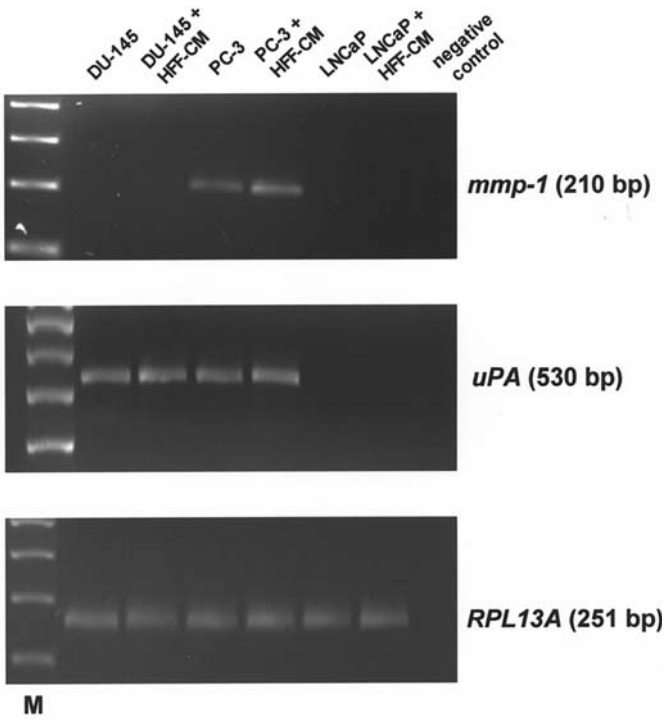

Figure 7. RT-PCR analysis of *mmp-1* and *uPA* expression in prostate carcinoma cell lines. RT-PCR for *mmp-1*, *uPA* and *RPL13A* was performed with RNA isolated from DU-145, PC-3 and LNCaP cells after incubation in either control media or HFF conditioned media. Size of amplification products were 210 bp for *mmp-1*, 530 bp for *uPA* and 251 bp for *RPL13A*. Transcripts of *mmp-1* and *uPA* were detected in PC-3 cells and transcripts of *uPA* in DU-145 cells. None of these transcripts were found in LNCaP cells. After incubation in fibroblast conditioned media the amount of *mmp-1* transcripts in PC-3 cells increased slightly. The amount of *uPA* transcripts in PC-3 and DU-145 cells was not affected by incubation in HFF conditioned media.

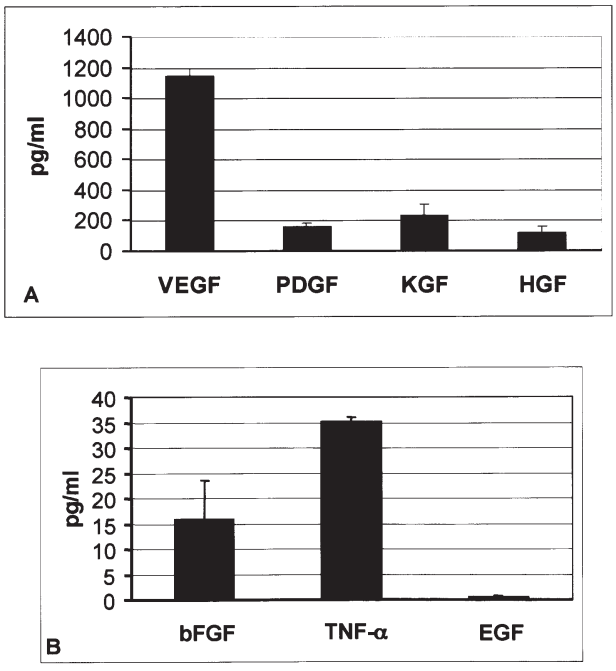

Figure 8. Concentrations of different growth-factors and cytokines in fibroblast (HFF) conditioned medium. Concentrations of VEGF, PDGF, KGF, HGF (A), bFGF, TNFα and EGF (B) were determined by ELISA in media conditioned by the HFF cell line (HFF-CM). Cells ($2x10^5$) were cultured in 24-well plates in 2 ml medium. After 24 h, medium was harvested and concentrations were determined by commercially available ELISAs. The mean value of four independent determinations is shown. KGF was secreted by fibroblasts at higher concentrations than by all three tumour cell lines. VEGF was secreted at higher amounts than by PC-3 and LNCaP cells (compare to Fig. 3).

Anchorage-independent growth of prostate carcinoma cell lines in soft agar assay. One significant feature of cancer cell lines is the ability to grow in an anchorage-independent way. Therefore, the three prostate carcinoma cell lines were analyzed under different growth conditions in soft agar. Again, the growth of cell lines incubated in medium with 0.5% FCS was used as an internal control. Du-145 cells showed an increased growth after treatment with fibroblast conditioned medium (Fig. 5). In contrast, fibroblast conditioned medium had no influence on the growth of LNCaP and PC-3 cells.

Effect of fibroblast conditioned media on prostate cancer cell migration and invasion. Cell migration and invasion are important features of tumour progression. Using the wound assay we found that fibroblast conditioned medium increased migration of PC-3 cells after 28 and 38 h (Fig. 6). In contrast, no clear effect was evident on migration of DU-145 and LNCaP cells. Fibroblast conditioned media had no effects on the invasive behaviour of the three cancer cell lines in the Boyden Chamber assay (data not shown).

Expression of proteases by prostate carcinoma cells. We also analyzed whether proteases are induced by fibroblast conditioned media in tumour cell lines. We first assessed expression of proteases in tumour cells in control media. We detected transcripts of *mmp-1* and *uPA* in PC-3 cells and transcripts of *uPA* in DU-145 cells. None of these transcripts were detected in LNCaP cells. After incubation in fibroblast conditioned media the amount of *mmp-1* transcripts in PC-3 cells increased slightly (Fig. 7). In contrast the amount of *uPA* in PC-3 and DU-145 cells was not affected by incubation in fibroblast conditioned media (Fig. 7).

Determination of growth factors and cytokines secreted by fibroblasts. We next determined by ELISA the concentrations of different growth factors and cytokines secreted by fibroblasts into the conditioned media. Results are shown in Fig. 8. We found KGF to be secreted by fibroblasts at higher concentrations than by all three tumour cell lines. In addition, fibroblasts secreted TNFα, bFGF, PDGF, HGF and VEGF. The latter was secreted at higher amounts by fibroblasts than by PC-3 and LNCaP cell lines.

Discussion

Previous work on tumour stroma interactions in the prostate has largely focused on the primary site (27,36). In the present study, we searched evidence for a cross-talk between metastatic prostate cancer cell lines and non-prostatic fibroblasts which are encountered by prostate cancer cells at different metastatic sites. We tried to characterize some functional interactions between tumour cells and fibroblasts, and to decipher messages exchanged between them in terms of secreted growth factors and cytokines.

Since dynamic interactions can hardly be deciphered *in vivo* we addressed these issues in cell culture systems. We used three established metastatic human prostate cancer cell lines on the one hand, and a human fibroblast line on the other which we had well characterized in previous studies (56,58). Cell isolation from fresh prostate tissues is possible (59,60) but has inherent problems such as preparation of 'pure' cell populations and short life-span of primary cells. In addition, the genetic background of different patients renders data difficult to interpret and to compare.

During metastatic progression prostate cancer cells have to pass through each step of the so-called metastatic cascade including proliferation, vascularization and invasion. Our results provide evidence that interactions between metastatic prostate cancer cells and fibroblasts have effects on several of these steps. For continuous growth and propagation at metastatic sites tumour cells first have to induce a supportive stroma. We found that media conditioned by all three metastatic prostate cancer cell lines are capable of inducing cultured fibroblasts to proliferate which corresponds to fibrous stroma induction *in vivo*. Degree and kinetics of proliferation differed, however, between media conditioned by the three cancer cell lines which is in line with the known fact that induction of fibroblastic stroma can differ between different tumours. We also identified in prostate cancer cell conditioned media growth factors (such as bFGF and PDGF) to which induction of fibroblast proliferation can be attributed (61,62). Basic fibroblast growth factor is already known to induce stromal growth within the human prostate (63). In the present study, both bFGF and PDGF were secreted at the highest amounts by PC-3 cells followed by LNCaP and DU-145 cells which did not reflect exactly the effects of tumour cell conditioned media on fibroblast proliferation. However, we did not display the whole 'secretome' (64,65) of the three tumour cells and these cells certainly secrete more factors than those determined in our study. We further found that not only tumour cells but also fibroblasts secrete bFGF and PDGF in line with additional autocrine effects of these factors on fibroblast proliferation.

Tumour cell proliferation is an essential step for metastasis formation and we found that fibroblasts in turn also stimulated proliferation of the DU-145 metastatic prostate cancer cell line while showing no significant effects on proliferation of the two other cell lines. This was well in line with an increase of anchorage-independent growth of DU-145 cells in soft agar assays after stimulation with fibroblast conditioned medium. In ELISA assays of fibroblast conditioned media we identified KGF as a known factor able to stimulate proliferation of normal and neoplastic prostate epithelial cells (66). The fact that all three metastatic prostate cancer cell lines secreted KGF similarly suggests again an autocrine effect on tumour cell proliferation.

Tumour cell proliferation alone is not sufficient for continuous growth of metastases which also requires tumour vascularization (30,56,67-70). VEGF is considered the most important factor for tumour vascularization (71). This factor was secreted at the highest amounts by DU-145 cells followed by PC-3 and LNCaP cells. Interestingly, we found that, besides the metastatic prostate cancer cells, fibroblasts also secrete VEGF. This suggests that both neoplastic and stromal cells can induce tumour vascularization in prostate cancer metastases through VEGF secretion. VEGF A is not only the most potent angiogenic factor, but is also thought to participate in the formation of fibroblastic stroma via a pronounced hyperpermeability effect on small blood vessels, leading to fibrin leakage with subsequent organization by granulation tissue which turns into fibroblastic connective tissue (72,73). Moreover, an autocrine function of VEGF on prostate cancer cell proliferation has been suggested by the demonstration of the high affinity receptors VEGFR-1 and VEGFR-2 on prostate cancer cells (74,75). VEGFR-1 has been found to be slightly expressed in PC-3 but not expressed in LNCaP and DU-145 cells (76).

Cell migration is an important part of tumour cell invasion at metastatic sites. Using the wound assay we found that fibroblast conditioned medium increased migration of PC-3 cells (77).

It has been shown in recent years that stromal fibroblasts participate in a major way in tumour invasion by secreting various matrix-degrading proteases (30,78), as well as their downstream activators such as uPA (31). Proteases carry out balanced degradation of extracellular matrix components facilitating tumour invasion (56,79-82). In quantitative light cycler analyses we found that media conditioned by DU-145 cells induced in fibroblasts expression of mmp-1 mRNA in contrast to LNCaP and PC-3 conditioned media. According to our previous findings bFGF, PDGF and TNFα are capable of inducing mmp-1 in the used fibroblast line probably via an activation of the Ets-1 transcription factor (56). However, these factors are secreted at only very low concentrations by DU-145 cells suggesting that other factors are responsible for mmp-1 induction in fibroblasts.

MMPs not only promote tumour invasion through matrix degradation but also release growth factors, angiogenic factors, and their inhibitors from the ECM and cell surfaces (83,84) thereby regulating bioavailability of growth factors. MMP-1 has been described to degrade perlecan and release bound FGF (85).

In vivo, cross-talk between metastatic prostate cancer cells and their stromal microenvironment includes more interactions than those demonstrated in this study. More than just tumour cells and fibroblasts are present in the microenvironment of metastatic sites including inflammatory, immune-competent cells and macrophages all of which participate in multidirectional communications (reviewed in ref. 30). Moreover, stroma tumour cell interactions probably also exhibit differences at different sites and finally functional properties of fibroblasts may be different at different metastatic sites.

However, the results of this study provide evidence that intercellular communications exist not only at the primary site but also between metastatic prostate cancer cell lines and their fibroblastic microenvironment. These interactions affect several steps of the metastatic cascade. Our results also suggest that the way and results of tumour-stroma communications differ according to particular features of metastatic tumour cell clones. This concerns particularly concentrations of growth factors and cytokines secreted by the neoplastic cells. Effects of different spectra of factors are probably not just the sum of effects of single factors but rather the result of non-linear potentiations or inhibitions.

Acknowledgements

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