The Prostate 50:38 – 45 (2002)

Differential Expression of Matrix Metalloproteinases and Their Tissue Inhibitors in Human Primary Cultured Prostatic Cells and Malignant Prostate Cell Lines

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BACKGROUND. The aim was to investigate the expression of matrix metalloproteinases (MMPs), membrane type MMPs (MT-MMPs), and their inhibitors (TIMPs) in human primary cultured prostatic cells and malignant prostate cell lines.
METHODS. Reverse transscription-polymerase chain reaction-based measurements of the mRNA levels of MMP-2, MMP-7, MT1-MMP, MT3-MMP, TIMP-1, and TIMP-2 in relation to the house-keeping gene glyceraldehyde phosphate dehydrogenase were performed in cancerous and non-cancerous prostatic tissue samples, in primary cell cultures of epithelial cells, in both fibroblasts, and smooth-muscle cells as stromal cells, and in the human malignant prostatic cell lines DU-145, LNCaP, and PC-3.
RESULTS. MMP-2 was mainly expressed in the stromal cells and MMP-7 showed their highest values in the epithelial cells. MT1-MMP, MT3-MMP, TIMP-1, and TIMP-2 were found both in the stromal and in the epithelial cells, but there were some differences between the expressions in fibroblasts and smooth-muscle cells. Different expressions were also observed between the cells deriving from the primary cell cultures, the benign cell line BPH-1, and the malignant cell lines LNCaP, D-145, and PC-3.
CONCLUSIONS. These exemplary results concerning different expressions of MMPs and TIMPs in cells from prostatic tissue suggest that a better insight into changes observed in prostatic tissue needs studies on cells cultured from the tissue. *Prostate 50: 38–45, 2002.*

KEY WORDS: **prostate cancer; epithelial cells; stromal cells; reverse transcriptional polymerase chain reaction**

INTRODUCTION

Both in vitro and in vivo investigations performed over the past few years have demonstrated the important role of matrix metalloproteinases (MMPs) in tumor invasion and metastasis [1]. A positive correlation between increased invasiveness of various

Grant sponsor: Deutsche Forschungsgemeinschaft; Grant numbers: Ju 365/3-1, Li 822/1-2, CHV-112/18/98; Grant sponsor: SONNENFELD-Stiftung Berlin, Germany.
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Received 24 April 2001; Accepted 10 September 2001

DOI 10.1002/pros.10030

human tumors and MMP expression could be shown [2]. The role of MMPs in prostatic malignancy was recently reviewed [3]. Few studies on MMPs and tissue inhibitors of metalloproteinase (TIMPs) have been performed in human prostate tissue samples, malignant prostatic cells, and prostate tumors grown in animals [4–13]. Strong correlations were found between the intensity of MMP-2 expression and the Gleason score [6,11,12]. Changed expressions of MMP-7 and MMP-9 were also observed in benign prostatic hyperplasia and prostate cancer [14,15]. Increased MMP-2 and MMP-9 but reduced TIMP concentrations were found in conditioned media of epithelial cultures from neoplastic prostate [5].

It is now recognized that several MMPs are synthesized by the peri-tumoral stroma rather than the tumor cells themselves, suggesting a strong cooperation between tumor cells and stromal cells with regard to the invasive properties [16]. The prostate consists of three major histological components: the stroma, epithelium, and luminal space [17]. However, scarce but contradictory data exist on the localization of the MMPs in these components. To get further insight into this problem, it was the aim of this study, using specific cell culture techniques, to estimate the occurrence of MMP-2, MMP-7, the membrane-type MMPs (MT-MMPs), MT1-MMP, MT3-MMP, TIMP-1, and TIMP-2 in the primary cultured prostatic stromal and epithelial cells in comparison to prostatic tissue and established human malignant prostatic cell lines.

MATERIALS AND METHODS

Tissue Samples

Prostate tissue samples were obtained from the cancerous and non-cancerous parts of the same prostates which had been surgically removed by radical prostatectomy so that matched pairs were used for tissue investigation and for growing primary cell cultures. Small pieces of tissue were dissected immediately after removal of the prostate and then stored in liquid nitrogen until analysis or used for cell culture experiments. The cut edges within the prostate were inked so that the dissected pieces could be easily assigned to the adjacent tissue examined histopathologically [18]. Histological analysis from all tissue pieces used was carefully performed as previously described by a clinical pathologist to determine tumor grade and tumor stage [19,20]. The use of this human tissue for research purposes has been approved by the Ethical Committee of the Charité Hospital, Berlin.

Preparation of Prostatic Epithelial Cell Cultures

The cultures of the prostatic epithelial cells were established either from the cancerous or from the non-cancerous parts of the same prostate that had been surgically removed by radical prostataectomy. The protocol for cell culture corresponded to a procedure previously described [21,22] according to a standard protocol [18]. Briefly, a cell suspension was prepared by finely chopping the prostatic portions with a scalpel and incubating 100 mg tissue in 2 ml KSFM medium (Gibco Life Technologies, Eggenstein, Germany; cat.no. 17005-34; with 5 μg/L epidermal growth factor and 50 mg/L bovine pituitary extract) supplemented with bovine serum albumin (final concentration: 250 mg/L; Behring AG, Marburg, Germany), transferrin (10 mg/L; Boehringer, Mannheim, Germany), dihydrotestosterone (5 μg/L; Sigma Chemical Company, St. Louis, MO), non-essential amino acid solution (1%; Gibco, cat.no. 11140-35) and penicillin/streptomycin (125 kU and 125 mg/L; Gibco); together with 2 mg collagenase type IA (245 U/mg; Sigma) and 2 mg hyaluronidase (1000 U/mg; Boehringer) on a rotator at 37°C for 16 hr. The suspension was forced through a 100 μm nylon sieve and washed with the same volume medium. The combined filtrate fraction was centrifuged at $270 \times g$ for 5 min. The pellet was resuspended in 6 ml medium, centrifuged, again resuspended in 5 mL medium, supplemented with 50 μg/ml ECL attachment matrix solution (Biozol, Echting, Germany), and maintained in 25 cm^2 Falcon-Primaria culture flasks. The cells were incubated in a humidified 5% CO_2/95% air mixture at 37°C and were fed fresh medium twice weekly until the cultures became confluent. The culture medium was removed and the monolayers were washed twice with 2 ml of phosphate buffered saline solution at room temperature and detached using trypsin/EDTA solution (0.05%, 0.02%) under microscopic control within 2–5 min. The cells were subcultured at a split ratio of 1:2 or 1:3. The collagenase digestion of the tissue pieces and the use of the special growth medium KSFM are useful methods to prevent fibroblasts or other non-epithelial cells from growing. This procedure guarantees a high purity of the cultured cells [18]. Immunohistochemical evaluation of cytokeratin expression and prostate-specific antigen were performed to confirm the epithelial nature and the prostatic origin of the cells [19,20]. For this study, three different cell strains were established and were used at third passages. Both malignant and non-malignant prostatic tissue samples were always investigated in pairs.

Preparation of Prostatic Stromal Cell Cultures

The approach previously described in detail in this journal was applied to culture prostatic smooth muscle and fibroblast cells [23]. Except for horse

serum, which was purchased from Biochrom (Berlin, Germany), all reagents and procedures were used as mentioned. The purity of cell cultures was controlled by immunohistochemical markers as previously described [23].

Culture of Permanent Human Prostatic Cell Lines

The human malignant prostatic cells PC 3, DU 145, LNCaP, and the cell line BPH-1 were obtained from the American Type Culture Collection. They were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (Biochrom), penicillin, and streptomycin as mentioned above. The PC 3 and DU 145 cells were cultured in plastic culture flasks from Costar, the LNCaP cells in Falcon-Primaria culture flasks.

RNA Isolation and RT–PCR

Total RNA was isolated from liquid nitrogen stored prostate tissue samples using the RNeasy Mini Kit from Quiagen (Hilden, Germany). Total RNA from cells were isolated using the High Pure RNA Isolation Kit from Roche (Mannheim, Germany). RNA yield and purity were checked by spectrophotometric measurement at 260 and 280 nm.

The first strand cDNA was synthesized from 1 μg total RNA after denaturation for 2 min at 72°C by use of 0.5 μg oligo(dT) primer, 0.5 mM dNTP, and 200 U M-MLV reverse transcriptase RNase H minus (all from Promega, Mannheim, Germany). The reaction was performed at 37°C for 2 hr in a 20 μl volume. For RT-PCR, a 25 μl reaction volume contained the following: 1 μl of sythesized cDNA, 1 μM of each specific primer pair, 0.75 U AmpliTaq Gold DNA polymerase with PCR reaction 1× buffer containing 1.5 mM $MgCl_2$ (Perkin Elmer, Foster City, CA), 0.2 mM of each dNTPs (Promega). The temperature profile of the PCR amplification consisted of activation of Taq polymerase at 93°C for 10 min, denaturation at 95°C for 1 min, primer annealing temperature for 2 min, elongation at 72°C for 1 min for the first five cycles, the following 16–40 cycles were performed in 1 min steps and finished by an extension step for 3 min. The gene-specific primers used are listed in Table I and were synthesized from MWG Biotech GmbH (Ebersberg, Germany). The amplifications were done as single-tube PCR in a UNO-Thermoblock (Biometra, Göttingen, Germany). The PCRs were optimized for each gene studied according to the specific annealing temperature (Table I). After that, PCRs of the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH) and of the MMPs and TIMPs were performed at the same annealing temperature in the same cycle run for all samples. The number of cycles used was adjusted in preliminary PCR runs and is given in Table I. This procedure allowed a comparison of the gene expression in different samples under the same conditions of amplification.

Semiquantitative Analysis of PCR Products

The PCR products for MMPs, TIMPs, and for GAPDH were analyzed by 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetat, 2 mM EDTA, pH 8.5). Products were lighted by ethidium bromide staining. The density of product bands was measured by digital imaging system Eagle Eye II and analyzed by the EagleSight version 3.1 image capture and analysis software (Stratagene, La Jolla, CA). Integrated density values of the band of the housekeeping gene of each sample were set as 100%; the

TABLE I. Characteristics of Primers Used for RT–PCR Analysis of MMPs and TIMPs

Gene	Accession number	Sequence 5′... 3′	PCR products (Bp)	Annealing temperature (°C)	Cycles
MMP-2	J03210	U: acctggatgccgtcgtggac L: tgtggcagcaccagggcagc	448	62	25
MMP-7	X07819	U: gtatggggaactgctgacatcatg L: ctgaatgcctttaatatcatcctg	324	56	35
MT1-MMP	U41078	U: gggcctgcctgcgtccatcaaca L: gccgccctcctcgtccacctcaat	517	64	26
MT3-MMP	D85511	U: tgcccccacaccgctctattcc L: tttcccgacgtcctcccaccaa	434	64	40
TIMP-1	M59906	U: agtcaaccagaccaccttat L: tttcagagccttggaggagc	386	58	23
TIMP-2	J05593	U: tgcagctgctccccggtgca L: ttatgggtcctcgatgtcga	585	58	30
GAPDH	AF261085	U: atggggaaggtgaaggtcggagtc L: gacgcctgcttcaccaccttcttg	797	56–64	19

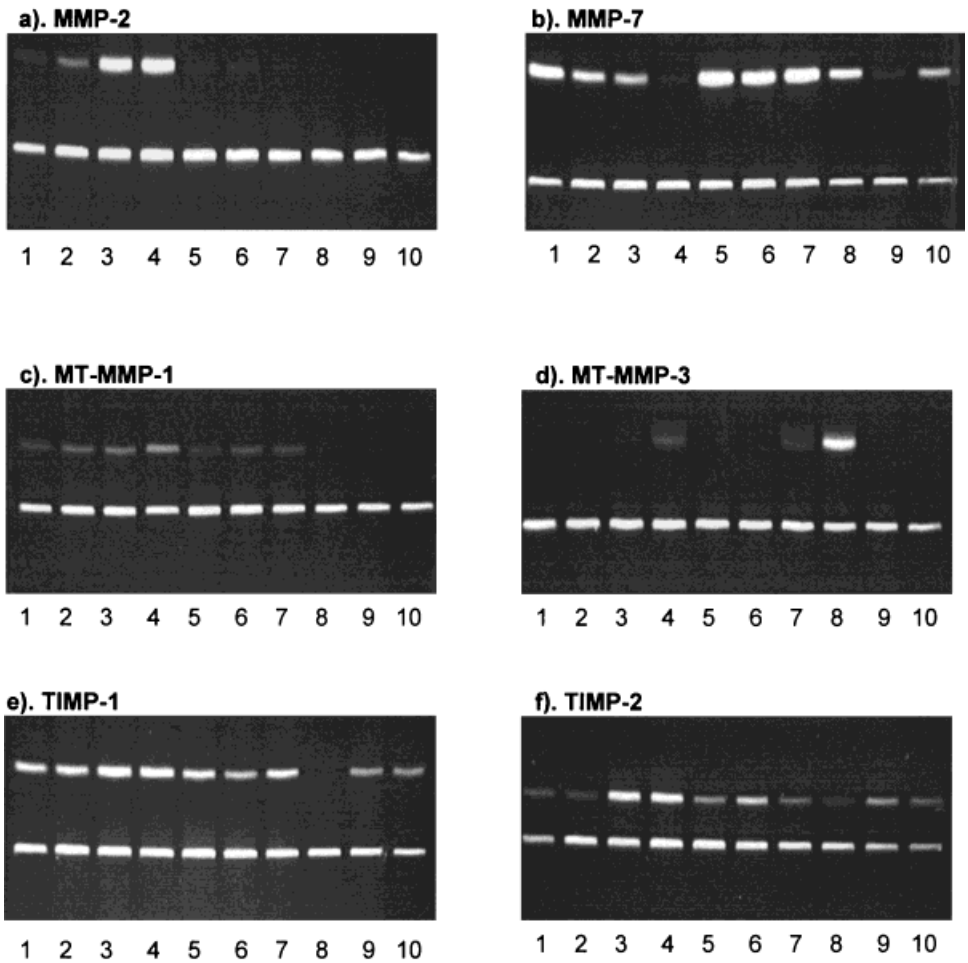

Fig. 1. RT–PCR analysis of MMP and TIMP transcripts in benign and malignant prostatic tissue, primary cell cultures from the prostate, and prostatic cell lines. The upper line shows the transcripts in the various sources corresponding the following lanes: **1**, benign prostatic tissue; **2**, malignant prostatic tissue; **3**, prostatic smooth muscle cell culture; **4**, prostatic fibroblast cell culture; **5**, prostatic epithelial cell culture (from benign tissue); **6**, prostatic epithelial cell culture (from malignant tissue); **7**, BPH-1 cell line; **8**, LNCaP; **9**, DU-145; **10**, PC-3. The lower line shows the expression of the house-keeping gene GAPDH.

density of band of the studied gene was related to this value.

RESULTS

Figure 1 shows a representative pattern of mRNA expressions of MMP-2, MMP-7, MT1-MMP, MT3-MMP, TIMP-1, and TIMP-2 in comparison to the house-keeping gene of GAPDH in the cancerous and non-cancerous prostatic tissue, epithelial and stromal prostatic cell strains, and in prostatic cell lines. Mean values of three experiments are given in Figure 2. For that purpose, three different epithelial cell strains cultured from the cancerous and the non-cancerous part of the prostate were used for all assays. Similarly, three different stromal prostatic cell strains were examined. MMP-2 was expressed both in epithelial and stromal cells. The expression was comparable both in fibroblasts and smooth muscle cells whereas the expression in the epithelial cell strains and in the cell lines BPH1, LNCaP, DU-145, and PC-3 was clearly lower.

In contrast, MMP-7 showed their highest values in the epithelial cells. These data corresponded with the expression in the BPH cell line BPH-1. A comparison between the two stromal cells proved a lower MMP-7 expression in the fibroblasts than in the smooth muscle cells.

MT1-MMP and MT3-MMP were found both in the stromal and in the epithelial cells; however, a higher expression was found in the fibroblasts.

TIMP-1 and TIMP-2 were also expressed both in the epithelial and the stromal cells while there was no difference between the expression in the fibroblasts and the smooth muscle cells.

Comparison of the expressions of MMPs showed differences between the cells deriving from the primary cell cultures, the benign cell line BPH-1, and the malignant cell lines LNCaP, DU-145, and PC-3.

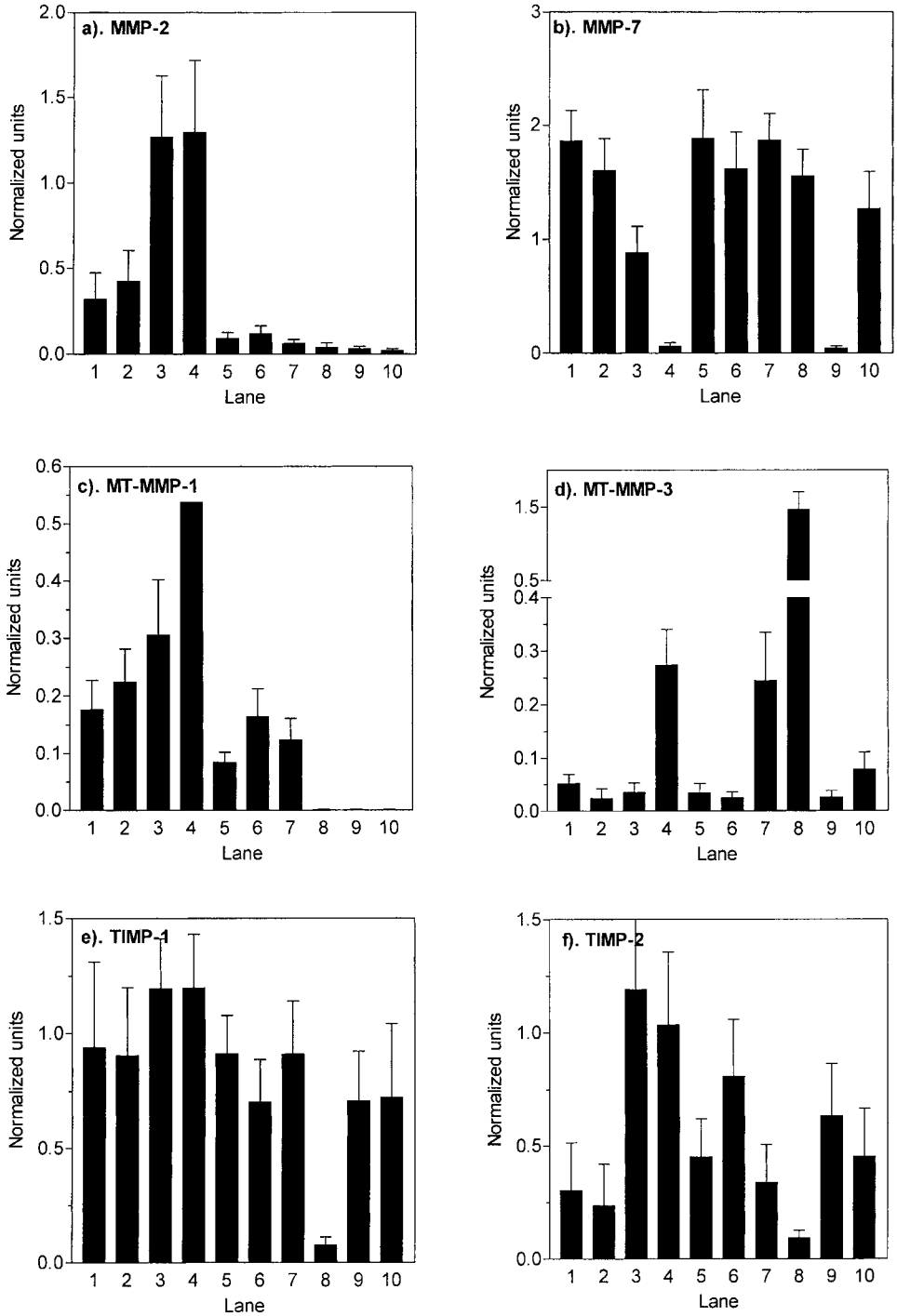

Fig. 2. Relative expression of the mRNA expressin of MMPs and TIMPs in benign and malignant prostatic tissue, primary cell cultures from the prostate, and prostatic cell lines in comparison to the house-keeping gene GAPDH. The columns represent the expression data (arithmetic means and SEM; n = 3) in relation to the GAPDH expression found in the various sources: **1**, benign prostatic tissue; **2**, malignant prostatic tissue; **3**, prostatic smooth muscle cell culture; **4**, prostatic fibroblast cell culture; **5**, prostatic epithelial cell culture (from benign tissue); **6**, prostatic epithelial cell culture (from malignant tissue); **7**, BPH-1 cell line; **8**, LNCaP; **9**, DU-145; **10**, PC-3. The expression of GAPDH was set 1. For further details, see text.

For example, very low expression was found for MT1-MMP in all malignant cell lines but higher expression in cells from primary cell cultures. MMP-7 in DU-145 cells (Figs. 1 and 2b, lane 9) showed a low expression in comparison with the higher expression in LNCaP and PC-3 cells (lanes 8 and 10) or in the cells cultured from the cancerous prostatic tissue. Similar divergencies were observed for MT3-MMP, TIMP-1, and TIMP-2. A comparison of the expressions in primary epithelial cells cultured from the malignant and non-malignant tissue specimen of the same prostate showed a tendency to higher values of MT1-MMP and TIMP-2 ($P = 0.084$ and 0.093; paired t-test) in cells cultured from the malignant prostatic tissue.

DISCUSSION

MMPs is a growing family of proteases now comprising more than 20 enzymes and having the common ability to degrade the extracellular matrix components such as collagen, elastin, and gelatin and to destroy the basement membrane. Depending on their different substrates, MMPs can be divided into subgroups such as gelatinases (MMP-2, MMP-9), collagenases (MMP-1, MMP-8, MMP-13), stromelysins (MMP-3, MMP-10; MMP-11, MMP-7). All MMPs secreted as inactive precursors and transformed into active forms can be modified in their activities by their specific inhibitors, the TIMPs. At present, four different TIMPs have been described (TIMP-1, 2, 3, 4). In addition to the classic MMPs, the new class of membrane type MMPs localized at the cell surface has been recently discovered and now includes five members. Therefore, to demonstrate the different occurrence of MMPs in the various types of prostatic cells, we restricted our mRNA measurements to some typical MMPs in relation to a house-keeping gene. This procedure allows to compare expression rates.

Our study showed that MMP-2, MMP-7, MT1-MMP, MT3-MMP, and the two tissue inhibitors TIMP-1 and TIMP-2 were expressed both in normal and cancerous prostatic tissue. However, there are different expression rates in the stromal and epithelial cells. In addition, the classical prostatic cell lines, both the benign cell line BPH-1 and the malignant cell lines LNCaP, DU-145, and PC-3 revealed different expressions among each other and in comparison with the primary cells. In addition, different expressions in cells cultured from the cancerous and the non-cancerous part of the same prostate were evident, for example, a tendency to increased expressions of MT1-MMP and TIMP-2 in cells from the malignant prostate. Differences between the benign and malignant tissue were not measured as the main aim of this study was to investigate the different expressions in the cell strains of the primary cell culture and in the established human benign and malignant cell lines. Up to now, such approach has not been performed for prostatic cells. Therefore, these results obtained from mRNA measurements in tissue and cell cultures allow to give more clarity into somewhat contradictory data of MMPs in prostatic tissue [24,25]. In comparison with studies on whole tissue, studies using isolated cell cultures have the advantage of showing whether the cells are able to synthesize these components.

We found MMP-2 both in epithelial and stromal cells, but the expression rate was stronger in stromal cells. Previous immunohistochemical data proved MMP-2 expression in cancerous prostatic tissue both in epithelial cells and in the stromal compartment [26]. However, using the technique of in situ hybridization, one study showed MMP-2 expression only in stromal cells whereas another study failed to detect that expression in stromal cells, but found it in only epithelial cells [24,25]. Our data of higher expression of MMP-2 in fibroblasts and smooth muscle cells both from benign and malignant prostatic tissue are also consistent with the predominant expression of MMP-2 observed in stromal areas of malignancies like breast, ovarian, and colorectal carcinomas [27–29]. Another study also showed MMP-2 secretion both in epithelial and fibroblastic cell cultures from benign and malignant prostate tissue.

Similarly, there are also divergent data with regard to the expression of TIMP-1 and TIMP-2 in the stromal area and the epithelium of the prostate [24,25]. Wood et al. [25] found both inhibitors only in the stromal areas while Still et al. [24] demonstrated TIMP-2 expression only in prostatic epithelial cells. TIMP-1 also was measured in the conditioned medium of prostatic epithelial cell cultures as proof of the occurrence of that component in epithelial cells [5,9]. Our cell culture studies have similarly provided evidence for the ability of both epithelial and stromal cells in the prostate to synthesize TIMP-1 and TIMP-2.

Concerning MMP-7, we found its predominant occurrence in epithelial cells, although fibroblasts and smooth muscle cells also expressed MMP-7. These data correspond with results found by in situ hybridization and immunohistochemistry showing high expression of MMP-7 in prostatic epithelial cells [10,14,30].

Up to now, there are no data on the localization of MT1-MMP and MT3-MMP expression in the prostate. Only one study pointed out a significant association between the pattern of MMP-2 and MT1-MMP staining within the epithelial components. The expression in the stromal components as shown in our study was not mentioned [31].

Recently, an overview of the expression of MMPs in the malignant prostatic cells LNCaP, DU-145, and PC-3 was given [32]. The expression data of MMP-2, MMP-7, MT1-MMP, and MT3-MMP roughly correspond with our results. This correspondence additionally corroborates the validity of our procedure applied in this study to measure and compare MMPs and TIMPs in tissue samples and primary stromal and epithelial cell cultures.

In summary, our data prove that a better understanding of the role of MMPs and their tissue inhibitors in invasion and metastatic spread of prostate cancer needs expression studies in cell cultures in addition to those in tissue. The different expression of MMPs in the prostatic epithelial and stromal cells, although only demonstrated on the mRNA level, has to be especially considered to study the inhibitory and stimulatory effects of cytokines on the transcriptional and translational regulation of MMPs.

ACKNOWLEDGMENTS

We thank Marianne Scholz for valuable technical assistance.

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