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mRNA expression of matrix metalloproteases and their inhibitors differs in subtypes of renal cell carcinomas

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Abstract

Altered expression of matrix metalloproteases (MMPs) and their inhibitors, the tissue inhibitors of matrix metalloproteases (TIMPs), has been demonstrated in various tumour tissues. mRNA expression patterns of *MMP-1*, *MMP-2*, *MMP-3*, *MMP-9*, *MMP-11*, *MMP-12*, *MMP-14* and *TIMP-1*, *TIMP-2, TIMP-3* and *TIMP-4* were evaluated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in 30 renal cell carcinomas (RCC), as well as in the surrounding tissues. Expression of the MMPs was significantly stronger in the carcinomas than in non-malignant tissues. High levels were demonstrated particularly in clear cell RCCs (CC-RCC). Except for *MMP-1*, MMP expression in the papillary RCCs (P-RCC) was, for most MMPs, significantly lower. Expression of the TIMPs in malignant cells of both subtypes was weak, with the exception of *TIMP-4* which was strongly expressed in the P-RCCs and downregulated in the CC-RCCs. The latter was correlated with chromosomal loss of 3p, harbouring the *TIMP-4* gene locus. In conclusion, deregulated expression of the MMPs and TIMPs in RCCs differs according to histology, grade, size and cytogenetic characteristics, suggesting that MMP and TIMP expression patterns play an important role for the typical histomorphological features of RCC subtypes and their respective biological behaviour. © 2001 Published by Elsevier Science Ltd.

Keywords: Renal cell carcinoma; Matrix metalloproteases; Tissue inhibitors of metalloproteases; Quantitative reverse transcriptase-polymerase chain reaction; Cytogenetics

1. Introduction

Matrix metalloproteases (MMPs) belong to a family of at least 21 human zinc-dependent, highly conserved endopeptidases (reviewed in Ref. [1]), capable of degrading components of the extracellular matrix (ECM). ECM degradation is essential for physiological processes where tissue remodelling is involved, such as wound healing and regulation of connective tissue growth during organ development. However, it has also been demonstrated to play a critical role for tumour invasiveness and metastasis (reviewed in Ref. [2]).

According to their substrate specificity and structure, MMPs are classified into five subgroups: collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11) as well as a metalloelastase (MMP-12), the membrane-type MMPs (MT1-MMP–MT5-MMP), and other MMPs (MMP-19, MMP-20) [2]. The substrate specifity of distinct MMPs has been determined by their ability to degrade different components of the ECM *in vitro*. Most MMPs are secreted as inactive zymogens (proMMPs) and are proteolytically activated extracellularly, with the exception of MMP-11 and MT1-MMP (= MMP-14), which are activated prior to secretion by Golgi-associated, furin-like proteases [3].

In the extracellular milieu, the activity of MMPs is controlled by tissue inhibitors of matrix metalloproteases (TIMPs), a family of natural inhibitors (reviewed in Ref. [4]). So far, four members of this family have been described. Although these inhibitors exhibit very similar inhibitory activities against most members of the MMP family, they differ in many aspects including interactions with proMMPs, solubility, regulation of expression, and tissue-specific expression [5–7]. While most of the cell biological effects of TIMPs are believed

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to be mediated indirectly by inhibition of MMPs, direct growth-promoting [8,9] as well as growth-inhibitory effects have been demonstrated for TIMP-2 [10]. TIMP-3 is involved in cell cycle regulation, differentiation and senescence [11].

Overexpression of MMPs has been reported in human carcinomas, such as those of the stomach [12], lung [13], prostate [14] and breast [15]. Clinicopathological studies have shown a correlation of MMP and TIMP production with malignant progression and with a shortened survival in various tumour types [16,17].

The aim of the present study was to investigate whether the expression patterns of the MMPs and TIMPs in renal cell carcinomas (RCC) differ according to the biological characteristics of the tumours. Therefore, the expression of seven MMPs (MMP-1,-2,-3,-9,-11,-12,-14) and four TIMPs (TIMP-1,-2,-3 and -4) was measured by quantitative reverse transcription-polymerase chain reaction (RT-PCR) in 24 clear cell RCCs (CC-RCC) and six papillary RCCs (P-RCC), as well as in the adjacent non-malignant tissue. Results were evaluated in relation to clinicopathological variables, such as histological subtype, tumour staging and grading as well as tumour cytogenetics.

2. Patients and methods

2.1. Patients

Results were derived from a series of 30 surgically removed primary RCCs. Tumours were classified, staged and graded according to the classification of renal cell carcinomas of the Union Internationale Contre Le Cancer and the American Joint Committee on Cancer [18–20]. Six of the 30 tumours (20%) showed the morphological features of P-RCCs, the remaining 24 (80%) belonged to the clear cell subtype. With regard to histological grading, 11 of the 30 samples (37%) were classified as G1, 13/30 (43%) as G2, and 6/30 (20%) as G3. Five of the 30 tumours (17%) were pT1, 9/30 (30%) were pT2, 14/30 (47%) were pT3 and 2/30 (7%) were pT4.

2.2. RNA Extraction and quantitative RT-PCR

Total RNA was extracted separately from the tumour and the surrounding non-malignant tissue using the guanidinium thiocyanate method [21]. Reverse transcription was performed from 2 μg of total RNA using oligo-dT primers and M-MLV reverse transcriptase (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Primers for *MMP-3*, *MMP-9* and *TIMP-4* were designed using the 'Primer3 program' (Whitehead Institute for Biomedical Research; Rozen S, Skaletsky HJ, 1998; http://www-genome.wi.mit.edu/genome_software/other/primer3.html) and synthesised by Biometra (Göttingen, Germany). The specific primers were as follows; for human *MMP-3* (forward primer 5′-GCAGTTTGCTCAGCCTATCC, reverse primer 5′-GAGTGTCGG-AGTCCAGCTTC) amplifying a 214-bp fragment (55 °C annealing temperature), for *MMP-9* (forward primer 5′-GGCGCT-CATGTACCCTATGT, reverse primer 5′-TCAAAG-ACCGAGTCCAGCTT) amplifying a 468-bp fragment (58 °C annealing temperature) and for *TIMP-4* (forward primer 5′-GACCAGTGACCATCACATCC, reverse primer 5′-ATGACATTCGCCATTTCTCC) amplifying a 349-bp fragment (62 °C annealing temperature). Primers for *MMP-1*, *MMP-2*, *MMP-11*, *MMP-12* and *MMP-14* were synthesised according to Giambernardi and colleagues [7], for *TIMP-1* according to Wilhelm and colleagues [22], for *TIMP-2* according to Stetler-Stevenson and colleagues [23], and for *TIMP-3* according to Lampert and colleagues [24].

Quantitative PCRs were performed on the Light Cycler PCR Analysis System® (Roche Diagnostics, Mannheim, Germany). The amount of generated DNA was measured by fluorescence detection of the double stranded-specific (ds)-DNA-binding dye SYBR Green I (Roche Diagnostics, Mannheim, Germany) and quantified in relation to serial dilutions of a known standard. The detailed procedure for quantification has been described previously in Ref. [25]. The PCR reaction contained a standard PCR buffer, SYBR Green I (1:20000), bovine serum albumin (0.05%) and 5 pmol of the specific sense and antisense primers. Forty cycles were performed, 0 s denaturation at 94 °C, 5 s annealing at the respective optimal annealing temperature (see above), 10 s extension at 72 °C and 5 s fluorescence detection at 72–84 °C. The melting curves were obtained at the end of amplification by cooling the sample at 20 °C/s to 72 °C and then increasing the temperature to 95 °C at 0.1 °C/s. Fluorescence was acquired every 0.1 °C. To confirm comparable efficiency of reverse transcription in the samples, *β-actin* was amplified and quantified according to the same protocol (primers from Clontech, Heidelberg, Germany). Experiments were performed at least three times.

2.3. Cytogenetic analysis

The tumours were processed for routine histological analysis and short-term cell culture. For cytogenetic analysis, viable tumour tissue and normal kidney tissue samples were minced, digested with collagenase (Sigma, Deisenhofen, Germany) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, Deisenhofen, Germany). Chromosome preparations were performed within the first weeks of primary cultures. Briefly, metaphase cells were arrested with colchicine (Sigma, Deisenhofen, Germany; final concentration 0.1 μg/ml) for 2–3 h and swollen in hypotonic 0.075 M KCl

solution for 20 min. Cells were then fixed in methanol/acetic glacial acid (3:1) and dropped on slides that were pre-rinsed with water. Chromosomes were banded using routine G-banding and 4′,6′-diamino-2-phenylindol (DAPI)-banding techniques. For each tumour approximately 15–20 metaphases were fully karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN) [26].

2.4. Statistical analysis

Graphs were calculated with the sigma plot for windows graphic system (version 1.1, Jandel Corporation, USA). Data were analysed with the Mann–Whitney test using the Analyse-it software (version 1.44, Analyse-it Ltd, UK) for Microsoft Excel (Microsoft Corporation, USA). A P value below 0.05 was considered significant. Results are expressed as medians with the 95% confidence interval (CI).

3. Results

3.1. Expression of MMP and TIMP mRNA in tumour versus non-malignant tissue

All examined MMPs and TIMPs were detectable in malignant and non-malignant tissues derived from CC-RCC-bearing kidneys, as well as from the papillary subgroup (Table 1). In the CC-RCC group, MMP mRNA expression was significantly stronger in the tumour than in the adjacent normal tissue. The same relationship could be observed in the P-RCCs. Differences between malignant and non-malignant tissues were smaller in the latter group, but were, still statistically significant, with the exception of *MMP-9* and *MMP-12* expression level.

Comparing the expression of the TIMPs in the CC-RCCs and in the adjacent normal tissue, *TIMP-1*, *TIMP-2* and especially *TIMP-3* and *TIMP-4* were expressed to a significantly higher degree in the non-malignant tissue compared with the tumour cells. In P-RCC-bearing kidneys, a comparable distribution was observed for *TIMP-1* and *TIMP-4*, while there was no significant difference for *TIMP-2* and *TIMP-3* expression in the non-malignant and tumour tissue. In contrast to the other TIMPs, mRNA concentration of *TIMP-4* was high in the P-RCCs, although still lower than in the respective non-malignant surrounding tissue.

3.2. MMP and TIMP expression profiles in clear cell versus papillary RCCs

The expression profiles of MMP mRNA were significantly different in the CC-RCCs and P-RCCs (Table 1). While the latter were associated with a predominant *MMP-1* expression, CC-RCCs were characterised by high mRNA concentrations of *MMP-1*, *MMP-9* and *MMP-11*. *MMP-1* was the only metalloprotease which was strongly upregulated in both subtypes, and there was significantly higher levels of *MMP-1* in the P-RCCs than in the CC-RCCs. For most of the other investigated MMPs, expression was stronger in the CC-RCCs than in the P-RCCs, with the exception of *MMP-2* and *MMP-14* which showed similar expression levels in both entities ($P=0.7$ and $P=0.9$; Fig. 1).

There was no differential expression of *TIMP-1* ($P=0.08$), *TIMP-2* ($P=0.06$) and *TIMP-3* ($P=0.55$) in tumour cells of both histologies, although *TIMP-3* expression was strongly upregulated in the non-malignant tissues adjacent to CC-RCCs compared with the normal cells surrounding the P-RCCs. A significant differential expression was demonstrated for *TIMP-4* which was detectable in high amounts in the P-RCCs and only weakly in the CC-RCCs (Fig. 1).

Table 1
Expression of MMPs and TIMPs in clear cell and papillary renal cell carcinomas

	mRNA expression in clear cell renal cell carcinomas (pg)			mRNA expression in papillary renal cell carcinomas (pg)		
	Non-malignant tissue	Tumour tissue	P value	Non-malignant tissue	Tumour tissue	P value
MMP-1	0.003 (0.001–0.006)	32.95 (13.04–38.53)	<0.0001	0.02 (0.01–0.04)	108.22 (36.16–282.91)	0.0022
MMP-2	0.005 (0.001–0.03)	11.09 (3.21–26.64)	<0.0001	0.05 (0.01–1.95)	14.17 (8.52–22.49)	0.0022
MMP-3	0.004 (0.00–0.017)	15.25 (5.95–69.0)	<0.0001	0.01 (0.01–0.14)	0.09 (0.06–0.23)	0.0411
MMP-9	0.08 (0.001–0.19)	138.18 (10.62–197.82)	<0.0001	1.56 (1.04–13.62)	6.44 (3.19–15.31)	0.0649
MMP-11	0.014 (0.003–0.03)	20.99 (14.4–28.38)	<0.0001	0.001 (0.00–0.09)	0.26 (0.002–0.52)	0.0152
MMP-12	0.17 (0.001–1.19)	1.4 (0.6–87.21)	0.0004	0.003 (0.002–0.004)	0.002 (0.001–0.25)	0.5887
MMP-14	0.02 (0.01–0.06)	0.62 (0.09–5.19)	<0.0001	0.001 (0.00–0.002)	0.52 (0.26–2.2)	0.0022
TIMP-1	5.21 (1.08–34.24)	1.22 (0.25–1.86)	0.0449	2.23 (1.46–12.7)	0.18 (0.02-1.5)	0.0043
TIMP-2	1.69 (0.13–4.93)	0.22 (0.07–0.36)	0.0205	0.95 (0.08–3.21)	0.69 (0.22–1.19)	0.6991
TIMP-3	688.14 (90.91–1847.9)	4.38 (0.68- 12.01)	0.0002	1.73 (0.05- 6.29)	3.7 (0.05- 9.23)	0.6991
TIMP-4	78.07 (38.16–128.25)	1.23 (0.83–3.29)	<0.0001	127.98 (98.01–138.03)	45.44 (33.47–51.14)	0.0079

MMP, matrix metalloprotease; TIMP, tissue inhibitor of matrix metalloprotease.

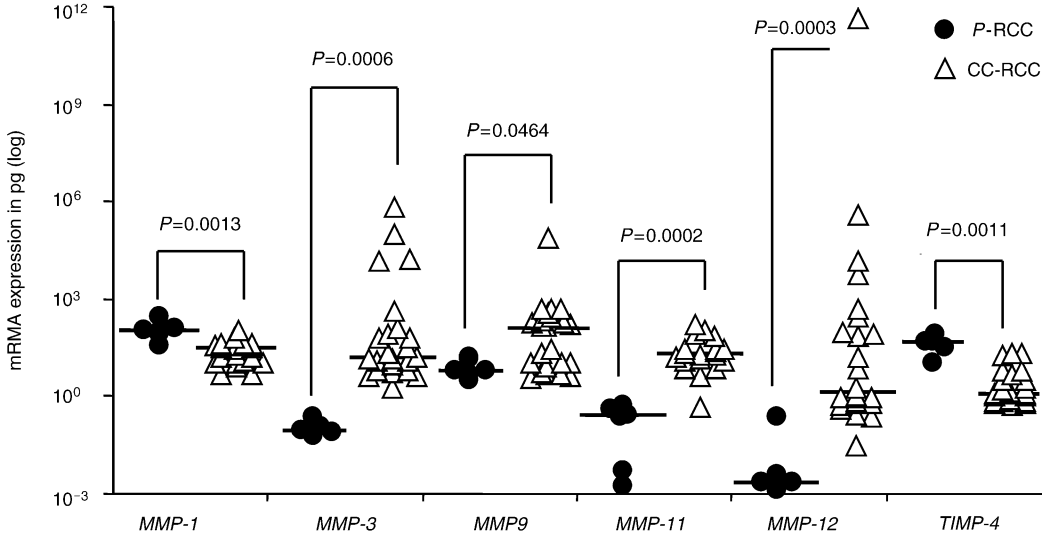

Fig. 1. *MMP* and *TIMP* mRNA expression in clear cell (CC-RCC) (white triangles) and papillary renal cell carcinomas (P-RCC) (black circles). Triangles and circles represent the mean of three determinations, median values are indicated as lines.

3.3. MMP and TIMP expression profiles in relation to histological grade and tumour stage

Comparing G1 tumours (11/30) on the one hand with G2 and 3 tumours (19/30) on the other, a statistically significant positive correlation of *MMP-9* mRNA expression and histological grade could be demonstrated (Fig. 2). Median values were significantly lower in the well differentiated RCCs (G1: 6.44 pg, 95% CI: 3.8–138.18 pg) than in the poorly differentiated group (G2/3: 156.24 pg, 95% CI: 10.88–334.07 pg: $P=0.0068$). The opposite was seen for *TIMP-4* expression (Fig. 3) with high median values in the G1 tumours (18.42 pg, 95% CI: 1.23–51.14 pg) and low levels in the G2/3 group (1.23 pg, 95% CI: 0.73–3.29 pg, $P=0.0034$). For the other investigated MMPs and TIMPs, there was no statistically significant difference between the subgroups.

A positive correlation of tumour size and MMP and TIMP expression could be demonstrated for *MMP-9* and *MMP-3* as well as for *TIMP-1*. Median *MMP-9* values were higher in pT3 and 4 stage tumours (85.5 pg, 95% CI: 10.88–237.33 pg) than in pT1/2 tumours (6.65 pg, 95% CI: 3.89–169.15 pg, $P=0.0437$). The same relationship was observed for *MMP-3* with 17.98 pg (95% CI: 10.04–352.02 pg) for the pT3/4 group and 3.0 pg (95% CI: 0.08–32.78 pg) for the pT1/2 group ($P=0.0222$), as well as for *TIMP-1* (pT3/4 group: 1.43

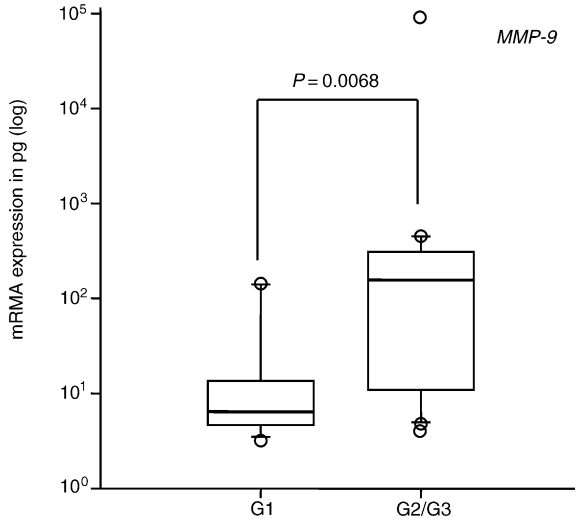

Fig. 2. *MMP-9* mRNA expression in correlation to histological grade. Data are given as box plots, the box encompasses the median value as well as the 25th through to the 75th percentile. Values outside the 10th and the 90th percentile (bars) are indicated as circles.

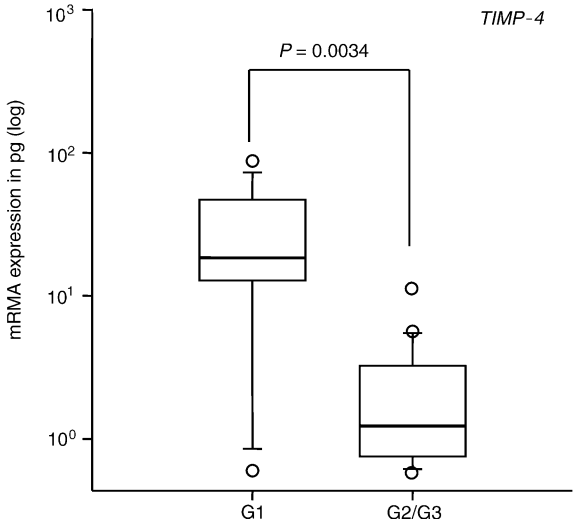

Fig. 3. *TIMP-4* mRNA expression in correlation to histological grade. Data are given as box plots, the box encompasses the median value as well as the 25th through to the 75th percentile. Values outside the 10th and the 90th percentile (bars) are indicated as circles.

pg, 95% CI: 0.13–2.71 pg; pT1/2 group: 0.22 pg, 95% CI: 0.03–1.5 pg, $P=0.0434$). *TIMP-4* levels, in contrast, were significantly higher in smaller (pT1/2: 17.12 pg, 95% CI: 1.23–45.44 pg) than in larger tumours (pT3/4: 1.23 pg, 95% CI: 0.72–3.29 pg, $P=0.0071$).

3.4. MMP and TIMP expression profiles and cytogenetic analysis

Cytogenetic data were available for 18 tumours. Only one displayed a normal karyotype, while 17 showed complex chromosomal aberrations. Histologically, 5 tumours belonged to the papillary subtype, graded as G1 (4/5) and G3 (1/5), each of them with complex chromosomal aberrations. 13 tumours were CC-RCCs, graded as G1 (2/13), G2 (8/13) and G3 (3/13). As typically found in tumours with a clear cell morphology (Fig. 4), 9 of the 13 CC-RCCs (7 G2 and 2 G3 tumours) showed aberrations of chromosome 3 involving the short arm 3p, which harbours the gene locus of *TIMP-4*. There was no correlation between cytogenetic characteristics and mRNA expression of the MMPs or TIMP-1,-2 and-3. However, measurement of *TIMP-4* expression, yielded significantly ($P=0.0003$) lower median values for tumours with chromosomal loss of 3p (1.23 pg, 95% CI: 0.72–1.73 pg) than for the tumours without such alterations (17.56 pg, 95% CI: 3.29–51.14 pg, Fig. 5). As all of the 3p alterations occurred in the CC-RCCs and none of them in the P-RCC subgroup, these results correspond well with the high expression levels of *TIMP-4* in P-RCCs compared with the CC-RCCs, as documented in Table 1 and Fig. 1.

4. Discussion

Apart from their role in the normal physiology of connective tissue during development and morphogenesis, MMPs and their naturally occurring inhibitors, the TIMPs, have been implicated in primary tumour growth as well as metastatic progression. Consistent with this, in the present study mRNA expression of *MMP-1*, -2, -3, -9, -11, -12 and -14 was significantly stronger in the RCCs of both clear cell and papillary morphology than in the surrounding non-malignant tissue, where MMP levels were generally low.

Comparison of the two histological entities yielded differential MMP expression profiles. Most of the

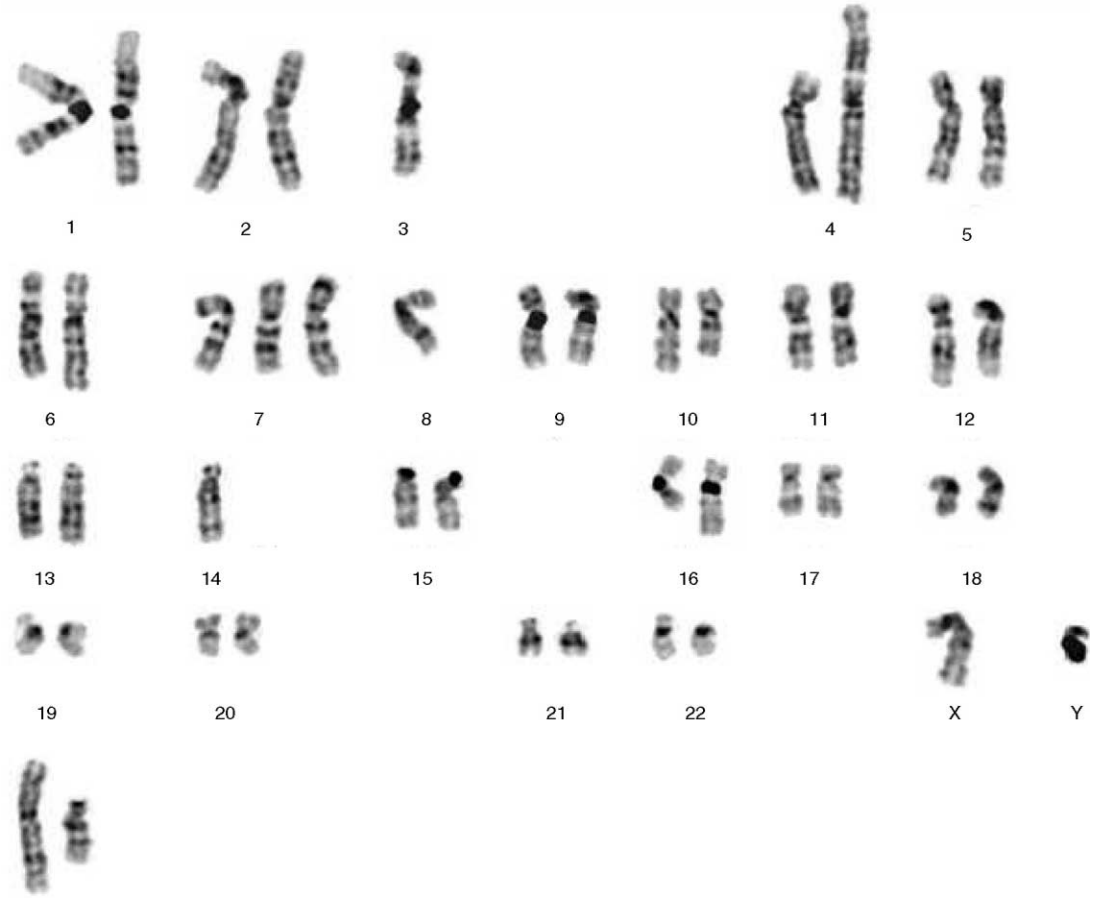

Fig. 4. Representative karyogram of a clear cell renal cell carcinoma with complex abnormalities involving chromosome 3 [46, XY, −3, der(4) t(4;5)(p16;q13), +7, −8, t(10;16)(q24;q24), −14, +mar1, +mar2].

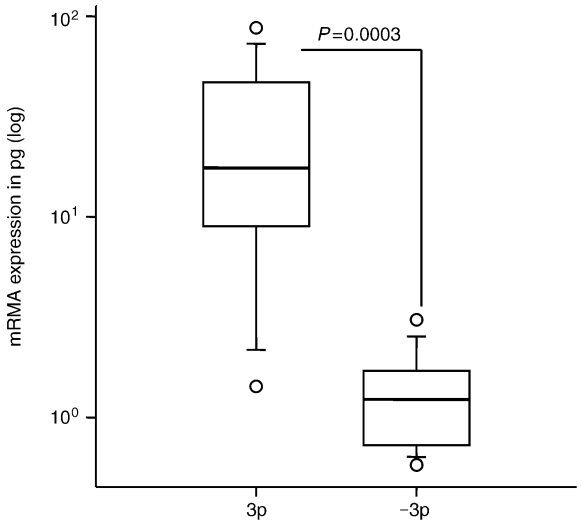

Fig. 5. *TIMP-4* mRNA expression in correlation to presence or absence of chromosomal loss of 3p. Data are given as box plots, the box encompasses the median value as well as the 25th through to the 75th percentile. Values outside the 10th and the 90th percentile (bars) are indicated as circles.

investigated MMPs showed a considerably stronger expression in the CC-RCCs than in the P-RCCs, with the exception of *MMP-2* and *MMP-14* for which there were no detectable differences. In particular, CC-RCCs were characterised by high mRNA concentrations of *MMP-9* and, to a lesser extent, *MMP-1* and *MMP-11*. Papillary morphology was associated with a pronounced expression of *MMP-1*. The latter MMP was unique in that it was overexpressed in both tumour subtypes, and mRNA levels were significantly higher in the P-RCCs than in the CC-RCCs. As non-malignant areas of P-RCC bearing kidneys also expressed more *MMP-1* mRNA than the respective tissues of the CC-RCC kidneys, the ratio of *MMP-1* mRNA in the tumour versus non-tumour tissues was still higher in the CC-RCCs. However, mRNA concentrations in the non-malignant parts of the CC-RCCs were close to the detection limit, and the papillary subgroup was comparably small. Thus, small differences might disproportionately alter the calculated ratio and should be interpreted with caution.

In general, the present data are in accordance with those of other authors. Increased *MMP-9* mRNA expression has been shown in colorectal cancers which was correlated with advanced tumour stage and the occurrence of synchronous metastases [27]. Augmented protein levels and activity of MMP-9 and -2 were associated with poor survival in gastric cancers [28] as well as with lymph-node involvement and unfavourable prognosis in breast cancer [17]. *MMP-11* has been shown to be upregulated in the majority of invasive breast cancers, in contrast to its absence in normal mammary tissue (reviewed in Ref. [29]). *MMP-11* and *MMP-14* were overexpressed in lung cancers [30], while high levels of *MMP-1*, *MMP-2*, *MMP-3* and *MMP-9* were detectable in oesophageal cancer and upregulation of *MMP-1* was a particularly strong predictor of poor prognosis [31]. In renal cell carcinomas, Kugler and coworkers demonstrated upregulation of *MMP-2*, *MMP-9* and *MMP-14* mRNA in tumour tissues compared with their non-malignant counterparts, and elevated activity of MMP-2 was associated with advanced tumours [32]. Moreover, Lein and colleagues [33] found significantly higher MMP-9 protein expression in malignant than in normal tissues. There was no difference in MMP-2 expression.

While the data unequivocally suggests an association of MMP overexpression and malignant progression in general, the relation between the expression of the TIMPs and malignancy is less clear. Early studies have shown a downregulation of TIMPs in tumour tissues, supporting the assumption that TIMPs as inhibitors of MMPs would exert an anti-invasive effect. Recent studies, however, point to a more complex role of TIMPs. Apart from their inhibitory function for tumour proliferation and angiogenesis, growth factor-like effects have been described for TIMP-1 and-2 [34]. TIMP-2 is involved in the activation of MMP-2 by formation of a trimolecular complex together with MT1-MMP (=MMP-14) [35], and TIMP-1 overexpression has been shown to be positively correlated with a shortened survival, at least in some tumours [36,37]. The biological function of TIMPs is additionally regulated by their relative expression in comparison to the respective MMPs. Miyake and coworkers [38] found a positive correlation between the MMP:TIMP ratio and the invasive potential in murine renal carcinoma cell lines with various MMP-2:TIMP-2 expressions. In aggressive renal carcinomas, Kugler and colleagues described a significantly increased MMP:TIMP ratio [32]. Other authors, however, could not confirm these data [33].

In our study, mRNA concentrations of *TIMP-1* and *TIMP-2* were generally low with a tendency to higher expression in benign than in malignant tissues. Of note, there was a significant upregulation of mRNA levels of *TIMP-3* and *TIMP-4* in the non-malignant surrounding tissue of CC-RCCs, while in the tissue surrounding the P-RCCs only *TIMP-4* was strongly expressed. Considering the relative expression of the investigated MMPs and TIMPs, the MMP:TIMP ratio was low in normal and high in malignant tissues. There was a particularly strong shift in favour of the MMPs in CC-RCCs which might contribute to the higher metastatic potential of this entity.

TIMP-4 was the only inhibitor strongly expressed in the tumour tissue, namely in the papillary subtype. In the CC-RCCs, median mRNA levels of *TIMP-4* were low. Upon evaluation of the *TIMP-4* expression in the 18 cytogenetically analysed tumours, a highly significant

correlation of low *TIMP-4* concentrations and chromosomal loss of 3p could be demonstrated. Alterations of the short arm of chromosome 3, especially within the region 3p21–26, are frequent findings in sporadic RCCs of clear cell morphology [39]. Furthermore, each of the carcinomas with 3p abnormalities belonged to the CC subtype. As the *TIMP-4* gene locus has been mapped to chromosome 3p25 [40], our data suggests that downregulation of *TIMP-4* transcription may represent a direct consequence of the structural genomic alteration. For the other detected mutations, no comparable connection could be established, possibly due to the relatively small number of cases.

Expression profiles of both MMP and TIMP mRNAs were surprisingly different in the two histological subtypes. CC-RCCs were characterised by strong upregulation of three MMPs as well as by general downregulation of TIMPs, while in P-RCCs only one MMP was substantially upregulated and the levels of *TIMP-4* were high. Only a few other studies have focused on the relationship between the MMP and TIMP expression profile and histology. Murray and coworkers described a correlation of *MMP-1* and *MMP-9* overexpression with stomach cancers of the intestinal subtype [31]. In breast cancers, *MMP-9* overexpression was characteristically associated with invasive lobular carcinomas in contrast to other invasive ductal tumours [41]. The detection of a higher frequency of MMP upregulation in adenocarcinomas of the lung compared with squamous cell cancers led the authors to assume a functional significance of the expression pattern for the biological behaviour of the tumours [30]. However, in 20 RCCs, 3 of them with papillary morphology, expression of MMP-2/-9 and TIMP-1/-2 protein was not correlated with the histological subtype [33]. The differing results may partly be explained by the differing composition of the investigated cases with regard to grade and tumour stage and the small size of the respective subgroups. Additionally, measurement of mRNA expression by quantitative RT-PCR, as used in the present study, is highly sensitive but does not provide information about the amount of protein expressed and its associated activity.

On the basis of the present data, it is tempting to speculate that the strong overexpression of multiple MMPs in CC-RCCs may explain the more aggressive phenotype of these tumours. In accordance with this hypothesis, upregulation of *MMP-9* was correlated with higher histological grade as well as with a larger size of the primary tumours, while an inverse correlation could be shown for *TIMP-4*. As less differentiated tumours were usually larger and more often found in the CC-RCC group, it remains unclear, whether these variables are statistically independent. Investigation of larger numbers by use of a multivariate analysis is necessary to clarify this question.

Taken together, clear cell and papillary subtypes of RCCs are characterised by differential MMP and TIMP expression profiles with more frequent overexpression of MMPs as well as downregulation of TIMPs in the CC-RCCs. Downregulation is particularly striking for TIMP-4, presumably as a direct consequence of the typical genomic alteration in CC-RCCs, the chromosomal loss of 3p. The impaired balance between MMPs and TIMPs with a preponderance of MMPs, especially in CC-RCCs, may provide an explanation for the differing growth patterns of P-RCCs and CC-RCCs and the higher tendency to vessel invasion and early metastatic dissemination of the CC-RCCs.

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