Disease Markers 34 (2013) 395–405
DOI 10.3233/DMA-130986
IOS Press

Expression of matrix metalloproteinases in human breast cancer tissues

Chellakkan Selvanesan Benson[a], Somasundaram Dinesh Babu[a], Selvi Radhakrishna[b], Nagarajan Selvamurugan[c] and Bhaskaran Ravi Sankar[a,*]
[a]*Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India*
[b]*Chennai Breast Centre, MRC Nagar, Chennai, India*
[c]*Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur, Chennai, India*

Abstract.
BACKGROUND: Breast cancer is the most common cancer affecting women in the world today. Matrix metalloproteinases (MMPs) are a family of endopeptidases that can degrade extracellular matrix proteins and promote cell invasion and metastasis. MMPs are differentially expressed and their expressions are often associated with a poor prognosis for patients.
OBJECTIVE: The aim of this study is to investigate and compare the expression of MMPs in different grades of human breast cancer tissues with normal breast tissues.
PATIENTS AND METHODS: We collected 39 breast cancer samples (24 grade II and 15 grade III) along with 16 normal breast tissues from outside the tumor margin during cancer removal surgery. The samples were analysed for the expression of all known MMPs using real-time quantitative PCR.
RESULTS: The results indicate that mRNA expressions of MMP-1, -9,-11,-15,-24 and -25 were upregulated in breast cancer tissues when compared to normal breast tissues. But, the mRNA expressions of MMP-10 and MMP-19 were downregulated in cancer tissue. In membrane associated MMPs like MMP-15 and MMP-24 we found a grade dependent increase of their mRNA expression.
CONCLUSION: Our studies demonstrate that MMPs are differentially regulated in breast cancer tissues and they might play various roles in tumor invasion, metastasis and angiogenesis. Thus, MMPs are of immense value to be studied as diagnostic markers and drug target.

Keywords: Matrix metalloproteinase, cancer grade, breast cancer, matrix degradation, diagnostic marker

Abbreviations

MMPs	Matrix metalloproteinases
PCR	Polymerase chain reaction
ECM	Extra cellular matrix
MMPIs	Matrix metalloproteinases inhibitors
EMT	Epithelial to mesenchymal transition
VEGF	Vascular endothelial growth factor
RT	Reverse transcription
ER	Estrogen Receptor
PR	Progesterone Receptor
HER2	Human Epidermal Growth Factor Receptor 2
TGFβ	Transforming growth factor β

1. Introduction

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases whose primary function is the degradation of proteins in the extracellular matrix (ECM) [1]. In humans, 23 members of MMPs have

*Corresponding author: B. Ravi Sankar, Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600113, India. Tel.: +91 44 24547044; Fax: +91 44 24540709; E-mail: bensoncs@gmail.com.

ISSN 0278-0240/13/$27.50

been reported so far and are classified based on their substrate specificity in to collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11), matrilysins (MMP-7 and -26), enamelysin (MMP-20), membrane-bound MMPs (MMP-14 to -17, MMP-24 and -25) and others (MMP-19, -21, -23, -27 and -28) [2]. MMPs are highly up-regulated and are active in malignant tissues compared to normal, benign or premalignant tissues, with highest expressions in tumor-stromal interface [3]. They are involved in various vital aspects of cancer progression including proliferation, invasion, epithelial to mesenchymal transformation, metastasis and angiogenesis.

Several studies have described the presence and role of MMPs in breast cancer. MMPs (MMP-1, -2, -8 to -13, -15, -19, -23, -24, -27 and -28) showed a stronger expression in breast cancer tissue compared to normal breast tissue [4]. Further, MMP-8, -10, -12 and -27 showed a tumor grade dependent expression [4]. MMP-1, 2, 3, 9 and 14 are implicated as key factors in tumor invasion, metastasis and angiogenesis [5]. MMP-2, -7, -9, -10, -11, -13, -14 and -15 have been involved in the progression of breast cancer [6]. MMP-1, -2, -7 and -9 have been linked to cancer cell proliferation [7–10], whereas MMP-1, -2, -3, -7 and -9 to -18 are involved in breast tumor metastasis [5,7,8,10–12]. MMP-13 is shown to dissolve bone matrix and favours breast-bone metastasis [8]. Further, MMP-1 to -4, -9 to -19 have been found to regulate the invasion of breast cancer [5–8,10–15]. Another key aspect in breast cancer is the epithelial to mesenchymal transformation which is facilitated by MMP-1, -2, -3 and -9 [16,17]. MMP-3 promotes late epithelial-to-mesenchymal phenotypic changes which are associated with aggressive malignancy [9]. MMPs -1, -2, -7 and -9 have been shown to play a role in tumor angiogenesis [5,8,10,11]. Twenty three members of MMPs are known so far, however all of their roles in breast cancer is not fully understood.

MMPs have been considered as potential targets for cancer therapy because of their enormous upregulation in cancer tissues and their exclusive ability to degrade ECM. MMPs are promising candidates to be used as potential cancer biomarkers with vast diagnostic and prognostic value. Evidence is emerging that MMPs can serve not only as potential markers for diagnosis and prognosis, early detection and risk assessment, but also as indicators of tumor recurrence, metastatic spread, and response to primary and adjuvant therapy for breast cancer [18]. The role of MMP-9 has been extensively studied in breast cancer [18]. In breast cancer patients, the levels of MMP-9 in tumor tissue, serum, plasma, urine and nipple aspirate fluid are highly elevated [19–21]. Further, MMP-9 levels significantly dropped following the surgical removal of primary breast tumours and in patients who responded well to adjuvant therapy [22]. Similar observations has been made in other types of cancers also including pancreatic [23], bladder [24], lung [25], colorectal [26], ovarian [27], prostate [28] and brain tumours [29]. MMP could be used as possible therapeutic target using MMP inhibitors (MMPIs). Its clinical use however is complicated. Similar to many anticancer drugs, MMPIs may actually be more effective when administered at earlier stages of cancer progression rather than when a patient is suffering from end stage disease having experienced failure with all other conventional therapies [18].

Although plenty of data is available on the role and expression of MMPs, no systematic attempt has been done so far to quantitatively survey the presence of all the known MMPs in different grades of human breast cancer tissues. In this study we are aiming to investigate expression levels of all human MMPs known so far in different grades of human breast cancer tissues along with normal human breast tissues.

2. Patients and methods

2.1. Tissue samples

The study was performed with approval of the Ethics Committee of the University of Madras, India, (Ref No: PGIBMS/CO/Human Ethical/2009-10/353) and was carried out in accordance with the Helsinki declaration of 2000 of the World Medical Association. Written informed consents were obtained from all patients according to the institutional regulations. All the samples were collected from ethnic Indians from the Indian sub-continent. Breast tumor removal surgeries were performed by a trained breast cancer surgeon (S.R) at Chennai Breast Centre, Chennai, India. Thirty nine samples of breast cancer tissue (24 grade II and 15 grade III tumours) and sixteen normal breast tissue samples were obtained between September 2009 and December 2011. These patients did not receive any form of chemotherapy or radiotherapy treatments prior to their surgery. Normal breast tissues were obtained from outside the tumor margin and these tissues were analysed histologically to exclude them from

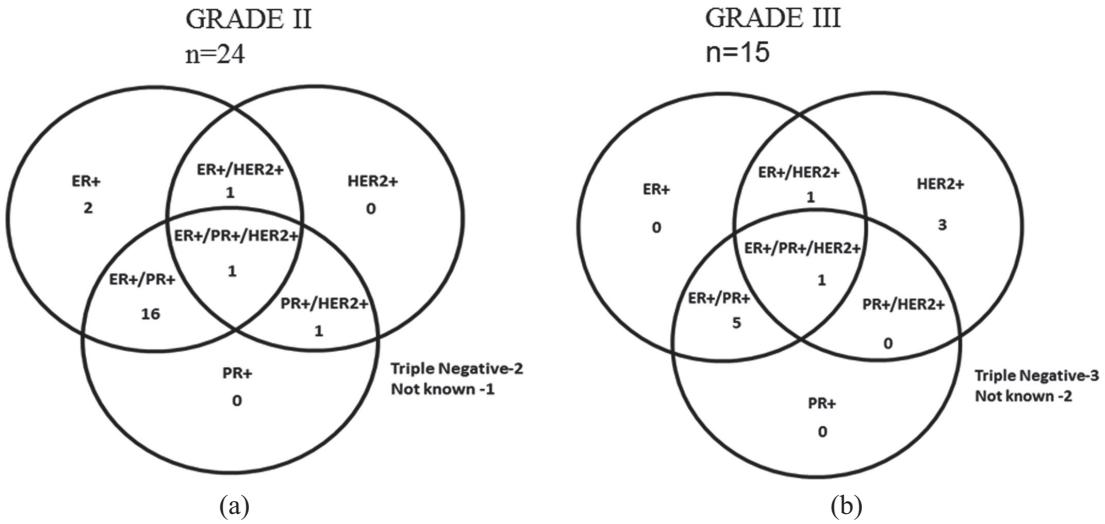

Fig. 1. Venn diagram shows the presence of ER, PR and HER2 and their distribution among the samples in Grade II (Fig. 1a) and Grade II (Fig. 1b) breast cancer tissues.

Table 1
Patients data

Age	Grade II	Grade III
Minimum	29	
Maximum	85	
Mean	56	
T (Tumor size)		
1	3	1
2	18	12
3	1	0
4	2	1
Not known	0	1
N (Nodal Status)		
0	11	5
1	5	3
2	5	1
3	2	4
X	1	1
Not known	0	1
L (Invasion of Lymphatic vessel)		
0	10	7
1	8	6
X	6	1
Not known	0	1

G II – moderately differentiated tumor; G III – poorly differentiated; T – tumor grade; N – nodal status; L – Invasion of lymphatic vessels.

any forms of malignancy or other pathological findings (Data not shown). The cancer tissues obtained during surgery were immediately placed in -20°C cryobox and brought to the laboratory and stored in -80°C freezers until used.

2.2. Total RNA extraction and real time RT-PCR

Total RNA was extracted by a single-step technique using TRI Reagent (Sigma, USA) according to the manufacturer's protocol. Reverse transcription (RT) was done using the iScript cDNA Synthesis kit (Bio-Rad, Richmond, CA, USA) following the manufacturer's procedures. For cDNA synthesis, 2 μg of total RNA was reverse-transcribed using random hexamer primers and MMLV RT enzyme. Real-time PCR was performed on a CFX 96 Touch Real Time PCR (Bio-Rad, USA) using the Mesa Green qPCR kit (Eurogentec, USA). The cycling conditions were 95°C 5 m, 45 cycles (95°C 30 s, 56°C 30 s). Under these conditions, no amplification was observed in the non-template or no RT controls. The list of primers and the internal control sequence are given in (Table 2). The specificity of the amplification product was determined by melting curve analysis for each primer pairs. The data was analysed by comparative CT method and the fold change was calculated by $2^{-\Delta\Delta CT}$ method using CFX Manager Version 2.1 (Bio Rad, USA).

2.3. Statistical analysis

The data were analysed using GraphPad Prism 5.0 Software (GraphPad Software, La Jolla, USA). Comparison of expression values between normal (control) and cancer were performed by the non-parametric t test followed by Mann-Whitney U test and comparison between normal, grade II and grade III were performed by the Kruskal-Wallis test followed by Dunn's multiple comparison test, and p-values < 0.05 were considered as statistically significant.

Table 2
The primers used in quantitative PCR reactions for human matrix metalloproteinases and GAPDH genes are as follow

Name	Accession number	Primer sequence
GAPDH	NM_002046	Forward 5'-GCATCCTGGGCTACACTGAG-3'
		Reverse 5'-TGCTGTAGCCAAATTCGTTG-3'
MMP-1	NM_002421	Forward 5'-GACAGAAAGAGACAGGAGAC-3'
		Reverse 5'-GAGTTATCCCTTGCCTATCC-3'
MMP-2	NM_004530	Forward 5'-GCTGGCTGCCTTAGAACCTTTC-3'
		Reverse 5'-GAACCATCACTATGTGGGCTGAGA-3'
MMP-3	NM_002422	Forward 5'-GAAGAGAAATTCCATGGAGCCAGG-3'
		Reverse 5'-AGAAATAAAAGAACCCAAATTCTTCAAAAACA-3'
MMP-7	NM_002423	Forward 5'-GGGACATTCCTCTGATCCTAATGC-3'
		Reverse 5'-GAATTACTTCTCTTTCCATATAGTTTCTGAATGC-3'
MMP-8	NM_002424	Forward 5'-CCACTTTCAGAATGTTGAAGGGAAG-3'
		Reverse 5'-TCACGGAGGACAGGTAGAATGGA-3'
MMP-9	NM_004994	Forward 5'-GCACGACGTCTTCCAGTACC-3'
		Reverse 5'-GCACTGCAGGATGTCATAGGT-3'
MMP-10	NM_002425	Forward 5'-ACATTGCTAGGCGAGATAGG-3'
		Reverse 5'-GGCTCATCTTCTTCAGTCAC-3'
MMP-11	NM_005940	Forward 5'-CAACATACCTCAATCCTGTCCC-3'
		Reverse 5'-CAATGGCTTTGGAGGATAGC-3'
MMP-12	NM_002426	Forward 5'-TTGAATATGACTTCCTACTCCAACG-3'
		Reverse 5'-GTGGTACACTGAGGACATAGCAAAT-3'
MMP-13	NM_002427	Forward 5'-GAC TTCCCAGGAATTGGTGA-3'
		Reverse 5'-TGA CGCGAACAATACGGTTA-3'
MMP-14	NM_004995	Forward 5'-GAGCTCAGGGCAGTGGATAG-3'
		Reverse 5'-GGTAGCCCGGTTCTACCTTC-3'
MMP-15	NM_002428	Forward 5'-CAGGCCACACCTTCTTCTTC-3'
		Reverse 5'-CCAGTATTTGGTGCCCTTGT-3'
MMP-16	NM_005941	Forward 5'-AGGTGTCAGTTCAGTGTTACTAGAG-3'
		Reverse 5'-AATGAGAAATGAAGCAGAAGGAGAA-3'
MMP-17	NM_016155	Forward 5'-ACTCATGTACTACGCCCTCA-3'
		Reverse 5'-GAGAAGTCGATCTGGATGTC-3'
MMP-19	NM_002429	Forward 5'-GGGTCCTGTTCTTCCTACAT-3'
		Reverse 5'-CAATCCTGCAGTACTGGTCT-3'
MMP-20	NM_004771	Forward 5'-TGAGAGGGGCACTGCTTACT-3'
		Reverse 5'-GTCTTCTGTGGCTCCCTGAG-3'
MMP-21	NM_147191	Forward 5'-AACAATAGGACACGCTATGG-3'
		Reverse 5'-CATCTCTTTTCCATGTCCAG-3'
MMP-23	NM_006983	Forward 5'-CCAGAAGATCCTCCACAAGA-3'
		Reverse 5'-CAGGTGTAGGTGCCCTCATT-3'
MMP-24	NM_006690	Forward 5'-GGCAAAAACACATCACCTAC-3'
		Reverse 5'-GGTCACTTTTGATCTCATGG-3'
MMP-25	NM_022468	Forward 5'-GATCAGCATGAGGACAGAAG-3'
		Reverse 5'-GAAACTGACAGAGGCCCAAATC-3'
MMP-26	NM_021801	Forward 5'-GGAATGGGACAGACCTACTT-3'
		Reverse 5'-AGTGTGCTTATTCCACTTGC-3'
MMP-27	NM_022122	Forward 5'-TCAGGCATATCTCAACCAGT-3'
		Reverse 5'-CTGGGTGTCTTCATGATCTC-3'
MMP-28	NM_024302	Forward 5'-GAGGCATTCCTAGAGAAGTACGGA-3'
		Reverse 5'-CTGAAACGCTCTGATGGCATC-3'

3. Results

3.1. Patient data

In the present study we analysed 24 grade II samples and 15 grade III samples from patients of age ranging from 29 to 85 with a mean age of 56 years. The patient data is summarized in Table 1. Patients with grade II tumour had bigger tumour size, but no differences were observed in the nodal status between the groups. More patients in grade III tumour had lymph node metastasis at the time of diagnosis. The distribution of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) statuses are shown in Figs 1a and 1b. The ER, PR and HER2 status was identified by immunohistochemistry.

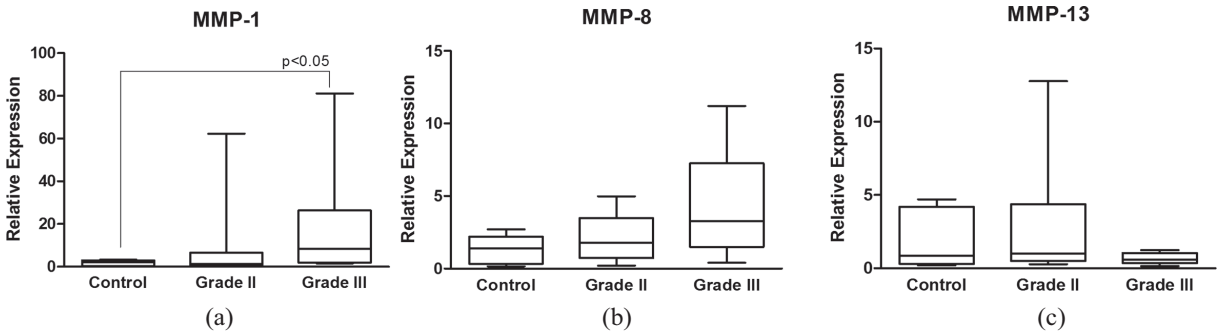

Fig. 2. Expression of collagenases in human breast cancer and in control tissues. Expression of MMP-1 (Fig. 2a), MMP-8 (Fig. 2b) and MMP-13 (Fig. 2c) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

3.2. *Expression of collagenases (MMP-1, -8 and -13) in breast cancer tissues*

The expression of collagenases tends to increase in the cancer tissues in comparison to the control breast tissues. In order to determine the quantitative changes of MMPs expression associated with human breast cancer progression, total RNA was isolated from tissues and subjected to real time PCR using the specific primers designed for MMPs. MMP-1 was significantly increased ($p < 0.05$) in grade III breast cancer when compared with control with around 4 times the median expression of the control, but in grade II the expression was equivalent to that of controls (Fig. 2a). MMP-8 expression showed an upregulation, however, it did not reach statistical significance (Fig. 2b). There was no change in the mRNA expression of MMP-13 in breast cancer tissues compared to normal breast tissues (Fig. 2c).

3.3. *Expression of gelatinases (MMP-2 and MMP-9) in breast cancer tissues*

MMP-2 did not show any significant increase in grade II or grade III when compared to controls (Fig. 3a). MMP-9 was found to be highly upregulated in grade II with more than 10 fold upregulation ($p < 0.01$) but its expression was significantly downregulated in grade III ($p < 0.05$) (Fig. 3b). MMP-9 expression was upregulated in cancer tissues compared to control ($p < 0.05$) in both grade II and grade III breast cancer tissues (Fig. 3c).

3.4. *Expression of stromelysins (MMP-3 and MMP-10) in breast cancer tissues*

There was no change in the mRNA expression level of MMP-3 in cancer tissues compared to normal breast tissue's (Fig. 4a). However, MMP-10 level was significantly decreased in grade II ($p < 0.001$) and in grade III ($p < 0.05$) compared with control to 6-fold and 5-fold respectively (Fig. 4b). MMP-10 level was dramatically downregulated ($p < 0.001$) with 95.83% downregulation when compared between normal human breast tissues and breast cancer tissues without taking grades into account (Fig. 4c).

3.5. *Expression of matrilysins (MMP-7, MMP-11 and MMP-26) in breast cancer tissues*

MMP-7 expression did not show any change at mRNA level between controls and human breast cancer tissues; however some samples in grade III showed several fold up regulation (Fig. 5a). The expression of MMP-11 significantly increased in grade II ($p < 0.05$) when compared with control but no increase could be seen in grade III cancer tissues (Fig. 5b). MMP-11 level was significantly increased 6-fold ($p < 0.05$) in grades II and III combined cancer tissues compared to normal human breast tissues (Fig. 5c). We did not observe any change in the expression of MMP-26 in human breast cancer tissues (Fig. 5d).

3.6. *Expression of membrane associated MMPs (MMP-14 to MMP-17, MMP-24 and MMP-25) in breast cancer tissues*

MMP-15 and -24 showed a grade dependent increase in their expression pattern in human breast cancer tissues. Their expressions were significantly increased in grade II ($p < 0.05$) and in grade III ($p < 0.01$) when compared to normal human breast tissues (Fig. 6a and 6b). MMP-25 expression level was significantly upregulated ($p < 0.05$) only in grade II but not in grade III human breast cancer tissues in com-

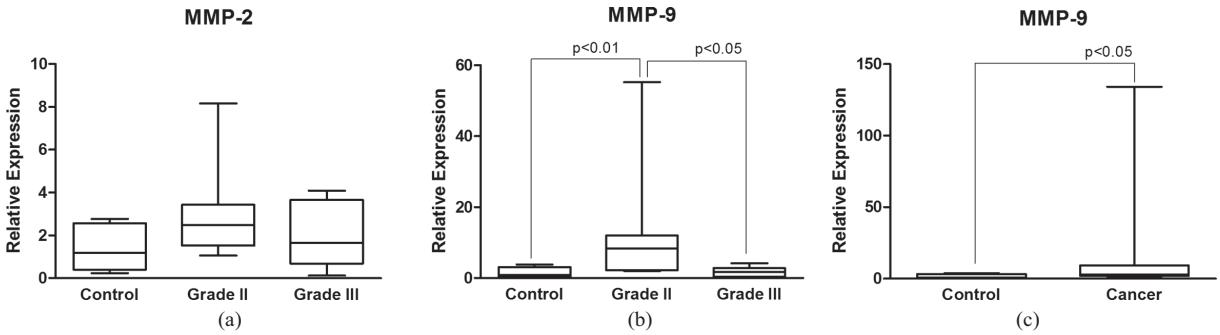

Fig. 3. The expression of gelatinases in breast cancer and in control tissue. Expression of MMP-2 (Fig. 3a) and MMP-9 (Fig. 3b) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

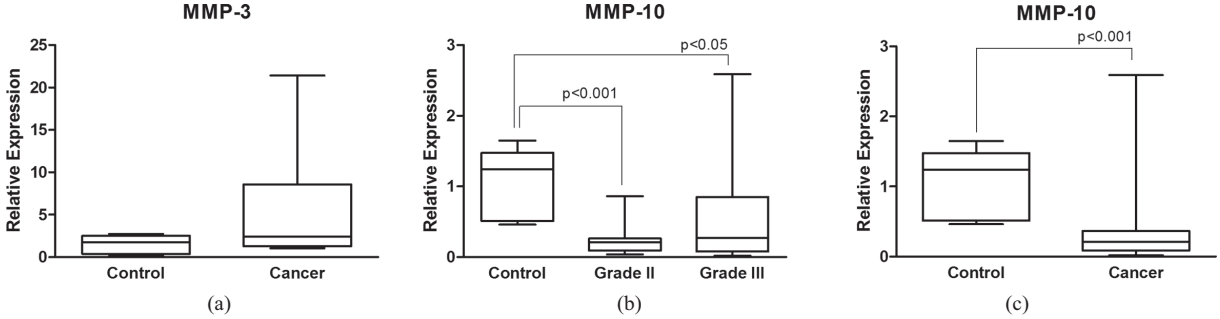

Fig. 4. The expression of stromelysins in breast cancer and in control tissue. Expression of MMP-3 (Fig. 4a) and MMP-10 (Fig. 4b) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

parison to control tissues (Fig. 6c). A significant upregulation of MMP-15 ($p < 0.05$), MMP-24 ($p < 0.01$) and MMP-25 ($p < 0.01$) expression was found in grades II and III combined human breast cancer tissues (Fig. 6d, e and f). There was no change in the expressions of MMP-14, MMP-16 and MMP-17 in cancer tissues compared to normal tissues (data not shown).

3.7. Expressions of other MMPs (MMP-12, MMP-19 to MMP-21, MMP-23, MMP-27 and MMP-28) in breast cancer tissues

There was a significant increase in the expression of MMP-12 in grade III when compared to grade II ($p < 0.01$), however, there was no difference in the expression level of MMP-12 between control and any of the cancer grade tissues (Fig. 7a). MMP-19 was significantly downregulated ($p < 0.05$) in grade combined cancer tissues when compared to normal breast tissue (Fig. 7b). MMP-20,-21,-23,-27 and -28 did not show any differences in their expression between normal breast and breast cancer tissues (data not shown).

4. Discussion

MMPs degrade virtually every component of the ECM and their substrates also include non-ECM molecules like growth factor precursors and cell surface adhesion molecules. MMPs have also been implicated in the epithelial to mesenchymal transition (EMT) leading to metastasis [30]. Further, they promote angiogenesis through their release of angiogenic factors such as vascular endothelial growth factor (VEGF) [31]. Our results show that MMPs are differentially regulated in breast cancer tissues compared to normal breast tissues. It is interesting to note that MMPs (collagenases, gelatinases, stromelysins and matrilysin) having different substrate specificities are regulated during breast cancer progression.

Our data show that among collagenases, MMP-1 alone is upregulated in breast cancer. The upregulation of MMP-1 in grade III breast cancer is similar to several previous observations [4,22,23]. It is known that MMP-1 promotes cancer invasion through matrix degradation, angiogenesis, osteoclast activa-

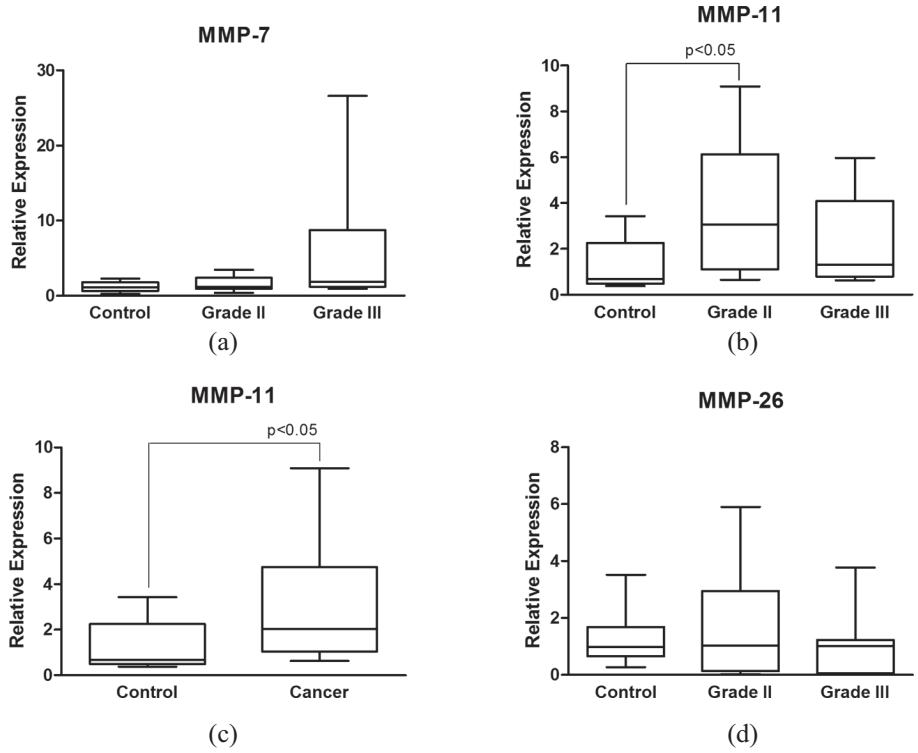

Fig. 5. The expression of matrilysin in breast cancer and in control tissue. Expression of MMP-7 (Fig. 5a), MMP-11 (Fig. 5b) and MMP-26 (Fig. 5c) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

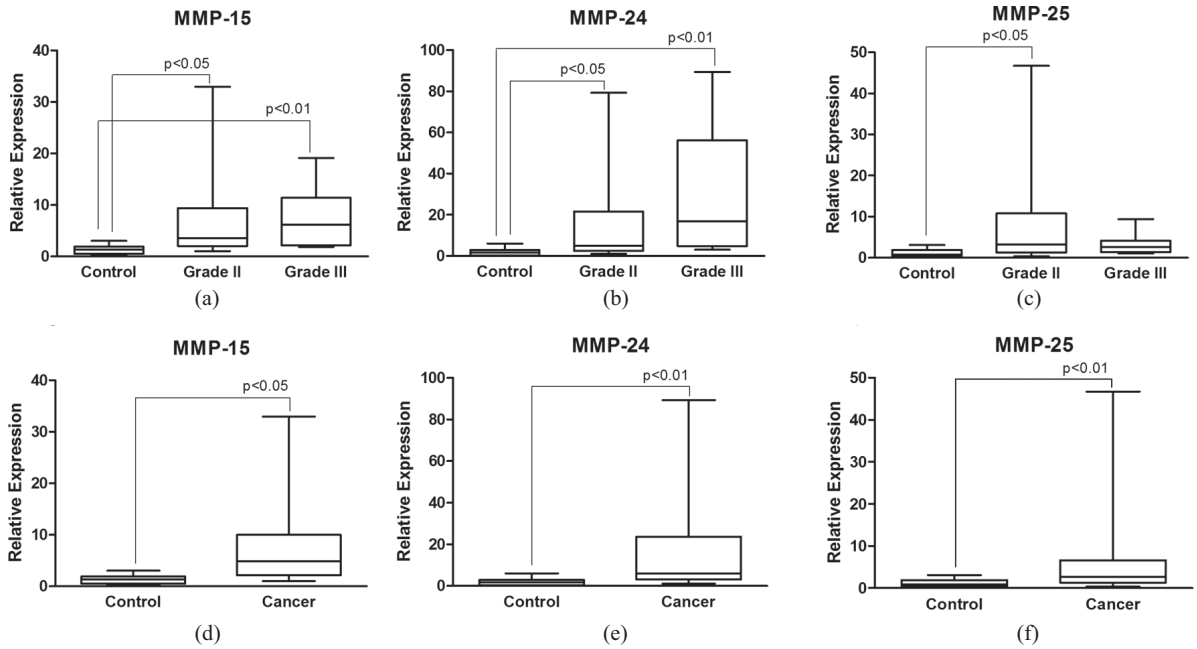

Fig. 6. The expression of Membrane Associated MMPs in breast cancer and in control tissue. Expression of MMP-14 (Fig. 6a), MMP-24 (Fig. 6b) and MMP-25 (Fig. 6c) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

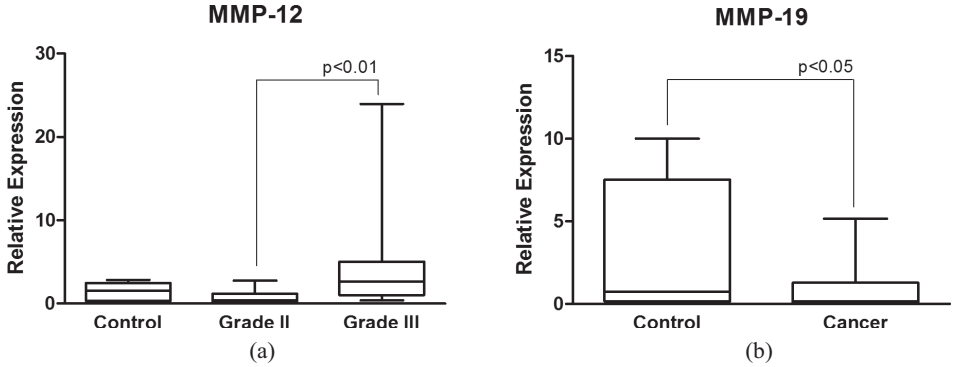

Fig. 7. The expression of Other MMPs in Control, Grade II and Grade III breast cancer tissues. Expression of MMP-12 (Fig. 7a) and MMP-19 (Fig. 7b) in control, grade II and grade III breast cancer. The expression level of each gene was normalized to corresponding expression of β-actin. Control, $n = 16$; Grade II, $n = 24$ and Grade III, $n = 15$.

tion and metastasis [24,25]. Recent studies demonstrated that MMP-1 is associated with poor clinical outcome showing significant negative correlation with survival, breast cancer progression and poor prognosis [26–28,32]. We did not observe any significant difference in MMP-8 mRNA levels and a similar observation has also been reported previously but at protein level [4]. MMP-13 is associated with increased malignancy and shorter overall survival and represents a poor prognosis marker in breast carcinomas [13,33, 34]. We previously reported that MMP-13 is expressed in highly metastatic and invasive human breast cancer cells (MDA-MB231) and its expression is upregulated by local bone growth factors such as TGF-β1 in these cells [35–37]. Although MMP-13 was shown to be upregulated in breast cancer [4], our data did not show any such tendency. Gelatinases (MMP-2 and MMP-9) are involved in tumor invasion, metastasis, tissue remodelling via extracellular matrix as well as basement membrane degradation and induction of angiogenesis [3]. Our data is also consistent that MMP-9 expression is upregulated in cancer tissues. MMP-9 plays a vital role in tumor angiogenesis, regulating the bioavailability of VEGF, which is a potent inducer of tumor angiogenesis and a therapeutic target [31, 38]. It has been shown that MMP-9 along with MMP-2 activates TGFβ signalling to promote tumor invasion, angiogenesis, cell survival and promotes metastasis [29,39]. In this study, MMP-2 mRNA expression did not show any change which is similar to a previous observation [4]. Experimental evidence has shown that MMP-3 may be involved in breast cancer invasion [15]. MMP-3 provides an example of a metalloproteinase that can be either protective or protumorigenic in relation to growth [40]. In our study expression level of MMP-3 did not show any change.

MMP-10 plays an important role in the invasion and metastasis of head and neck squamous cell carcinoma [41] but its role on breast cancer is largely unknown. We found that MMP-10 expression was decreased in comparison with control suggesting that MMP-10 could act as tumor suppressor. In matrilysin family, MMP-7 expression has been shown to be upregulated [4]. MMP-7 has been implicated in inflammation, proliferation, apoptosis, invasion and angiogenesis [37]. Our results show an upregulating tendency but did not reach statistical significance. MMP-11 was upregulated in our study in grade II breast cancer patients. Other reports also support our data [4, 38]. It is reported that overexpression of MMP-11 correlates with poorly differentiated tumours and lymph node metastasis [38]. Unlike a previous report [4], we observed the presence of MMP-26, however there was no change in its expression. Membrane associated MMPs like MMP-15, MMP-24 and MMP-25 showed upregulation with MMP-15 and MMP-24 showing a grade dependent increase. A recent study indicate that MMP-15 is associated with tumorogenesis, invasion and metastasis [42]. MMP-24 and MMP-25 have been shown to be present in MDA-MB-231 breast cancer cells [15] and breast cancer tissue [4]. Similarly, our data also show a grade dependent increased in the expression of MMP-24. MMP-24 has also been shown to promote invasiveness in ovarian cancer cells [43]. Although no reports are available on role of MMP-24 in breast cancer, there is a possibility that MMP-24 may perform a similar action. MMP-25 has been shown to express highly in colon cancer and promotes tumor growth [44].

MMP-12 has been reported to play a role in the degradation of extracellular matrix [45]. In this study, we found that MMP-12 expression was increased be-

Table 3
Summary table

MMPs	Cancer*	Grade II	Grade III
MMP-1	↔	↔	↑
MMP-2	↔	↔	↔
MMP-3	↔	↔	↔
MMP-7	↔	↔	↔
MMP-8	↔	↔	↔
MMP-9	↑	↑	↓
MMP-10	↓	↓	↓
MMP-11	↑	↑	↔
MMP-12	↔	↔	↑
MMP-13	↔	↔	↔
MMP-14	↔	↔	↔
MMP-15	↑	↑	↑
MMP-16	↔	↔	↔
MMP-17	↔	↔	↔
MMP-19	↓	↔	↔
MMP-20	↔	↔	↔
MMP-21	↔	↔	↔
MMP-23	↔	↔	↔
MMP-24	↔	↑	↑
MMP-25	↔	↑	↔
MMP-26	↔	↔	↔
MMP-27	↔	↔	↔
MMP-28	↔	↔	↔

↑ – Up regulated; ↓ –Down regulated; ↔ – Up or Down regulated but no statistical significance; *Both grade II and grade III cancer.

tween grade II and grade III of breast cancer. A similar observation was reported earlier [4]. Although the role for MMP-19 in breast cancer is not understood, it is involved the development of colorectal cancer [46]. We also show that the expression of MMP-19 is downregulated in breast cancer compared to control samples. Further, it has also been reported that MMP-19 is downregulated during neoplastic transformation and histological dedifferentiation [47] supporting our observation in this study.

5. Conclusion

Overall, our study shows that expression of MMP-1, -9,-11,-15,-24 and -25 are upregulated in human breast cancer tissues compared to normal human breast tissues. Further, membrane associated MMPs like MMP-15 and MMP-24 displayed a grade dependent increase of their expression, whereas the expression of MMP-10 and MMP-19 were downregulated (Table 3). During breast cancer progression, there is differential expression of MMPs and this is the first study to systematically report expression of all 23 MMPs at mRNA level in human breast cancer tissues using a quantitative approach. Since expressions of these MMPs are altered during breast cancer progression, these regulated MMPs could be of enormous value to be studied as diagnostic markers and novel drug targets.

Acknowledgements

The work was supported by Department of Science and Technology, INSPIRE, IF 10052 (to C.S.B). Financial assistance was provided by UGC-SAP, DST PURSE, Susan G. Komen Breast Cancer Foundation (to N.S) and University of Madras is gratefully acknowledged.

Conflict of interest

None.

References

[1] Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nature Reviews Cancer 2(3): 161-174.
[2] Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovascular Research 69(3): 562-573.
[3] Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annual Review of Cell and Developmental Biology 17: 463-516.
[4] Kohrmann A, Kammerer U, Kapp M, Dietl J, Anacker J (2009) Expression of matrix metalloproteinases (MMPs) in primary human breast cancer and breast cancer cell lines: New findings and review of the literature. BMC Cancer 9: 188.
[5] Mark D Sternlicht GB (2000) Matrix metalloproteinases as emerging targets in anticancer therapy: Status and prospects. Emerging Therapeutic Targets 4: 609-633.
[6] Eiseler T, Doppler H, Yan IK, Goodison S, Storz P (2009) Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. Breast Cancer Research: BCR 11(1): R13.
[7] Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P (1990) A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348(6303): 699-704.
[8] Pivetta E, Scapolan M, Pecolo M, Wassermann B, Abu-Rumeileh I, Balestreri L, Borsatti E, Tripodo C, Colombatti A, Spessotto P (2011) MMP-13 stimulates osteoclast differentiation and activation in tumour breast bone metastases. Breast Cancer Research: BCR 13(5): R105.
[9] Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ (1997) Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. The Journal of Cell Biology 139(7): 1861-1872.
[10] Keshgegian AA, Cnaan A (1996) Estrogen receptor-negative, progesterone receptor-positive breast carcinoma: Poor clinical outcome. Archives of Pathology and Laboratory Medicine 120(10): 970-973.

[11] Surowiak P, Dziegiel P, Zabel M, Matkowski R, Kornafel J (2001) Analysis of estrogen receptor (ER) and estrogen-dependent pS2 protein expression in cells of mammary ductal carcinoma. Folia histochemica et cytobiologica/Polish Academy of Sciences, Polish Histochemical and Cytochemical Society 39(2): 141-142.

[12] Chabottaux V, Sounni NE, Pennington CJ, English WR, van den Brule F, Blacher S, Gilles C, Munaut C, Maquoi E, Lopez-Otin C, Murphy G, Edwards DR, Foidart JM, Noel A (2006) Membrane-type 4 matrix metalloproteinase promotes breast cancer growth and metastases. Cancer Research 66(10): 5165-5172.

[13] Balduyck M, Zerimech F, Gouyer V, Lemaire R, Hemon B, Grard G, Thiebaut C, Lemaire V, Dacquembronne E, Duhem T, Lebrun A, Dejonghe MJ, Huet G (2000) Specific expression of matrix metalloproteinases 1, 3, 9 and 13 associated with invasiveness of breast cancer cells *in vitro*. Clinical and Experimental Metastasis 18(2): 171-178.

[14] Delassus GS, Cho H, Park J, Eliceiri GL (2008) New pathway links from cancer-progression determinants to gene expression of matrix metalloproteinases in breast cancer cells. Journal of Cellular Physiology 217(3): 739-744.

[15] Hegedus L, Cho H, Xie X, Eliceiri GL (2008) Additional MDA-MB-231 breast cancer cell matrix metalloproteinases promote invasiveness. Journal of Cellular Physiology 216(2): 480-485.

[16] Lynch CC, Vargo-Gogola T, Matrisian LM, Fingleton B (2010) Cleavage of E-Cadherin by Matrix Metalloproteinase-7 Promotes Cellular Proliferation in Nontransformed Cell Lines via Activation of RhoA. Journal of Oncology 2010: 530745.

[17] Meric F, Hung MC, Hortobagyi GN, Hunt KK (2002) HER2/neu in the management of invasive breast cancer. Journal of the American College of Surgeons 194(4): 488-501.

[18] Roy R, Yang J, Moses MA (2009) Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. Journal of clinical oncology: Official Journal of the American Society of Clinical Oncology 27(31): 5287-5297.

[19] Wu ZS, Wu Q, Yang JH, Wang HQ, Ding XD, Yang F, Xu XC (2008) Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. International Journal of Cancer Journal International du Cancer 122(9): 2050-2056.

[20] Fernandez CA, Yan L, Louis G, Yang J, Kutok JL, Moses MA (2005) The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients. Clinical cancer research: An official Journal of the American Association for Cancer Research 11(15): 5390-5395.

[21] Mannello F, Malatesta M, Sebastiani M, Battistelli S, Gazzanelli G (1999) Molecular forms and ultrastructural localization of prostate-specific antigen in nipple aspirate fluids. Clinical Chemistry 45(12): 2263-2266.

[22] La Rocca G, Pucci-Minafra I, Marrazzo A, Taormina P, Minafra S (2004) Zymographic detection and clinical correlations of MMP-2 and MMP-9 in breast cancer sera. British Journal of Cancer 90(7): 1414-1421.

[23] Yokoyama M, Ochi K, Ichimura M, Mizushima T, Shinji T, Koide N, Tsurumi T, Hasuoka H, Harada M (2002) Matrix metalloproteinase-2 in pancreatic juice for diagnosis of pancreatic cancer. Pancreas 24(4): 344-347.

[24] Roy R, Louis G, Loughlin KR, Wiederschain D, Kilroy SM, Lamb CC, Zurakowski D, Moses MA (2008) Tumor-specific urinary matrix metalloproteinase fingerprinting: Identification of high molecular weight urinary matrix metalloproteinase species. Clinical cancer research: An official journal of the American Association for Cancer Research 14(20): 6610-6617.

[25] Koc M, Ediger D, Budak F, Karadag M, Oral HB, Uzaslan E, Ege E, Gozu RO (2006) Matrix metalloproteinase-9 (MMP-9) elevated in serum but not in bronchial lavage fluid in patients with lung cancer. Tumori 92(2): 149-154.

[26] Hilska M, Roberts PJ, Collan YU, Laine VJ, Kossi J, Hirsimaki P, Rahkonen O, Laato M (2007) Prognostic significance of matrix metalloproteinases-1, -2, -7 and -13 and tissue inhibitors of metalloproteinases-1, -2, -3 and -4 in colorectal cancer. International Journal of Cancer Journal International du Cancer 121(4): 714-723.

[27] Lengyel E, Schmalfeldt B, Konik E, Spathe K, Harting K, Fenn A, Berger U, Fridman R, Schmitt M, Prechtel D, Kuhn W (2001) Expression of latent matrix metalloproteinase 9 (MMP-9) predicts survival in advanced ovarian cancer. Gynecologic Oncology 82(2): 291-298.

[28] Morgia G, Falsaperla M, Malaponte G, Madonia M, Indelicato M, Travali S, Mazzarino MC (2005) Matrix metalloproteinases as diagnostic (MMP-13) and prognostic (MMP-2, MMP-9) markers of prostate cancer. Urological Research 33(1): 44-50.

[29] Choe G, Park JK, Jouben-Steele L, Kremen TJ, Liau LM, Vinters HV, Cloughesy TF, Mischel PS (2002) Active matrix metalloproteinase 9 expression is associated with primary glioblastoma subtype. Clinical cancer research: An official journal of the American Association for Cancer Research 8(9): 2894-2901.

[30] Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. Nature Reviews Cancer 2(6): 442-454.

[31] Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nature Cell Biology 2(10): 737-744.

[32] Mannello F (2011) What does matrix metalloproteinase-1 expression in patients with breast cancer really tell us? BMC Medicine 9: 95.

[33] Luukkaa M, Vihinen P, Kronqvist P, Vahlberg T, Pyrhonen S, Kahari VM, Grenman R (2006) Association between high collagenase-3 expression levels and poor prognosis in patients with head and neck cancer. Head and Neck 28(3): 225-234.

[34] Zhang B, Cao X, Liu Y, Cao W, Zhang F, Zhang S, Li H, Ning L, Fu L, Niu Y, Niu R, Sun B, Hao X (2008) Tumor-derived matrix metalloproteinase-13 (MMP-13) correlates with poor prognoses of invasive breast cancer. BMC Cancer 8: 83.

[35] Kwok S, Rittling SR, Partridge NC, Benson CS, Thiyagaraj M, Srinivasan N, Selvamurugan N (2009) Transforming growth factor-beta1 regulation of ATF-3 and identification of ATF-3 target genes in breast cancer cells. Journal of Cellular Biochemistry 108(2): 408-414.

[36] Selvamurugan N, Kwok S, Partridge NC (2004) Smad3 interacts with JunB and Cbfa1/Runx2 for transforming growth factor-beta1-stimulated collagenase-3 expression in human breast cancer cells. The Journal of Biological Chemistry 279(26): 27764-27773.

[37] Selvamurugan N, Kwok S, Alliston T, Reiss M, Partridge NC (2004) Transforming growth factor-beta 1 regulation of collagenase-3 expression in osteoblastic cells by cross-talk between the Smad and MAPK signaling pathways and their components, Smad2 and Runx2. The Journal of Biological Chemistry 279(18): 19327-19334.

[38] Pratap J, Javed A, Languino LR, van Wijnen AJ, Stein JL, Stein GS, Lian JB (2005) The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. Molecular and Cellular Biology 25(19): 8581-8591.

[39] Yu Q, Stamenkovic I (2004) Transforming growth factor-beta facilitates breast carcinoma metastasis by promoting tumor cell survival. Clinical and Experimental Metastasis 21(3): 235-242.

[40] McCawley LJ, Crawford HC, King LE, Jr., Mudgett J, Matrisian LM (2004) A protective role for matrix metalloproteinase-3 in squamous cell carcinoma. Cancer Research 64(19): 6965-6972.

[41] Deraz EM, Kudo Y, Yoshida M, Obayashi M, Tsunematsu T, Tani H, Siriwardena SB, Keikhaee MR, Qi G, Iizuka S, Ogawa I, Campisi G, Lo Muzio L, Abiko Y, Kikuchi A, Takata T (2011) MMP-10/stromelysin-2 promotes invasion of head and neck cancer. PloS One 6(10): e25438.

[42] Strong AL, Semon JA, Strong TA, Santoke TT, Zhang S, McFerrin HE, Gimble JM, Bunnell BA (2012) Obesity-associated dysregulation of calpastatin and MMP-15 in adipose-derived stromal cells results in their enhanced invasion. Stem Cells 30(12): 2774-2783.

[43] Luo YP, Zhong M, Wang LP, Sun GQ, Li J (2009) Inhibitory effects of RNA interference on MMP-24 expression and invasiveness of ovarian cancer SKOV(3) cells. Journal of Southern Medical University 29(4): 781-784.

[44] Sun Q, Weber CR, Sohail A, Bernardo MM, Toth M, Zhao H, Turner JR, Fridman R (2007) MMP25 (MT6-MMP) is highly expressed in human colon cancer, promotes tumor growth, and exhibits unique biochemical properties. The Journal of Biological Chemistry 282(30): 21998-22010.

[45] Shapiro SD, Kobayashi DK, Ley TJ (1993) Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. The Journal of Biological Chemistry 268(32): 23824-23829.

[46] Sena P, Mariani F, Marzona L, Benincasa M, Ponz de Leon M, Palumbo C, Roncucci L (2012) Matrix metalloproteinases 15 and 19 are stromal regulators of colorectal cancer development from the early stages. International Journal of Oncology 41(1): 260-266.

[47] Impola U, Toriseva M, Suomela S, Jeskanen L, Hieta N, Jahkola T, Grenman R, Kahari VM, Saarialho-Kere U (2003) Matrix metalloproteinase-19 is expressed by proliferating epithelium but disappears with neoplastic dedifferentiation. International Journal of Cancer Journal International du Cancer 103(6): 709-716.