Nat. Volatiles & Essent. Oils, 2021; 8(4): 13165-13177

NVEO
Natural Volatiles &
Essential Oils

Assessment Of Matrix Metalloproteinase- 2 &Tissue Inhibitor -1 In Human Prostate Cancer

Azhar A. Ameen[1], Dhamia K.Suker[2] and Rafid . Abood[3]

[1,2]Department of Biology, College of Science, Basrah University

[3]Medical College , Basrah University

Abstract

Matrix metalloproteinases (MMPs) are a group of zinc -dependent enzymes that have a role in the tumor cell invasion and metastasis, so involved in different malignancies ,MMPs are essential in the degradation process and also promote cellular migration, regulate growth factors and cytokines, influence apoptosis and collaborate in neovascularization , the expression of these proteins is regulated by their physiological inhibitors :tissue inhibitors of metalloprotein (TIMPs) ,the expression levels of these proteins in tumoral, stromal, and inflammatory cells can be considered potential tumoral microenvironment invasion markers.

The expression level of MMP2 and TIMP1 in serum wasdetermined by Eliza ,and in tissues by Immunohistochemistry.

The present studyrevealed that MMP2&Timp1 levels were significantly higher in prostate cancer serum and tissue compared to controls, and their levels were positive associated with Gleason grades and stages in patients.

Key words: MMP2 ,TIMP1,Prostate cancer, Immunohistochemistry

Introduction

Cancer is a type of disease**characterized by uncontrolled cell proliferation, invasion, and metastasis of cells from the primary site to other parts of the body (Aghajani et al., 2020)**

Prostate cancer is the most commonlydiagnosed malignancies cancer and the leading cause of cancer death in men, with morbidity and mortality caused mostly by invasion and metastasis (Jemal et al.,2009).

Matrix metalloproteinases (MMPs) are Ca^{2+}–Zn^{2+}-dependent endopeptidases family and that play important role in proliferation, migration, angiogenesis and death of cells (Abdul-Muneer et al.,2016) ,MMPproduced by neoplasticandstromal cells, are divide into five categories:gelatinases collagenases,stromelysins ,matrilysins, and membrane-type metalloproteinases (Vihinen and Kahari,2002).

MMPs can stimulate the growth ofa tumor,cell invasion,angiogenesis ,metastasis ,and vascularization by regulating the expression and activity ofcytokines, growth factors,, and chemokines(Yadav et al.,2014).Several of MMPs protiens,including MMP2,have been associated to poor outocomes and can be used as prognostic indicators for solid tumors,with elevatedexpression levels of MMP in tumors orplasma \serum of patients (Tantai et al,2016).

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous proteins could decrease cell proliferation and migration by blockingthe function of Matrix metalloproteinases (MMPs). Previous studies have revealed that disrupting the equilibriumbetween MMPs and TIMPs was disrupted can lead to the breakdown of the extracellular matrix and stimulate tumor cell migration, invasion, or other receptor-mediated modulation (Tauro and Lynch , 2018).

MMPs and there tissue inhibitors have a dynamic balance that have a role in extracellular matrix degradation, repair, angiogenesis, and tissue remodeling (Sato and Takino,2010), Several studies have detected the role matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in PCa metastasis and tumorigenesis majority of the studies conducted by looking at in situ expression of the metalloproteinases,via immunohistochemistry with biopsy or postoperative tissue or through transcriptional measures, Wilson.,et al.2002RevealsMMP-2 is secreted by the human prostate gland, both in vivo and in vitro, higher expression levels of MMP-2 are associated with rising Gleason scores, tumor metastasis, and more aggressive prostate cancer (El-Chaeret al.,2018)

The aim of this study was to evaluate the expression levels of MMP-2 and TIMP-1 in the serum and tissue, determine correlation between their levels withadvanced Gleason score &stage in order to achieve a better knowledge of the role of MMP-2&TIMP1 in prostate cancer and possibility to be a biomarker for cancer

Materials and Methods

The groups of the current study were included100 serum sample (50 healthy &50 patients) , and 60 sample offormaline fixed paraffin embedded tissue(FFPE)(30 benign and 30 malignant) from AL-Sader Hospital

The range of ages was 50-80 in all samples, the protocol of this study was confirmed by an ethical committee, information on cancer histopathology, and other information was included age, weight, sport, smoking, job, from pathology reports.

The PC samples were divided into two categories according to their PC grades (grade 2-5)and (grade 6-9) and according to stages divide tostage stage1,stage2,stage3,stage4.

Serum levels of circulating MMP2&TIMP1 was assessed by using Immunosorbent assay (ELISA) kit (BioSource company ,USA) on 96-well microtiter plates using 8-well stripes according to manufactures instructions.

Assessment of MMP2&TIMP1 in prostatic tissue was assayed by Immunohistochemistry assay with FFPE tissue procedure(Cuello,1993).

Formaline fixed paraffin embedded tissue was cutting to 4 mm in thickness ,then deparaffinized , rehydrated,immunostaining for MMP-2&TIMP-1 involved heat-based antigen retrieval(5 min at 95C) was conducted ,followed by endogenous peroxidase blocking with 3% hydrogen peroxide for 5 min , 100 µl primary antibody solution was added to the slides at 37 °C for at least 60 minutes with humidified, Secondary Antibody Reaction: 100 µl of biotinylated secondary antibody was applied to every Slide, a humidity of chamber for at least 30 minutes. at room temperature, Substrate Preparation: One drop (approximately 20µl) from DAB chromogen added to each 1ml of substrate buffer ,mixed immediately and applied to tissue sections, Counterstaining with Mayer's hematoxylin was applied to cover the sections then incubated for 5 minutes, then rinsed gently with distilled water for 5 minutes mounted and examined.

The immunostaining MMP2 &TIMP-1 in the samples was detected by founding or not of brown granules in the cytoplasm of the cells to detect positive &negative staining, then scored by semi-quantitative according to the evaluating intensity& percent of positively stained cells, due to the staining intensity ,the samples were categories into **0 : negative 1:Weak, 2:intermediat, and 3: Strong**,and according to the percent of positive staining, the cases were grouped to 3 categories as follow score 0 :0% negative score , and (1:<10% ,2:10-50% , :3 >50%) positive score , in positive staining the highest score was:6 and the

minimum:1, in the final we have a threshold value of 3, so a value≥3 considered as a high score and <3 as a low score(Nagel et al .,2004).

The statically analysis for all data was performed by using SPSS.23 ,independent T-test ,one way a nova and Chi-square test were performed to detect correlation coefficient betweenexpression of MMP2&MMP9 in differentvariables,statisticalcal significance (P<0.05) was consideredastatistically significant and all data were represented by mean ± Standard deviation.

Results

Serum levels of MMP2&MMP9

The results showed that the comparison between serum levels of the circulating MMP-2between patients and controlswere significantly difference(p <0.05) ,since in patient group was :(19.9686±5.9068) whereas in controls group was :(11.0762±4.20832), also there was significant difference in serum level ofTIMP1 between patients and controls group which was: (1756.984±162.0659) and : (1601.681±331.5612) respectively (figure 1:A&B) .

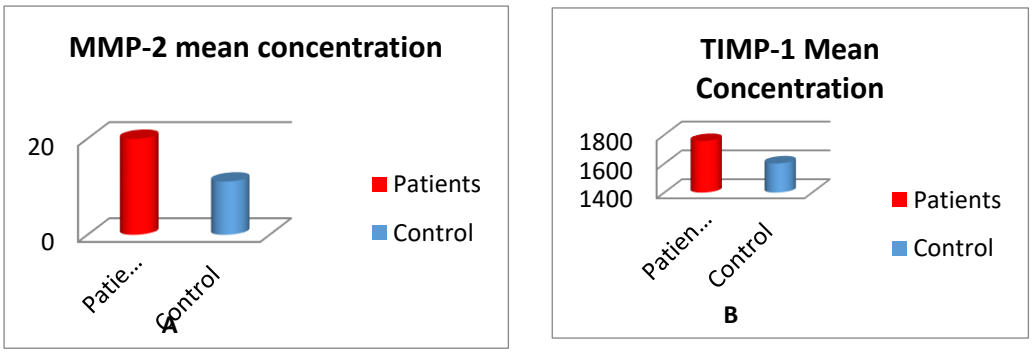


Figure (1) Serum level of MMP-2(A)&TIMP1 (B) in patients and controls groups ,as determined by independent t-test .

Expression levels of MMP2 and timp1according to different PC Grades

The results reveals that there were significant differences(P<0.05) in serum levels of MMP2according to different grades of prostate cancer, since the expression level of MMP2 was (12.2003 ±4.2727)in (Grade 2-5) group whereas it was (19.9229± 6.77136) in (grade 6-9)groupfigure 4(A)

The expression level of TIMP-1 also showed significant differences(P<0.05) between grades groups, which was (1633.9765 ± 335.89899)in(Grade 2-5) and was (1832.2786± 140.70016) in (grade 6-9) figure 4(B)

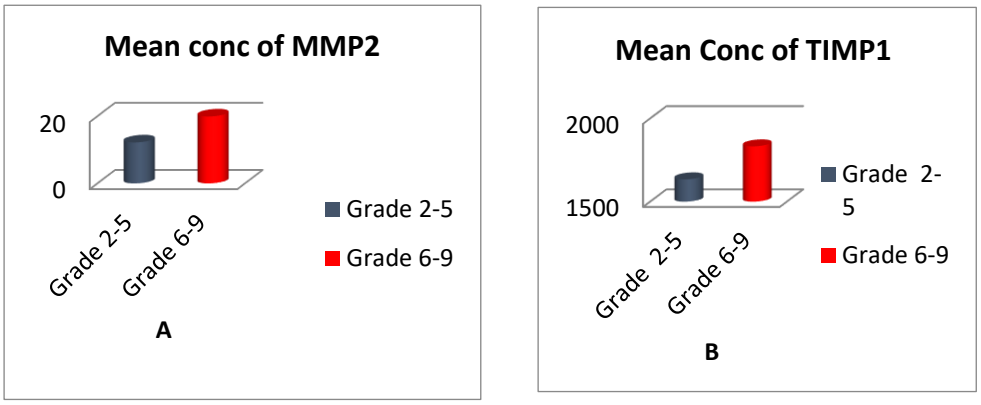


Figure (4:A&B)Comparisonexpression levels of MMP2(A) and timp1(B)between twoGleason score groups in PC patients

Comparison serum levels of MMP2 & TIMP-1 according to different PC stages

Theresults showed that there were differences in serum level of MMP-2between different stages groups of PC which was (7.06 ± 1.83521)in stage1, (11.004±5.55248) in stage 2, (11.3666±5.50291) in stage 3 and (22.4136 ±6.60953) in stage 4,these different were not significant between stage 1,stage 2 and stage 3, statistically significant different (P<0.05)was only between stage4 when compared with other stages figure (5)

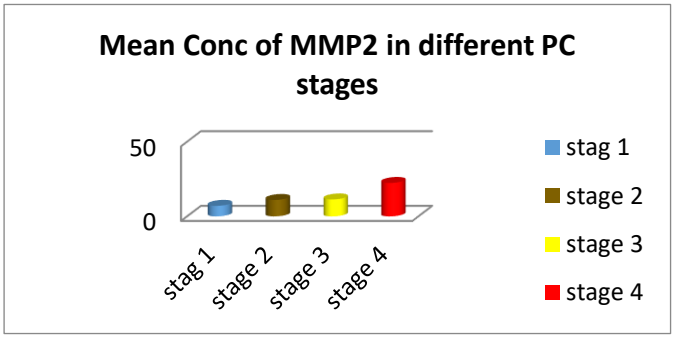


Figure (5) : Correlation expression level of MMP-2 between different stages of PC patients samples,the differencewas significantly only between stage 4 and other stage

Also results showed there were different inThe expression level of TIMP-1 between different stages of PC which was (1503.2435 ±232.618) in stage1,(1599.1373±250.6076) in stage 2 , (1730.8312± 84.47494) in stage 3 and (1849.7651± 106.1139),statistically significant different(P<0.05) was only between stage4 and stage 1,figure (6)

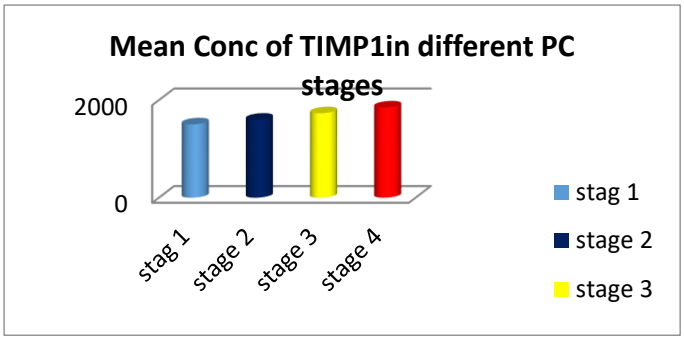


Figure (6) : Correlation expression level of TIMP-1 between different stages of PC patients samples,the different was significantly only between stage 4 and stage1 , (P<0.05) as determined by one way a nova.

Quantitative detection of MMP-2&TIMP-1

The expressionofMMP2 &TIMP-1 was checked in prostate cancer and benign prostatichyperplasia tissues by using the Immunohistochemistry staining technique , the expression was classified into positive when staining strong,moderate,low , and negative when there are no staining(7&8)

Semi quantitative detection of MMP-2&TIMP-1

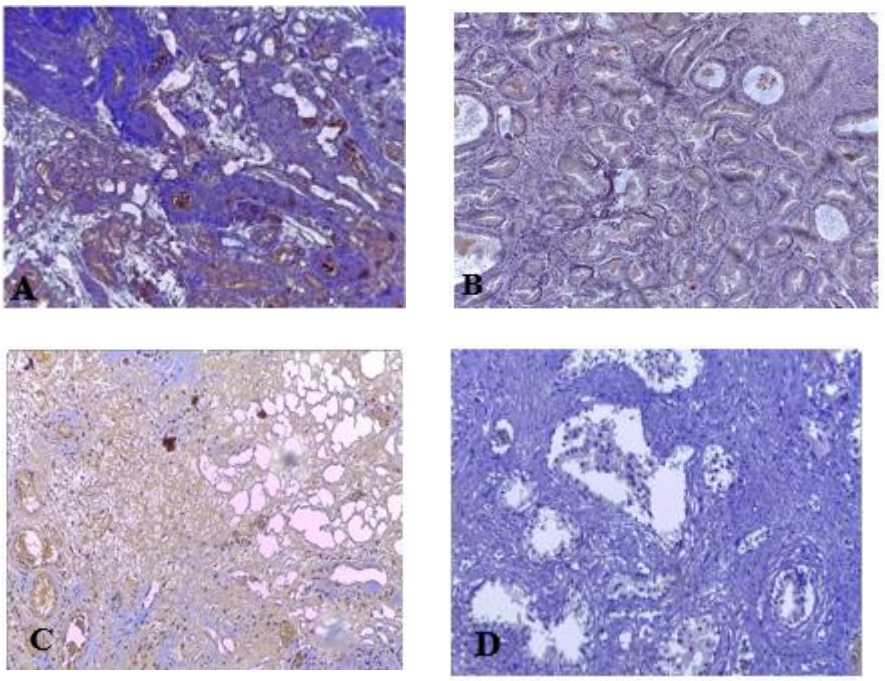
The expression of MMP-2 &TIMP-1 were resides in the cytoplasm or interstitial , resultsrevealed that there were significant differences in MMP2 &TIMP-1expression level between benign and malignant of prostate tumor at (P value <0.05), table (1) & (2) based on the immune positive tumoral percentage and intensity of staining

Table 1: Immunoscore of MMP2 between benign and malignant tumor showed significantly different between malignant and benign tumor tissue,as determined by Chi-squaretest

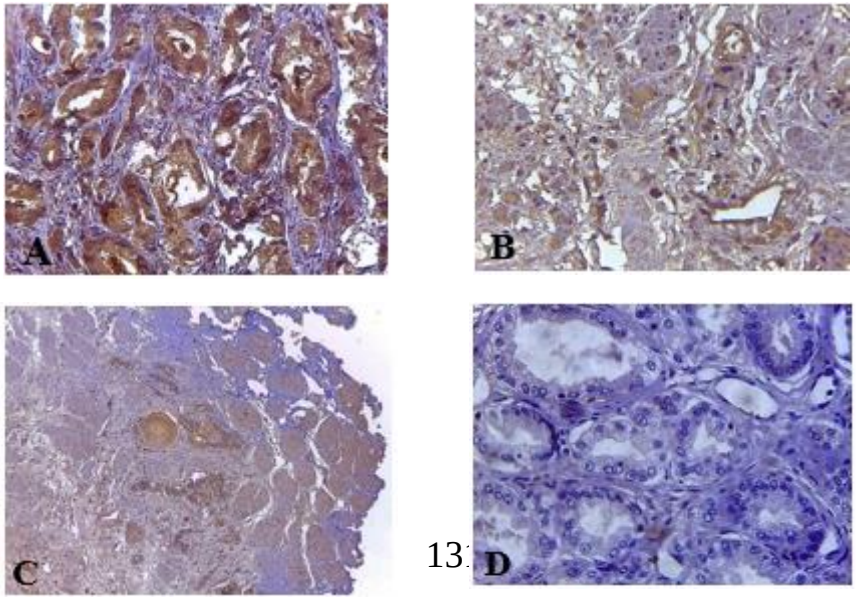
MMP2 Immunoscore cross tabulation							
			Immunoscore				
			<3	>3	Total	person chi squar	P-value
Prostate tumor	Malignant prostate tumor	**Count**	6	24	30	7.177a	0.007
		%within	20%	80%	100%		
	Benign	**Count**	16	14	26		
		%within	53.30%	46.70%	100%		
		prostate tumor					

Table2: Immunoscore of TIMP-1 between benign and malignant tumor showed significantly different between malignant and benign tumor tissue, as determined by Chi-square.

TIMP1 Immunoscore cross tabulation							
			Immunoscore				
			<3	>3	Total	person chi squar	P-value
		Count	0	20	20		
		%within					
	Malignant	PC			100%	14.118a	0.05
Prostate tumor			26.70%	73%			
		Count	17	13	30		
	Benign	%within					
		BH	56.70%	43.30%	100%		



Figure(7): Immunohistochemistry expression of MMP2 in human Prostate tissue with different intensity,(A) positive strong staining(B)positive moderate staining ,(c)positive weak staining ,(D) negative staining .



Figure(8): Immunohistochemistry expression of TIMP-1 in human Prostate tissue with different intensity,(A) positive strong staining(B)positive moderate staining ,(c)positive weak staining ,(D) negative staining

Discussion

Matrix metalloproteinases play a key role in the destruction ofthe extracellular matrix (ECM) and their bioactivities are control by the (TIMPs)(Bourboulia and Stetler-Stevenson.,2010).

Both MMPs and TIMPs have been reported to be changed in malignant and benign tumors, as well as during invasion and metastasis, which require the degradation and removal of extracellular matrix (Schröpfer et al .,2010).

The present study detected the MMP-2 ,and TIMP-1 levels in cancerous and non-cancerous in serum by Eliza technique and in tissue by Immunohistochemistry staining and showed that there significantly different in their expression : higher expression in patientscompare to controls serum and tissue of malignant tumor compared to a benign tumor of samples .

Our study agrees with (Gohji et al.,1998)who detected MMP-2 in serum specimens and show that the level of MMP-2 was associated with the advance and progression of prostate cancer and that MMP-2 serum level refers to the degree of prostate cancer progression.

Also,(Lichtinghagen et al .,2002) observed high expression of MMP-2 in cancer tissue by using immunohistochemistry .

The levels of MMPs in tissueandserum are high expression, significant prognostic, implicated in many aspects in the progression of prostate cancer and effect on their microenvironment which refers to their role in prostate cancer molecular biology (Gong et al ,2014).

Several MMP proteins ,including MMP2, are related topoor outcomes, and can be employed as prognostic indicators for the solid tumors, with rising MMP expression levels in tumor or serum of patients (Tantai et al .,2016).

Many studies refer to an increase in circulating levels of most MMPs with cancer progression,making their analysis promising for use in the diagnosis and prognosis of various types of tumors, although research in this fieldhave been down , results are

conflicting, and it has not been possible so far to define a given MMP with the potential to distinguish any type of cancer(Hadler-Olsen et al .,2013).

During cancer progression, it is important that cancer cells properly connect with and successfully modify their surrounding host microenvironment,cancer cells communicate with their surrounding microenvironment via the receptors of cell-surface adhesion and the receptors for the extracellular matrix (ECM) and modify their surroundings largely by activities of the (MMPs)(Walker et al.,2018).

(Gong et al.,2013) performed immunohistochemistry (IHC) on a panel of human samples of prostate cancer and normal prostate tissues ,the results showed that TIMP-1 protein is more elevated in the stroma of prostate cancer compared to its normal counterpart.

(Reis et al.,2015) refer to that MMP2 is positive in prostate cancer but its regulators (TIMPs) are negative in the majority of cases.

Current study showed that the expression of MMP2 and TIMP1 is significant different and positive association with advanced Gleason scoregrade degree and thecomparison between different stagesof pc groups show there was significant different between stage 4 and other stages , while TIMP1 concentration has significant different only between stage1 &stage 4 .

(XIE et al .,2015) refer to that the meta-analysis revealed that MMP-2 level in the PCa group was significantly higher than that in the benign prostatic hyperplasia (BPH) group also, the level of MMP-2 was significantly related to Gleason

Score and clinical stages ,so MMP2 can used as an indicator in PCa patients, several investigators have confirmed that the expression of TIMP1 in tumor tissue is lower than the unchanged one(Brehmer et al 2003., Lichtinghagen et al.,2002).

There is an imbalanced expression of MMPs and TIMPs in the prostate cancer tissue, represented bya reduction in expression of TIMPs and an a high level of MMPs, as such, so generally thought that MMPs are more active in higher stages of prostate cancer, also most MMPs show increase in their expression in higher Gleason score tumors (Miyake et al .,2010)

Studies showed the role of TIMP-1 on cancerprogression have yielded contradictory results ,Sinceit is an endogenous inhibitor of MMPs so high expression ofTIMP-1 were postulated to block metastases indeed, several research showed that high expression of TIMP1 was related to tumor suppression incell lines and mice that genetically engineered (chen et al .,2011 ; Bloomston et al ;2002., Ikenaka.,2003)

Conclusion

1. Overexperssion of MMP1 &TIMP1 protein correlates significantly to prostate cancer
2. The correlated assessment of MMP2 and TIMP1 is useful in the evaluation of their potential prognostic capacities

3.The circulating levels of MMP-2&TIMP-1 aresignificantly high in prostate cancer patients when compare to healthy controls and this indicator to these role in prostate cancer.

3.The significant correlation between MMP-2&TIMP-1 expression and the tumor stage or grade ofProstate cancer may indicate their importance in progression of this cancer.

References :

Abdul-Muneer, P. M., Pfister, B. J., Haorah, J., & Chandra, N. (2016). Role of matrix metalloproteinases in the pathogenesis of traumatic brain injury. Molecular neurobiology, 53(9), 6106-6123.

Bloomston, M., Shafii, A., Zervos, E. E., & Rosemurgy, A. S. (2002). TIMP-1 overexpression in pancreatic cancer attenuates tumor growth, decreases implantation and metastasis, and inhibits angiogenesis. Journal of Surgical Research, 102(1), 39-44.

Bourboulia, D., & Stetler-Stevenson, W. G. (2010, June). Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. In Seminars in cancer biology (Vol. 20, No. 3, pp. 161-168). Academic Press.

Brehmer B, Biesterfeld S, Jakse G .(2003) .(TIMP-1 and -2) in prostate cancer tissue.Prostate Cancer Prostatic Dis. ; 6(3):217-22

Chambers, A. F., & Matrisian, L. M. (1997). Changing views of the role of matrix metalloproteinases in metastasis. Journal of the National Cancer Institute, 89(17), 1260-1270.

Chen, Y., Wei, X., Guo, C., Jin, H., Han, Z., Han, Y & Fan, D. (2011). Runx3 suppresses gastric cancer metastasis through inactivation of MMP9 by upregulation of TIMP-1. International journal of cancer, 129(7), 1586-1598.

Cho, N. H., Shim, H. S., Rha, S. Y., Kang, S. H., Hong, S. H., Choi, Y. D., ... & Cho, S. H. (2003). Increased expression of matrix metalloproteinase 9 correlates with poor prognostic variables in renal cell carcinoma. Eur Urol, 44(5), 560-566.

El-Chaer, W. K., Moraes, C. F., & Nóbrega, O. T. (2018). Diagnosis and prognosis of prostate cancer from circulating matrix metalloproteinases and inhibitors. J Aging Res.,

Gohji, K., Fujimoto, N., Hara, I., Fujii, A., Gotoh, A., Okada, H., ... & Nakajima, M. (1998). Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer, 79(1), 96-101.

Gong, Y., Chippada-Venkata, U. D., & Oh, W. K. (2014). Roles of matrix metalloproteinases and their natural inhibitors in prostate cancer progression. Cancers, 6(3), 1298-1327.

Gong, Y., Scott, E., Lu, R., Xu, Y., Oh, W. K., & Yu, Q. (2013). TIMP-1 promotes accumulation of cancer associated fibroblasts and cancer progression. PloS one, 8(10), e77366.

Ikenaka Y, Yoshiji H, Kuriyama S, Yoshii J, Noguchi R,Tsujinoue H, Yanase K, Namisaki T, Imazu H, Masaki T, FukuiH. (2003) .Tissue inhibitor of metalloproteinases-1 (TIMP-1) inhibitstumor growth and angiogenesis in the TIMP-1 transgenicmouse model. Int J Cancer ,105:340–346.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ(2009): Cancer statistics. CA Cancer J Clin.9: 225-49. 10.3322/caac.20006

Liao, X., Thrasher, J. B., Pelling, J., Holzbeierlein, J., Sang, Q. X. A., & Li, B. (2003). Androgen stimulates matrix metalloproteinase-2 expression in human prostate cancer. Endocrinology, 144(5), 1656-1663.

Lichtinghagen R, Musholt PB, Lein M, Römer A, Rudolph B, Kristiansen G, Hauptmann S, Schnorr D, Loening SA, Jung K .(2002). Different mRNA and protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinases 1 in benign and malignant prostate tissue. Eur Urol ; 42(4):398-406.

Miyake H, Muramaki M, Kurahashi T, Takenaka A, Fujisawa M.(2010.)Expression of potential molecular markers in prostate cancer: correlation with clinicopathological outcomes in patients undergoing radical prostatectomy.

Nagel, H., Laskawi, R., Wahlers, A., & Hemmerlein, B. (2004). Expression of matrix metalloproteinases MMP-2, MMP-9 and their tissue inhibitors TIMP-1,-2, and-3 in benign and malignant tumours of the salivary gland. Histopathology, 44(3), 222-231.

Reis, S. T. D., Viana, N. I., Iscaife, A., Pontes Junior, J., Dip, N., Antunes, A. A., ... & Leite, K. R. M. (2015). Loss of TIMP-1 immune expression and tumor recurrence in localized prostate cancer. International braz j urol, 41(6), 1088-1095.

Sato,H and Takino,T. (2010).Coordinate action of membrane-type matrix metalloproteinase-1 (MT1-MMP) and MMP-2 enhances pericellular proteolysis and invasion. Cancer Science; 101(4):843-7.

Sbardella, D., Fasciglione, G. F., Gioia, M., Ciaccio, C., Tundo, G. R., Marini, S., & Coletta, M. (2012). Human matrix metalloproteinases: an ubiquitarian class of enzymes involved in several pathological processes. Molecular aspects of medicine, 33(2), 119-208.

Simi, L., Andreani, M., Davini, F., Janni, A., Pazzagli, M., Serio, M., & Orlando, C. (2004). Simultaneous measurement of MMP9 and TIMP1 mRNA in human non small cell lung cancers by multiplex real time RT-PCR. Lung Cancer, 45(2), 171-179.

Tantai, J. C., Zhang, Y., & Zhao, H. (2016). Heterophyllin B inhibits the adhesion and invasion of ECA-109 human esophageal carcinoma cells by targeting PI3K/AKT/β-catenin signaling. Molecular medicine reports, 13(2), 1097-1104.

Tauro, M., & Lynch, C. C. (2018). Cutting to the chase: how matrix metalloproteinase-2 activity controls breast-cancer-to-bone metastasis. Cancers, 10(6), 185.. Urol Oncol. ; 28(2):145-51.

Vihinen, P., & Kähäri, V. M. (2002). Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer. 99(2),:157-166.

Walker, C., Mojares, E., & del Río Hernández, A. (2018). Role of extracellular matrix in development and cancer progression. International journal of molecular sciences, 19(10), 3028.

Wilson, M. J., Sellers, R. G., Wiehr, C., Melamud, O., Pei, D., & Peehl, D. M. (2002). Expression of matrix metalloproteinase-2 and-9 and their inhibitors, tissue inhibitor of metalloproteinase-1 and-2, in primary cultures of human prostatic stromal and epithelial cells. . J Cell Physiol, 191(2), 208-216.

Xie, T., Dong, B., Yan, Y., Hu, G., & Xu, Y. (2016). Association between MMP-2 expression and prostate cancer: A meta-analysis. Biomed Rep, 4(2), 241-245.

Yadav, L., Puri, N., Rastogi, V., Satpute, P., Ahmad, R., & Kaur, G. (2014). Matrix metalloproteinases and cancer-roles in threat and therapy. Asian Pacific journal of cancer prevention, 15(3), 1085-1091..