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Regulation, Function, and Expression of Matrix Metalloproteinase-9 in Colon, Lung, and Breast Cancers *In Silico* and Experimental Methods From Iraqi Metastatic Patients

Research Article

Hamad Ali Hamad[1,5], Yamama Farouk Shakir[2], Omar Tareq Zaidan[3], Aymen Adnan Rafea[4], Nur Fariesha Md Hashim[5]*

[1]*Department of Pathological Analysis, College of Applied Sciences, University of Fallujah, Alanbar,31002, Iraq*
[2]*Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq*
[3]*Department of Applied Chemistry, College of Applied Sciences, University of Fallujah, Alanbar,31002, Iraq*
[4]*Department of Medical Oncology, Anbar Cancer Centre, Anbar Health Directorate, Ramadi 31001, Iraq*
[5]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43300, Malaysia*

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Abstract:

Background: Various molecular cancer variables, including K-RAS, B-RAF, and HER2/neu, are essential in the growth and advancement of tumors. Matrix metalloproteinase-9 (MMP-9) is suggested as a biomarker that is excessively produced in different types of malignancies and plays a critical role in chemotherapy resistance that leads to tumor progression and metastasis.

Patients and Methods: The study used two different methods to evaluate the expression level of *MMP9.* First, we sought to find its expression and interaction with other proteins or genes using *in slico* techniques. Second, we evaluated the protein levels of MMP-9 in 80 metastatic patients with breast, colon, and lung cancer using enzyme-linked immunosorbent assay (ELISA) techniques.

Results: *In silico* techniques showed a high expression of *MMP9* gene in all three types of cancer. Furthermore, genes and proteins that interact the most with *MMP9* were discovered using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and Human Protein Atlas (HPA). It was found that the genes associated with *MMP9* gene in colon, lung, and breast cancers are *MMP2* and *TIMP2*, along with MAPK, PI3K, and NF-κB pathways as the most significant genes and pathways across all three types of cancer that could be linked to the *MMP9* gene in cancer metastasis. In addition, ELISA techniques showed that *MMP9* was at the highest level in metastatic colon cancer patients at 0.62 ± 0.25, followed by breast and lung metastatic patients.

Conclusion: *MMP9* appears to play a crucial role in progression of particularly colon metastatic cancer and could be targeted alongside other genes as a possible therapeutic target for this type of cancer in patients with chemotherapy resistance and metastasis.

Keywords: *MMP9 gene • tumor progression marker • genes implicated with MMP9 and TIMP2 as biomarkers • colon cancer, and breast cancer*

1. Introduction

Colon cancer ranks as the third most prevalent cancer and the second leading cause of cancer-related deaths worldwide, with its incidence showing a concerning upward trend[1]. Similarly, lung cancer stands as the most prevalent and deadliest form of cancer globally, contributing to high rates of mortality and morbidity[2]. With its classification into small cell lung carcinoma (SCLC) and non-small cell lung cancer (NSCLC), the latter comprises approximately two-thirds of lung cancer cases, further subdivided into distinct subgroups[3]. In addition, breast cancer remains a significant health burden, particularly among women, accounting for numerous deaths annually on a global scale[4].

The molecular landscape of lung, colon, and breast malignancies is intricate, with key players such as *KRAS*, *BRAF*, *RET*, *ALK*, *BRCA1/2*, HER2-neu, and

* *E-mail: nurfariesha@upm.edu.my*

PDL-1 shaping disease progression. Among these, matrix metalloproteinase-9 (MMP-9) emerges as a critical matricellular protein belonging to the gelatinase family of matrix metalloproteinases (MMPs)[5,6,7]. Its multifaceted role encompasses protein cleavage, immune modulation, tumor metastasis, and various physiologic processes including wound healing and tissue remodeling, significantly impacting cancer progression across lung, breast, and colon cancers[8].

Metastasis stands as a pivotal determinant of cancer-related mortality, with MMPs orchestrating tumor cell invasion and dissemination through intricate pathways. Notably, MMP-9's proteolytic activity plays a pivotal role in degrading extracellular matrix (ECM) components, facilitating tumor cell migration, invasion, and angiogenesis. Elevated MMP-9 levels have been consistently implicated as a biomarker in various aggressive tumor types, correlating with poor prognosis and diminished survival rates, particularly evident in breast cancer[9,10].

Moreover, tissue inhibitor of metalloproteinase-1 (TIMP1), a primary antagonist of MMP-9, has been associated with cancer growth promotion and resistance to apoptosis, exacerbating the aggressive behavior of tumor cells while impairing MMP-9 functionality[11,12]. Dysregulation of key signaling pathways such as the epidermal growth factor receptor (EGFR) pathway in breast cancer and the PI3K/AKT signaling pathway further accentuates MMP-9's role in tumor progression[13,14]. In addition, genes like *EZH2* and *CTHRC1* have been implicated in various cancer types, including lung, colon, and breast cancers, contributing to advanced tumor stages and adverse prognostic outcomes[15]. The expression of metalloproteinase family genes increases under hypoxic conditions due to hypoxia-inducible factor-1alpha (HIF-1α), which can enhance the invasiveness of cancer cells *in vitro* through the formation of invadopodia. These structures contain members of the metalloproteinase family as components to facilitate invasion of the surrounding tissue, specifically ECM, and subsequent metastasis to other sites[16,17]. Thus, MMPs, particularly MMP-9, have a promising role in drugs targeting cancer invasion.

Against this backdrop, this study aims to comprehensively analyze *MMP9* expression levels in lung, colon, and breast cancers, shedding light on associated genes and pathways. Furthermore, it endeavors to elucidate MMP-9's functional role within these cancers, exploring its significance in modulating other tumorigenic genes. Through experimental analyses, this research aims to uncover potential regulatory mechanisms influencing *MMP9* expression and activity, thus providing valuable insights into tumor proliferation and progression in lung, colon, and breast cancers that can help in drug discovery.

2. Methodology

2.1. In silico exploration of *MMP9* expression at gene and protein levels in lung, colon, and breast cancers

The *in silico* expression of *MMP9* at gene and protein levels in lung, colon, and breast cancers was evaluated by different computational techniques. Firstly, an extensive literature review was performed, followed by the identification of functional partners of the *MMP9* gene using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and its expression at gene and protein levels was analyzed using databases such as Gene Expression Profiling Interactive Analysis (GEPIA) and The Human Protein Atlas (HPA), respectively. This was performed to identify the genes involved in the progression of lung, colon, and breast cancers, either by the *MMP9* gene inducing the function of other genes or by the *MMP9* gene getting induced by other genes during tumor progression.

2.2. Literature review to identify genes and pathways implicated with *MMP9*

A comprehensive review of the literature was performed to gain insights into the genes playing a crucial role in the development and progression of genes. Several different search terms such as "*MMP-9* expression," "*MMP-9* lung cancer," "*MMP-9* colon cancer," "*MMP-9* breast cancer," "role of *MMP-9* in cancers," "the role of *MMP-9* in lung, colon, and breast cancers," and "genes inducing *MMP-9* in lung, colon, and breast cancers" were used on "Google Scholar" to identify the genes significantly involved in lung, colon, and breast cancers.

2.3. Protein–protein interaction analysis using STRING

The functional partners of *MMP9* gene were identified using the STRING database, which identifies both physical interactions and functional associations while aiming to integrate all known and predicted associations between proteins[18,30]. This was performed to identify the known and predicted functional partners of *MMP9* at default parameters such as network type (full STRING network), required score (median confidence [0.400]),

and size cutoff (no more than 10 interactions), resulting in the interacting genes that showed the highest interactions with *MMP9* gene.

2.4. *MMP9* gene expression analysis

The gene expression analysis for *MMP9* gene was performed using GEPIA, a web-based tool based on TCGA and GTEx data providing interactive and customizable functions such as differential expression analysis, profiling plotting, correlation analysis, patient survival analysis, similar gene detection, and dimensionality reduction analysis[19]. It was utilized to identify the expression levels of *MMP9* gene in normal tissue compared to tumor tissues, resulting in various expression values of the *MMP9* gene in a range of cancers provided in GEPIA, including lung, colon, and breast cancers.

2.5. *MMP9* protein expression analysis

MMP9 protein expression analysis was performed using HPA https://www.proteinatlas.org, an open resource providing the spatial expression levels of transcripts and proteins across cells, tissues, and organs[20]. The HPA analysis identified various aspects of genome-wide analysis of MMP-9 protein, including protein–protein interactions (PPIs), concentration in cancers, expression clustering, and prognostic probability in provided cancer types in HPA, including lung, colon, and breast cancers.

2.6. Sample collection

Five milliliters of blood samples were collected from late-stage colon, lung, and breast cancer patients after the clinical evaluation of each participant in Anbar Cancer Center. Blood was incubated in a water bath for 15 min until it clotted. Then, centrifugation of the samples was performed for 15 min to obtain clear serum to be used in the enzyme-linked immunosorbent assay (ELISA) technique. The serum of patients was stored at −80 °C for 2 months before use for evaluation.

2.7. ELISA technique using *MMP9*

The *MMP9* kit comprising all the reagents as well as the samples was equilibrated to room temperature for optimal temperature conditions. The reagents were prepared according to the manufacturer's protocol, which commenced with the addition of 100 µL of standard or sample in each well, followed by incubation for 90 min at 37 °C. After removal of the standard or sample from each well, 100 µL of biotinylated detection Ab/Ag was added, which was followed by three wash cycles; in addition, 100 µL of horseradish peroxidase (HRP) conjugate was added and incubated for 30 min. Thenceforth, to five washes, a substrate reagent of 90 µL was added, whereas a stop solution of 50 µL was introduced, and the optical density (OD) value was determined at 450 nm.

2.8. Statistical analysis of clinical data

The statistical analysis involved expressing *MMP9* as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to assess significant differences among groups. Furthermore, Duncan test was utilized to identify variations in the means of *MMP9* indicators within the study groups. The sensitivity and specificity of *MMP9* were determined using the receiver operating characteristic (ROC) curve. A significance level of $P \leq 0.05$ was applied to identify statistically significant differences. In addition, the data was processed using the statistical software program Statistical Package for the Social Sciences (SPSS) v. 23.0 and GraphPad Prism v. 6.

3. Results

3.1. Patient characteristics

The study data showed that the participants were predominantly males (33.3%), with most belonging to the age groups of 45–54 years (30.0%) and 55–64 years (33.3%). A significantly large percentage of participants were found to have been diagnosed with lung and breast cancer, making up 33.3% and 38.9% of the total, respectively. Most of these instances were detected in the advanced stage (91.25%), suggesting significant disease progression (91.25%). The statistical analysis showed significant differences among participants ($P < 0.05$), which corresponded with anthropometric and clinical parameters detailed in Table 1.

3.2. Expression level analysis of *MMP9*

The highest levels of *MMP9* expression were observed in patients with colon cancer (0.62 ± 0.25), followed by lung cancer (0.46 ± 0.22) and then breast cancer (0.35 ± 0.16), compared to controls (0.30 ± 0.15), with

Table 1: Anthropometric and clinical features of participants.

		Count	Percentage	*P*-value
Age groups (years)	25–34	3	3.30%	*P*<0.001***
	35–44	7	7.80%	
	45–54	27	30.00%	
	55–64	31	33.30%	
	>64	24	25.60%	
Gender	Males	30	33.30%	*P*<0.001***
	Females	60	66.70%	
Groups	Colon cancer	15	16.70%	*P*<0.001***
	Lung cancer	30	33.30%	
	Breast cancer	35	38.90%	
	Controls	10	11.10%	
Stage	Late	73	91.25%	*P*<0.001***
	Early	7	8.75%	
Response to chemotherapy	Disease progression	73	91.25%	*P*<0.001***
	Stable disease	7	8.75%	

Table 2: Comparative mean levels of *MMP9* between late- and early-stage diseases.

Stages		***n***	**Mean**	**Std deviation**
MMP9	Late	73	0.43	0.21
	Early	7	0.36	0.19
***P*-value**			*P*>0.05	

Table 3: Comparative mean levels of *MMP9* between disease progression and stable disease.

Response to chemotherapy		***n***	**Mean**	**Std deviation**
MMP9	Disease progression	73	0.41	0.21
	Stable disease	7	0.6	0.29
***P*-value**			*P* > 0.05	

statistically significant differences ($p < 0.05$). These findings are presented in **Figure 1A and B.** Moreover, there were no significant differences ($p > 0.05$) in the levels of *MMP9* concerning cancer stages and response to chemotherapy, as detailed in **Tables 2** and **3**.

Furthermore, *MMP9* exhibited its highest sensitivity (80%) and specificity (90%) at a cutoff value of 0.39, demonstrating significant differences ($p < 0.05$) in screening patients with colon cancer. Conversely, for screening patients with lung cancer, *MMP9* showed lower sensitivity (60%) but maintained high specificity (90%) at a cutoff value of 0.37. Notably, for the diagnosis of breast cancer, *MMP9* proved to be less efficient, with a sensitivity of 49% and specificity of 50% at a cutoff value of 0.30, as shown in **Table 4** and **Figure 1C–E**.

3.3. MMP9 gene expression analysis using GEPIA

This study utilized GEPIA to examine the regulation of *MMP9* in lung adenocarcinoma (LUAD), colorectal adenocarcinoma (COAD), and breast invasive carcinoma tumors. *MMP9* was significantly upregulated in tumor tissues compared to normal tissues, with substantial upregulation in LUAD (30.52 in tumor vs. 10 in normal), COAD (18.86 in tumor vs. 0.69 in normal), and BRCA (29.67 in tumor vs. 2.34 in normal) tumors. The differential expression of *MMP9* in LUAD, COAD, and BRCA tumors is shown in **Figure 1F**.

Table 4: ROC curve, sensitivity, and specificity of *MMP9* in screening cancer patients.

Cancer type	AUC	Std error	*P*-value	Cutoff	Sensitivity %	Specificity %
Colon	0.867	0.075	0.05*	0.39	80%	90%
Lung	0.69	0.093	0.08	0.37	60%	90%
Breast	0.577	0.099	0.46	0.3	49%	50%

AUC: area under the curve

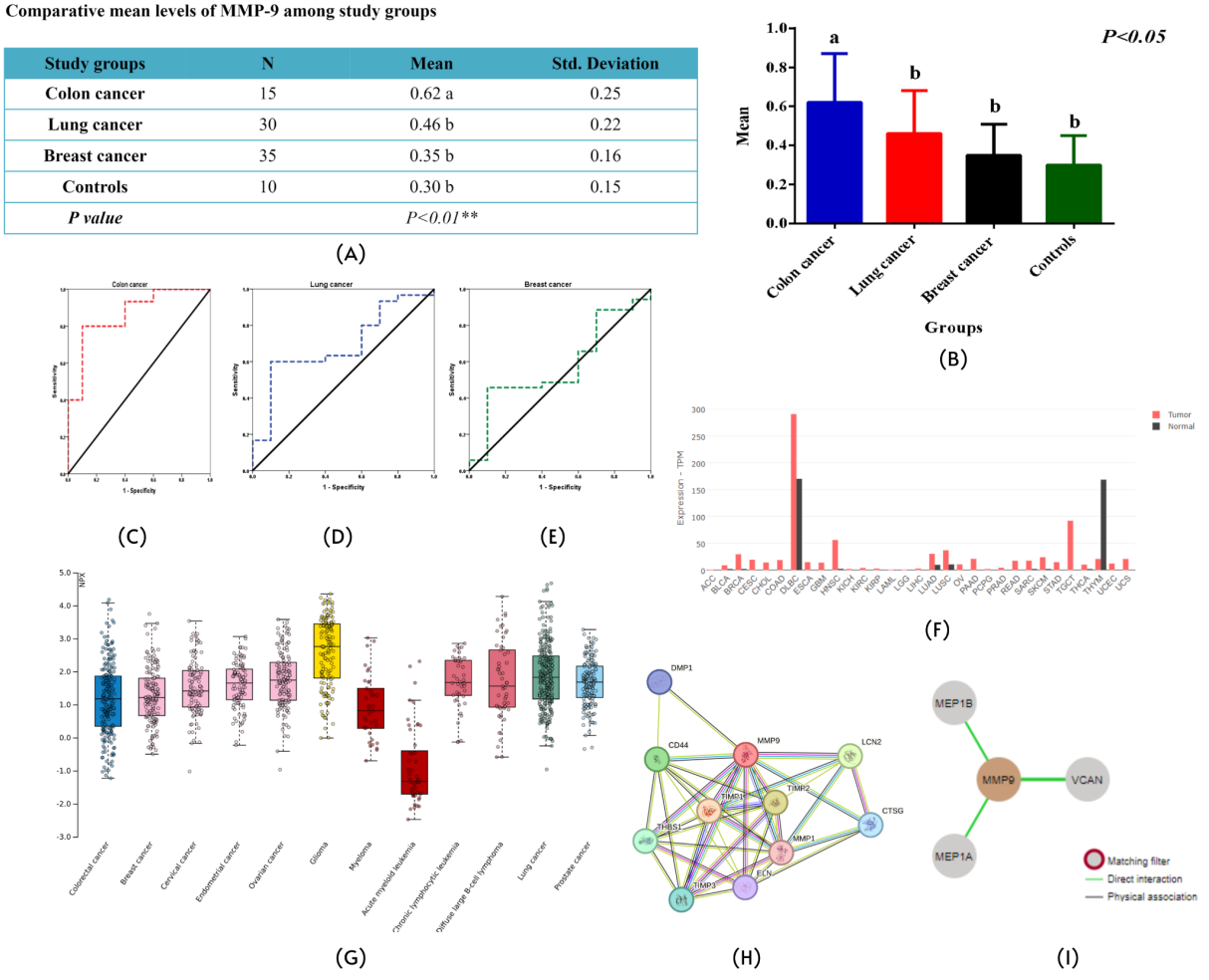

Figure 1: Expression and protein analysis of *MMP9*. (A) Means of the study groups when compared using the Duncan test; [a,b] Significant differences ($p < 0.05$). (B) Comparative mean levels of *MMP9* among study groups. (C) Sensitivity and specificity graph of colon cancer. (D) Sensitivity and specificity graph of lung cancer. (E) Sensitivity and specificity graph of breast cancer. (F) Differential gene expression of *MMP9* in LUAD, COAD, and BRCA tumors. (G) Protein concentrations of *MMP9* in each cancer. (H) PPI analysis through STRING. (I) PPI analysis through protein atlas.

3.4. *MMP9* protein expression analysis using HPA

MMP9 protein concentrations were observed through clustering-based analysis on the tissue-based expression dataset, which revealed higher concentrations of *MMP9* in both breast and lung cancers. However, in colon cancer, *MMP9* concentrations exhibited a more varied pattern, with some samples showing high concentrations while others displayed lower concentrations. The varying concentrations of *MMP9* in each cancer are depicted in **Figure 1G.**

3.5. Literature review to identify genes and pathways implicated with *MMP9*

An extensive literature review was performed to identify, filter, and target the genes and pathways that are implicated with *MMP9* gene dysregulation in breast, lung, and colon cancers. A comprehensive analysis identified a total of 34 genes and 21 pathways for the aforementioned cancer types. Specifically, in lung cancer, notable genes, including *CRKL, URGCP, SDF-1α, SEMA4b, ATDC, PTTG1, FAK, TIMP2, MMP3, TCF2 (HNF1β)*, and *KISS1*, were implicated alongside crucial pathways, namely the plasmin-dependent pathway, NF-κB, CXCR4/ERK/NF-κB, EGFR signaling pathway, and JNK pathways. Furthermore, genes such as *TSP1,* Clusterin *(CLU), RasGRF2,* piwi-like protein 2 *(Piwil2), CTHRC1, VEGF, TIMP2, MMP2*, and *uPA*, alongside pathways such as Src/PI 3-kinase, NF-κB pathway, MAPK/ERK, PI3K/Akt, JNK-activated AP-1, ROS-dependent ERK1/2, p38-MAPK-activated, and NOTCH1 signaling pathways were implicated in colon cancer[21,22,23].

Similarly, genes such as Heregulin-β1 *(NRG-1), p53, CDC42, CD44, EGF, Ets-1, TGFβ, EGFR, TNF-β, MMP2, TIMP1, TIMP2,* syndecan-2, and syndecan-4, along with pathways such as ERK, MAPK, PI3K, PKC, p38 kinase, JAK3/ERK, PI3K/AKT, and NF-κB pathways were implicated in breast cancer[24,25,26]. Notably, *TIMP2* gene and NF-κB pathway were found to be implicated across all three cancers. *MMP2* gene, along with the MAPK and PI3K pathways, were identified in both colon and breast cancers[27,28,29]. The genes and pathways implicated with *MMP9* in aforementioned cancers are listed in **Table 5**.

Table 5: Genes and pathways implicated with *MMP9* in lung, colon, and breast cancers.

Lung cancer	
Genes	**Pathways**
1. *CRKL*	
2. *URGCP*	
3. *SDF-1*	
4. *SEMA4b*	1. Plasmin-dependent pathway
5. *ATDC*	2. NF- B
6. *PTTG1*	3. CXCR4/ERK/NF- B
7. *FAK*	4. EGFR signaling pathway
8. *TIMP2*	5. JNK pathways
9. *MMP3*	
10. *TCF2 (HNF1)*	
11. *KISS1*	
Colon cancer	
Genes	**Pathways**
1. *TSP1*	1. Src/PI 3-kinase
2. Clusterin *(CLU)*	2. NF- B pathway
3. *RasGRF2*	3. MAPK/ERK
4. *piwi-like protein 2 (Piwil2)*	4. PI3K/Akt
5. *CTHRC1*	5. JNK-activated AP-1
6. *VEGF*	6. ROS-dependent ERK1/2
7. *TIMP2*	7. p38-MAPK-activated
8. *MMP2*	8. NOTCH1 signaling
9. *uPA*	
Breast cancer	
Genes	**Pathways**
1. Heregulin- 1 *(NRG-1)*	
2. *P53*	
3. *CDC42*	
4. *CD44*	1. ERK
5. *EGF*	2. MAPK
6. *Ets-1*	3. PI3K
7. *TGF*	4. PKC
8. *EGFR*	5. p38 kinase
9. *TNF-*	6. JAK3/ERK
10. *MMP2*	7. PI3K/AKT
11. *TIMP1*	8. NF- B
12. *TIMP2*	
13. Syndecan-2	
14. Syndecan-4	

3.6. PPI and clustering analysis

Identification of the *MMP9* functional partners was conducted through the STRING database. Genes such as *TIMP1, TIMP2, LCN2, CD44, THBS1, TIMP3, CTSG, DMP1, ELN*, and *MMP1* exhibited the highest interactions with *MMP9*, with scores ranging from 0.999 to 0.974, respectively. The detailed list of genes and their corresponding scores is provided in **Table 6**. A visual representation of interactions between *MMP9* and its functional partners can be observed in **Figure 1H**.

Moreover, direct interactions of *MMP9* with *VCAN, MEP1A,* and *MEP1B* were identified. A high-confidence interaction was observed with *VCAN*, with an MI score of 0.6, while medium-confidence interactions were noted

Table 6: *MMP9* functional partners and their respective scores.

Functional partners	Score
TIMP1	0.999
TIMP2	0.998
LCN2	0.998
CD44	0.998
THBS1	0.997
TIMP3	0.995
CTSG	0.992
DMP1	0.982
ELN	0.979
MMP1	0.974

Table 7: Top 15 nearest neighbors based on tissue RNA expression.

Neighbor	Description	Correlation	Cluster
NKG7	Natural killer cell granule protein 7	0.9842	6
CLEC12A	C-type lectin domain family 12 member A	0.9825	81
CTSW	Cathepsin W	0.9825	81
NFE2	Nuclear factor, erythroid 2	0.9789	81
GNLY	Granulysin	0.9772	77
PIK3R5	Phosphoinositide-3-kinase regulatory subunit 5	0.9772	77
RETN	Resistin	0.9754	6
PADI4	Peptidyl arginine deiminase 4	0.9754	6
IL18RAP	Interleukin 18 receptor accessory protein	0.9737	81
FMNL1	Formin-like 1	0.9737	81
FOLR3	Folate receptor gamma	0.9684	6
SLFN14	Schlafen family member 14	0.9667	6
ADGRG3	Adhesion G protein-coupled receptor G3	0.9667	6
HK3	Hexokinase 3	0.9632	81
GYPE	Glycophorin E (MNS blood group)	0.9632	77

with *MEP1A* and *MEP1B*, each with an MI score of 0.56. The interaction network is illustrated in **Figure 1I**.

Furthermore, *MMP9* tissue RNA expression clustering was performed to identify the nearest neighboring genes correlated with the annotated functions. The analysis revealed that *MMP9* is part of the "*lymphoid tissue & bone marrow – innate immune response*" cluster with confidence 1. The top 15 neighbor genes *NKG7, CLEC12A, CTSW, NFE2, GNLY, PIK3R5, RETN, PADI4, IL18RAP, FMNL1, FOLR3, SLFN14, ADGRG3, HK3*, and *GYPE* showed correlation scores ranging from 0.984 to 0.963 in clusters 6, 81, and 77. The top neighboring genes, their description, along with their respective correlation scores and cluster numbers are listed in **Table 7**.

4. Discussion

MMP9 gene plays a critical role in tumor invasion, metastasis, and tissue remodeling, as well as angiogenesis[8]. The experimental analysis showed that the highest expression levels of *MMP9* were in colon cancer compared to lung and breast cancer patients, indicating a more significant role of *MMP9* in colon cancer patients compared to lung and breast cancer patients. Furthermore, a thorough study of the literature on *MMP9* gene and its effects on lung, colon, and breast cancers showed that this gene is involved in many important pathways and is linked to other genes that affect how tumors grow. Notably, a significant increase in *MMP9* expression is observed in patients with lung, colon, and breast cancers, predicting poorer progression-free survival[2,11,21]. Thus, the overexpression of MMPs and *MMP9* might be promising candidates for diagnostic biomarkers and drug targets for tumor invasion and metastasis.

Furthermore, several different studies on breast cancer reported that genes such as heregulin-β1 (*NRG-1*), *p53*, *CDC42, CD44, EGF, Ets-1,* and *TGFβ* induce the expression of *MMP9* gene, regulating its activity, while genes such as *EGFR* and *TNF-β* are reported to be induced by *MMP9* gene in breast cancer progression; however, the genes, *MMP2, TIMP1, TIMP2,* syndecan-2, and syndecan-4 are also reported to be implicated with *MMP9* gene in the development and progression of breast cancer. Lastly, the pathways including *ERK, MAPK, PI3K, PKC,* p38 kinase*, JAK3/ERK, PI3K/AKT*, and *NF-κB* are reported to be implicated with the *MMP9* gene expression in breast cancer[22,24].

In addition, studies have investigated the role of genes and pathways implicated with *MMP9* in lung cancer, where the genes including *CRKL, URGCP, SDF-1α, SEMA4b, ATDC,* and *PTTG1* are known to influence *MMP9* by regulating its expression, while the expression and function of *FAK* gene are found to be regulated by *MMP9* gene in lung cancer. However, *TIMP2, MMP3, TCF2 (HNF1β)*, and *KISS1* have been reported to play crucial roles in lung cancer cells' growth and expansion. Finally, the pathways, including plasmin-dependent pathway, NF-κB, CXCR4/ERK/NF-κB, EGFR signaling

pathway, and JNK pathway, are associated with lung cancer advancement by dysregulating the crucial genes in their respective pathways[25,28].

Furthermore, the genes and pathways that are implicated with *MMP9* gene in colon cancer are reported to be *TSP1, CLU, RasGRF2, Piwil2,* and *CTHRC1,* which are involved in the induction of *MMP9* gene, regulating its activity, while *VEGF* gene expression is regulated by *MMP9* gene. In addition, *TIMP2, MMP2,* and *uPA* genes are also reported to be involved in colon cancer with *MMP9* gene. However, pathways such as Src/PI 3-kinase, NF-κB pathway, MAPK/ERK, PI3K/Akt, JNK-activated AP-1, ROS-dependent ERK1/2, p38-MAPK-activated, and NOTCH1 signaling are reported to be implicated with *MMP9* gene and are involved in colon cancer development and aggression[29,31].

Still, *TIMP2* gene and the NF-κB pathway were found in all three cancers. *TIMP2* gene was also found in the STRING analysis results, with a score of 0.998. This suggests that *TIMP2* gene and the NF-κB pathway may be involved in the development of lung, colon, and breast cancers and may be the most important biomarker and pathway in their progression. A study reported that different sets of immune markers are strongly correlated with *TIMP2* expression and are associated with the prognosis and level of immune infiltration in colon and gastric cancers[32]. Moreover, high levels of *MMP2* and *TIMP2* are associated with a higher risk of lung cancer[33]. Another study reported the expression of *TIMP2* in breast cancer, which is associated with advanced disease, a decrease in survival time, increased tumor size, node-positive status, and tumor recurrence[34].

In addition, *MMP2* gene and the MAPK and PI3K pathways were linked to both colon and breast cancers. This means that, like the *TIMP2* gene, *MMP2* is linked to both colon and breast cancers, suggesting that there is another biomarker that is common between the two types of cancer. *MMP2* gene is reported to be involved in tumor invasion and metastasis in colon cancer[17,35].

Several previous studies have reported *MMP9* as the therapeutic target in lung, colon, and breast cancers and have used various drugs to inhibit its expression and prevent tumor metastasis and invasion[36,37]. Comparatively, our study has identified specific biomarker genes (*MMP2* and *TIMP2*) and pathways (MAPK, PI3K, and NF-κB) that are implicated with *MMP9* gene, influencing its activity, or getting influenced by *MMP9*, which might be playing a crucial role in the invasion, progression, and metastasis of each respective cancer.

Subsequently, it can be concluded that several genes and pathways, such as *MMP2* and *TIMP2* genes and MAPK, PI3K, and NF-κB pathways, might be implicated with *MMP9* gene in lung, colon, and breast cancers, suggesting crucial biomarkers that can be explored and investigated as therapeutic targets to prevent or cure the development and progression of lung, colon, and breast cancers.

5. Conclusion

Bioinformatics data and clinical data from Iraqi metastatic cancer patients showed that *MMP9* plays a significant role in tumor invasion, metastasis, angiogenesis, and in mediating tumor microenvironments. Furthermore, this study revealed several genes and pathways for lung, colon, and breast cancers, which are reported to be implicated with *MMP9* gene in tumor progression and development. *MMP2* and *TIMP2* genes, as well as the MAPK, PI3K, and NF-κB pathways, may be important biomarkers that may be linked to *MMP9* gene. These pathways can be studied further at the clinical level as therapeutic targets or monitoring targets to find out what role the *MMP9* gene plays in lung, colon, and breast cancers.

Research ethics

The method was approved by the ethics committee at the Division of Biotechnology (Department of Applied Sciences, University of Fallujah) and the Fallujah Teaching Hospital of Maternity and Children (Iraqi Ministry of Health and Environment) approval No. UF 20-06/2021. The study complied with the ethical standards of the Helsinki Declaration of the World Medical Association in 2013. All participants were informed of the study objectives, and they provided written consent letter.

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Research ethics

This research was approved by ethics committee at the department of pathological department, College

of Applied Sciences, University of Fallujah, approval number UOF842A13. The study complied with the ethical standards of Helsinki Declaration of World Medical Association, 2013. All participants agreed to participate in the study.

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