

TARGETING MATRIX METALLOPROTEINASES 11 (MMP11) AS A PROMISING APPROACH IN HER2 POSITIVE BREAST CANCER CELLS

PROJECT WORK (PHASE-II)

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

SHOBANA.A (39230043)

TIVIYA THANGASWAMY (39230053)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC
JEPPIAAR NAGAR, RAJIV GANDHI SALAI,
CHENNAI - 600 119

MAY-2023



SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of TIVIYA THANGASWAMY(39230053), SHOBANA.A (39230043), who carried out the project **"TARGETING MMP-11 AS A PROMISING APPROACH IN HER2 POSITIVE BREAST CANCER"** under our supervision from **June 2022 to May 2023**.

Internal Guide

DR.OVIYA.R.P

Head of the Department

DR. V. RAMESH KUMAR

Submitted for Viva-voce Examination: 11/05/2023

Internal Examiner

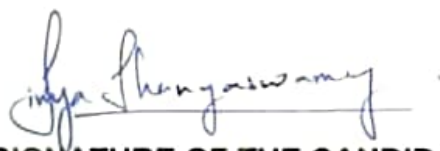
External Examiner

DECLARATION

I, **TIVIYA THANGASWAMY (39230053)** hereby declare that the Project report entitled "**TARGETING MATRIX METALLOPROTEINASES 11 (MMP11) AS A PROMISING APPROACH IN HER2 POSITIVE BREAST CANCER CELLS**" done by me under the guidance of **Dr.OVIYA** at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of a Bachelor of Technology degree in **Biotechnology**.

DATE: 10/05/2023

PLACE: CHENNAI

A handwritten signature in blue ink, reading 'Tiviya Thangaswamy', written over a horizontal line.

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr.V.RAMESH KUMAR, M. Phil., Ph.D.**, Head of the Department, Dept. of Biotechnology for providing me with necessary support and details at the right time during the progressive reviews.

I express my precious thanks to my internal Guide, **Dr.OVIYA, Ph.D.**, Assistant Professor, Department of Biotechnology, for her constant support and encouragement during the advancement of my project work.

I would like to express my sincere and deep sense of gratitude to my external Guide, **Dr.OVIYA, Ph.D.**, Department of Biotechnology for her valuable guidance, suggestions, and constant encouragement paved way for the successful completion of my review work.

I also express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the work.

I would like to express my heartfelt gratitude to my family and friends for their keen interest, constant support, and encouragement throughout my project work and the almighty, whose grace and blessings helped me to achieve my goal.

ABSTRACT

Our study involves identifying the potential of MMP11 in pancreatic cancer and epigenetic status of MMP11. Our study showed hypermethylation and hypomethylation in cancer such as thyroid carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, glioblastoma multiforme, breast invasive carcinoma, lung squamous cell carcinoma, uterine corpus endometrial carcinoma, sarcoma, liver hepatocellular carcinoma, head and neck squamous carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, stomach carcinoma, lung adenocarcinoma, kidney renal papillary cell carcinoma, colon carcinoma and esophageal carcinoma. In case of breast cancer GOBO analysis reveals upregulation and breast cancer in summary MMP11 expression was modulated in various cancer. Upregulation breast cancer was observed and methylation phases a direct role modulating the gene expression MMP11 in cancer. We also analysed therapeutic potential in breast cancer using pimozide and anticycotic drug. Our invitro analysing her2 positive breast cancer showed effective cytotoxicity of pimozide against her2 positive breast cancer cells. Further effect of pimozide treatment was studied to identify its effect on Epithelial-to-Mesenchymal , apoptosis and necroptosis.

TABLE OF CONTENTS

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	v
	CONTENTS	vi
	LIST OF ABBREVIATIONS	ix
	LIST OF FIGURES	x
	LIST OF TABLES	xi
1	INTRODUCTION	1
	1.1 ANGIOGENESIS	4
	1.2 EPIGENETIC CONTROL OF MECHANISM	6
	1.3 HYPOMETHYLATION AND HYPERMETHYLATION	6
2	LITERATURE SURVEY	8
	2.1 TARGETING MMP11 IN CANCERS	8
	2.2 PANCREATIC CANCER	9
	2.3 PROSTATE CANCER	12
	2.4 CERVICAL CANCER	13
	2.5 COLORECTAL CANCER	14
	2.6 HEPATOCELLULAR CARCINOMA	15
	2.7 BREAST CANCER	16
	2.8 GASTRIC CANCER	17
	2.9 LUNG CANCER	17
	2.10 HEAD AND NECK CANCER	18
	2.11 ENDOMETRIAL CANCER	19
3	AIM AND SCOPE	20
4	MATERIALS AND METHODS	21
	4.1 MAMMALIAN CELL CULTURE	21
	4.2 COLONY FORMATION ASSAY	22
	4.3 TOTAL RNA ISOLATION	22
	4.4 AGAROSE GEL ELECTROPHORESIS	22
	4.5 cDNA CONVERSION	23
	4.6 PCR (POLYMERASE CHAIN REACTION)	23

	4.7 DESIGNING OF REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION(qRT-PCR) primers	23
	4.8BIOINFORMATICS WORK OF ANALYSIS OF METHYLATION OF MMP11	24
	4.8.1 METHYLATION STATUUS OF MMP11	24
	4.8.2 MMP11 EXPRESSION ANALYSIS IN BC AND ITS SUBTYPES	25
	4.8.3 VALIDATION OF MMP11 EXPRESSION PROFILE	25
	4.8.4 GENETIC CHANGES TO MMP11	26
	4.8.5 CLINICAL OUTCOME VALIDATION FOR BC SUBTYPES	26
	4.8.6 MMP11 TRANSCRIPT LEVELS AND CLINICAL OUTCOMES IN BC SUBTYPES	27
	4.8.7 STATISTICAL SIGNIFICANCE	27
5	RESULTS AND DISCUSSION	28
	5.1 VALIIDATION OF MMP11 MRNA EXPRESSION IN BC SUBTYPES	28
	5.2 MMP11 MUTATION STUDY USING COSMIC AND CBIOPORTAL ANALYSIS OF THE NUMBER OF ALTERATIONS	29
	5.3 CORRELATION OF MRNA EXPRESSION WITH COPY NUMBER ANALYSIS AND MMP11 PROMOTER METHYLATION	30
	5.4 RT-PCR RESULTS	31
	5.5 MMP11 EXPRESSION PROFILE IN BC	32
	5.6 CELL CULTURE	33
	5.6.1 MTT ASSAY	33
	5.6.2 COLONY FORMATION ASSAY	34
	5.7 METHYLATION OF MMP11	35
	5.8 RNA ISOLATION (AGAROSE GEL ELECTROPHORESIS)	37
	5.9 DISCUSSION	38
6	SUMMARY AND CONCLUSIONS	39
	REFERENCES	40
	ANNEXURE	51

LIST OF ABBREVIATIONS

1. ABC	Advanced Breast Cancer
2. BCC	Basal Cell Carcinoma
3. BSE	Breast Self Examination
4. Ca	Cancer; carcinoma
5. c-DNA	Complementary DNA
6. DALY	Disability-Adjusted Life Year
7. DNA	Deoxyribonucleic acid
8. ECM	Extracellular matrix
9. EMT	Epithelial-to-mesenchymal transition
10.ERs	Estrogen receptors
11.GCT	Germ Cell TumourPBSC
12.GEO	Gene Expression Omnibus
13.HCC	Hepatocellular Carcinoma
14.HDC	High Dose Chemotherapy
15.HER2	Human epidermal growth factor receptor 2
16.MMP11	Matrix Metalloproteinase-11
17.MPNST	Malignant Peripheral Nerve Sheath Tumour
18.PR	Progesterone receptor

LIST OF FIGURES

FIGURE NO.	FIGURE NAME	PAGE NO.
Figure 1.1	Normal vs Abnormal Cell growth and Development	1
Figure 1.2	Cross-section of female breast with Breast Cancer	4
Figure 5.1	Validation of MMP11 expression levels using individual datasets	28
Figure 5.2	Validation of MMP11 expression levels using the Oncomine database	29
Figure 5.3	Genetic alterations in MMP11	30
Figure 5.4	rt-PCR RESULTS	31
Figure 5.5	Analysis of expression levels of MMP11 using GOBO	32
Figure 5.6	Analysis of expression levels of MMP11 using bc-GenEx-miner	33
Figure 5.7	Cytotoxicity% results	33
Figure 5.8	Colony formation assay results	34
Figure 5.9	Methylation (p-value) Analysis	36
Figure 5.10	Agarose gel electrophoresis	37

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
4.1	LIST OF CELL LINES USED	21
4.2	LIST OF PRIMERS USED FOR QRT-PCR ANALYSIS	24

CHAPTER-1

1. INTRODUCTION

Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. Approximately 12,00,000 new cancer cases are diagnosed in India every year. As per the latest National Cancer Registry data, one in eight men and one in nine women in India will develop some form of cancer. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women.

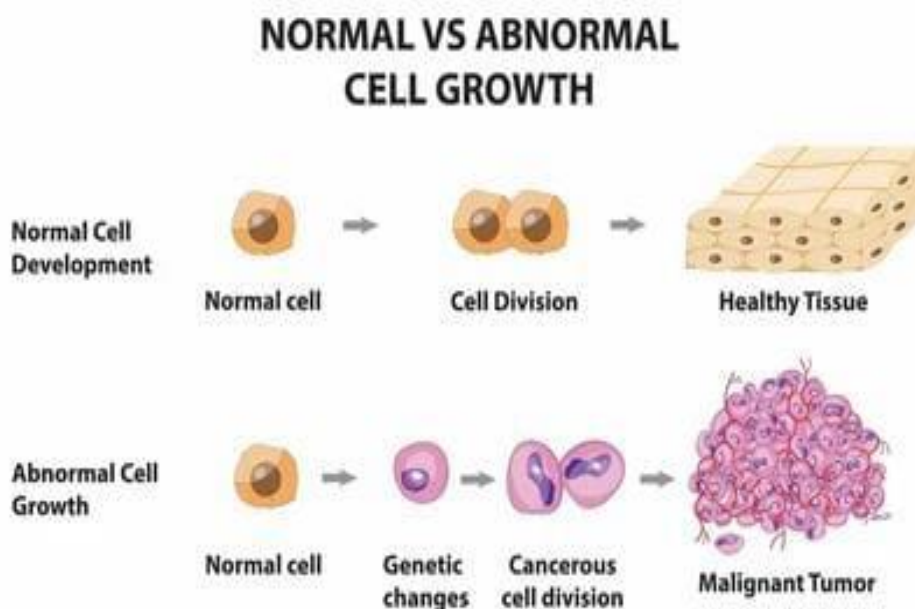


Fig1.1:Normal vs Abnormal Cell growth and Development

(Credits: <https://www.shutterstock.com/image-vector/normal-vs-abnormal-cell-growth-development-2207801345>)

One of the most prevalent malignancies in women is breast cancer (BC). BC formation involves a complicated interaction between the matrix metalloproteinase (MMP) enzyme family and other factors. There is growing indication that MMP11 is important in BC, but the underlying processes are unclear. The current research verified, through analysis of clinical samples and TCGA data sets, that a high expression of MMP11 in clinical samples of BC was highly correlated with a poor prognosis in BC patients(zhuang, 2021). Additionally, MTT and colony formation tests showed that changes in MMP11 expression had an impact on BC's ability to

proliferate. The main cause of cancer-related deaths in women globally is breast cancer (BC). BC also has the highest prevalence and fatality rates of all female malignant tumors, seriously endangering the health and lives of women. Despite major advancements in BC therapy over the past few years, metastasis and recurrence have had an adverse impact on patients' total mortality rates, and the prevalence of BC in China has increased over the past ten years. As a result, there has been an increase in focus on investigating pertinent possible molecular markers in order to better understand the pattern of BC proliferation and to create new therapeutic strategies to slow BC development. MMP11, a important member of the matrix metalloproteinase (MMP) family, regulates a number of physiological processes and signalling events, manipulates some biologically active molecules on the cell surface, and modifies the biological behaviour of cells, playing a significant role in the tumour microenvironment. Additionally, several studies have shown that MMPs are strongly linked to carcinogenesis and that MMP11, which is overexpressed in tumours and is involved in the proliferation and malignant growth of cancer cells, is the most functionally relevant MMP. According to a prior study, individuals with BC who had high expression of MMP11 had a bad prognosis. MMP11 was found to increase cancer malignancy by suppressing apoptosis and encouraging migration and invasion, however the underlying mechanism is yet unknown. MMPs, or matrix metalloproteinases, are zinc-dependent endopeptidases that play a key role in a variety of physiological and pathological processes, such as tissue remodeling, inflammation, and tumour metastasis(Kang. S, 2022). We concentrated on MMP11 to create a vaccination against stromal antigens produced by fibroblasts linked with cancer. (orstromelysin 3)(peruzzi, 2009). MMP11 expression is correlated with the invasiveness and aggressiveness of several forms of cancer. A highly expressed protein called MMP11 (stromelysin-3) was originally discovered in the stromal cells of breast cancer11. It distinguishes itself from other MMPs by its distinctive traits. However, MMP11 is secreted as an active enzyme12 and catalyses the degradation of the serine protease inhibitors 1-antitrypsin and insulin-like growth factor binding protein-1, rather than hydrolyzing conventional MMP substrates, such as laminin and fibronectin. Most MMPs are secreted as inactive zymogens and are activated by extracellular proteinase cleavage. Despite stromal MMP11 expression in cancer, earlier research concentrated on the function of MMP11 in cancer cells and showed that MMP11 promotes the development of

oral, gastric, and colon cancers, among others. The surrounding microenvironment has a significant impact on the biological processes of cancer cells, such as carcinogenesis, proliferation, angiogenesis, apoptosis, and invasion. An essential property that allows solid malignant tumours to acquire certain functionalities necessary for their malignant biological behaviours is the capacity to change the microenvironment. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that can remodel extracellular matrix (ECM) by breaking down nearly all ECM proteins. These enzymes play crucial roles in the invasion and metastasis of solid malignant tumours by enabling tumour cells to alter ECM components and release cytokines, which in turn promotes protease-dependent tumour progression. MMP-11, also known as stromelysin-3, is a member of the MMPs superfamily's stromelysin subgroup and has been found in stromal cells, nearby microenvironment, and cancer cells. While MMP-11 has a dual function in the formation of cancer by limiting metastasis in animal models, it also serves a dual role in the promotion of cancer growth by blocking apoptosis and promoting cancer cell motility and invasion. MMP-11 was shown to be overexpressed in cancer patients' sera as compared to a healthy control group, as well as in several tumour tissue specimens, including those from pancreatic, breast, and gastric cancer. Currently, there is some evidence to suggest that MMP-11 may function as a major tumour biomarker for early cancer diagnosis, tumour staging, prognostic analysis, tracking recurrence during follow-up, as well as a possible target for cancer immunotherapy. A greater knowledge of how MMP-11 influences tumour growth and investigating its potential therapeutic use are urgently needed given the significance of MMP-11 in altering the tumour microenvironment and its significant antitumoral effects on solid tumours.

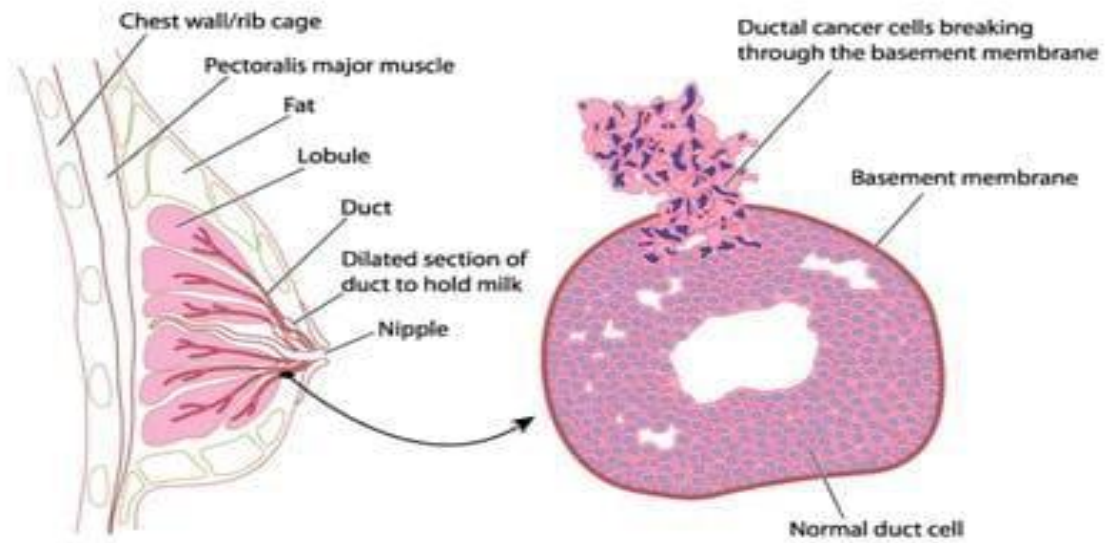


Fig1.2: Cross-section of female breast with Breast Cancer

(Credits: <https://www.shutterstock.com/image-vector/cross-section-female-breast-detail-cancer-101583709>)

1.1 ANGIOGENESIS:

The growth and spread of solid tumours depend on angiogenesis and invasion. A number of signalling pathways, including those triggered by growth factors, may be necessary for the formation of tumours. Although it has been shown that microRNAs play a crucial role in carcinogenesis, their functions in cancer angiogenesis, invasion, and other vital signalling pathways for tumour formation remain unclear from the perspective of tumour biology. Using an extremely aggressive breast cancer model *in vitro* and *in vivo*, we looked at the function of microRNA miR-98 in controlling tumour development, invasion, and angiogenesis. The proliferation, survival, tumour development, invasion, and angiogenesis of breast cancer cells were all decreased by miR-98 expression, according to our research. On the other hand, endogenous miR-98 suppression increased tumour development, invasion, and angiogenesis as well as cell proliferation and survival. It appeared that miR-98 restricted endothelial cells' abilities to disseminate, invade, and form tubules, hence preventing angiogenesis. It's interesting to note that miR-98 suppressed the expression of ALK4 and MMP11, both of which were thought to be potential targets. Both targets' expression was elevated after anti-miR-98 construct transfection. We verified that miR-98 specifically targeted the 3'-untranslated regions of MMP11 and ALK4. Last but not least, breast cancer cell survival, proliferation, and angiogenesis were suppressed by siRNAs that targeted

ALK4 and MMP11. MiR-98's inhibitory effects on proliferation, invasion, and angiogenesis were reversed by rescue tests using ALK4 and MMP11 constructs.

Furthermore, angiogenesis may be influenced by microRNAs(Jeyapalan Z, 2011). Our research shows that miR-378 stimulates angiogenesis by suppressing the expression of Sufu and Fus1 and that miR-93 participates in angiogenesis by targeting integrin-8 (Fang L, 2011). ALK4 is an activin type 1 receptor, and miR-24 has been found to influence erythroid development by targeting its expression. This finding suggests that miR-24 may be involved in activin signalling. In addition, Hebert and colleagues discovered that miR-98 expression controls the expression of HMGA2 (High Mobility Group A2) in head and neck squamous cell cancer (HNSCC) cells. MiR-98 has also been linked to controlling the production of the CIS (Cytokine-inducible Src homology 2-containing protein) protein in human cholangiocytes in response to the microbial challenge. It is currently unknown how miR-98 affects tumour angiogenesis. According to certain studies, miR-98 expression is linked to the expansion of tumour cells. Also, according to clinical investigations, miR-98 expression influences the growth of head and neck cancers and is underexpressed in nasopharyngeal carcinoma. Microarrays and real-time PCR, however, also discovered that miR-98 was up-regulated in primary breast cancer tissues. These findings imply that miR-98 may have a role in tumour suppression. Clinical analysis findings imply that miR-98 may behave differently in various cancer types. MiR-98 expression seems to be pro-apoptotic. When tumour sections were analysed, it was shown that the miR-98-expressing cells had more evidence of cell death than the control and anti-miR-98 tumours did. The miR-98-expressing tumours experienced significant cell death because they were unable to grow as rapidly as the anti-miR-98-expressing tumours. In vitro tests were used to support these findings. Anti-miR-98-transfected cells outlived miR-98-transfected cells and control vector cells when 4T1 cells were transfected with either miR-98 or anti-miR-98 and maintained in a serum-free environment. A number of in vitro studies demonstrating that miR-98 reduced endothelial cell activities, including cell spreading and tubule formation, confirmed the significance of miR-98 in suppressing angiogenesis. In the trials involving cellular spreading, the endothelial cells YPEN were able to spread quickly on top of the anti-miR-98-transfected cells but not on the GFP or miR-98 transfected cells. These findings clearly showed that the anti-

miR-98-transfected cells' surfaces differed from those of GFP- or miR-98-transfected cells. Endothelial cell spreading and intimate contact with cells that have been transfected with miR-98 may both be inhibited by miR-98's potential to restrict the production of cell surface proteins. In cells that have been transfected with miR-98, this function may also be connected to the prevention of invasion.

1.2 EPIGENETIC CONTROL OF MECHANISM:

Physically active people are frequently afflicted by patellar tendinopathy (PT), a severe disorder. A various types of gene variation DNA methylation is an epigenetic component that has just recently been shown to play a function in human tendinopathy. While comparing patellar tendinopathy to healthy tendon, we used a case-control strategy to see if there were any variations in the methylation state of the MMP11 gene promoter. Four CpG sites (regions of the genome rich in C/G nucleotides) upstream of the MMP11 gene were examined for methylation patterns using PCR and pyrosequencing in DNA from individuals with PT (n = 10) and those with healthy tendon (n=10) respectively. Histologically, neovascularization, an increase in collagen type III fibres, and an accumulation of glycosaminoglycans are all linked to the development of PT. It has been demonstrated that matrix metalloproteinase (MMP) enzymes have a significant role in controlling tendon extracellular matrix (ECM) homeostasis, which suggests that these alterations may, in part, be caused by changing expression of MMP enzymes.

Recently, researchers suggested that epigenetic changes like DNA methylation might account for the inconsistent association signals occasionally observed in research on human patellar tendinopathy. In fact, we showed that patellar tendinopathy was connected to methylation alterations at a CpG site upstream of the ADAMTS4 promoter. Yet as far as we are aware, no other studies have been published to date that discuss how DNA methylation affects the chance of developing human tendinopathy.

1.3 HYPOMETHYLATION AND HYPERMETHYLATION:

A number of extracellular matrix (ECM) constituents, such as aggrecan, fibronectin, and laminin, are catalytically degraded by the MMP11 enzyme(Araki and Mimura, 2017). Although the precise function of MMP11 in maintaining ECM homeostasis is not well established, Achilles tendinopathy significantly alters

MMP11 expression. Particularly, hypomethylation is linked to the transcriptional upregulation of MMP11. In view of this, we proposed that DNA extracted from patellar tendinopathy and normal tendon would have different levels of methylation at the MMP11 promoter. If this were the case, these variations in methylation might provide an explanation for why MMP11 expression is significantly changed in tendinopathy and open the door to innovative tactics that could lower the frequency of these injuries. In comparison to controls, we observed a remarkable hypermethylation (rather than hypomethylation) event at the MMP11 promoter in PT samples. According to Roach et al. lower gene expression is frequently linked to hypermethylation. As a result, it seems sense that MMP11 substrates would build up in PT since there would be less MMP11 enzyme available for catalysis. During PT, proteoglycan buildup has been seen. Among the tendon samples from patients utilised in this investigation, Parkinson and colleagues discovered elevated amounts of proteoglycans such as aggrecan. While it assists in hydration and lessens the effects of excessive compression, given especially often can prevent tendon from harm. Yet, tendinopathy can cause swelling when people drink too much water and it's possible that hypermethylation of the MMP11 promoter could make this situation worse. Although we are unable to currently comment on the importance of this result, it is plausible that as a person matures, an increase in methylation in the MMP11 gene promoter may be required to allow for ECM adaptations. The lack of a relationship between age and CpG methylation that we found in the PT group may thus point to an aberrant promoter methylation pattern that occurs before tendinopathy. However, further investigation is required to confirm this. DNA from males with PT was examined for the methylation status of multiple CpG sites upstream of the MMP11 gene and compared to DNA from controls.

CHAPTER-2

2. LITERATURE SURVEY

2.1 TARGETING MMP11(MATRIX METALLOPROTEINASE 11) IN CANCERS

MMP11 (Matrix Metalloproteinases) is a well known that immune cells can influence cancer cells to increase their proliferative and invasive properties. It is also known that Matrix metalloproteinases (MMPs) are crucial for remodelling the extracellular matrix and are also involved in the invasion of tumor cells. MMPs targeting may be effective as an additional therapeutic strategy in the treatment of cancer and there are 25 matrix metalloproteinases in humans, which are zinc-dependent endopeptidases with a powerful ability to break down extracellular matrix proteins¹(Abdel-Hamid &Abass, 2021). Except for certain processes like tissue healing and remodelling, inflammation, and neoplasia, the extracellular matrix functions as a barrier to prevent cell movement(Wieczorek et al., 2015). MMP-11, also known as stromelysin-3, is a member of the MMPs superfamily's stromelysin subgroup and has been found in stromal cells, nearby microenvironment, and cancer cells(B. Ma *et al.*, 2021). When compared to cancer cells, stromal cells in tumor tissues are genetically more stable, but they differ from their counterparts in normal tissues in terms of the types and concentrations of specific proteins(Khare *et al.*, 2021; Rundhaug, 2005). This trait implies that stromal antigens may serve as antitumoral targets. Currently, tumour endothelial cells, infiltrating macrophages/histiocytes, and fibroblasts linked to cancer are the targets of immune interventions(Kim *et al.*, 2021). Numerous studies have shown that MMPs are immune targets. MMP-11 is a perfect antigen target for immunotherapy because it is only expressed in the majority of primary solid cancer tissues and metastatic lesions. Several studies also showed that MMP-11 has therapeutic potential. In a colon adenocarcinoma mouse model, Peruzzi et al. found that a genetic vaccine against MMP-11 based on DNA electro-gene-transfer technology was able to overcome immune tolerance and have anti-tumoreffects(Peruzzi *et al.*, 2009). In order to control the progression of the disease, therapy that specifically targets MMP-11 may be effective. Various tumour types have elevated MMP11 expression. In cases of breast carcinoma, pancreatic tumors, and colon cancer, the expression

levels of MMP11 can be used to pinpoint patients who are most at risk of cancer recurrence. MMP-11 was found to be overexpressed in cancer patients' sera when compared to a healthy control group, as well as in numerous tumor tissue specimens, including those from pancreatic, breast, and gastric cancer. Because it is important to develop effective treatment plans, identifying prognostic indicators has always been a focus of cancer research. Breast carcinoma(Cheng *et al.*, 2012), pancreatic tumours(Wang *et al.*, 2022), and colon cancer patients may be identified using the expression levels of MMP-11 as being at higher risk for cancer recurrence. Less time was spent alive for patients with breast, non-small cell lung cancer, and colon cancer(Cheng *et al.*, 2012),(Taetuet *et al.*, 2006) when higher levels of MMP-11 expression were found in poorly differentiated high-grade thyroid carcinoma(X. Zhang *et al.*, 2016)and breast carcinoma(Ma *et al.*, 2021). Cancer progression is influenced by the tumour microenvironment, which is influenced by angiogenesis, reactive stromal fibroblasts, the immune system, and proteinase production(Ribeiro Franco *et al.*, 2020). Through ECM modification, the MMP family of zinc-dependent endopeptidases can facilitate the stromal invasion of cancer cells(Winer *et al.*, 2018). The stromelysin-3 subunit of MMP-11, which was first discovered in breast cancer tissue, is one of the MMP family members. Through proteolytic degradation of the endothelial basement membrane, MMP-11 may accelerate the development and spread of cancer. Numerous studies have demonstrated that high MMP-11 expression is linked to poor clinical outcomes in various cancers, including breast, prostate, hepatocellular carcinoma, colorectal, lung, and osteosarcoma(Korbeckiet *et al.*, 2021)-(Taheri *et al.*, 2021). High MMP-11 expression was linked to a worse prognosis as well as a decline in DFS and DSS in our analysis of the HUGH cohort and TCGA database. Previous research showed that a high MMP-11 level was linked to a poor prognosis for breast cancer(Eiroet *et al.*, 2019)-.The information on the pattern of MMP-11 expression in various human cancers and its corresponding effects on the biological characteristics of tumor cells that are malignant are all summarised in the sections that follow.

2.2 PANCREATIC CANCER

Pancreatic cancer tissues were the only ones to express the MMP11 protein; neither normal nor other cancerous tissues did. Patients with pancreatic cancer had a poor prognosis when their MMP11 levels were high(Singhiet *et al.*, 2019). Pancreatic

cancer has a poor prognosis, a very high mortality rate, and challenges with early detection. When significant invasion of large peritumoral vessels and distant metastases are present, which is common for the majority of patients with pancreatic cancer, surgery—the only likely curative option—is restricted (B. Ma *et al.*, 2021). MMP-11 has not been found in the mesenchymal or epithelial cells of healthy pancreatic tissues. However, more than 80% of pancreatic cancer specimens show strong signals for MMP-11 located in the epithelial cancer cells as well as in the stromal cells surrounding them. One of the most aggressive diseases in the world is pancreatic ductal adenocarcinoma, also referred to as pancreatic cancer. With a median survival of roughly 6 months, the 5-year survival rate for patients with this cancer is under 7%. (Cao *et al.*, 2021). It is the fourth most common cause of cancer-related death despite having a low prevalence [(Neoptolemos *et al.*, 2018)]. Furthermore, despite notable increases in overall survival rates for other cancers, the prognosis for patients with pancreatic cancer has remained unchanged, which suggests that there may be a greater significance to understanding pancreatic cancer (Aghamir *et al.*, 2021). High resistance of pancreatic cancer to traditional cancer treatments like chemotherapy and radiation therapy is one of its main characteristics. Early distant metastasis and local abnormal progression of pancreatic cancer, which together contribute to the relative rarity of surgery, are another serious aspect of the disease (Akter *et al.*, 2022). The absence of a reliable biomarker may be a major contributing factor to the extremely poor prognosis (Lee *et al.*, 2019). A total of 18,430 genes were sorted from nine data sets of the Gene Expression Omnibus (GEO) public microarray database to choose a candidate gene as a pancreatic cancer biomarker, as previously described. In order to find a potential candidate for a pancreatic cancer biomarker using The Human Protein Atlas database, a number of genes that were significantly up-regulated in pancreatic cancer compared to those of normal pancreas were successively eliminated. The need for effective biomarkers for pancreatic cancer is growing due to the lack of surgical options and the poor prognosis. Although many researchers have proposed challenging treatments to prevent pancreatic cancer, overall survival for patients with pancreatic cancer has not increased in recent years (Hasan *et al.*, 2019). Lack of a reliable biomarker is linked to such a poor prognosis (Hou *et al.*, 2021; Sidaway, 2017). MMP11 may be a promising prognostic biomarker in pancreatic cancer, according to new *in silico*, *in vitro*, and *in vivo* clinical evidence obtained in the

current study. We have access to a wealth of data about pancreatic cancer thanks to the impressive advancement of cancer-related bioinformatics (Bergholtz *et al.*, 2021). With the aid of analysis of gene or protein expression levels in public databases, eleven potential biomarkers for pancreatic cancer were found using the consecutive eliminating method. According to our research, the consecutive eliminating method stood out from other open database approaches based on three characteristics, which is consistent with earlier reports using open databases to choose biomarkers. First, the UPC normalisation method allowed a total of 18,430 genes from nine data sets for pancreatic cancer and seven data sets for normal pancreas in the public GEO and AE microarray database to be adequately pooled. Utilizing the most prevalent and recent genes from open-data sources, the selection of pancreatic cancer biomarkers was carried out in the current study. Second, both mRNA and protein expression patterns were taken into account when using the consecutive eliminating method in an organised manner. Different methods have been used to choose cancer biomarkers, each reflecting a focus on mRNA or protein expression in a particular areas. However, we identified potential pancreatic cancer biomarkers that, at the mRNA level, were markedly up-regulated in pancreatic cancer as compared to healthy pancreas. Using the Human Protein Atlas database, these were subsequently eliminated one at a time based on their protein content. As a result, candidates for pancreatic cancer biomarkers were chosen using sources of mRNA and protein levels that were organically combined. Finally, 8 nominees (42%, including mesothelin; gene ID, 10232) coincided with earlier reports, giving the final 11 candidates scientific credibility (Truong & Pauklin, 2021). Using real-time RT-PCR and immunohistochemistry analysis, nine MMPs [1-3, 7-9, 11, 12, and 14] expression levels in pancreatic cancer have recently been reported. The expression levels of MMP7, -8, -9, and -11 were up-regulated, according to immunohistochemistry. When compared to healthy pancreas, real-time RT-PCR revealed that the mRNA levels of MMP11 in pancreatic cancer were significantly higher (Hsieh *et al.*, 2020). In addition, pancreatic cancer specifically had higher levels of the MMP11 protein than did other cancers and healthy tissues, suggesting that MMP11's distinct patterns of expression may make it a potential biomarker for pancreatic cancer. Overall, our findings offer the first proof that soluble MMP11 could be specifically found in whole blood samples from patients with pancreatic cancer. Furthermore, there was a strong correlation between its high expression

and a poor prognosis for pancreatic cancer. Our findings offer compelling evidence that MMP11 might be a useful prognostic biomarker in pancreatic cancer.

2.3 PROSTATE CANCER

A common malignant tumour around the world is prostate cancer. A crucial part of the development and metastasis of cancer is played by the enzyme matrix metalloproteinase (MMP)-11, which is involved in the remodelling and degradation of the extracellular matrix(Pak *et al.*, 2022). Prostate cancer has grown to be a significant health burden for men everywhere, especially for elderly men. MMP-11 may change the stromal microenvironment to make it suitable for the growth, survival, and metastasis of prostate cancer cells because its expression is restricted to stromal fibroblasts in prostate cancer tissues(Kanharat&Tuamsuk, 2015). Furthermore, Kanharat *et al.* showed that MMP-11 may promote tumour angiogenesis in prostate cancer by changing the interstitial microenvironment. Nonsrijun *et al.* showed that the expression of MMP-11 in prostate cancer was positively correlated with Gleason grade pathological tumour stage 4 (pT4) and bone metastasis and was closely correlated with survival time. Additionally, Wan *et al.* (Rosclillet *et al.*, 2014) discovered that knocking out the MMP-11 gene significantly reduced the migration of prostate cancer cells. As a result, MMP-11 can be thought of as a potential tumour marker and therapeutic target for advanced prostate cancer. Patients with higher levels of MMP-11 had significantly shorter overall survival times than patients with lower levels(Toropovsky *et al.*, 2019). Prostate biopsies were performed more frequently as a result of the widespread adoption of prostate specific antigen (PSA) testing into clinical practise(Siegel *et al.*, 2019). The second most common cancer to be diagnosed worldwide is prostate cancer, which is also one of the leading causes of cancer death in men(Luo *et al.*, 2022). Even in Asian nations where the incidence was previously reported to be low, prostate cancer incidence has recently increased worldwide (Lowderet *et al.*, 2022). Prostate cancer carcinogenesis has been linked to risk factors including race, family history, diet, smoking, and somatic genomic alterations. Men's risk of developing prostate cancer rises with age, and the majority of cases are detected after age 65 [47]. Currently, prostate cancer is identified, tracked, and assessed using serum levels of the prostate-specific antigen (PSA). Additionally, PSA is a crucial tool for detecting prostate cancer recurrence. In particular, elevated serum PSA levels following

treatment are linked to the definition of biochemical recurrence (BCR) in patients with prostate cancer. The genotype distributions of MMP-11 polymorphisms in patients with prostate cancer were also examined. According to a prior study, prostate cancers with high levels of MMP-11 expression were significantly associated with a higher likelihood of biochemical recurrence. Additionally, a recent study by Escaffet *et al.* revealed that MMPs, such as MMP-11, were involved and played a significant role in the tumorigenesis and biochemical recurrence of prostate cancer (Sopylloet *et al.*, 2021). The five MMP-11 SNPs chosen for the current study did not significantly differ in their associations with biochemical recurrence, however, suggesting that the direct influence of these SNPs on biochemical recurrence may be limited. MMP-11 overexpression was significantly linked to poor survival, according to a study carried out in Thailand, and it may be used to predict a poor prognosis for prostate cancer. In addition, we examined the connections between MMP-11 mRNA expression levels and prostate cancer carcinogenesis, clinicopathological features, and biochemical recurrence using data from The Cancer Genome Atlas (TCGA)(Sudha *et al.*, 2022). Together, these results suggest that MMP-11 rs131451 polymorphisms may contribute to the effect of MMP-11 overexpression on prostate cancer patients poor prognosis and biochemical recurrence. The absence of prostate cancer patient tumour samples or data regarding MMP-11 expression levels is one of the study's limitations.

2.4 CERVICAL CANCER

With roughly 5.7 million new cases and 3.11 million fatalities globally in 2018, cervical cancer is the fourth most prevalent cancer among women(Okyere *et al.*, 2021). Cervical cancer continues to rank third among all cancers in developing nations despite extensive screening programmes. Since dysplasia and tumour epithelial cells express MMP-11, this protein may be used as a potential marker for the development of cervical cancer (Herbsteret *et al.*, 2018). According to Valdivia et al.(Tian *et al.*, 2015), MMP-11 was expressed at rates of 77% and 66% in low and high squamous lesions, respectively. MMP-11 expression may be an early occurrence in cervical neoplasms because it was linked to HPV infection as well as the diagnosis of precancerous lesions. Previous studies have shown that MMP-11 does not cause tumorigenesis by increasing the frequency of angiogenesis or the proliferation of cancer cells, but rather by reducing the death of cancer cells through

apoptosis and necrosis. The MMP11 protein may be involved in the neoplastic epithelial process as evidenced by the MMP11 expression showing a significantly increased relative fold change that is correlated with the type of lesion. In several human invasive tumours, including the breast, gastric, and renal, as well as in some lesions that are precursors to breast and oral cancer, an increased expression of MMP11 has been reported. Similar to this, MMP-11 expression was found in the tissues of cervical carcinoma, which has also been shown to be associated with the emergence of malignancy. MMP-11 expression was not found in the normal cervical mucosa tissues, but it was found in about 50% of dysplasia, 70% of carcinoma in situ, and 100% of invasive carcinomas. According to this discovery, MMP-11 is involved in a cancer-specific development that requires further investigation. However, histologically normal cervix tissues did not express MMP11, which we found in cervical precancerous lesions. Invasive carcinomas showed a markedly higher MMP11 expression. Unlike other tumour tissues where MMP11 expression is only found in stromal cells that surround the neoplastic area, the cytoplasm of cervix epithelium tumour cells showed signs of a positive immunoreaction.

2.5 COLORECTAL CANCER

The third most common cause of cancer-related death worldwide and the most common cancer in Taiwan is colorectal cancer (CRC) (Bray et al., 2018). With an incidence rate of 10.2% and a mortality rate of 9.2% , colorectal cancer ranks third among all cancers in the world(Sharma *et al.*, 2022). Invasion, metastasis, and postoperative recurrence are factors in its poor prognosis. Most human cancers, including colorectal cancer, have high levels of MMP expression and activity when compared to normal tissues(Morini *et al.*, 2020). MMP-11 can be found in colon adenocarcinoma cell lines derived from three different epithelial sources, in addition to stromal cells, according to research by Barrasa et al. According to Porte *et al.*, the development of local invasion and liver metastasis of colorectal tumours is correlated with the high expression of MMP-11 transcripts. The presence of colorectal cancer has also been linked to the immunohistochemical detection of MMP-11 expression (Shi *et al.*, 2021). A recent study found that CD147 was a standalone prognostic factor for patients with CRC who survived without developing disease. In research, discovered that CD147 expression was associated with lymph node metastasis, distant metastasis, and TNM stage for CRC. In addition, it is a

standalone prognostic factor for colorectal cancer patients. According to findings, CD147 plays a significant role in the metastasis of colorectal cancer and has prognostic value for patients, both of which point to the clinical importance of this protein. Also recently discovered are some novel functions for CD147 in cancer. In order to cause breast cancer cells to undergo the epithelial-to-mesenchymal (EMT) transition, Xu *et al.* found that CD147 was necessary for the transformation of fibroblasts into cancer-associated fibroblasts. Finally, this study showed a positive correlation and co-localization between the proteins CD147 and MMP-11, which are both overexpressed in colorectal cancer. MMP-11 and CD147 may both function as tumour promoters as colorectal cancer patients tumours develop. These suggested that CD147 and MMP-11 might serve as potential therapeutic targets for colorectal cancer patients as well as prognostic markers.

2.6 HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC), one of the most prevalent solid cancers, is the main reason for cancer-related deaths globally. Approximately 840,000 new cases and more than 780,000 deaths worldwide are caused by liver cancer each year, according to WHO statistics for 2018(Saad *et al.*, 2020). It ranks sixth in terms of morbidity and fourth in terms of mortality. In HCC, MMP-11 is overexpressed(di Palo *et al.*, 2020). A potential target for liver cancer therapy is MiR-125a, a novel biomarker for tumour aggressiveness.expression is markedly downregulated in tumour tissues along with these changes(Scheauet *et al.*, 2019). In the epithelial-mesenchymal transition (EMT) of HCC, MMP-11 has been discovered to have a role. Additionally, it has been determined that EMT is a crucial transformation process for both the local and distant progression of HCC(Hadjimichaelet *et al.*, 2020). According to pre-clinical research, MMP-11 regulates tumour metastasis, including MMP-11 expression, which controls the local or distant invasion of HCC cells in transgenic mice(Duong *et al.*, 2017). The mouse hepatoma cell line HCa-F can also be prevented from proliferating and metastasizing to the lymph nodes by downregulating MMP-11 expression(Rahat, 2022). Therefore, the exDownregulation of MMP-11 may be a useful tactic for the management of lymph node metastasis in HCC (Geervliet& Bansal, 2020). MiR-125a expression is highly significant in tumour tissues. According to preclinical research, MMP-11 regulates local or distant invasion in transgenic mice, as well as how cancer metastasizes.

The mouse hepatocarcinoma cell line Hca-metastatic F's proliferation to lymph nodes is additionally inhibited by MMP-11 expression knockdown(Kamimura *et al.*, 2019). When investigated the effect of the MMP-11 gene on the metastatic phenotype of HCC, we found that patients with the rs28382575 C/T+C/C genotype were significantly more likely to develop lymph node metastasis than T/T carriers. These findings imply that MMP-11 knockdown may be a useful therapeutic approach for HCC lymph node metastasis. A reliable biomarker for the development of the disease and a significant predictor of early occurrence of HCC may be genetic variations in the MMP-11 gene.

2.7 BREAST CANCER

MMP-11, also known as stromelysin-3, was first identified in breast cancer tissue. On malignancies, MMP-11 has two unique actions. MMP-11 inhibits apoptosis and the proteolytic breakdown of the endothelium basement membrane, which on the one hand promotes the formation of cancer(X. Zhang *et al.*, 2016). A crucial step in deciding how to treat breast cancer and determining the effectiveness of treatment is identifying the prognostic biomarkers of the illness. Tumor biological characteristics and molecular genetic traits have lately been acknowledged as important prognostic biomarkers due to the development of molecular approaches(Savci-Heijink *et al.*, 2019). Finding innovative therapeutic and prognostic indicators at the molecular level is essential for treating breast cancer patients effectively and learning more about the disease. MMP11 may serve as a potential downstream target of the TGF-signaling pathway. MMP11 may significantly affect the levels of Smad2 and Smad3 protein synthesis in the TGF-signaling pathway and may prevent Smad2 from being destroyed by the ubiquitin proteasome(Georgopoulou *et al.*, 2021). The proliferation of breast cancer cells is promoted by MMP-11, which contributes in the progression of the condition. MMP-11 was expressed greater in primary cancer tissue than in healthy breast tissue. High MMP-11 expression has been associated with reduced tumour antigenicity, decreased TILs, including CD8+ T cells, CD4+ memory T cells, and memory B cells, as well as poor dendritic cell activation. Mechanistically, MMP11's cancer-promoting actions in breast cancer are connected to the increased stability of the Smad2 protein and the activation of the TGF- signalling pathway. On the other hand, MMP11 inhibition greatly inhibited breast cancer cells' ability to proliferate and

develop. The outcomes demonstrated that MMP11 knockdown could counteract its pro-tumorigenic effects in vivo. However, it is recommended that the MMP11 gene be the focus of BC therapy. Pictilisib and AZ960 had an effect on breast cancer cell lines with high MMP-11 expression, we found. These possible drugs might be used to treat patients who express high levels of MMP-11 and are resistant to chemotherapy.(Nardouet *et al.*, 2022)(Georgopoulou *et al.*, 2021)

2.8 GASTRIC CANCER

The fifth most frequent cancer in the world is gastric cancer. Despite improvements in surgical techniques and treatment strategies, the overall survival rate of individuals with stomach cancer is still dismal(Chen *et al.*, 2016) because to tumour recurrence and metastasis.(Cirri & Chiarugi, 2011)(Z. Ma *et al.*, 2018)(B. Ma *et al.*, 2021). According to gene expression profiling, MMP-11 has a significant role in the development of cancer and has demonstrated significance as a potential biomarker for the diagnosis and prognosis of human cancers, including gastric cancer. This is because patients' poor prognoses are associated with MMP-11 overexpression(Szászet *et al.*, 2016 Yang *et al.*, 2008). Extracellular MMP-11, which was found to be significantly expressed in exosomes isolated from plasma and tumour tissue of patients with gastric cancer, was used by Xu *et al.* (Xu *et al.*, 2019)to show that cancer-associated fibroblasts helped gastric cancer cells migrate in part. The overall survival of these patients was correlated with this enhancement of migration. The expression of MMP-11 was unaffected by age, sex, tumour size, pathological stage, lymph node metastasis, or any other clinicopathological factors. However, serum MMP-11 levels are connected to lymph node metastases and could serve as a stand-alone prognostic marker in patients with advanced gastric cancer(Yan *et al.*, 2011). Inhibiting MMP-11 expression may prevent the growth and invasion of human gastric adenocarcinoma cells by inhibiting the IGF-I signalling pathway, making it a potential target for the treatment of stomach cancer(Kou *et al.*, 2013).

2.9 LUNG CANCER

The two types of lung cancer—small-cell lung carcinomas and non-small cell lung carcinomas—have continuously been among the most malignant malignancies(Knight *et al.*, 2017). Rodak *et al.*, 2021). MMP11 may express in both

tumour cells and stromal cells, contrary to the initial hypothesis that it is expressed by fibroblasts in tumour stroma, which was later refuted(von Marschallet *al.*, 1998),(Barrasaet *al.*, 2012). Strong MMP11 staining was found in LUAD-transformed cells within. tumour cell masses, but the stroma in the area showed minimal to no staining. This shows that LUAD tumour cells may actively exert their biological endopeptidase activity in digesting ECM while releasing MMP11 protein in an autocrine manner to enhance invasion of neighbouring tissues. Since MMP11 is a secreted protein, a rise in its blood levels could be a sign that cancer is developing. Therefore, MMP11 concentrations in serum from LUAD patients were compared to those in healthy people. The serum of LUAD patients had considerably higher levels of MMP11 protein, indicating that MMP11 might be useful as a serum biomarker for LUAD diagnosis. MMP11, a vital component of the cancer microenvironment that is secreted by LUAD tumour cells, may change ECM to encourage carcinogenesis and the growth of cancer(Barrasaet *al.*, 2012). The proliferation, migration, and invasion of LUAD cells were considerably reduced when MMP11 was reduced in vitro, which was consistent with slower tumour growth in xenograft models. For a number of solid tumours, MMP11 has the potential to be a therapeutic target.

2.10 HEAD AND NECK CANCER

Head & neck cancer was the sixth most common disease in the world in 2018 (890,000 new cases and 450,000 deaths), accounting for 3% of all cancer diagnoses (51,540 new cases) and more than 1.5% of all cancer fatalities (10,030 deaths). MMP-11 overexpression was shown to promote in vitro cell migration of oral squamous cell carcinoma (OSCC) cells by Hsinet *al.* In example, lymph node metastases and the prognosis for OSCC patients can be predicted based on the expression of MMP-11 in OSCC samples. In patients with laryngeal cancer, those with low MMP-11 expression outlived those with high MMP-11 expression by a substantial margin. Additionally, patients with low MMP-11 expression had a considerably decreased chance of dying compared to those with high MMP-11 expression(Hsinet *al.*, 2017). MiR-125a-3p can reduce thyroid papillary carcinoma cell viability, motility, and invasion by inhibiting MMP-11 expression in vitro . MMP-11 is a highly effective prognostic marker for oesophageal cancer. Overexpression of MMP-11 may alter the behaviour of invading tumours by promoting ECM

disintegration, tumour invasion, and metastasis(Song *et al.*, 2020), (Yamashita *et al.*, 2004). The expression of stromelysin-3 (ST3) in oral epithelial cells and stromal fibroblasts undergoing the mesenchymal transition may be associated with the progression of phenotypic defects acquired early in the malignant transformation pathway of this tissue. ST3 may therefore be a useful target for the development of molecular therapies for early intervention in the development of oral tumours.

2.11 ENDOMETRIAL CANCER

MMP-11 is overexpressed in 75% and underexpressed in 25% of endometrial carcinoma tissues, and its increased expression can be used as a prognostic marker for patients with type 1 endometrial carcinoma . MMP types 1, 2, 7, and 9 all showed overexpression and were connected to a poor prognosis in EC, according to studies done on the expression of MMP in this tissue(Bozhenkoet *al.*, 2018)]. The findings of the present study indicate that, after data validation, EC might be included because, in terms of progression and prognosis, the findings of the present study are consistent with those of a previous study. The majority of cases in the current study (83.8%) had a moderately differentiated histological grade (G2) and were discovered in the pT1a and pT1b early stages (83.7%), which is consistent with findings from earlier literature(Rugoet *al.*, 2021). This is a limitation of the current study because there aren't many late stages and there aren't many carcinomas that are well and poorly differentiated. In summary, type I EC has higher MMP11 expression, which is linked to a poor prognosis. In patients with type I EC, overexpression may be used aa prognostic biomarker, but this hypothesis needs to be supported by studies with a larger sample.(El-Tananiaet *al.*, 2022)

CHAPTER-3

3.1 AIM

- To identify the gene expression pattern of MMP11 in pancreatic cancer and gene expression changes in various breast cancer subtypes
- To identify the epigenetic changes of MMP11 in pancreatic cancer and especially in breast cancer
- To identify therapeutic potential of MMP11 in HER2 positive breast cancer cells.
- To study the effect of small molecular inhibitor, Pimozide on important signalling pathway including Epithelial to Mesenchymal, apoptosis and necroptosis

3.2 SCOPE

- Our study involves identifying the potential of MMP11 in pancreatic cancer and epigenetic status of MMP11.
- MMP11 expression was modified in a variety of cancers, according to GOBO study in the instance of breast cancer, which shows overexpression in this disease.
- We also analysed therapeutic potential in breast cancer using pimozide and anticycotic drug.
- Our invitro analysing her2 positive breast cancer showed effective cytotoxicity of pimozide against her2 positive breast cancer cells.
- Effect of pimozide treatment was studied to identify its effect on Epithelial-to-Mesenchymal , apoptosis and necroptosis.

CHAPTER-4

4.MATERIALS AND METHODS:

4.1 MAMMALIAN CELL CULTURE:

DMEM (HiMedia, India) or Roswell Park Memorial Institute -1640 (RPMI-1640) (HiMedia, India) supplemented with 10% Fetal Bovine Serum (FBS) (GIBCO, USA) containing 100 mg/L penicillin and 100 mg/L streptomycin (HiMedia, India) were used to maintain human-derived cell lines (Table 4.1) at 37°C and 5% CO₂ in a humid incubator. For sub-culturing, 80 to 90% confluent cells were rinsed in sterile phosphate-buffered saline (PBS) before 0.5 to 2 mL of a Trypsin-EDTA solution [0.25% w/v Trypsin (Sigma Aldrich, India) and ethylene diamine tetraacetate (EDTA) (MB biomedical, India)] was added. The cells were then centrifuged at 5000 rpm for 2 minutes to inactivate the trypsin. The pellet was then suspended in a growth medium containing 10% FBS. Thermo Scientific's (USA) Countess II FL Automatic Cell Counter was used to count the cells after staining a 10 L cell suspension with 10 L of 0.4% trypan-blue solution. A freezing combination of 70% FBS, 20% DMEM, and 10% dimethylsulfoxide was used to freeze cell line supplies before they were placed in liquid nitrogen. Using a mycoplasma PCR ELISA kit, the analysis for contamination was regularly evaluated (Roche, Switzerland)

Table 4.1 List of cell lines used:

TYPE OFCELL LINE	CELL LINE	MEDIUM USED	SOURCE OF CELL LINE
Breastcancer	SKBR3	DMEM with 10% FBS	Adyar Institute of Science and Technology, Chennai

4.2 COLONY FORMATION ASSAY:

In a 6-well plate, 1000 attached cells were seeded per well for a total of 6000 cells, and then the cells were treated with necessary amount of chemical or radiation. And was incubate for 9 days. The medium was removed. Cells were washed with PBS. The cells were fixed by using (Methanol: Acetic Acid = 3:1). For five minutes, it was incubate. The fixings agent was eliminated. Cells were stained with 0.5% crystal violet (in methanol) for 15 minutes. And was washed with tap water.

4.3 TOTAL RNA ISOLATION:

Trizol reagent was used to isolate total RNA (Invitrogen, USA). Equal parts of trizol-suspended cells and chloroform were combined gently, and the mixture was centrifuged at 10,000 rpm for 15 minutes at 4 °C. Without touching the liquid, the aqueous phase was pipetted out and transferred to a brand-new 1.5 mL tube, which was then set on ice. After the addition of isopropanol, the aqueous phase was centrifuged at 10,000 rpm for 15 min at 4°C. The resulting white pellet was then given two washes in 70% ethanol. When the pellet had been air-dried, 20 litres of sterile, nuclease-free water were added to dissolve it. A Nanodrop ND 2000 spectrophotometer was used to measure the amount of RNA (Thermo Scientific, USA). The concentration of RNA in 1 L of the sample was estimated in ng/L using 20µL sterile, nuclease-free water as the blank. The quality of the extracted RNA was determined by a 260 nm/280 nm absorbance ratio of 1.8 to 2.

4.4 AGAROSE GEL ELECTROPHORESIS:

Agarose gel electrophoresis was used to check the purity of the RNA. A solution of 0.8% agarose (Cytvia, USA) in 100 mL of 1x Tris/Borate/EDTA (TBE) buffer (0.13 M Tris, 5 mM boric acid, and 2.5 mM EDTA, pH 7.6) was made. The agarose was cooked in the oven until it was entirely dissolved, then it was put onto the gel tray, where the comb was set. The gel tray was placed in the gel tank containing 1x TBE buffer after the agarose had 30 minutes to solidify. The agarose gel was loaded with 1 g of total RNA and 6x clear safe dye (Green Biosciences, India), and ran at 100 V for 30 minutes. Using the ChemiDoc™ MP imaging equipment, the gel was seen and recorded (Biorad, USA) under UV absorption.

4.5 cDNA CONVERSION

1 µg of total RNA was converted to cDNA, using GeNei™ RT-PCR Kit according to the manufacturer's protocol in a two-step procedure. 12 µL of total RNA was mixed with 12 µL of gDNA wipeout buffer to eliminate genomic DNA contamination. The mixture's volume was adjusted to 84 µL with sterile nuclease-free water. The master mix was then prepared using 13 µL reverse transcriptase, 52 µL 5x RT buffer, 13 µL RT primer mix, and 14 µL of gDNA eliminated template RNA. The resulting mix was aliquoted into 4 tubes with 23 µL each. The tubes were incubated at 42°C for 15 min, and then at 95°C for 3 min to inactivate reverse transcriptase. The tubes were stored at -20°C for further use.

4.6 PCR (POLYMERASE CHAIN REACTION)

Using an online infusion primer design tool, the primers for each MRPS gene's ORF were created (DSS Takara Clontech, Japan). (Table 4.1). PrimeScript™ RT-PCR Kit (DSS Takara Clontech, Japan) was used to set up the PCR reaction, which contained HEK293 cDNA, 12.5 µL of 2 X PrimeScriptmaster mix, and 0.40 µL of each forward and reverse primer in a 25 µL reaction volume. The PCR cycle parameters included a single cycle of a 1 minute initial denaturation of the template DNA at 95°C, followed by 30 cycles of a 30 second template denaturation at 95°C before each extension step, 30 seconds at the annealing temperature, an extension step at 72°C with a time calculated based on the length of the amplification product (1 min for each kb of DNA), and a final extension of 5 minutes at 72°C. Using a nucleotide removal column (Promega), the products were purified in accordance with the manufacturer's instructions.

4.7 DESIGNING OF REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (qRT-PCR) PRIMERS

The primers for four oncogenes, tumour suppressor genes, were created using the primer designing programme from Integrated DNA Technologies (Coralville, Iowa, USA). They are listed in Table 4.2. Prior to being delivered to Eurogentec, Belgium, for synthesis, the primers were verified using primer BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The primers were diluted to a working concentration of 10 M for further usage after being dissolved in sterile, nuclease-free water

Table 4.2 List of primers used for qRT-PCR analysis

Gene	Primers for qRT-PCR
e-cad	FP: 5'CTAACCAACGACAAAGCCC3 RP: 5'CCTCTCTCTGAAGCATCTCC3'
Vimentin	FP: 5'CTAACCAACGACAAAGCCC3 RP: 5'CCTCTCTCTGAAGCATCTCC3'
MMP11	FP: 5'CGACTATGATGAGACCTGGAC3' RP: 5'GGGTAGCGAAAGGTGTAGAAG3'
RIPK3	FP: 5'AATATGAATGCTGCTGTCTCC3' RP: 5'CCATCCATTTCTGTCCCTCC3'
TP53	FP: 5'ACCACCATCCACACAACACTAC3' RP: 5'AAACACGCACCTCAAAGC3'

4.8.BIOINFORMATICS WORK OF ANALYSIS OF METHYLATION OF MMP11

4.8.1 METHYLATION STATUS OF MMP11

UALCAN (RRID SCR 015827) was used to examine the promoter methylation of MMP11 in TCGA samples. The TCGA gene expression data may be thoroughly analysed with the help of the interactive web programme UALCAN. It includes RNA-seq information and pertinent clinical information from 31 different cancer types. The portal enables the investigation of the relative expression of one or more genes, across tumour samples (including various stages, grades, and subtypes), and normal samples. It also enables the analysis of the impact of expression levels on overall survival (OS) and the identification of the most up- and down-regulated genes in specific cancers (Chandrashekar et al., 2017). Between normal (N = 97) and BC samples (N = 793) the level of methylation was shown as beta values on the y-axis. Between 0 (unmethylated) and 1 (methylated), the Beta value denotes the degree of DNA methylation (fully methylated). Several beta value

cut-offs have been proposed to signify hypermethylation [Beta value: 0.7-0.5] or hypomethylation [Beta-value: 0.3-0.25].

4.8.2 MMP11 EXPRESSION ANALYSIS IN BC AND ITS SUBTYPES

Gene expression-based Outcome for Breast Cancer Online (GOBO) v1.0.3 (<http://co.bmc.lu.se/gobo>) was used to measure the mRNA expression levels of MMP11 in relation to several clinical characteristics (Ringnér et al., 2011). A platform for the predictive validation of Affymetrix U133A arrays were used to analyse a combined dataset of 1881 breast cancer patients from eleven public data sets (GSE11121, GSE7390, GSE2034, GSE5327, GSE2603, GSE3494, GSE1456, GSE6532, GSE4922, GSE12093, and Chin). Based on histological grade, molecular subtype(PAM50), gene modules, ER states and p53 status of BC, the different amounts of MMP11 transcripts were observed. One-way ANOVA was performed to quantify the expression differences between subtypes and stages, and the correlation of MMP-11 with ER status was verified using a Student's t-test. Using BC Gene-Expression Miner v4.4 (bc-GenExMiner) (<http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1>), the GOBO-derived data was additionally verified. (2013) (Jézéquel et al.)

4.8.3. VALIDATION OF MMP11 EXPRESSION PROFILE

Using different datasets with no overlapping samples from the preliminary analyses, the outcomes from GOBO and bc-GeneExMiner were verified. The PanCancer Atlas (Koboldt et al., 2012) and Nature 2012 studies, as well as two overlapping TCGA (RRID SCR 003193) investigations, were used to validate the expression profile of MMP11. The datasets were classified into molecular subtypes in accordance with the PAM50 categorization, and a scatter plot was created using the RNA expression z-scores for each subtype. One-way ANOVA with Dunnett's test and GraphPad Prism V5.0 software (RRID SCR 015807) were used to analyse the eA web-based data mining tool called Oncomine (RRID SCR 007834) was also utilised to compare MMP11 expression levels across pre-defined BC datasets to normal breast samples in order to verify MMP11 expression levels (Rhodes et al., 2007, 2004). For BC and normal samples, a fold change cut limit was chosen at >5. Expression of MMP11 in the PAM50 subtypes. The control group was treated as normal-like BC.

4.8.4 GENETIC CHANGES TO MMP11

The Catalogue of Somatic Mutations in Cancer (COSMIC) database (RRID SCR 002260) was used for the investigation of MMP11 mutations. The database offers comprehensive and in-depth details on how somatic mutations affect human cancer. The database also contains information on non-coding mutations, gene fusions, copy-number variations, and drug-resistant mutations in addition to data on coding mutations (Tate et al., 2019). cBioPortal was used to determine the frequency of MMP11 mRNA changes in BC (Cheng et al., 2015). The MMP11 mutation and changed expression in the METABRIC, TCGA, Nature 2012, and TCGA, PanCancer BC datasets served as the basis for the database search. The TCGA, Nature 2012 BC dataset on cBioPortal was used to analyse the relationship between MMP11 mRNA expression and copy number variation. The various copy number variants found in the MMP11 gene were plotted against the MMP11 mRNA expression.

4.8.5 CLINICAL OUTCOME VALIDATION FOR BC SUBTYPES

The survival outcomes of BC and its subtypes were estimated using the Kaplan-Meier Plotter(<https://kmplot.com/analysis/>) based on the varied MMP11 expression levels. The platform enables meta-analysis that results in the identification and verification of prognostic signals. It enables the examination of 54,000 genes related to survival in 21 different cancer types. Now, the breast dataset has more than 6000 samples (Györfy et al., 2010). The hazard ratio and log-rank p-value were used to plot the Kaplan-Meier survival curves. GraphPad prism V5.0 software was used to further investigate the data exported from the cBioPortal (RRID SCR 014555) for the expression analysis. The third quartile value of MMP11 mRNA expression, or 11.70 in METABRIC and 0.866 in METABRIC, was used as the cut-off for the study. The samples were divided into high and low MMP11 groups.

4.8.6. MMP11 TRANSCRIPT LEVELS AND CLINICAL OUTCOMES IN BC SUBTYPES

Using the GOBO outcome analysis module, the relationship between MMP11's mRNA expression in PAM50 subtypes and clinical outcome in BC patients was examined to determine the predictive value of the enzyme. Based on MMP11 expression, the patients were divided into three quantiles: low (log2 expression: 4.702 to 0.527), medium (log2 expression: 0.527 to 0.665), and high (log2 expression 0.665 to 3.290). By employing OS and distant metastasis-free survival (DMFS) as endpoints and a 10-year censoring grade, Kaplan-Meier analysis was used to examine the association with outcome. Molecular subtype and grade were used to estimate individual results.

4.8.7 STATISTICAL SIGNIFICANCE

P-values and false discovery rate (FDR) 0.05 were regarded as statistically significant for all analyses.

CHAPTER-5

5.RESULTS AND DISCUSSION

5.1 VALIDATION OF MMP11 MRNA EXPRESSION IN BC SUBTYPES

Using separate, publicly accessible datasets from the cBioportal and Oncomine databases, the expression data from GOBO was verified. We collected the data from these studies and performed an expression analysis. According to the three BC datasets, MMP11 was expressed more highly in the luminal A, luminal B, and HER2 subtypes than in the basal and normal-like subtypes (ANOVA, $p < 0.0001$). According to data from Oncomine, MMP11 was overexpressed in both tumour and stroma compared to normal. There was no increase in the MMP11 expression pattern across the several Oncomine experiments. This could be explained by differences in sample sizes and inclusion standards for the BC datasets on the website.

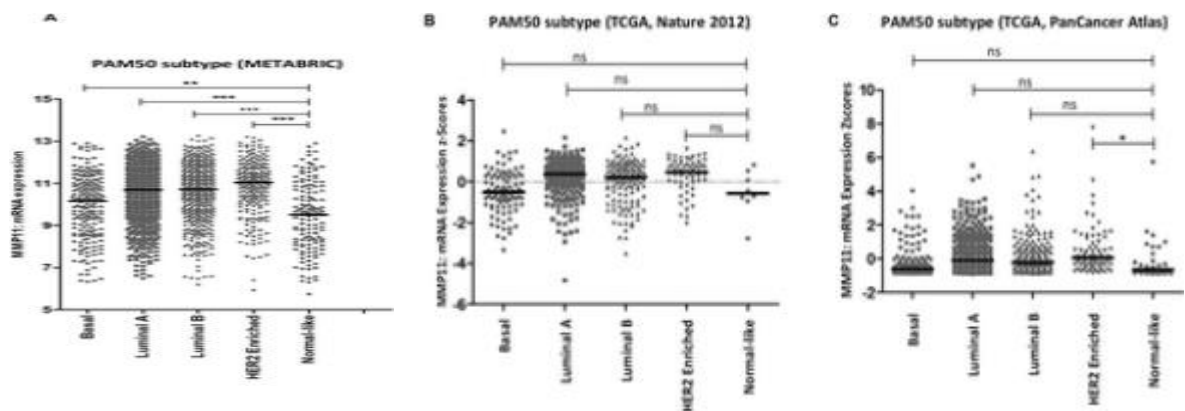


Fig 5.1: Validation of expression levels ofMMP11 using independent datasets. mRNA expression levels ofMMP11 in PAM50 subtypes, validated using METABRIC (A), TCGA (Nature, 2012 and PanCancer Atlas) datasets (B and C).

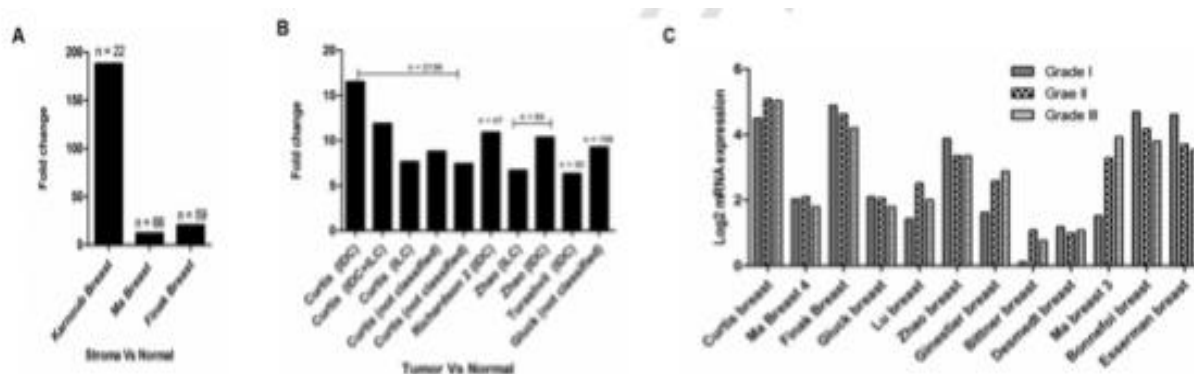


Fig 5.2:Validation of MMP11 expression levels using the Oncomine database.

Many BC studies were analysed for the fold change in MMP11 mRNA expression levels in the stroma and tumour when compared to normal. The bars show the total number of samples analysed in each analysis. Similarly, MMP11 log2 mRNA expression levels in breast cancer were determined by the grade (C), nodal status (N0- nodes negative, N1+ - nodes positive), and ER status.

5.2 MMP11 MUTATION STUDY USING COSMIC AND CBIOPORTAL ANALYSIS OF THE NUMBER OF ALTERATIONS

Pie charts from COSMIC were used to illustrate the facts on MMP11 gene mutations (Fig. 8A). In BC, substitution genetic variations made up over 50% of all changes, whereas substitution similar mutations made up nearly 16% of all changes. A>C, A>G, C>T, C>G, and G>A were among the other replacements that made up more than 30% of the total (Fig. 8B). In all three datasets, cBioPortal's analysis of the modification frequency of the MMP11 mutations in BC found that it was >0.75%. (Fig. 8C). At the protein level, METABRIC and TCGA revealed three mutations (E396K, Q441H, and F170Lfs*63) and one truncating (F170Lfs*63) changes.

5.3 CORRELATION OF MRNA EXPRESSION WITH COPY NUMBER ANALYSIS AND MMP11 PROMOTER METHYLATION

Using the cBioPortal database, the relationship between MMP11 mRNA expression and other copy number alterations was studied. Diploid and shallow deletions were the most common alterations detected; changes in copy number were not found to have an impact on the levels of mRNA (Fig. 8E). When compared to normal, data from the TCGA dataset examined by UALCAN showed hypomethylation at the MMP11 promoter in tumours ($p = 1.82E-12$) (Fig. 8F). One of the underlying causes for MMP11 overexpression in cancers compared to normal may be promoter hypomethylation.

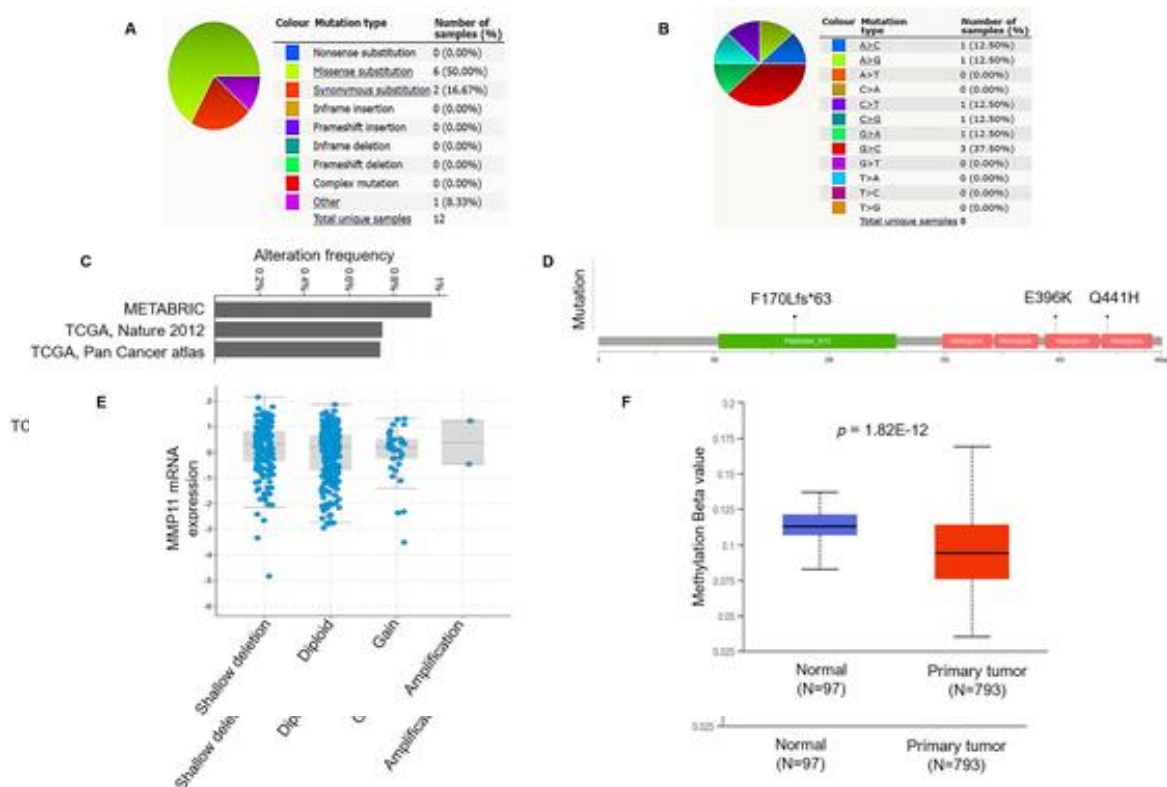


Fig. 5.3: Genetic alterations in MMP11. The pie-chart shows distribution of different type of mutations in MMP11, among BC cases analysed by COSMIC database (A). Various missense mutations found in MMP11 gene (B). cBioPortal analysis of MMP11 revealed alteration frequency in METABRIC, TCGA (Nature, 2012 and PanCancer Atlas) datasets (C and D). cBioPortal analysis of association between copy number variations and mRNA expression levels of MMP11 (E). UALCAN analysis of correlation between promoter methylation and mRNA expression levels of MMP11 (F).

5.4 rt-PCR RESULTS

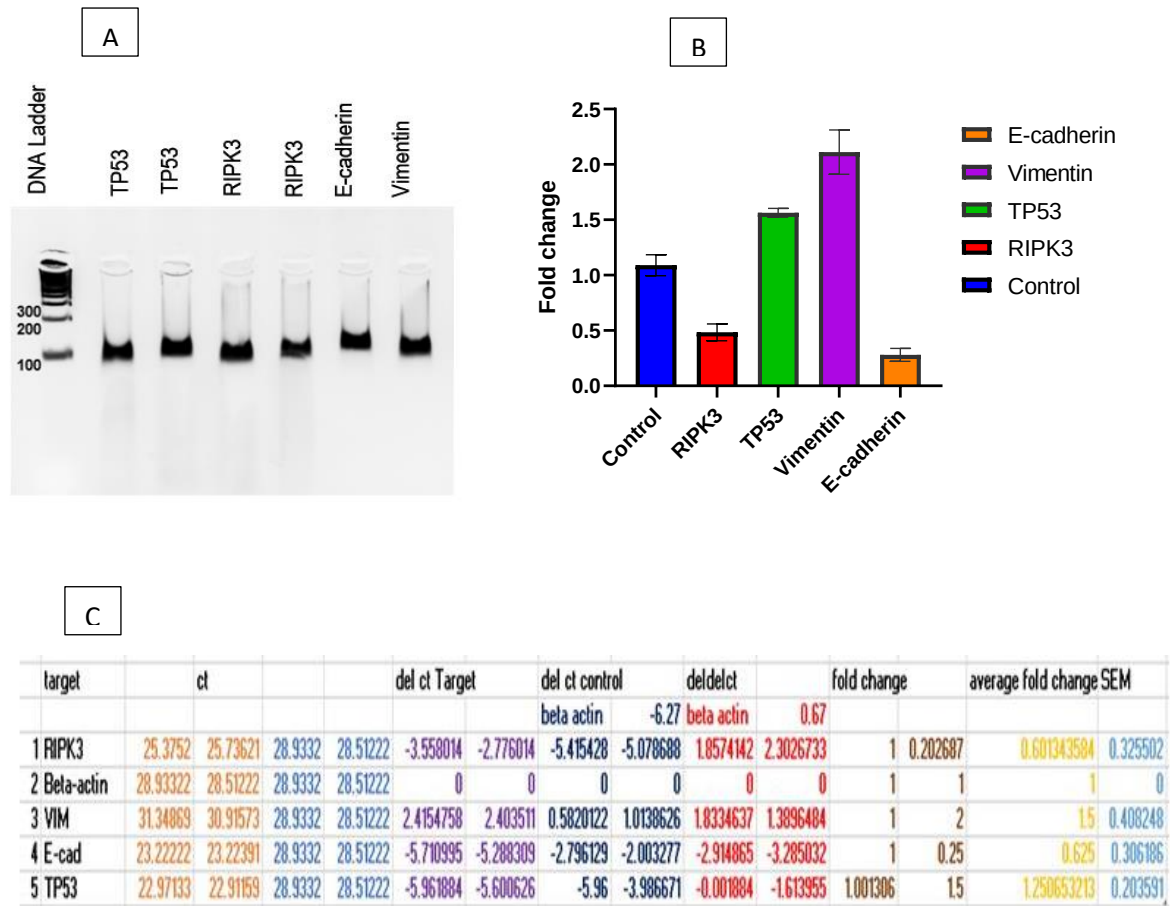


Fig 5.4: rt-PCR results (A) 2% AGE showing PCR amplification of 4 MMP11 genes run along with 100 bp DNA ladder (B) Fold change results (C) Fold change table

Results from RT-PCR on the MMP11 genes can reveal important details on the efficiency, specificity, and levels of gene expression. The gene expression levels between various samples or treatments may be compared using these findings, and the PCR reaction parameters can be improved. The difference in gene expression levels between the experimental and control groups is indicated by a larger fold change. A fold change larger than 1 specifically denotes upregulation of the target gene, i.e., higher levels of gene expression in the experimental group in comparison to the control group. A fold change smaller than 1, on the other hand, denotes downregulation of the target gene, i.e., a lower level of expression of the gene in the experimental group in comparison to the control group. Therefore, TP53 and

Vimentin are upregulated and RIPK3 and E-cadherin are downregulated and the same results have been confirmed by agarose gel electrophoresis.

5.5 MMP11 EXPRESSION PROFILE IN BC

In comparison to basal [N = 304] and normal-like subtypes [N = 304], the GOBO gene expression module found that MMP11 expression was enhanced in HER2 enriched [N = 240], luminal A [N = 465], and luminal B [N = 471] subtypes. (p 0.00001). (Fig. 1A). ER-positive cancers (N = 1225) and ER-negative tumours (N = 395) both showed higher expression of MMP11 (p 1e-05) (Fig. 1B). As opposite to grade I [N = 239] and grade II [N = 677] cancers, grade III [N = 495] tumours had very low MMP11 expression (p 0.0001) (Fig. 1C). Additional analysis performed with bc-GenExMiner revealed a significant increase in MMP11 transcript levels in patients with invasive prostate cancer (IDC) [N = 4276] (p = 0.0157) among histological types (Fig. 2A), HER2 absorbed PAM50 intrinsic subtype [N = 452] (p = 0.022) (Fig. 2B), and nodal spread.

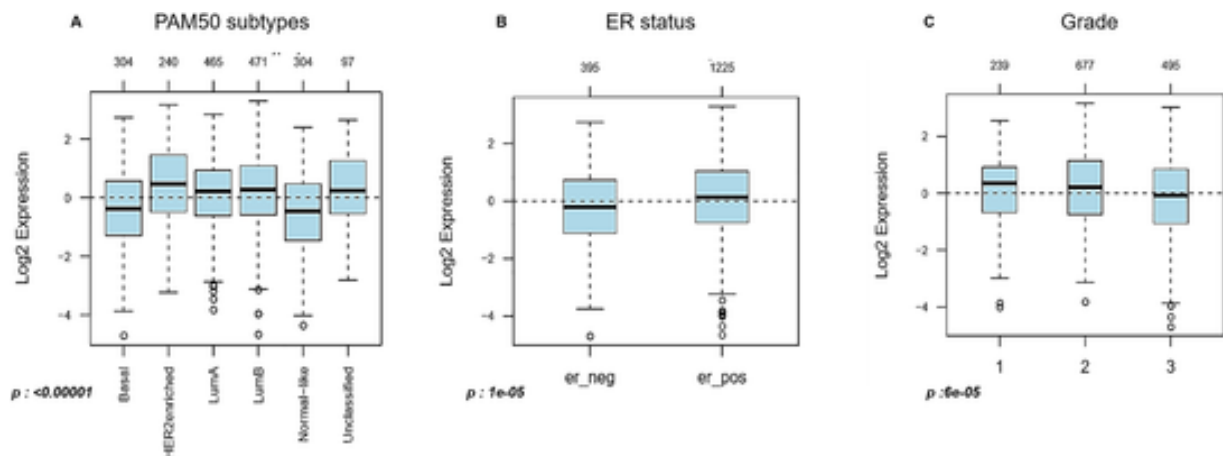


Fig 5.5:Analysis of expression levels of MMP11 using GOBO. The mRNA expression levels of MMP11 in Breast cancer according to PAM 50 subtypes (A), ER status (B), and histological grade (C) was analysed on GOBO. The number of patients is shown on top of the bars of in each panel.

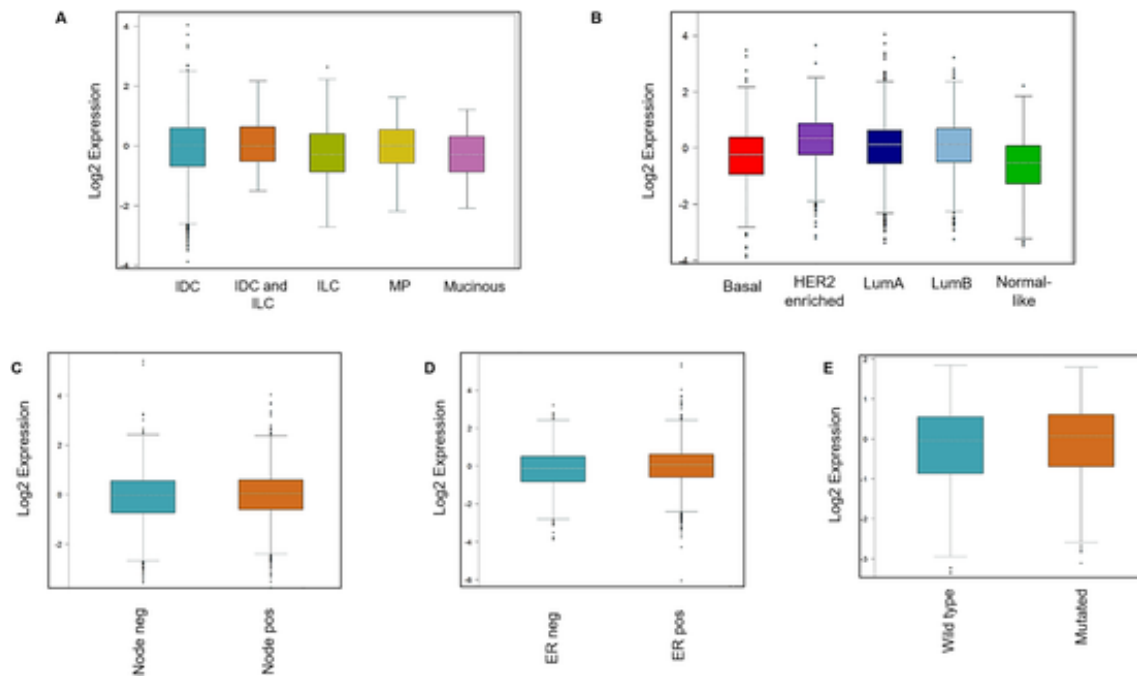


Fig 5.6: Analysis of expression levels of MMP11 using bc-GenEx-miner. The mRNA expression levels of MMP11 in Breast cancer according to histological type (A), PAM 50 subtypes (B), Nodal status (C), ER status (D) and p53 mutation status (E).

5.6 CELL CULTURE:

5.6.1 MTT assay:

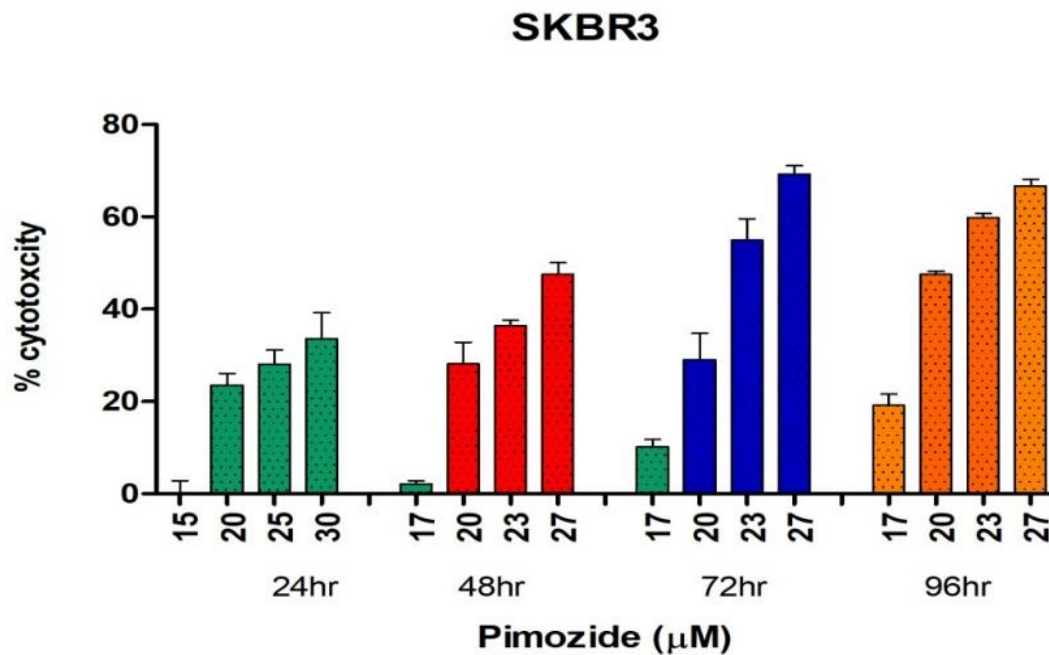


Fig 5.7: Cytotoxicity% results

A typical technique for assessing cell viability and proliferation is the MTT test. In this test, cells are exposed to a substance or medicine (Pimozide) at various doses, and its capacity to cause the formation of formazan crystals from MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is evaluated. The quantity of live cells directly correlates with the production of formazan crystals. After 4 days of Pimozide treatment on SKBR3 cell line, the IC₅₀ value was found to be 20µM, which indicates that a lower concentration of Pimozide is enough to achieve a therapeutic effect. A lower potency is indicated by a greater IC₅₀ value, which means that a lower concentration of the drug is needed to achieve a given amount of inhibition.

5.6.2. COLONY FORMATION ASSAY

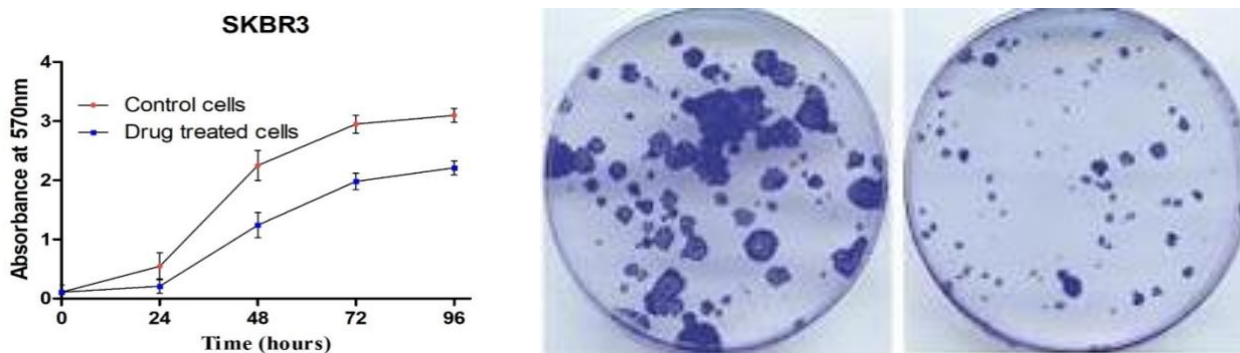


Fig 5.8: Colony Formation assay results

When compared to the control group, treatment with the test substance caused a considerable drop in the number of colonies. This implies that the test substance may have cytotoxic or anti-proliferative effects on the SKBR3 cells. There was no discernible change in the number of colonies following treatment with the test substance compared to the control group. This shows that, under the investigated experimental circumstances, the test chemical may not significantly affect the capacity of SKBR3 cells to form colonies. When compared to the control group, treatment with the test substance significantly increased the number of colonies. This shows that under the examined experimental settings, the test

substance may have an impact on the SKBR3 cells that is pro-proliferative or growth-promoting.

5.7 METHYLATION OF MMP11

To check if Methylation P-value plays important role in MMP11 differential expression among cancer. The methylation status analysis is done by UALCAN with the TCGA datasets which revealed MMP11 is Hypermethylated in Lung adenocarcinoma, Kidney renal clear cell carcinoma and Breast invasive carcinoma, etc are Hypermethylated. However, Hypomethylated in Pancreatic adenocarcinoma, Prostate adenocarcinoma and Uterine Corpus Endometrial Carcinoma, etc and it is significant which indicates increased expression in cancer.

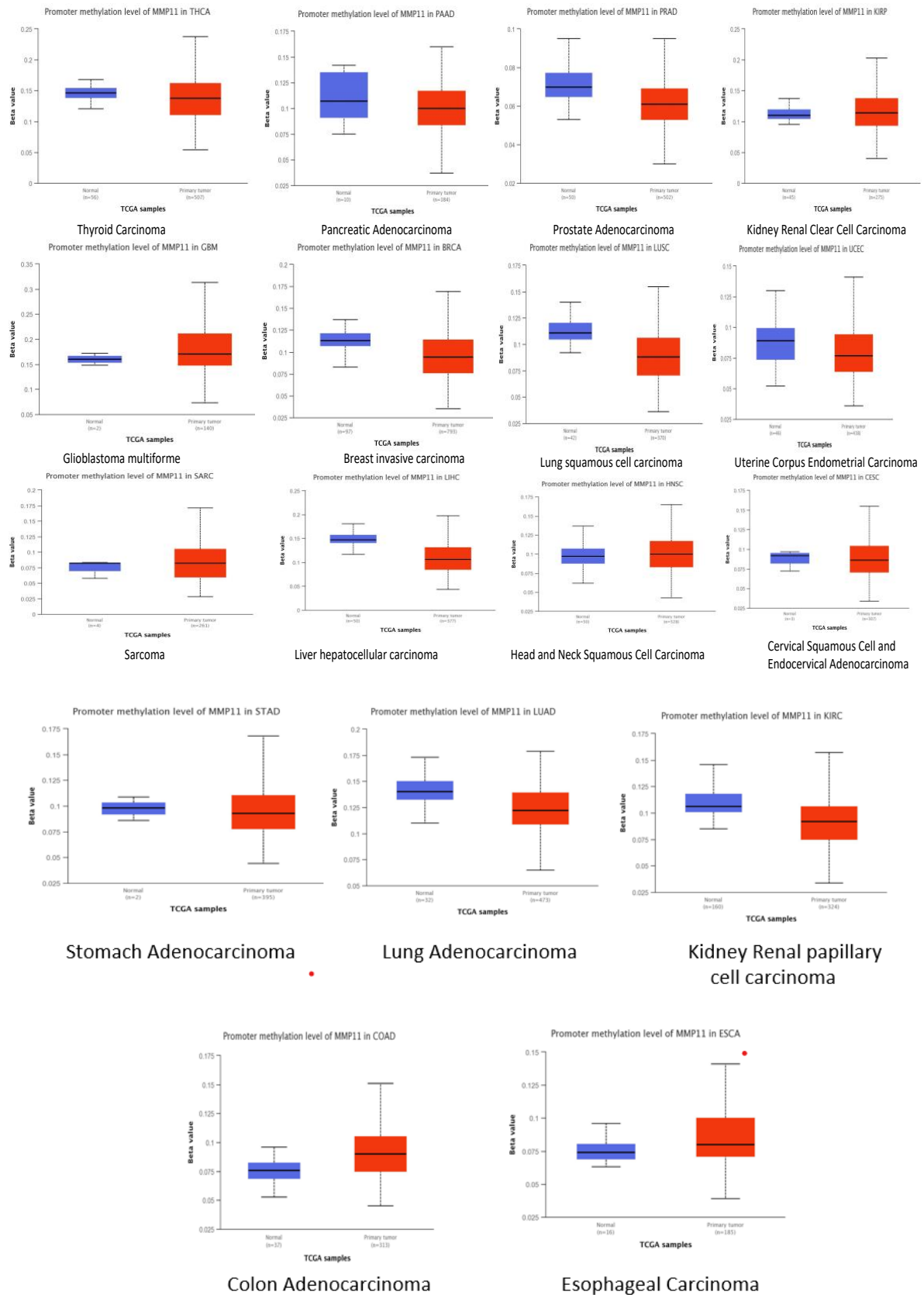


Fig5.9: Methylation (P-value) Analysis

5.8 RNA ISOLATION(AGAROSE GEL ELECTROPHORESIS):

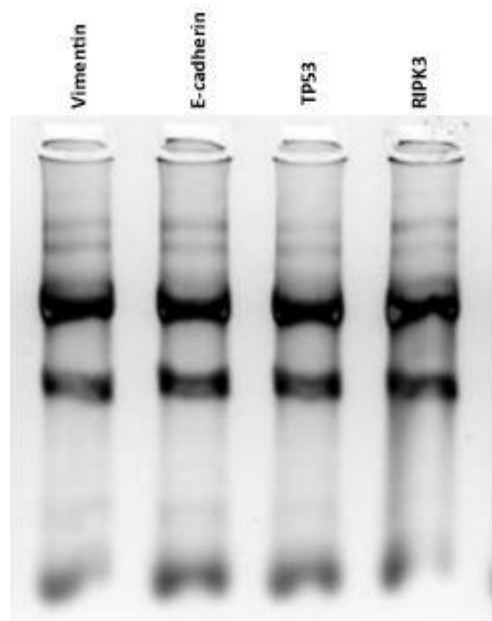


Fig 5.10: Agarose Gel electrophoresis of total RNA isolated from cell lines

RNA extracted from MMP11 may be electrophoresed on an agarose gel, which can reveal important details about the sample's integrity, quality, and presence of impurities. This data may be used to judge if the RNA sample is suitable for later uses like gene expression analysis. Agarose Gel Electrophoresis of total RNA isolated from cell lines (2% agarose gel electrophoresis), showing PCR amplification of 4 MMP11 genes run along with 100 bp DNA ladder is seen in Figure 5.8. The results showed that all MMP11 genes showed differential expression relative to control cells. All genes except RIPK3 and E-cadherin were found to be upregulated in the cell line. Taken together gene expression analyses indicate a widespread alteration in the expression levels of the MMP11 genes in the cancer cell line.

5.9. DISCUSSION

Differential expression of MMP11 of pancreatic cancer was showed. GOBO analysis reveals upregulation and breast cancer in summary MMP11 expression was modulated in various cancer. Invitro cytotoxicity of SKBR3 significance was observed. Effect of pimozide treatment was analysed in a signalling pathway genes such as vimetin, E-cadherin, TP53, RIPK3. The difference in gene expression levels between the experimental and control groups is indicated by a larger fold change. A fold change larger than 1 specifically denotes upregulation of the target gene, i.e., higher levels of gene expression in the experimental group in comparison to the control group. A fold change smaller than 1, on the other hand, denotes downregulation of the target gene, i.e., a lower level of expression of the gene in the experimental group in comparison to the control group. Therefore, TP53 and Vimentin are upregulated and RIPK3 and E-cadherin are downregulated. Changes in cell signalling genes TP53 has no change, however further western blot studies will be followed. Our invitro analysing her2 positive breast cancer showed effective cytotoxicity of pimozide against her2 positive breast cancer cells. Effect of pimozide treatment was studied to identify its effect on Epithelial-to-Mesenchymal , apoptosis and necroptosis. However, to analyse the potential in a therapeutic targeting in-vivo cytotoxicity analyse using mouse model is essential to confirm the same.

CHAPTER 6

6. SUMMARY AND CONCLUSIONS

MMPs play a crucial role in cancer cell proliferation, invasion, and metastasis. Among the MMPs, MMP11 is unique with its distinct functions and association with various cell signaling pathways. Differential expression of MMP11 was reported in the majority of the cancers, less evidence are present to understand the role and mechanism by which MMP11 is involved in cancer. MMP11 was also reported to be associated with eliciting immune response as a tumor-associated antigen, indicating its potential in cancer immunotherapy. Reports show several microRNAs target MMP11 in cancer and its therapeutic potential, but yet the exact role and mechanism by which microRNAs act upon MMP11 is not known in cancer. However further studies are required to understand its potential in the development of gene therapy.

REFERENCES

1. Abdel-Hamid, N. M., &Abass, S. A. (2021). Matrix metalloproteinase contribution in management of cancer proliferation, metastasis and drug targeting. In *Molecular Biology Reports* (Vol. 48, Issue 9). <https://doi.org/10.1007/s11033-021-06635-z>
2. Aghamiri, S., Raei, P., Talaei, S., Mohammadi-Yeganeh, S., Bayat, S., Rezaee, D., Ghavidel, A. A., Teymouri, A., Roshanzamiri, S., Farhadi, S., &Ghanbarian, H. (2021). Nonviral siRNA delivery systems for pancreatic cancer therapy. In *Biotechnology and Bioengineering* (Vol. 118, Issue 10). <https://doi.org/10.1002/bit.27869>
3. Akter, S., Rahman, M. A., Hasan, M. N., Akhter, H., Noor, P., Islam, R., Shin, Y., Rahman, M. H., Gazi, M. S., Huda, M. N., Nam, N. M., Chung, J., Han, S., Kim, B., Kang, I., Ha, J., Choe, W., Choi, T. G., & Kim, S. S. (2022). Recent Advances in Ovarian Cancer: Therapeutic Strategies, Potential Biomarkers, and Technological Improvements. In *Cells* (Vol. 11, Issue 4). <https://doi.org/10.3390/cells11040650>
4. Araki, Y. and Mimura, T. (2017) Matrix Metalloproteinase Gene Activation Resulting from Disordred Epigenetic Mechanisms in Rheumatoid Arthritis.
5. Barrasa, J. I., Olmo, N., Santiago-Gómez, A., Lecona, E., Anglard, P., Turnay, J., &Lizarbe, M. A. (2012). Histone deacetylase inhibitors upregulate MMP11 gene expression through Sp1/Smad complexes in human colon adenocarcinoma cells. *Biochimica et BiophysicaActa - Molecular Cell Research*, 1823(2). <https://doi.org/10.1016/j.bbamcr.2011.12.010>
6. Bergholtz, H., Carter, J. M., Cesano, A., Cheang, M. C. U., Church, S. E., Divakar, P., Fuhrman, C. A., Goel, S., Gong, J., Guerriero, J. L., Hoang, M. L., Hwang, E. S., Kuasne, H., Lee, J., Liang, Y., Mittendorf, E. A., Perez, J., Prat, A., Pusztai, L., ... Warren, S. E. (2021). Best practices for spatial profiling for breast cancer research with the GeoMx® digital spatial profiler. *Cancers*, 13(17). <https://doi.org/10.3390/cancers13174456>
7. Bozhenko, V. K., Stanojevic, U. S., Trotsenko, I. D., Zakharenko, M. v., Kiseleva, Y. Y., &Solodkiy, V. A. (2018). Comparison of matrix proteinase mRNA expression in morphologically normal, neoplastic, andmetastatic colon

- tissue and colon biopsies from healthy donors. *Biomeditsinskaya Khimiya*, 64(1). <https://doi.org/10.18097/PBMC20186401046>
8. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68(6). <https://doi.org/10.3322/caac.21492>
 9. Cao, W., Chen, H. da, Yu, Y. W., Li, N., & Chen, W. Q. (2021). Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chinese Medical Journal*, 134(7). <https://doi.org/10.1097/CM9.0000000000001474>
 10. Chen, W., Zheng, R., Baade, P. D., Zhang, S., Zeng, H., Bray, F., Jemal, A., Yu, X. Q., & He, J. (2016). Cancer statistics in China, 2015. *CA: A Cancer Journal for Clinicians*, 66(2). <https://doi.org/10.3322/caac.21338>
 11. Cheng, C. W., Wang, H. W., Chang, C. W., Chu, H. W., Chen, C. Y., Yu, J. C., Chao, J. I., Liu, H. F., Ding, S. L., & Shen, C. Y. (2012). MicroRNA-30a inhibits cell migration and invasion by downregulating vimentin expression and is a potential prognostic marker in breast cancer. *Breast Cancer Research and Treatment*, 134(3). <https://doi.org/10.1007/s10549-012-2034-4>
 12. Cirri, P., & Chiarugi, P. (2011). Cancer associated fibroblasts: the dark side of the coin. *American Journal of Cancer Research*, 1(4).
 13. Devarakonda, S., Morgensztern, D., & Govindan, R. (2015). Genomic alterations in lung adenocarcinoma. In *The Lancet Oncology* (Vol. 16, Issue 7). [https://doi.org/10.1016/S1470-2045\(15\)00077-7](https://doi.org/10.1016/S1470-2045(15)00077-7)
 14. di Palo, A., Siniscalchi, C., Mosca, N., Russo, A., & Potenza, N. (2020). A novel cerna regulatory network involving the long non-coding antisense rna spaca6p-as, mir-125a and its mrna targets in hepatocarcinoma cells. *International Journal of Molecular Sciences*, 21(14). <https://doi.org/10.3390/ijms21145068>
 15. Duong, M. N., Geneste, A., Fallone, F., Li, X., Dumontet, C., & Muller, C. (2017). The fat and the bad: Mature adipocytes, key actors in tumor progression and resistance. In *Oncotarget* (Vol. 8, Issue 34). <https://doi.org/10.18632/oncotarget.18038>
 16. Eiro, N., Cid, S., Fernández, B., Fraile, M., Cernea, A., Sánchez, R., Andicoechea, A., DeAndrésGaliana, E. J., González, L. O., Fernández-Muñiz,

- Z., Fernández-Martínez, J. L., & Vizoso, F. J. (2019). MMP11 expression in intratumoral inflammatory cells in breast cancer. *Histopathology*, 75(6). <https://doi.org/10.1111/his.13956>
17. El-Tanani, M., al Khatib, A. O., Aladwan, S. M., Abuelhana, A., McCarron, P. A., & Tambuwala, M. M. (2022). Importance of STAT3 signalling in cancer, metastasis and therapeutic interventions. In *Cellular Signalling* (Vol. 92). <https://doi.org/10.1016/j.cellsig.2022.110275>
 18. Fang L, Deng Z, Shatseva T, Yang J, Peng C, Du WW, Yee AJ, Ang LC, He C, Shan SW and Yang BB. MicroRNA miR-93 promotes tumor growth and angiogenesis by targeting integrin-beta8. *Oncogene*. 2011; 30(7):806-821. <https://www.onscotarget.com/article/717/text>
 19. Geervliet, E., & Bansal, R. (2020). Matrix Metalloproteinases as Potential Biomarkers and Therapeutic Targets in Liver Diseases. In *Cells* (Vol. 9, Issue 5). <https://doi.org/10.3390/cells9051212>
 20. Georgopoulou, D., Callari, M., Rueda, O. M., Shea, A., Martin, A., Giovannetti, A., Qosaj, F., Dariush, A., Chin, S. F., Carnevalli, L. S., Provenzano, E., Greenwood, W., Lerda, G., Esmailshirazifard, E., O'Reilly, M., Serra, V., Bressan, D., Ali, H. R., al Sa'd, M., ... Caldas, C. (2021). Landscapes of cellular phenotypic diversity in breast cancer xenografts and their impact on drug response. *Nature Communications*, 12(1). <https://doi.org/10.1038/s41467-021-22303-z>
 21. Hadjimichael, A. C., Foukas, A. F., Savvidou, O. D., Mavrogenis, A. F., Psyrris, A. K., & Papagelopoulos, P. J. (2020). The anti-neoplastic effect of doxycycline in osteosarcoma as a metalloproteinase (MMP) inhibitor: a systematic review. *Clinical Sarcoma Research*, 10(1). <https://doi.org/10.1186/s13569-020-00128-6>
 22. Hasan, S., Jacob, R., Manne, U., & Paluri, R. (2019). Advances in pancreatic cancer biomarkers. *Oncology Reviews*, 13(1). <https://doi.org/10.4081/oncol.2019.410>
 23. Herbster, S., Paladino, A., de Freitas, S., & Boccardo, E. (2018). Alterations in the expression and activity of extracellular matrix components in HPV-associated infections and diseases. In *Clinics* (Vol. 73). <https://doi.org/10.6061/clinics/2018/e551s>

24. Hou, J., Li, X. T., & Xie, K. P. (2021). Coupled liquid biopsy and bioinformatics for pancreatic cancer early detection and precision prognostication. In *Molecular Cancer* (Vol. 20, Issue 1). <https://doi.org/10.1186/s12943-021-01309-7>
25. Hsieh, C. Y., Chou, Y. E., Lin, C. Y., Wang, S. S., Chien, M. H., Tang, C. H., Lin, J. C., Wen, Y. C., & Yang, S. F. (2020). Impact of matrix metalloproteinase-11 gene polymorphisms on biochemical recurrence and clinicopathological characteristics of prostate cancer. *International Journal of Environmental Research and Public Health*, 17(22). <https://doi.org/10.3390/ijerph17228603>
26. Hsin, C. H., Chou, Y. E., Yang, S. F., Su, S. C., Chuang, Y. T., Lin, S. H., & Lin, C. W. (2017). MMP-11 promoted the oral cancer migration and FAK/Src activation. *Oncotarget*, 8(20). <https://doi.org/10.18632/oncotarget.15824>
27. <https://doi.org/10.1158/1078-0432.CCR-08-3226>
28. <https://www.researchgate.net/publication/33130327>
29. Jeyapalan Z, Deng Z, Shatseva T, Fang L, He C and Yang BB. Expression of CD44 3'-untranslated region regulates endogenous microRNA functions in tumorigenesis and angiogenesis. *Nucleic Acids Res.* 2011; 39(8):3026-3041 <https://doi.org/10.18632/oncotarget.717>
30. Kamimura, K., Yokoo, T., Abe, H., & Terai, S. (2019). Gene therapy for liver cancers: Current status from basic to clinics. In *Cancers* (Vol. 11, Issue 12). <https://doi.org/10.3390/cancers11121865>
31. Kang, S. U., Cho, S. Y., Jeong, H., Han, J., Chae, H. Y., Yang, H., Sung, C. O., Choi, Y. L., Shin, Y. K., & Kwon, M. J. (2022). Matrix metalloproteinase 11 (MMP11) in macrophages promotes the migration of HER2-positive breast cancer cells and monocyte recruitment through CCL2-CCR2 signaling. *Laboratory investigation; a journal of technical methods and pathology*, 102(4), 376–390. <https://doi.org/10.1038/s41374-021-00699>
32. Kang, S. U., Cho, S. Y., Jeong, H., Han, J., Chae, H. Y., Yang, H., Sung, C. O., Choi, Y. I., Shin, Y. K., & Kwon, M. J. (2022). Matrix metalloproteinase 11 (MMP11) in macrophages promotes the migration of HER2-positive breast cancer cells and monocyte recruitment through CCL2–CCR2 signaling. *Laboratory Investigation*, 102(4). <https://doi.org/10.1038/s41374-021-00699-y>

33. Kanharat, N., & Tuamsuk, P. (2015). Correlation between microvascular density and matrix metalloproteinase 11 expression in prostate cancer tissues: A preliminary study in Thailand. *Asian Pacific Journal of Cancer Prevention*, 16(15). <https://doi.org/10.7314/APJCP.2015.16.15.6639>
34. Khare, T., Bissonnette, M., & Khare, S. (2021). Cxcl12-cxcr4/cxcr7 axis in colorectal cancer: Therapeutic target in preclinical and clinical studies. In *International Journal of Molecular Sciences* (Vol. 22, Issue 14). <https://doi.org/10.3390/ijms22147371>
35. Kim, H. S., Kim, M. G., Min, K. W., Jung, U. S., & Kim, D. H. (2021). High MMP-11 expression associated with low CD8+ T cells decreases the survival rate in patients with breast cancer. *PLoS ONE*, 16(5 May). <https://doi.org/10.1371/journal.pone.0252052>
36. Knight, S. B., Crosbie, P. A., Balata, H., Chudziak, J., Hussell, T., & Dive, C. (2017). Progress and prospects of early detection in lung cancer. *Open Biology*, 7(9). <https://doi.org/10.1098/rsob.170070>
37. Korbecki, J., Bajdak-Rusinek, K., Kupnicka, P., Kapczuk, P., Simińska, D., Chlubek, D., & Baranowska-Bosiacka, I. (2021). The role of cxcl16 in the pathogenesis of cancer and other diseases. *International Journal of Molecular Sciences*, 22(7). <https://doi.org/10.3390/ijms22073490>
38. Kosvira, A., Maramis, C., & Chouvarda, I. (2019). Developing an integrated genomic profile for cancer patients with the use of NGS data. *Emerging Science Journal*, 3(3). <https://doi.org/10.28991/esj-2019-01178>
39. Kou, Y. B., Zhang, S. Y., Zhao, B. L., Ding, R., Liu, H., & Li, S. (2013). Knockdown of MMP11 inhibits proliferation and invasion of gastric cancer cells. *International Journal of Immunopathology and Pharmacology*, 26(2). <https://doi.org/10.1177/039463201302600209>
40. Lee, J., Lee, J., & Kim, J. H. (2019). Identification of matrix metalloproteinase 11 as a prognostic biomarker in pancreatic cancer. *Anticancer Research*, 39(11). <https://doi.org/10.21873/anticancer.13801>
41. Lin, J. J., Riely, G. J., & Shaw, A. T. (2017). Targeting ALK: Precision medicine takes on drug resistance. In *Cancer Discovery* (Vol. 7, Issue 2). <https://doi.org/10.1158/2159-8290.CD-16-1123>
42. Lowder, D., Rizwan, K., McColl, C., Paparella, A., Ittmann, M., Mitsiades, N., & Kaochar, S. (2022). Racial disparities in prostate cancer: A complex

- interplay between socioeconomic inequities and genomics. *Cancer Letters*, 531. <https://doi.org/10.1016/j.canlet.2022.01.028>
43. Luo, L. S., Jiang, J. F., Luan, H. H., Zi, H., Zhu, C., Li, B. H., & Zeng, X. T. (2022). Spatial and temporal patterns of prostate cancer burden and their association with Socio-Demographic Index in Asia, 1990–2019. *Prostate*, 82(2). <https://doi.org/10.1002/pros.24258>
 44. Ma, B., Ran, R., Liao, H. Y., & Zhang, H. H. (2021). The paradoxical role of matrix metalloproteinase-11 in cancer. In *Biomedicine and Pharmacotherapy* (Vol. 141). <https://doi.org/10.1016/j.biopha.2021.111899>
 45. Ma, Z., Chen, M., Yang, X., Xu, B., Song, Z., Zhou, B., & Yang, T. (2018). The Role of Cancer-associated Fibroblasts in Tumorigenesis of Gastric Cancer. *Current Pharmaceutical Design*, 24(28). <https://doi.org/10.2174/1381612824666180601094056>
 46. Morini, S. R., Denadai, M. V., Waisberg, J., Filho, G. de J. L., Matos, D., & Saad, S. S. (2020). Metalloproteinases and colorectal cancer. Correlation of gene expression and clinical-pathological parameters. *ActaCirurgicaBrasileira*, 35(7). <https://doi.org/10.1590/s0102-8650202000700000007>
 47. Nardou, K., Nicolas, M., Kuttler, F., Cisarova, K., Celik, E., Quinodoz, M., Riggi, N., Michielin, O., Rivolta, C., Turcatti, G., & Moulin, A. P. (2022). Identification of New Vulnerabilities in Conjunctival Melanoma Using Image-Based High Content Drug Screening. *Cancers*, 14(6). <https://doi.org/10.3390/cancers14061575>
 48. Neoptolemos, J. P., Kleeff, J., Michl, P., Costello, E., Greenhalf, W., & Palmer, D. H. (2018). Therapeutic developments in pancreatic cancer: Current and future perspectives. In *Nature Reviews Gastroenterology and Hepatology* (Vol. 15, Issue 6). <https://doi.org/10.1038/s41575-018-0005-x>
 49. Okyere, J., Duodu, P. A., Aduse-Poku, L., Agbadi, P., & Nutor, J. J. (2021). Cervical cancer screening prevalence and its correlates in Cameroon: secondary data analysis of the 2018 demographic and health surveys. *BMC Public Health*, 21(1). <https://doi.org/10.1186/s12889-021-11024-z>
 50. Pak, S., Jung, K. W., Park, E. H., Ko, Y. H., Won, Y. J., & Joung, J. Y. (2022). Incidence and mortality projections for major cancers among Korean men until

- 2034, with a focus on prostate cancer. *Investigative and Clinical Urology*, 63(2). <https://doi.org/10.4111/icu.20210405>
51. Peruzzi, D., Mori, F., Conforti, A., Lazzaro, D., De Rinaldis, E., Ciliberto, G., La Monica, N., & Aurisicchio, L. (2009). MMP11: a novel target antigen for cancer immunotherapy. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 15(12), 4104–4113.
 52. Peruzzi, D., Mori, F., Conforti, A., Lazzaro, D., de Rinaldis, E., Ciliberto, G., la Monica, N., & Aurisicchio, L. (2009). MMP11: A novel target antigen for cancer immunotherapy. *Clinical Cancer Research*, 15(12). <https://doi.org/10.1158/1078-0432.CCR-08-3226>
 53. Rahat, M. A. (2022). Mini-Review: Can the Metastatic Cascade Be Inhibited by Targeting CD147/EMMPRIN to Prevent Tumor Recurrence? In *Frontiers in Immunology* (Vol. 13). <https://doi.org/10.3389/fimmu.2022.855978>
 54. Ribeiro Franco, P. I., Rodrigues, A. P., de Menezes, L. B., & Pacheco Miguel, M. (2020). Tumor microenvironment components: Allies of cancer progression. In *Pathology Research and Practice* (Vol. 216, Issue 1). <https://doi.org/10.1016/j.prp.2019.152729>
 55. Rodak, O., Peris-Díaz, M. D., Olbromski, M., Podhorska-Okółów, M., & Dzięciel, P. (2021). Current landscape of non-small cell lung cancer: Epidemiology, histological classification, targeted therapies, and immunotherapy. *Cancers*, 13(18). <https://doi.org/10.3390/cancers13184705>
 56. Roscilli, G., Cappelletti, M., de Vitis, C., Ciliberto, G., di Napoli, A., Ruco, L., Mancini, R., & Aurisicchio, L. (2014). Circulating MMP11 and specific antibody immune response in breast and prostate cancer patients. *Journal of Translational Medicine*, 12(1). <https://doi.org/10.1186/1479-5876-12-54>
 57. Rugo, H. S., Lerebours, F., Ciruelos, E., Drullinsky, P., Ruiz-Borrego, M., Neven, P., Park, Y. H., Prat, A., Bachelot, T., Juric, D., Turner, N., Sophos, N., Zarate, J. P., Arce, C., Shen, Y. M., Turner, S., Kanakamedala, H., Hsu, W. C., & Chia, S. (2021). Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. *The Lancet Oncology*, 22(4). [https://doi.org/10.1016/S1470-2045\(21\)00034-6](https://doi.org/10.1016/S1470-2045(21)00034-6)

58. Rundhaug, J. E. (2005). Matrix metalloproteinases and angiogenesis. In *Journal of cellular and molecular medicine* (Vol. 9, Issue 2). <https://doi.org/10.1111/j.1582-4934.2005.tb00355.x>
59. Saad, H., Zahran, M. A. H., Hendy, O., Abdel-Samiee, M., Bedair, H. M., & Abdelsameea, E. (2020). Matrix Metalloproteinase-11 Gene Polymorphisms as a Risk for Hepatocellular Carcinoma Development in Egyptian Patients. *Asian Pacific Journal of Cancer Prevention*, 21(12). <https://doi.org/10.31557/APJCP.2020.21.12.3725>
60. Savci-Heijink, C. D., Halfwerk, H., Koster, J., Horlings, H. M., & van de Vijver, M. J. (2019). A specific gene expression signature for visceral organ metastasis in breast cancer. *BMC Cancer*, 19(1). <https://doi.org/10.1186/s12885-019-5554-z>
61. Scheau, C., Badarau, I. A., Costache, R., Caruntu, C., Mihai, G. L., Didilescu, A. C., Constantin, C., & Neagu, M. (2019). The role of matrix metalloproteinases in the epithelial-mesenchymal transition of hepatocellular carcinoma. In *Analytical Cellular Pathology* (Vol. 2019). <https://doi.org/10.1155/2019/9423907>
62. Sharma, V., Letson, J., & Furuta, S. (2022). Fibrous stroma: Driver and passenger in cancer development. In *Science Signaling* (Vol. 15, Issue 724). <https://doi.org/10.1126/scisignal.abg3449>
63. Shi, F., Dai, Y., Bai, Y., & Li, Q. (2021). Expression and Clinical Significance of Ki67 in Common Gastrointestinal Tumours. *Open Journal of Gastroenterology*, 11(12). <https://doi.org/10.4236/ojgas.2021.1112029>
64. Sidaway, P. (2017). Pancreatic cancer: New biomarkers improve standard screening. *Nature Reviews. Clinical Oncology*, 14(5). <https://doi.org/10.1038/nrclinonc.2017.35>
65. Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, 69(1). <https://doi.org/10.3322/caac.21551>
66. Singhi, A. D., Koay, E. J., Chari, S. T., & Maitra, A. (2019). Early Detection of Pancreatic Cancer: Opportunities and Challenges. *Gastroenterology*, 156(7). <https://doi.org/10.1053/j.gastro.2019.01.259>
67. Song, M., Wang, N., Li, Z., Zhang, Y., Zheng, Y., Yi, P., & Chen, J. (2020). miR-125a-3p suppresses the growth and progression of papillary thyroid

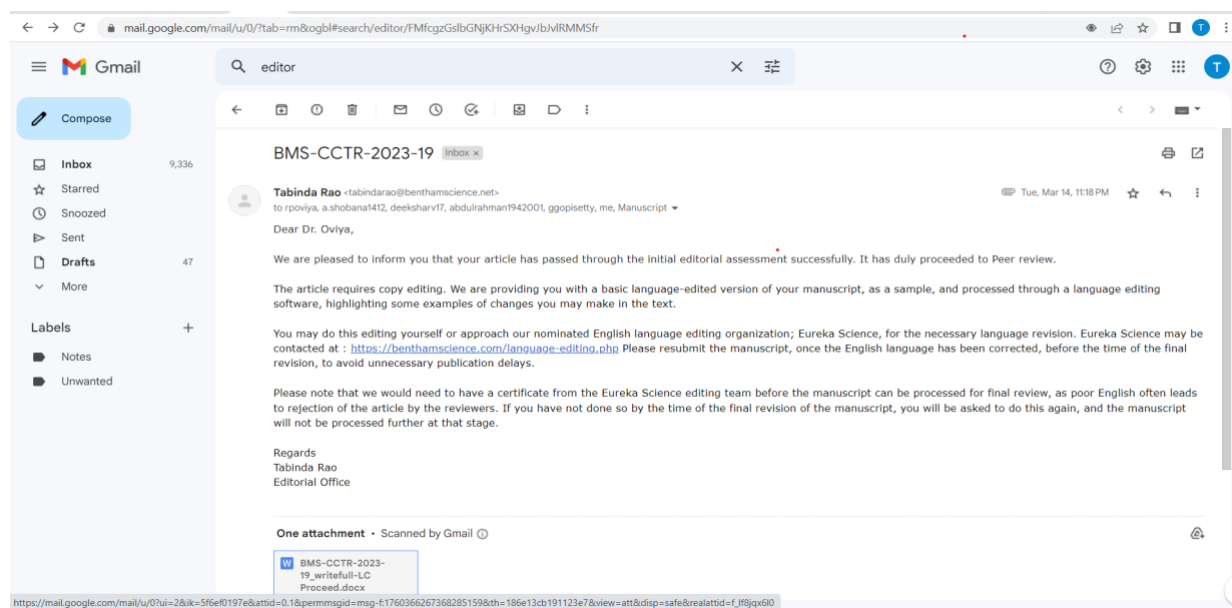
- carcinoma cell by targeting MMP11. *Journal of Cellular Biochemistry*, 121(2). <https://doi.org/10.1002/jcb.29333>
68. Sopyllo, K., Erickson, A. M., & Mirtti, T. (2021). Grading evolution and contemporary prognostic biomarkers of clinically significant prostate cancer. In *Cancers* (Vol. 13, Issue 4). <https://doi.org/10.3390/cancers13040628>
 69. Sudha, B., Kumar, N. S., & Sumathi, S. (2022). Absence of Knowledge and Awareness About Cervical Cancer Among Educated Women: A Need for Education About Cervical Cancer. *Indian Journal of Gynecologic Oncology*, 20(1). <https://doi.org/10.1007/s40944-022-00611-6>
 70. Szász, A. M., Lánczky, A., Nagy, Á., Förster, S., Hark, K., Green, J. E., Boussioutas, A., Busuttil, R., Szabó, A., & Györffy, B. (2016). Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget*, 7(31). <https://doi.org/10.18632/oncotarget.10337>
 71. Taetu, B., Trudel, D., & Wang, C. S. (2006). [Proteases by reactive stromal cells in cancer: an attractive therapeutic target]. *Bulletin Du Cancer*, 93(9).
 72. Taheri, M., Khoshbakht, T., Jamali, E., Kallenbach, J., Ghafouri-fard, S., & Baniahmad, A. (2021). Interaction between non-coding rnas and androgen receptor with an especial focus on prostate cancer. In *Cells* (Vol. 10, Issue 11). <https://doi.org/10.3390/cells10113198>
 73. Tian, X., Ye, C., Yang, Y., Guan, X., Dong, B., Zhao, M., & Hao, C. (2015). Expression of CD147 and matrix metalloproteinase-11 in colorectal cancer and their relationship to clinicopathological features. *Journal of Translational Medicine*, 13(1). <https://doi.org/10.1186/s12967-015-0702-y>
 74. Toropovsky, A. N., Nikitin, A. G., Pavlova, O. N., & Viktorov, D. A. (2019). Perspectives of improvement of the diagnosis of prostate cancer based on analysis of PCA3 gene expression. *Urologiia*, 2019(2). <https://doi.org/10.18565/urology.2019.2.82-86>
 75. Truong, L. H., & Pauklin, S. (2021). Pancreatic cancer microenvironment and cellular composition: Current understandings and therapeutic approaches. In *Cancers* (Vol. 13, Issue 19). <https://doi.org/10.3390/cancers13195028>
 76. von Marschall, Z., Riecken, E. O., & Rosewicz, S. (1998). Stromelysin 3 is overexpressed in human pancreatic carcinoma and regulated by retinoic acid

- in pancreatic carcinoma cell lines. *Gut*, 43(5). <https://doi.org/10.1136/gut.43.5.692>
77. Wang, H., Lu, L., Liang, X., & Chen, Y. (2022). Identification of prognostic genes in the pancreatic adenocarcinoma immune microenvironment by integrated bioinformatics analysis. *Cancer Immunology, Immunotherapy*, 71(7). <https://doi.org/10.1007/s00262-021-03110-3>
 78. Wieczorek, E., Jablonska, E., Wasowicz, W., & Reszka, E. (2015). Matrix metalloproteinases and genetic mouse models in cancer research: a mini-review. *Tumor Biology*, 36(1). <https://doi.org/10.1007/s13277-014-2747-6>
 79. Winer, A., Adams, S., & Mignatti, P. (2018). Matrix metalloproteinase inhibitors in cancer therapy: Turning past failures into future successes. In *Molecular Cancer Therapeutics* (Vol. 17, Issue 6). <https://doi.org/10.1158/1535-7163.MCT-17-0646>
 80. Xu, G., Zhang, B., Ye, J., Cao, S., Shi, J., Zhao, Y., Wang, Y., Sang, J., Yao, Y., Guan, W., Tao, J., Feng, M., & Zhang, W. (2019). Exosomal miRNA-139 in cancer-associated fibroblasts inhibits gastric cancer progression by repressing mmp11 expression. *International Journal of Biological Sciences*, 15(11). <https://doi.org/10.7150/ijbs.33750>
 81. Yamashita, K., Tanaka, Y., Mimori, K., Inoue, H., & Mori, M. (2004). Differential expression of MMP and uPA systems and prognostic relevance of their expression in esophageal squamous cell carcinoma. *International Journal of Cancer*, 110(2). <https://doi.org/10.1002/ijc.20067>
 82. Yan, D., Dai, H., & Liu, J. W. (2011). Serum levels of MMP-11 correlate with clinical outcome in Chinese patients with advanced gastric adenocarcinoma. *BMC Cancer*, 11. <https://doi.org/10.1186/1471-2407-11-151>
 83. Yang, Y. H., Deng, H., Li, W. M., Zhang, Q. Y., Hu, X. T., Xiao, B., Zhu, H. H., Geng, P. L., & Lu, Y. Y. (2008). Identification of matrix metalloproteinase 11 as a predictive tumor marker in serum based on gene expression profiling. *Clinical Cancer Research*, 14(1). <https://doi.org/10.1158/1078-0432.CCR-07-1179>
 84. Zhang, C., Zhang, G., Sun, N., Zhang, Z., Zhang, Z., Luo, Y., Che, Y., Xue, Q., & He, J. (2020). Comprehensive molecular analyses of a TNF family-based signature with regard to prognosis, immune features, and biomarkers

- for immunotherapy in lung adenocarcinoma. *EBioMedicine*, 59. <https://doi.org/10.1016/j.ebiom.2020.102959>
- 85.** Zhang, X., Huang, S., Guo, J., Zhou, L., You, L., Zhang, T., & Zhao, Y. (2016). Insights into the distinct roles of MMP-11 in tumor biology and future therapeutics (Review). *International Journal of Oncology*, 48(5). <https://doi.org/10.3892/ijo.2016.3400>
- 86.** Zhuang, Y., Li, X., Zhan, P., Pi, G., & Wen, G. (2021). MMP11 promotes the proliferation and progression of breast cancer through stabilizing Smad2 protein. *Oncology reports*, 45(4), 16. <https://doi.org/10.3892/or.2021.7967>

ANNEXURE

Submission and acceptance of review article titled "Current perspectives of Matrix Metalloproteinases as a diagnostic and therapeutic target for cancer" in Current Cancer Therapy Reviews, Bentham Science Publishers.



PATENT FILING

Submission of new invention for patent filing to HumCen Global Pvt. Ltd on March 20, 2023

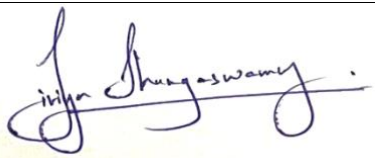
1. Invention title: **Development of facial serum with natural fruit extracts**

Inventors: Sathyabama Institute of Science and Technology, Tiviya Thangaswamy, A.Shobana, Dr. R.P Oviya

. **TITLE** : Development of facial serum with natural fruit extracts

ALLOTTED REF.NO – B4

WE NEED THE FOLLOWING DETAILS (MUST) FOR EVERY SINGLE APPLICATION

S.NO	INVENTOR NAME	AFFILIATION WITH FULL ADDRESS	SIGNATURE SOFTCOPY (USE BLUE PEN WITH WHITE BACKGROUND)
1	Sathyabama Institute of Science and Technology		
2	Tiviya Thangaswamy	Sathyabama Institute of Science and Technology, Chennai	
3	A. Shobana	Sathyabama Institute of Science and Technology, Chennai	
4	Dr.R.P.Oviya	Sathyabama Institute of Science and Technology, Chennai	

2. Invention title: **Formulation of Protein-soup-mix “ a plant-based health supplement**

Inventors: Sathyabama Institute of Science and Technology. Deeksha RV, A.Shobana , Dr. R.P Oviya

TITLE : Formulation of Protein-soup-mix “ a plant-based health supplement

ALLOTTED REF.NO – B2

WE NEED THE FOLLOWING DETAILS (MUST) FOR EVERY SINGLE APPLICATION

S.NO	INVENTOR NAME	AFFILIATION WITH FULL ADDRESS	SIGNATURE SOFTCOPY (USE BLUE PEN WITH WHITE BACKGROUND)
1	Sathyabama Institute of Science and Technology		
2	R V Deeksha	Sathyabama Institute of Science and Technology, Chennai	
3	A. Shobana	Sathyabama Institute of Science and Technology, Chennai	
4	Dr.R.P.Oviya	Sathyabama Institute of Science and Technology, Chennai	

