

EFFECTS OF CHLOROTOXIN ON THE MATRIX METALLOPROTEINASE-2 (MMP-2) EXPRESSION IN BREAST CANCER AND MELANOMA CANCER

ABSTRACT

Matrix metalloproteinases-2 belongs to gelatinase group of MMP family which are endopeptidases involved in the degradation of extracellular matrix proteins such as collagen, elastin, fibronectin or proteoglycans. MMPs play crucial functions in physiological processes such as embryogenesis, wound healing, and pathogenesis of many diseases such as cancer inflammatory, liver and neurological diseases. Scorpion venom peptides are promising drug candidates for cancer treatment, especially chlorotoxin commonly extracted from Israeli *Leiurus quinquestriatus* scorpion giant. Chlorotoxin specifically binds to the surface of glioma cells through its principal Cltx receptor – MMP-2 and impairs their ability to invade. This study investigated the expression of MMP-2 in breast cancer cell lines (MDA-MB-436) and melanoma cancer cell lines (A375). Although both of cell lines (A375 and MDA-MB-463) were cultured in DMEM and RPMI media containing fetal calf serum and penicillin/streptomycin, A375 cells grew faster than MDA-MB-436 cells because A375 cells covered 80% of the area of the T-75 culture flash within only 2 culture days compared to 3 days of MDA-MB-436 cells. Enzyme collagenase was used in gel electrophoresis with pre-made zymogram gel. The catalytic cleavage of the gelatin in gel zymography indicated pro-MMP-2 at 72kDa and activated MMP-2 at 68kDa. In addition, there was no active response of MMP-2 in the presence of chlorotoxin recorded on pre-made zymogram gel, which highlights inhibitory action of chlorotoxin on MMP-2.

Key words: matrix metalloproteinase-2, MMP-2, breast cancer, melanoma cancer, gel electrophoresis, SDS-PAGE/Zymography, coomassie blue

1. INTRODUCTION

Cancer is an uncontrollable cell growth. Cancer cells have the ability to migrate or invade another part of the host, which can be termed as metastasis. Deaths are caused majorly because of metastasis which may be a result of drug resistance or the presence of tumor in critical organs such as kidney, brain, liver and lungs [14]. Tumor metastasis is a process which requires multi-steps such as local infiltration of tumor cells into the adjacent tissue, movement of the cancer cells through the endothelial tissue into vessels known as intravasation, survival in the circulatory system, extravasation and subsequent proliferation in capable organs leading to colonization, all these processes must be done before metastasis can occur therefore a deficit in one of the processes eliminates the migration of the tumor to a

secondary sites and understanding this role could disable the migration of this tumor [23]. A large number of physiological, biological and chemical limitation governs metastasis, the biological includes regulations by cytokines and chemokines and secretion of MMPs [13]. 350,000 new cases of breast cancer have been diagnosed each year in Europe and mortality rate of about 130,000 has been recorded. It is the most common malignancy in women [29]. The ability of the tumour to metastasize from the primary site to a distance site forming new tumor by spreading through the bloodstream to the other part of the body such as liver, lung, kidney and brain. Detection of tumour and metastasis is essential to evade the death rate. Over-expression of P53 or Bcl₂, estrogen level, lymph node status, mutation in BRCA1 and BRCA 2, HER-2 amplification are crucial markers useful for the diagnosis of breast cancer [19].

MMP-2 abbreviated to matrix metalloproteinase-2, a zinc-dependent enzyme can degrade structural proteins of the extracellular matrix during cancer invasion of normal tissue and has also been considered as receptor of chlorotoxin (CTX) on glioma cells [7]. The expression of MMP-2 was detected in breast carcinoma cells and the presence of activated MMP-2 in the culture media of breast carcinoma tissue reveals the presence of pro-MMP-2 [12]. As a result of immunohistochemical studies of breast tumor, MMP-2 has been suggested to have a possibly prognostic value and also may be used to detect early breast cancer, monitoring, treatment and disease classification [12]. Also, HER-2 oncogene has been shown to be enormously expressed which increases the invasive ability and are to some extent related to the expression of MMP-2 [25]. Recently, liposomes modified with CITx increase the uptake of cells and cytotoxicity in murine metastatic cell lines in vitro, suggesting the potential to inhibit metastasis of breast tumor [24].

Melanoma on the other hand is noted for its rapid metastasis, progression, and poor prognosis and it arises from the basal stratum in the epidermis of the skin [4,17]. 80% of skin cancer death is as a result of melanoma, even though it is responsible for 4% of skin cancer and 14% of patients diagnosed with metastatic melanoma survive up to 5 years [17]. 20% of patients develop tumor that is metastatic because melanoma rapidly metastasize and its resistant to chemotherapy and radiotherapy [28]. Patient can survive up to 10 years if diagnosed early and surgical excision [15,17]. A lot of progress has been made to understand the biology of melanoma and its prognostic markers that could help increase the survival rate [2]. Evidence have suggested the role of MMPs invasion and metastasis and 26 MMPs has been discovered out of which MMP-2 plays an important role in the progression of melanoma [15,17]. Chlorotoxin (CTX) is classified into the peptide family of insectotoxins with the selective paralytic activity on insects and other invertebrates. Chlorotoxin which consist of 36 amino acid has been reported to inhibit the enzymatic activity of Matrix metalloproteinase-2 expressed in glioma cells in a dose-dependent pattern and reduce MMP-2 surface expression [7,21].

A375 and MDA-MB-436 cell lines are known to possess MMP-2 on their cell surface while chlorotoxin is known to bind to MMP-2 which it serves as the receptor to and inhibit its catalytic activities, this research study was carried out to analyse the mechanism effect of

chlorotoxin on the MMP-2 secreted in the cancer cell line which directly reduces or block cell invasion and metastasis. The aim of this study is to examine the effect of chlorotoxin on MMP-2 produced by breast cancer cell lines (MDA-MB-436) and melanoma cancer cell lines (A375) which are adherent cells and derived from 43-year-old breast cancer females and 54-year-old malignant melanoma females, respectively. SDS-PAGE/Zymography using enzyme collagenase was also used to examine the expression of MMP-2 in breast and melanoma cancer cell lines.

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87 **2. MATERIALS AND METHODS**

88 **2.1. MATERIALS**

Sterile pipette and pipette tips, sterile microfuge tubes, measuring cylinder, 96 well plates, 6 well plates, haemocytometer, 6ml viva spin tubes, covers lips, bleach pot, 1 aliquot of penicillin/ streptomycin, glutamine (6ml), 2 aliquot of fetal calf serum (10%), phosphate buffer saline (PBS), 70% ethanol, trypsin-EDTA are all from sigma Aldrich except for viva spin tube from Novex. Centrifuge, shaker, microscope, stirrer, pH meter were obtained from the biomedical laboratory Sheffield Hallam University, UK. Breast cancer cell line (MDA-MB-463) and melanoma cancer cell line (A375) were obtained from Biomedical Research Centre (BMRC).

The SDS apparatus including the gel tank, power pack, cassette, gel, and spatula are all from the biomedical laboratory Sheffield Hallam University. 2x sample buffer (250mM tris HCL pH 6.8, 8% SDS), separating buffer (250mM Tris, 1.92 glycine, 1% SDS), stacking gel (1.25M tris-HCl pH 6.8), Ammonium persulphate (APS), TEMED, acrylamide. Fetal calf serum as a positive control, color plus protein marker are all obtained from sigma Aldrich. For the zymography, Enzyme developing buffer (1M NaCl, 50mM cac12, 50mM tris-HCl pH 7.5, 2.5 triton x-100, 1mM Zncl2), 25% Triton x-100, 1 g coomassie blue, 50% methanol, 10% glacial acetic acid, 40% deionized water. All were obtained from Thermo Fischer except for the deionized water which is from Biomedical Laboratory Sheffield Hallam University.

106 **2.2. PREPARATION OF REAGENT**

107 *2.2.1. Preparation of media*

To each 500 ml of RPMI and DMEM media, 2 x 30 ml of Fetal calf serum (10%) 6 ml of penicillin/streptomycin (1%) which serves as antibiotics and prevents microbial contamination and 6 ml of glutamine (1%) is added, each bottle was labeled and stored at 4°C.

112 *2.2.2. Preparation of SDS-PAGE*

Tank buffer was prepared by adding 10 g of SDS, 30.28 g of Tris and 144 g of Glycine and making it up to 1 litre with deionized water to make 10x tank buffer, 1 g of SDS was added to 10mls of deionized water to make 10% solution of SDS, 1 g of APS was added to 10ml of

deionized water to get 10% solution of APS, separating gel buffer was prepared by dissolving 181.71g of Tris in 500mls of water, adjusting the pH to 8.8 with hydrochloric acid ,then it was made up to 1litre to make 1.5M Tris pH 8.8, stacking gel buffer was prepared by dissolving 121.14g in 500mls of water in a beaker, then the pH was adjusted to 6.8 with hydrochloric acid, then it was made up to 1 litre to make 1.0 M Tris pH 6.8. Sample buffer was prepared by adding 2ml of 20% glycerol, 6.58ml of 100Mm Tris pH 6.8 . To prepare the separating gel, 100 µl of 10% SDS, 100 µl of 10% APS,4 µl of TEMED, 2.5ml of separating gel buffer, 4.0 ml distilled water and 3.3ml of 30% acrylamide solution was aliquoted in a 50ml beaker and then poured within the glass plate within an inch to the notched area, neat ethanol was then overlaid on the gel and left to polymerise for 20 minutes, while waiting for the gel to set, stacking gel was prepared by adding 50 µl of 10% SDS, 50 µl of APS, 5 µl of TEMED, 0.63ml of stacking gel buffer, 3.4ml distilled water and 830 µl of 30% acrylamide solution to a 50ml beaker and then the neat ethanol present on the separating gel was removed, and the gel surface was rinsed with water and replaced with the stacking gel, the comb was gently placed in position and allowed to set for 30 minutes, then the comb and rubber gasket was removed. 500 ml of tank buffer was prepared by diluting the already prepared 50 ml of the already prepared 10x concentrated with 450mls of deionized water to make 1x concentrate of tank buffer. The tank buffer was then poured into the two electrode compartments, then the glass plate was gently secured to the electrophoresis tank, then the tank buffer is poured into the central compartment of the tank making sure the gel is completely immersed, the samples are the aliquoted into each well and the power pack is set to 150 V to run.

2.3. EXPERIMENTAL SECTION

2.3.1. Cell passaging

The T-75 flask containing MDA-MB-436 cell lines was removed out of the incubator (37⁰C), the media was then decanted into the bleach pot, the T-75 flask containing the adherent cells was then washed with 10 ml PBS to completely remove the serum present in the media and allow trypsin to detach the cell and poured into the bleach pot, 3ml of trypsin/EDTA was then added to the flask and the T-75 flask was incubated for 5 minutes to dislodge the cells, after then, the T-75 flask was then tapped against the hand to completely dislodge the cells and then the flask is viewed under the microscope to ensure that the adherent cells are detached. Following this, 6 ml of complete medium was added to the T-75 flask to inhibit the effect of trypsin. The content in the T-75 flask is then poured into a 50 ml falcon tube and the tube was then centrifuged at 1000rpm for 5 minutes to get the cell pellet. The supernatant was then poured into the bleach pot, 5ml of complete media was added to the falcon tube containing the pellet and 1 ml of the mixture is pipette and added to a new T-75 flask and 25 ml of complete media was added as well to get the 1 in 5 ratios. The new T-75 flask was viewed under the microscope to ensure the cells are present and then placed in the incubator. The same procedure was carried out for the A375 melanoma cell line except that the media used was DMEM splitting ratio was 1 in 8. Regarding the pilot experiment, concerning the rate of

growth and doubling time, the cells were harvested and dislodged with trypsin and PBS every three days. The cells are then split into 1:6 and 1:8 ratio respectively in a new T-75 flask.

The haemocytometer was sterilized using ethanol and covered with a cover slip. 50 µl of the cell suspension was pipette under the cover slip to the chamber of the haemocytometer, the haemocytometer is then viewed under the microscope at x10 objective.

2.3.2. *Detection of MMP-2 using fetal calf serum (FCS) as a positive control*

Fetal calf serum (FCS) possesses a lot of proteins out of which MMP-2 is part of them. Different dilution was carried out by pipetting 10 µl of 1 in 100 µl dilutions of FCS and water, 1 µl of FCS in 19 µl of water, 2 µl of FCS and 18 µl of water, 5 µl of FCS and 15 µl of water, 10 µl of FCS and 10 µl of water in each microfuge tube. 5 µl of sample buffer was added to each tube and vortex for 30 seconds. 15 µl of each dilution set is pipette into the SDS gel and it was run at 150 V for 1 hour and 30 minutes, the cassette was opened, the gel was taken out and washed with distilled water and stained with coomassie blue on a shaker for 30 minutes and then destained on a shaker until blue bands as appeared on a white background.

2.3.3. *Cell culture condition for the expression of MMP-2*

The breast cancer cell line (MDA-MB-436) and melanoma cell line (A375) was studied for the expression of MMP-2 in serum free media. This was done by splitting the cells in a serum containing media T-75 flask in 1:6 and 1:8 ratios respectively for 24 hours and the media was removed and replaced by a serum-free media to prevent the ensure the MMP-2 produced is from the cell line and not Fetal calf serum and the flask was further incubated for 24 hours. To evaluate the MMP-2 expression, the serum free media from each cell line (MDA-MB-436 and A375) was collected in a 6ml viva spin tube and centrifuged at 4000 rpm for 12 and 25 minutes respectively, this was done in order to collect proteins with higher molecular weight that cannot pass through the filter in the viva spin tube. The concentrated supernatant was run on a zymogram gel.

2.3.4. *Expression of MMP-2 by in-gel zymography*

2.3.4.1. *Collagenase enzyme as positive control*

Enzyme collagenase was used as a positive control to get the technique working and to examine the presence of MMP-2. 1 in 10 dilution was carried out using 5 µl of collagenase and 45 µl of water, then 2 µl (set 1), 5 µl (set 2), 10 µl (set 3), 15 µl (set 4) of the 1 in 10 dilution was pipette in different sterile microfuge tube and 5 µl of sample buffer was added and vortex for 30 seconds, 10µl of each set (set 1, 2, 3 and 4) was pipetted into each well (well 1, 2 and 3, 4, respectively) of the 10% zymogram gel and run at 150 V for 1hour, the gasket was cut open to remove the gel. The gel was then rinsed in 300 ml of water three times followed by incubation time of 30minutes in 25% triton-100 then, the gel was transferred to a weighing bowl containing 200 ml of enzyme buffer for another 30 minutes, then the gel was further incubated in 200 ml enzyme buffer for 20 hours, stained with coomassie blue for 20

minutes on a shaker, followed by destaining until white bands are seen on the gel indicating proteolytic activity.

2.3.4.2. Expression of MMP-2 using concentrated supernatant from MDA-MB-436 and A375

Serial dilution was carried out with concentrated supernatant from MDA-MB-436 and A375. Firstly, 5 µl of each supernatant and 45 µl of water was used to prepare 1 in 10 dilution followed by pipetting 5µl from each dilution and adding 5µl of sample buffer.

The gelatinolytic activity of A375 and MDA-MB-436 cell line, in gel gelatin zymography was performed by initially growing the cells in serum containing media for 24 hours at 37°C and removing the serum out of media before incubation within 24 hours at 37°C. The media was then collected in a Viva spine tube, concentrated by centrifuging at 4000rpm for 20 and 12 minutes respectively. 10 µl of concentrated serum-free media from A375 and MDA-MB-436 respectively was loaded on a pre-made zymogram gel at different concentration and run at 150 V for 1 hour 30 minutes, the gel was washed in 25% triton-100 for 30 minute to remove the SDS and further incubated in enzyme buffer for 20 hours followed by staining with coomassie blue for 20 minutes on a shaker, then destained till a white band appears on a blue background.

2.3.5. Effect of chlorotoxin on MMP-2 produced by MDA-MB-436 and A375

1 x 10⁶ cells/ml of MDA-MB-436 and A375 in medium without fetal calf serum was pipette into different 6 wells and 10 µl of chlorotoxin was pipette into 3 wells of the 6-well plate. After overnight incubation of the 6-well plates, the media was removed from cells incubated with and without chlorotoxin, placed in a 6ml viva spin tube and centrifuged at 4000rpm for 12 and 20 minutes respectively, the concentrated supernatant was then transferred to a sterile microfuge tube and run on a pre-made zymography gel.

3. RESULTS

3.1. DETECTION OF MMP-2 USING FETAL CALF SERUM AS A POSITIVE CONTROL

After SDS-PAGE electrophoresis with different dilutions of FCS as the positive control for MMP-2, the gel was stained with coomassie blue to identify protein components. In figure 1, from the left to the right, well 1 (lane 1) contained 10µl of 1 in 100µl dilutions of FCS and water, well 2 contained 1µl of FCS in 19µl of water, well 3 contained 2 µl of FCS and 18µl of water, well 4 contained 5µl of FCS and 15µl of water, well 5 contained 10µl of FCS and 10µl of water, and well 6 was the colour plus marker. Each well also contained 5µl of sample buffer. The staining result showed bands of MMP-2 and other proteins at the molecular weight of 54kDa, 62kDa, 72kDa and 190kDa. Well 1, 2, 4 showed over expression of FCS with less distinct bands of FCS with molecular weight of 42KDa, 62kDa, 135kDa, 190kDa and 245kDa respectively. This indicates the presence of different proteins in fetal calf serum and only one well showed a distinct band of about 50kDa.

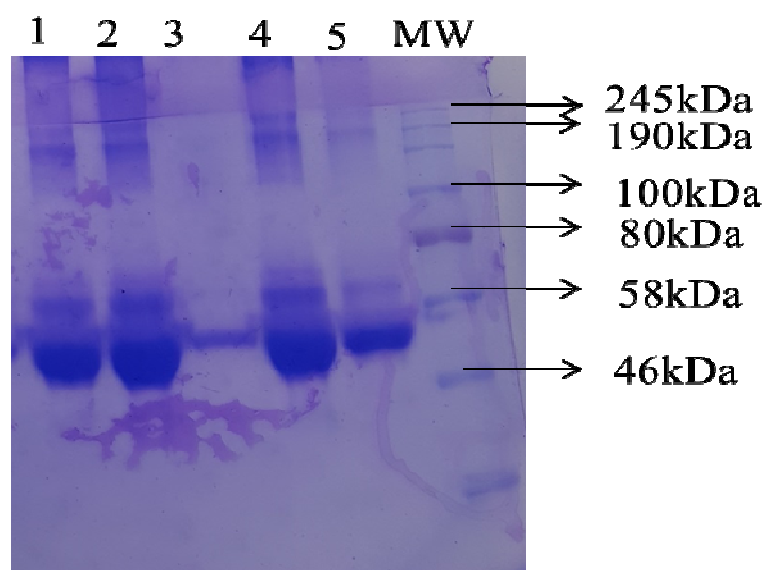


Figure 1. SDS-PAGE of FCS stained in coomassie blue

3.2. EXPRESSION OF MMP-2

A375 and MDA-MB-463 cell lines were cultured in DMEM and RPMI media containing fetal calf serum and penicillin/streptomycin. A375 cell achieve 80% confluence on the second day of incubation whereas MDA-MB-463 achieve 80% confluence on the third day of incubation. As observation of their growing pattern in T-75 flasks, A375 cells grew faster than MDA-MB-436 cells because they take 2 and 3 days respectively to reach confluence which is a state of growth or area covered by the cells, their images were seen under the microscope at x40 and x10 magnifications as in figure 2 and 3 respectively.

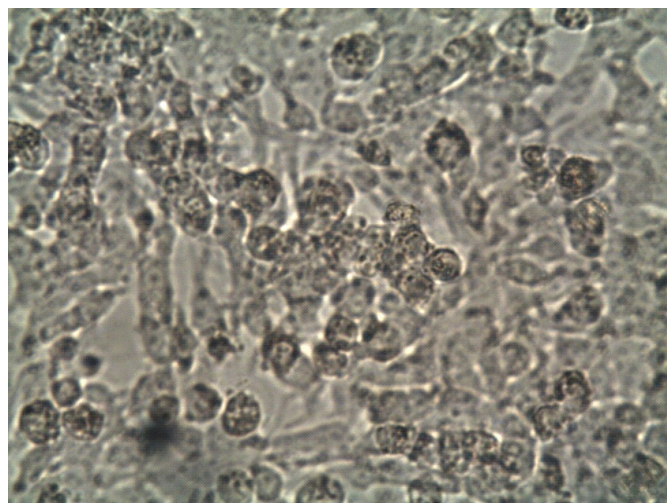


Figure 2. A375 melanoma cancer cell line at x40 magnification

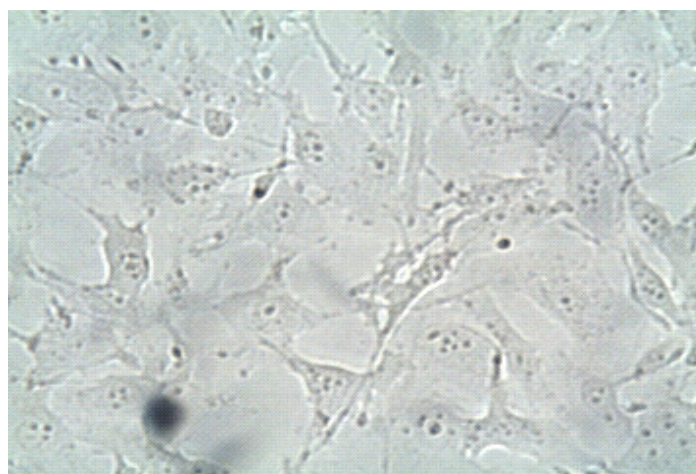


Figure 3. MDA-MB-436 cancer cell line x10 magnification

3.2.1. Collagenase enzyme as positive control

Pre-made zymogram gel with a different dilution of the enzyme collagenase was loaded and ran for an hour, washed in water, then incubated in enzyme buffer for 20 hours followed by staining with coomassie blue for 30 minutes on a shaker. The dye was then destained from the gel on a shaker till a white band appears on a blue background indicating catalytic cleavage of the gelatin in the zymograph gel, indicating the pro-MMP-2 at 72kDa and activated MMP-2 at 68kDa as shown in figure 4. Distinct bands at 72kDa correlate with the molecular weight of pro-MMP-2, other bands which locate at 72kDa represent overexpressed MMP-2 and there are less distinct bands of other forms of MMP-2 at 68kDa. This result was similar to the previous review of Kurzepa et al. 2014 that the proenzyme of MMP-2 has molecular weight of 72 kDa.

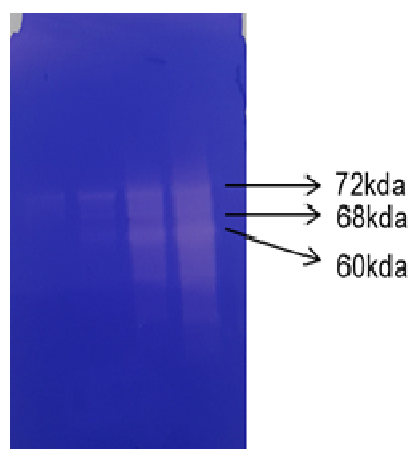


Figure 4. Zymography gel of collagenase enzyme

Therefore, SDS-PAGE electrophoresis of concentrated supernatant from A375 and MDA-MB-463 cell lines respectively was then carried out using different dilutions in the next experiment.

3.2.2. Expression of MMP-2 using concentrated supernatant from MDA-MB-436 and A375

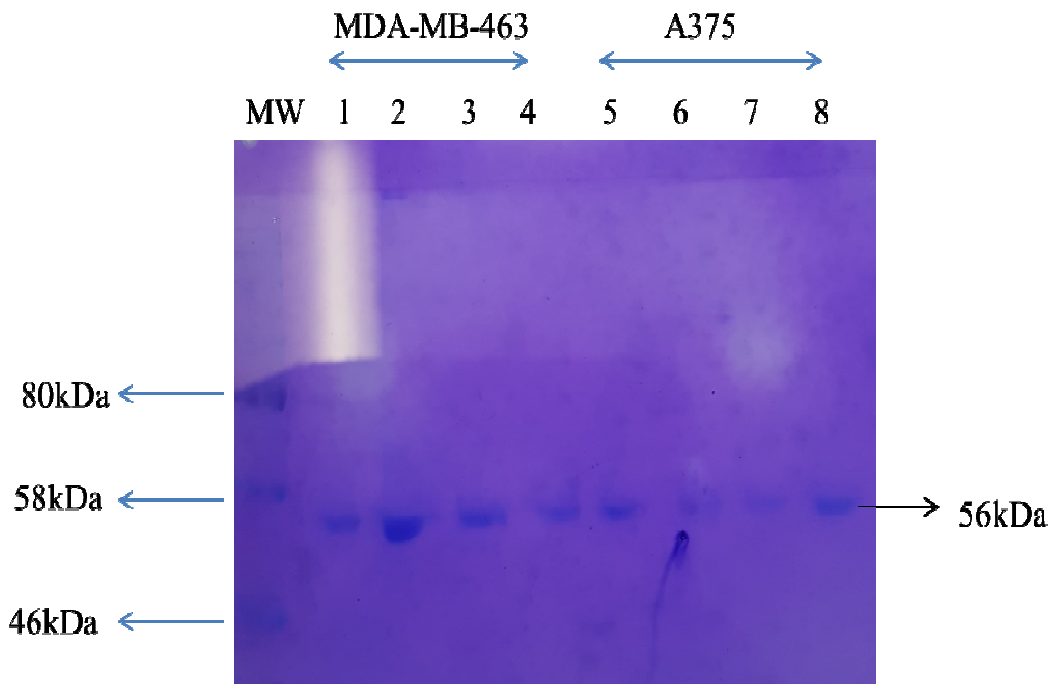


Figure 5. Zymography gel of concentrated supernatant from A375 and MDA-MB-436 cell lines

In figure 5, the first well (marked as MW on the gel image) was 5 μ l of molecular marker (Colour plus). Wells from 1 to 4 contained different volumes of solutions of MDA-MB cell lines at the 1:10 dilution, particularly, 5 μ l, 4 μ l, 3 μ l and 2 μ l. Wells from 5 to 8 contained different volumes of solutions of A375 cell lines at the 1:10 dilution, particularly, 5 μ l, 4 μ l, 3 μ l and 2 μ l. The cell lines expressed a distinct band at molecular weight of 56kDa. As a result, the pro-form of MMP-2 was detected in A375 and not in MDA-MB-436 cell line, while the active form of MMP-2 was not detected in either the A375 or the MDA-MB-436.

3.3. Effects of chlorotoxin

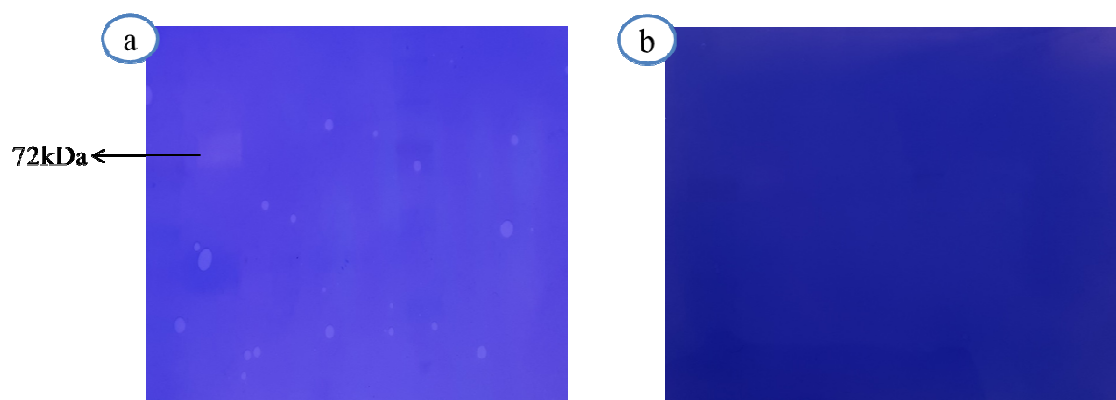


Figure 7. Inhibitory action of chlorotoxin on MMP-2 secreted by A375 cell lines
a) Without chlorotoxin; b) With chlorotoxin

As we can see, when chlorotoxin was absent in the samples (figure 7a), MMP-2 molecules with the molecular of 72kDa were still expressed as normal in MDA-MB-436 and A375 cells. In contrast, when adding chlorotoxin in the mixture solution (figure 7b), there was no active response recorded on the pre-made zymogram gel, which may be a result of the inhibitory action of chlorotoxin on the proliferation of cancer cells. However, no signals on the gel might be caused by too low amount of samples loaded on the gel, which therefore needs further optimization.

DISCUSSION

MMP-2 has been strongly linked to metastasis and progression of many forms of cancer such as breast, melanoma, gliomas [7,26]. This research shows that MMP-2 is present in both FCS which serves as a positive control and melanoma cell line (A375) where it degrades the extracellular matrix and allows migration from the primary tumor site to the new site, as mentioned in previous study of Caputo et al. [4]. This data also supports the early discovery that MMP-2 is present in both pro and activated forms [7]. Evidence has also been provided that the difference in the expression of various MMPs in various cancer cell lines of different tumorigenicity agrees with the biological characteristics of these cells, that is, the more malignant cells expresses more MMPs than the less malignant ones as observed in the A375 melanoma cell line compared to the MDA-MB-436 breast cancer cell line. The A375 cell line possesses more MMP-2 than MDA-MB-436 cell line as seen in the zymography gel. Loading low amount of samples into the gel could result in poor detection. This research also discovered that A375 grew faster than MDA-MB-436 as the time to concentrate the supernatant of A375 cell line was longer than that of MDA-MB-436 cell line. This may be due to lesser protein concentration in the supernatant derived from the cell line which therefore needs more optimization. Addition of more volume of the concentrated supernatant to the gel may lead to blurred band and saturation because the protease can digest a certain amount of substrate in the band area, in contrast, loading too little will prevent detection as observed with the MDA-MB-436 cell line [11].

The SDS-PAGE analysis of both MDA-MB-436 and A375 cell line gave a band at 56kDa similar to the molecular weight of AKT. AKT also known as protein kinase B (PKB) is a proto-oncogene discovered by Bellaosa et al [3] as a cellular homolog of the V-AKT oncogene [3]. AKT re-localizes from the membrane to the nucleus or cytosol when phosphorylated upstream kinase which activates the enzyme AKT [20]. It is important, in a lot of human cancers especially aggressive cancers such as gastric and breast cancer possess hyper-activated AKT [20,27]. Akt is a protein that has been identified to promote cell invasion and metastasis. Biopsies from metastatic melanoma has an increased AKT amount compared to normal skin tissue which is linked to poor survival of the patient [18,25]. As Akt is linked with the survival of tumours, invasion and proliferation [33], it is suggested that therapies inhibiting Akt would reduce proliferation and invasion of tumors. A tumour suppressor gene (PTEN) frequently activated by a serine/threonine phosphatase PHLPP [22] directly suppresses tumour growth. In a recent study, when phosphatase and tension homolog

(PTEN) protein negatively regulates phosphorylation of AKT and upregulates activity of MMP-2, a transcription factor (SP1) is upregulated [5,20].

PKB/AKT pathway has been discovered to be involved in its ability to phosphorylate estrogen receptor (ER) which increases the transcription of genes regulated by the ER. It is known that estrogen promotes the survival and growth of ER. Once ER is bounded to the ligand, it recruits its coactivator proteins prompting the transcription of C-myc and C-fos which are target gene through their promoters [22]. ER-positive breast tumors is supported by the ability of tamoxifen which is an angiogenic drug to suppress such tumors, but the ability of ER-positive tumor to develop resistance to tamoxifen is due to ligand-independent activation through growth factors such as EGF and IGF-1. Cellular resistance to tamoxifen correlates to the phosphorylation of ER by PKB/AKT, suggesting that antiestrogen resistance is a result of PKB/AKT [27]. Overexpressing the protein (PKB/AKT) in different host systems addresses the question whether PKB/AKT is tumorigenic, overexpression of wildtype PKB β /AKT2 transforms NIH-3T3 fibroblast but not in rat-1 fibroblast, whereas constitutively active PKB/AKT which is membrane-targeted is responsible for the oncogenic transformation of both cells. At least in mammary gland overexpression PKB/AKT is not tumorigenic as observed in the phenotype of PKB/AKT in transgenic mice formation of tumor maybe a result of constitutive activation of PKB/AKT, nevertheless, the PKB/AKT cooperates with some oncogenic pathway to enhance progression of tumor. Enzyme collagenase serving as another positive control because collagenase is a substrate of MMP-2, the result shows the correlation between both latent and activate form of MMP-2 [1].

Chlorotoxin that is a 36-amino-acid peptide has been shown to inhibit cell migration of glioma cells by binding and reducing enzymatic activity of MMP-2 [8]. Sontheimer et al. showed that MMP-2, the membrane type metalloprotease-1 macromolecular complex, and the CLC-3 chloride ion channel are targets for CTX on human glioma cells surface [16]. In this study, CTX was observed to partially inhibit the expression of MMP-2 in A375 cell line as there was no activity recorded on the gel. This result highlights that CTX could be used as therapeutics for these tumors, CTX could also be fluorescently labelled to enable imaging of theses tumours. Breast and melanoma cancer are shown to overexpress MMP-2 [6,10] correlating to rate of tumor invasion. Veiseh et al. [31] also reveals that MMP-2 aids the binding of CTX to MCF-7 breast cancer cells.

Washing after electrophoresis gel is a crucial step to remove SDS out of the gel because SDS would interact and denature protein. Faint bands may be due to inadequate washing of the gels or the period for activation of proteins. 24-hour protein activation in this study was contrary to study of Frankowski et al. [9] in which more time (48 hours) for renaturing the denatured protein together with activating both the pro and active enzyme. In addition, both the staining and destaining time had affect the visualization of the gels [9]. For this and other problems, further studies would optimize the concentrates from MDA-MB-436 to detect the presence of MMP-2 by gel zymography or alternatively using Western blotting as examined in the study of Van Wart and Birkedal-Hansen [30]. Followed by incubating the MDA-MB-436 with different concentrations of chlorotoxin to detect the effect on MDA-MB-436 cell

line as done in the study of Deshane et al.[7]. In addition, cell surface biotinylation experiment with Western blotting should be performed to demonstrate if the CITX-binding protein is either on the surface or in the intracellular membrane.

CONCLUSION

To draw a conclusion, MMP-2 has been strongly linked to metastasis and progression of many forms of cancer including breast and melanoma, thus considered as a prognostic biomarker. This study provides evidence that expression of MMP-2 is significantly increased in melanoma cell line compared to breast cancer cell line. The difference in the expression of various MMPs in various cancer cell lines corresponds to the biological characteristics of these cells.

CTX has been found to inhibit the activity of MMP-2 present in these cancer cell lines as with the gliomas. Therefore, MMP-2 can be used as a good therapeutic option for both types of cancer. AKT is a protein that has been identified to promote cell invasion and metastasis. Biopsies from metastatic melanoma has increased AKT compared to normal skin tissue. PKB/AKT phosphorylates promotes resistance against tamoxifen which is an anti-estrogen drug.

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