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# Characterization of Collagenase 3 (MMP-13) Expressions in Skin Melanoma, Breast Cancer and Cervical Cancer *in Vitro*

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**Abstract.** Matrix metalloproteinases (MMPs) comprise of a family of secreted and membrane-bound endopeptidases that hydrolyze extracellular matrix proteins. MMPs are one of many proteins which are undergoing scientific analysis in order to discover their therapeutic potentials. Collagenase 3 (MMP-13), a type of collagenase from the MMP family, has been previously reported to be involved in the development and metastasis of cancers. However, the depth of its involvement in the underlying molecular mechanisms of cancer remains to be poorly known. This study aims to determine the degree of MMP-13 protein expressions in several human cancer cell lines and to ascertain its probable involvement in cancer development. From the study, it was hypothesized that the expression of MMP-13 are varied in several human cancer cell lines (MCF-7, HeLa and A375) and that its expression plays an important role in cancer progression and thus, by understanding such interactions, targeted therapeutic treatments could be developed over time. The quantitative expressions of MMP-13 in skin melanoma (A375), breast cancer (MCF-7) and cervix cancer (HeLa) cells were evaluated through ELISA test. The results showed a marked expression of MMP-13 proteins in MCF-7 (12900 pg/ml), followed by HeLa (8109 pg/ml) and A375 (7515 pg/ml). A375 showed the least amount of expression, which is supported by the fact that only certain forms of skin cancer have been found to express a significant amount of MMP-13. Overall, although variations were found in the expression of the collagenase in various human cancer cell lines, the results depict a prominent presence and probable involvement of MMP-13 in cancer.

**Keywords:** Matrix metalloproteinase, MMP-13, human cancer

## 1. Introduction

Cancer, as one of the leading causes of disease and mortality worldwide [1], has been, and remains to be, the focus of much research endeavors for the past decades. For the past several years, biomedical researchers have been focused on cancer and the various mechanisms that may have influenced its succession. Among these studies, the involvement of matrix metalloproteinases (MMPs) in cancer progression has been found to be of importance. The MMPs family of enzymes is comprised of critically important extracellular matrix (ECM) remodeling proteases or membrane-bound endopeptidases that hydrolyse extracellular matrix proteins [2], whose activity has been implicated in a number of key pathological processes including those associated with cancer [3]. Consequently, these proteins play a crucial role in tumor invasion and the building of metastatic formations due to their ability to degrade extracellular matrix proteins [4]. Based on their preferred substrates and on the structural features, MMPs can be divided into collagenases, gelatinases, stromelysins, and membrane-type matrix metalloproteinases [5], [6]. Recently, several studies have asserted the probable high involvement of Collagenase 3, or MMP-13, in the development and metastasis of cancer [2]-[8]. Under physiological conditions, the activities of these proteins are precisely regulated in order to prevent tissue disruption [4]. Consequently, the physiological balance seems to be disrupted in cancer, allowing the cancerous cells the capability of invading its adjoining tissues [2]. This study is designed with

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the objective of analyzing the expressions of MMP-13 in several human cancer cell lines and to ascertain its probable involvement in cancer development, in the hope that it will help to further enhance the progression in the search for a treatment in cancer.

# 2. Research Methodology

## 2.1. Materials and samples

The materials used are as follows: Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, USA), Fetal Bovine Serum (FBS) (Invitrogen, USA), penicillin-streptomycin (PenStrep) (GIBCO, USA), Phosphate Buffer Saline (PBS) – magnesium and calcium free (Fisher Scientific; New Hampshire, USA), HEPES solution (GIBCO, USA), sodium pyruvate (Invitrogen, USA), RIPA Lysis Buffer (Millipore, USA), MMP-13 Standard (Calbiochem, USA), Biotinylated antibody (Calbiochem, USA), Assay Buffer (Calbiochem, USA), Detection Buffer (Calbiochem, USA).

## 2.2. Cell culture

Three different types of human cancer cell lines; breast cancer (MCF-7), cervix cancer (HeLa) and skin melanoma (A375) which are derived from American Type Culture Collection (ATCC, USA), were used in this study. The cells were established *in vitro* by culturing them separately in tissue culture flasks in the appropriate growth media (DMEM supplemented with sodium bicarbonate, HEPES buffer, PenStrep, FBS) and maintained at 37 ℃ in a humid incubator with an air mixture containing 5 % (v/v) $CO_2$.

## 2.3. Preparation of Total Cell Lysate

Adherent cells were trypsinized before being transferred into test tubes for centrifugation at 250 *g* for 15 minutes. The pellet was washed twice with 1X PBS buffer before being counted by using a haemocytometer. The standard seeding density ($1.0 \times 10^5$ cells/$cm^2$) was established via the cell counting. The PBS was then drained and the cells were resuspended in RIPA lysis buffer. Lysis of cells was maintained overnight, which was then followed by centrifugation at 13000 *g* for 30 minutes. Supernatant resulted from the centrifugation was transferred into a sterile vial and stored at -80 ℃ until further use.

## 2.4. MMP-13 ELISA (Enzyme-Linked Immunosorbent Assay)

Preparation of solutions and buffers as well as the processing of the ELISA assay was conducted according to the instructions provided by the manufacturer. Cell lysates from MCF-7, HeLa and A375 were diluted at ratio 1:6 with Assay Buffer before undergoing analysis. The concentrations of MMP-13 in these samples were determined by MMP-13 ELISA Kit (Calbiochem, USA) according to the manufacturer's instructions. This assay is an *in vitro* quantitation assay that is based on a two-sided sandwich format. Based on the standard curve generated (plotted using MMP-13 Standard), the concentrations of MMP-13 in the cancer lysates were determined.

## 2.5. Statistical Analysis

In order to determine the absorbance reading in each well, a microplate reader was utilized to read the plate, which was read at 450 nm, with 620 nm as the reference filter. The data was analyzed using Microsoft Professional Excel 2010 where the significant difference (S.D.) within the test groups were calculated, as well as the standard error of the mean (S.E.M.) of the test groups' mean.

# 3. Results and Discussion

## 3.1. Western blot and ELISA Analysis

The analyzed MMP-13 could be identified in all three types of human cancer cell lines (MCF-7, HeLa and A375) examined through MMP-13 ELISA test. The results from the ELISA test were utilized in order to generate a standard curve (Figure 1) and to formulate/calculate the regression coefficient formula of the curve: y=0.0004x-0.3837

The results showed varying degrees of MMP-13 protein absorbance in all the human cancer cell lines applied in the study; MCF-7, HeLa and A375 (Table 1). The MMP-13 protein concentration values were calculated by using the regression coefficient formula obtained from the standard curve (Table 2). The

calculated concentration values were then multiplied by the dilution factor of the samples, which resulted in the following formula:

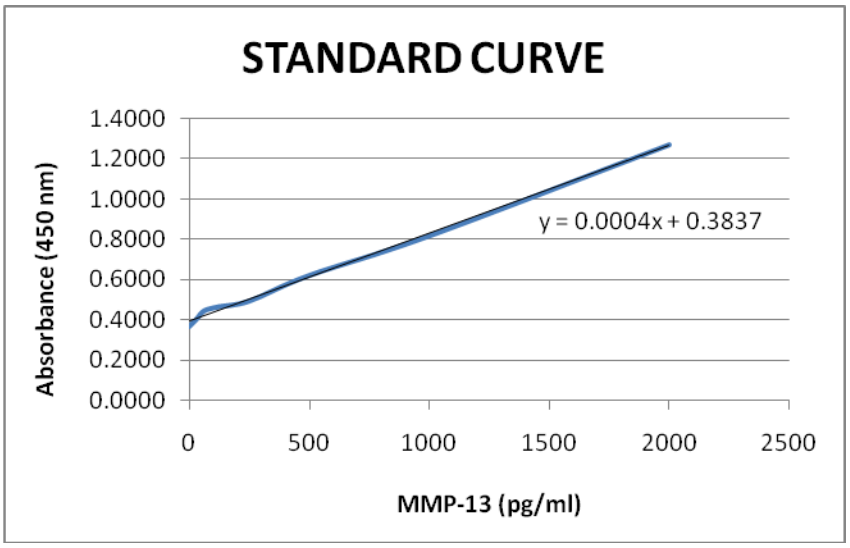


Fig. 1: Standard curve generated by plotting the mean absorbance (y axis) against MMP-13 (pg/ml) (x axis).

Final concentration value of each samples = [(y-0.3837)/0.0004] x 6

Table 1. Analysis of absorbance in breast cancer (MCF-7), cervix cancer (HeLa) and skin melanoma (A375) cells.

| Human Cancer Cell Line | Mean Absorbance (450 nm) ± S.D. (n=3) | | | | | Mean Absorbance (450 nm) |
|---|---|---|---|---|---|---|
| | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | |
| MCF-7 | 1.2924 ± 0.0172 | 1.2647 ± 0.0272 | 1.2500 ± 0.0271 | 1.1987 ± 0.0183 | 1.2127 ± 0.0101 | 1.2437 ± 0.0171* |
| HeLa | 1.0840 ± 0.0099 | 0.9094 ± 0.0193 | 0.8377 ± 0.0233 | 0.8654 ± 0.0259 | 0.9250 ± 0.0153 | 0.9243 ± 0.0428* |
| A375 | 1.0300 ± 0.0110 | 0.7970 ± 0.0127 | 0.7967 ± 0.0165 | 0.7690 ± 0.0178 | 1.0307 ± 0.0151 | 0.8847 ± 0.0597* |

*Note:* *Mean absorbance ± S.E.M. (n=5); S.E.M. = Standard Error of Mean; S.D. = Standard Deviation

Table 2. Summary of ELISA readings of MMP-13 protein expression in breast cancer (MCF-7), cervix cancer (HeLa) and skin melanoma (A375) cells.

| MMP-13 Quantification via MMP-13 ELISA | | |
|---|---|---|
| Human Cancer Cell Line | Absorbance (450 nm) | MMP-13 (pg/ml) |
| MCF-7 | 1.2437 ± 0.0382* | 12900 |
| HeLa | 0.9243 ± 0.0958* | 8109 |
| A375 | 0.8847 ± 0.1335* | 7515 |

*Note:* *Mean absorbance ± S.E.M (n=5); S.E.M. = Standard Error of Mean

## 3.2. Expression of MMP-13 in Several Human Cancer Cell Lines

MMP-13 is principally a type of secreted proteinase that is capable of cleaving native fibrillar collagens of types I, II, III, V and IX. The protein has been implicated in the processes of growth, tumor progression, promoting invasion, metastasis [3]; are frequently over-expressed in malignant tumors; and have been associated with an aggressive malignant phenotype and adverse prognosis in patients with cancer [9].They have also been reported to be expressed by numerous types of cancerous cells [2], [8]. In the study conducted, MCF-7, HeLa and A375 (each cell respectively represents a type of breast, cervical and skin cancer cell line), were utilized in order to determine the expressions of MMP-13 protein in these various cell lines.

From the results of the absorbance readings shown in Table 2, it can be implicated that all three of the cultivated cell lines secrete/expresse diverse amount of MMP-13 protein in cells' protein lysates. Furthermore, the analysis of MMP-13 protein concentration in MCF-7 cells showed the highest expression

compared to the other cancer cell lines, where strong expression of MMP-13 proteins were observed in HeLa cells and a very weak expression was found in A375 cells. By applying the formula previously stated ([(y-0.3837)/0.0004] x 6) and incorporating the mean value of absorbance into the equation (y = Mean Absorbance), the final protein concentration value of each samples were calculated. Generally, concentration of MMP-13 was of different levels for each cell lines, with MCF-7 cells expressing the highest amount of protein concentration (12900 pg/ml). This is followed by HeLa cells with protein concentration readings of 8109 pg/ml and A375 cells which showed the least amount of MMP-13 protein concentration with a reading of 7515 pg/ml.

These findings correlate to previous studies where it has been found that the expressions of MMP-13 in several breast cancer cell lines are either highly or overly expressed [7]-[10]. Moreover, researches on MMPs' expression in cervical cancer are vast and many and these studies have shown that, depending on the type of cervical cancer cell lines used, MMP-13 proteins are found to be either moderately or strongly expressed [11]. In contrast, up till now, only a few studies have been published concerning the distribution of MMPs in human melanocytic tumor progression, though most have reported a weak or non-expressions of the protein in several skin cancer cell lines [12].

### 3.3. Comparison with other studies

There has been an extensively increasing interest on MMPs and its potential role in cancer development. Though much of MMPs' characteristics and functions in carcinoma progression have not been explored, nevertheless, several have been found to correspond to the study conducted. A study conducted by Hui-Jen Chang and colleagues (2009) showed that in breast cancer tissues, several of its proteins and genes, which includes MMP-13 proteins, are found to be over-expressed as compared to normal tissues [7]. In another study, the expression patterns of MMPs in human gynecological cancer cell lines were observed. From the results, they concluded that, as compared to the other two cervical cancer cell lines used, HeLa cells expressed the strongest expression of MMP-13 in Western Blot [11]. In addition, a study pertaining to the expressions of MMP-1 and MMP-13 in malignant melanoma have shown that MMP-1 was expression was of more prominence than MMP-13 as it was found to have a weak expression at certain stages of melanocytic tumor progression [12]. These findings support the qualitative and quantitative expressions of MMP-13 protein observed in MCF-7, HeLa and A375 cells as the strength of each cell's expression correlates to that of previous studies.

## 4. Conclusion

This study demonstrated the probable constitutive expression of MMP-13 on protein level in breast, cervical as well as skin cancer cell lines. The results showed that different types of human cancer cell lines have differing outcomes in respect to its MMP-13 protein expressions. These diverse expressions of MMP-13 could reflect on many possibilities that might be occurring during cancer progression. Based in its many expression patterns, MMP-13 shows high prospect as potential predictive and/or prognostic markers in the effort to determine the best course of treatment in human cancer. The determination of its relative contribution in the context of tumor progression is critical since evidence has suggested that the same MMP can have opposing effects based upon the cell type in which it is expressed. With further studies, better understanding of the protein and its effects toward cancer progressions could be elucidated in the hopes of finding potentially therapeutic and diagnostic targets for the treatment and detection of human cancers.

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