

CHARACTERIZATION OF COLLAGEN (IV) MRNA IN CELL LINES OF BREAST CANCER

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Abstract

Breast cancer incidence rate has increased in the 5 recent years with 14% increases in mortality. The structural change in the collagen chain has led to alterations in the cancer cells. Various biological processes, such as differentiation or gene expression, are regulated through extracellular matrix (ECM)[1]. The restructuring of the collagenous architecture in the hypoxic microenvironment may influence the invasive growth of the cancer cells. With the increased stress within the cell, the invasion of cancer cells into the ECM was triggered. This cell lines model would enable the exploration of the relationship between the extracellular matrices component and the tumor proliferation. The aim of this study is to characterize the collagen (IV) mRNA expression in the breast cancer cell. Breast cancer (MCF7) cell lines were cultured and harvested upon confluent. The RNA was extracted from the cell lines and then the cDNA were synthesized. The collagen (IV) mRNA levels in breast cancer cell lines were measured using real time PCR and *GAPDH* was used as an internal control. The level of *COL4A2* (IV) mRNA expression was higher compared with *COL4A1* (IV) mRNA. The level of *COL4A5* (IV) mRNA was reduced significantly in breast cancer cell lines. Overall, the expression of *COL4A1-A6* (IV) was reduced. The reduced amount of collagen (IV) in breast cancer cell lines suggested that the collagen was restructured and this has triggered the tumor invasion into the ECM.

Keyword: Collagen, type IV collagen, breast cancer cell lines, gene expression

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1.0 INTRODUCTION

Breast cancer is the most common cancer which causes death in women worldwide [2]. The incidence of breast cancer has increased more than 20% with 14% increases in mortality in five recent years. Among the general population in Malaysia, occurrence of breast cancer is within the highest statistics of five leading cancers [3].

Breast structure is composed of epithelial and stromal components. The ductal-lobular system in

breast is made up of a dual layer of epithelial resting on a basement membrane enveloped by the extracellular matrix (ECM) and stromal cell. While, the mammary glands of the breast consist of a complex interaction network of laminin, fibronectin and collagen which responsible for the development and functioning of the organ [4].

Basement membranes is a thin layer sheet of fiber that underlies the epithelium or endothelium, which composed of collagen IV, laminin, sulfate proteoglycan and fibronectin [5]. The deposition of

ECM structure gives support on which epithelial and endothelial cells resides [6]. The ECM and basement membranes are extensively cross-linked for it to function. Hence, the remodeling of the ECM may lead to cancer.

Restructuring of collagenous architecture in the hypoxic microenvironment may influence invasive growth of cancer cells. This is because, collagens and laminin undergone independent intermolecular self-assembly to form a sheet-like structure that interacts with each other [7]. Cancer cells are stimulated to secrete lysyl oxidase that triggered collagen type IV crosslinking cascades as the tumor mass expanded [8, 9]. This process produced irregular, dense and sheet-like deposition at basement membrane [10] (which undergoes a series of structural changes based on type IV collagen uncoiling for degradation and this facilitates tumorigenesis [11]).

The stiffness of the tissues enhanced the proliferation, invasion and migration of the cancer cells and tumor angiogenesis [12]. In the extracellular matrix (ECM), the stress increased, triggering the invasion of cancer cells into ECM to reduce the stress. The secretion of matrix metalloproteinase by cancer cells along with uncoiling of collagen triple helix caused collagen degradation into fragment at invasion front [13]. The shedding of cancer cells formed new cluster protrusions as the collagen disappeared. On the other hand, the involvement of lysyl oxidase will increase the stiffness of tissues in ECM [14]. The stiff and rigid of collagen type IV act as highway for cancer cells migration.

Mutations and interruptions in collagen type IV chains have been shown to cause diseases. The inheritance of X-linked mutation in COL4A5 gene or in autosomal recessive patients, either heterozygous or homozygous mutations in COL4A3 and COL4A4 caused Alport's Syndrome [15]. In addition, diffuse leiomyomatosis are reported to cause by deletions involving the adjacent COL4A5 and COL4A6 genes [16].

Type IV collagen is a member of the collagen superfamily that contains 29 different triple helical molecules assembled from 42 genetically distinct α chains. Collagen is classified into nine subfamilies based on their supramolecular organization and other features [17, 18, 19]. To date, extensive studies have been focusing on collagen type VI that has a similar structural features and roles as collagen type IV [20,21]. It has been shown that the collagen VI was upregulated in breast cancer, generating a microenvironment that promotes tumour progression and metastasis in breast and was recognized as a potential biomarker for cancer diagnosis [22, 23].

Yet, little is known about the expression of type IV collagen in breast cancer cells. Therefore, we used real time qPCR to identify the expression of type IV collagens mRNA in breast cancer.

2.0 EXPERIMENTAL

2.1 Samples

The breast adenocarcinoma cell line, Michigan Cancer Foundation-7 (MCF 7) was obtained from ATCC® HTB-22 (ATCC, Virginia) and the fibroblast cell line was obtained from Research Laboratory of Kulliyah of Pharmacy, IIUM, Kuantan. The complete growth medium (CGM) used to expand the cell lines of breast cancer and fibroblast and harvested upon confluence.

2.2 RNA Extraction and cDNA Synthesis

The RNA was extracted using RNeasy Mini kit according to manufacturer instructions (Qiagen). The purified mRNA of the ratio of absorbance at 260/280 equal to 2.0 was selected and subjected to complementary DNA (cDNA) synthesis by using SensiFAST cDNA synthesis Kit (Bioline) following the manufacturer's protocol. The cDNA obtained were then used in the qPCR.

2.3 Real Time PCR (qPCR)

PCR amplification and detection were performed using the CFX96 qPCR machine, C1000 Touch® Thermal Cycler (Bio-Rad, USA). Briefly, each qPCR reaction comprised 5 μ l of 2x SensiFAST SYBR® & Fluorescein Kit (Bioline), 0.7 μ l of each 20ng/ μ l sense and antisense primer and 2 μ l of 100ng/ μ l cDNA template, made up to a total volume of 10 μ l. The gene for GAPDH was amplified in parallel as an internal control. The levels of collagen type IV α 1 to α 6 mRNA were compared in breast cancer cell lines. The primer pairs were optimized before the amplifications. The assays were done in triplicate and also run in two separate experiments.

3.0 RESULTS AND DISCUSSION

3.1 Collagen Type IV mRNA Expression in Breast Cancer Cell Lines

The study observed α 1(IV), α 2(IV), and low levels of α 4(IV) and α 6(IV) in breast cancer cell lines (Table 1 and Figure 1). The level of α 3(IV) and α 5(IV) could not be observed. This is consistent with the collagen distribution in the mammary gland of breast. In normal breast, α 1(IV) and α 2(IV) were found in all basement membrane whereas the distribution of α 3(IV), α 4(IV), α 5(IV) and α 6(IV) were restricted to certain area [24]. The α 5(IV) and α 6(IV) were localized in the basement membrane of the mammary gland in a linear pattern, but form a second network in the membrane of cancer cells[25].

Table 1 Intra- and inter-assay coefficients of variation for mRNA quantitation of COL4A1-COL4A6 (IV) in breast cancer cell lines

| Collagen type IV | CV of intra-assay (%) | CV of inter-assay (%) |
|------------------|-----------------------|-----------------------|
| COL4A1 | 3.11 | 2.09 |
| COL4A2 | 1.97 | 2.02 |
| COL4A3 | 9.11 | 0.38 |
| COL4A4 | 5.75 | 5.22 |
| COL4A5 | 2.44 | 2.48 |
| COL4A6 | 3.17 | 3.54 |
| GAPDH | 1.34 | 1.22 |

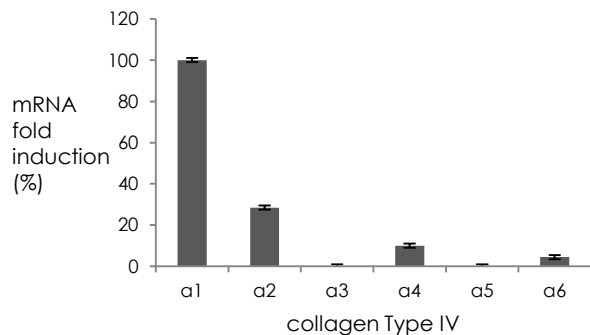


Figure 1 Collagen type IV mRNA expression in breast cancer cell lines (MCF-7) corresponding to COL4A1-COL4A6

This study has characterized the mRNA expression of collagen type IV in breast cancer cells with the use of real-time PCR for quantification. The intra- and inter-assay value of correlation coefficients (%CV) of the real time assay of breast cancer cell line were less than 10% based on C_q -value. The value obtained show that the quantification of mRNA was highly reproducible [26].

It was found in the study that the level of expression for $\alpha 2$ (IV), $\alpha 4$ (IV) and $\alpha 6$ (IV) was lower when compared to $\alpha 1$ (IV). Lack of studies focusing in quantification of the collagen type IV expression level in breast has made the result of this study important. Previous study has demonstrated by in situ hybridization and immunohistochemistry that $\alpha 5$ (IV) and $\alpha 6$ (IV) were defective in the invasive stages of breast cancer [25] and that the normal production as well as the basement membrane assembly was disrupted during cancer progression [27]. Thus the lower levels of collagen have been associated with the cancer mechanism of proliferation and metastasis [28, 29].

Collagen type IV was mainly expressed at basement membrane to constrain the cells from escaping out by encapsulated the tumor nest. By doing this, it produced irregular, dense and sheet-like deposition at basement membrane [30]. A series of structural changes based on type IV collagen uncoiling for degradation were developed thus indicates the importance of this collagen during tumorigenesis.

4.0 CONCLUSION

This study has established the mRNA expression assay using real-time PCR and represents an effort to quantify the level of COL4A1-COL4A6 mRNA chain of type IV collagen in breast cancer cell lines.

The results of the study imply that the collagen expression was altered in the cancer cell lines because certain network of the type IV chain ($\alpha 1$ - $\alpha 6$) was observed in a low amount as compared to its composition in normal breast cell that been reported in the literature.

Previous study has suggested that structural change in collagen leads to modification of the cancer cells since its morphology is different at difference stages [31]. Therefore future study involving collagen type IV chains is sought to determine whether a change in the expression would be also observed in its secretion in extracellular membrane of the cell at protein level.

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