Jurnal Teknologi

Full Paper

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

Afzan Mat Yusof[a,b*], Mardhiah Mohammad[a,b], Sharifah Norbaizura Syed Bahrom[a], Syahirah Kaja Mohideen[a], Ridhwan Roshdi[a], Adibah Parman[a]

[a]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University of Malaysia, Indera Mahkota Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia

[b]Integrated Cellular and Molecular Biology Cluster (iMolec), Integrated Centre for Animal Care and Use, International Islamic University Malaysia, Indera Mahkota Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia

Article history
Received
28 Jun 2015
Received in revised form
15 September 2015
Accepted
15 October 2015

*Corresponding author:
afzan@iium.edu.my

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 extracelullar 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 cells 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

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 5µl of 2x SensiFAST SYBR® & Fluorescein Kit (Bioline), 0.7µl of each 20ng/µl sense and antisense primer and 2µl of 100ng/µl cDNA template, made up to a total volume of 10µl. The gene for *GAPDH* was amplified in parallel as an internal control. The levels of collagen type IV α1 to α6 mRNA were compared in breast cancer cell lines. The primer pairs were optimized before the amplications. 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 α1(IV), α2(IV), and low levels of α4(IV) and α6(IV) in breast cancer cell lines (Table 1 and Figure 1). The level of α3(IV) and α5(IV) could not be observed. This is consistent with the collagen distribution in the mammary gland of breast. In normal breast, α1(IV) and α2(IV) were found in all basement membrane whereas the distribution of α3(IV), α4(IV), α5(IV) and α6(IV) were restricted to certain area [24]. The α5(IV) and α6(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

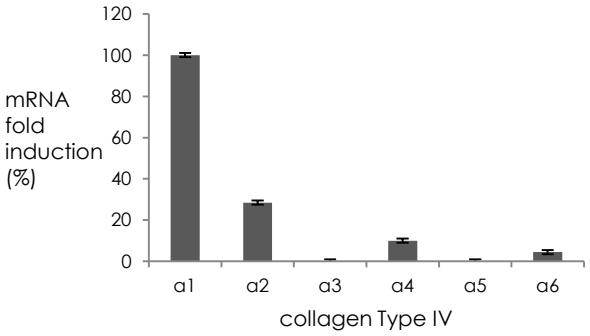

Figure 1 Collagen type IV mRNA expression in breast cancer cell lines (MCF-7) corresponding to *COL4A1-COL4A6*

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 α2(IV), α4(IV) and α6(IV) was lower when compared to α1(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 α5(IV) and α6(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 tumorgenesis.

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 (α1-α6) 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.

Acknowledgement

We would like to thank Br. Muhammad Afif Musa and Br. Syahril Ismail for their technical assistance in the study. This study was supported by the Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University of Malaysia, and iMolec Research Cluster, Integrated Centre for Research Animal Care and Use (ICRACU), IIUM. We would also like to acknowledge the helpful resources from Laboratory of Kulliyah of Pharmacy, IIUM Kuantan.

References

[1] Jarvelainen H., Sainio, A., Koulu, M., Wight, T. N., & Penttinen, R. 2009. Extracellular Matrix Molecules: Potential Targets In Pharmacotherapy. *Pharmacol. Rev*. 61: 198-223.
[2] Latest World Cancer Statistics. Geneva, France: Lyon. 2013. International Agency for Research on Cancer, WHO.
[3] National Cancer Registry Report: Malaysia Cancer Statistics-Data and Figure. 2007. National Cancer Registry Malaysia, Ministry of Health Malaysia.
[4] Polyak, K. & Kalluri, R. 2010. The Role of Microenvironment in Mammary Gland Development and Cancer. *Cold Spring Harbor Perspectives in Biology*. 1-12.
[5] Miner, J. H. 1999. Renal Basement Membrane Components. *Kidney International*. 56: 2016-2024.

[6] Kalluri, R. 2003. Basement Membranes: Structure, Assembly and Role in Tumor Angiogenesis. *Nature Reviews Cancer*. 3: 422-433.
[7] Luparello, C, 2013. Aspect of Collagen Changes in Breast Cancer, *Carcinogenesis & Mutagenesis*. S13: 007.
[8] Erler, J. T., Bennewith, K. L., Cox, T. R., Lang, G., Bird, D., Koong A., Le, Q. T. & Giaccia, A. J. 2009. Hypoxia-Induced Lysyl Oxidase is a Critical Mediator of Bone Marrow Cell Recruitment to Form the Premetastatic Niche. *Cancer Cell*. 15: 35-44.
[9] Levental, K. R., Yu, H., Kass, L., Lakins, J. N., Egeblad, M., Erler J. T., Fong, S. F., Csiszar, K., Giaccia, A., Weninger, W., Yamauchi, M, Gasser, D. L. and Weaver, V. M. 2009. Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling. *Cell.* 139: 891-906.
[10] Egeblad, M., Rasch, M. G. & Weaver, V. M. 2010. Dynamic Interplay between the Collagen Scaffold and Tumor Evolution. *Current Opinion in Cell Biology*. 22: 697-706.
[11] Ng, M. R. and Brugge, J. S. 2009. A Stiff Blow from the Stroma: Collagen Crosslinking Drives Tumor Progression. *Cancer Cell*. 16(6): 455-457.
[12] Torzili, P. A., Bourne, J. W., Cigler., T.& Vincent, C. T. A New Paradigm for Mechano-biological Mechanisms in Tumor Metastasis. *Seminars in Cancer Biology*. 22(5-6): 385-395.
[13] Zeng, Z. S., Cohen, A. M. and Guillem, J. G. 1999. Loss of Basement Membrane Type IV Collagen is Associated with Increased Expression of Metalloproteinase 2 and 9 (MMP-2 and MMP-9) during Human Colorectal Tumorigenesis. *Carcinogenesis*. 20(5): 749-755.
[14] Fang, M., Jing-Ping, Y., Chun-Wei, P., Dai-Wen, P. & Li, Y. 2013. Quantum Dots-based in situ Molecular Imaging of Dynamic Changes of Collagen IV during Cancer Invasion. *Biomaterials.* 34: 8708-8717.
[15] Hudson, B. G., Tryggvason, K., Sundaramoorthy, M. & Neilson, E. G. 2003. Alport's Syndrome, Good Pasture's Syndrome, and Type IV Collagen. *The New England Journal of Medicine*. 348: 2543-2556.
[16] Zhou, J., De Paepe, A., Antignac, C., Smeets, H., Tryggvason, K., Laurila, P., de Paepe, A., Tryggvason, K., & Reeders, S. T. 1993. Deletion of the Paired Alpha 5(IV) and 6(IV) Collagen Genes in Inherited Smooth Muscle Tumors, *Science*. 261: 1167-1169.
[17] Myllyharju, J. & Kivirikko, K. I. 2001. Collagens and Collagen-Related Diseases. *Ann Med.* 33(1): 7-21.
[18] Rautavuoma, K. 2003. Human .lysyl hydroxylases.
[19] Canty, E. G. & Kadler, K. E. 2005. Procollagen Trafficking, Processing and Fibrillogenesis. *J Cell Sci.* 118(Pt 7): 1341-53.
[20] Jander, R.,, Rauterberg, J., & Glanville, R. W. 1983. Further Characterization of the Three Polypeptide Chains of Bovine and Human Short-Chain Collagen (Intima Collagen). *European Journal of Biochemistry*. 133(1): 39-46.
[21] Bonaldo, P., & Colombatti, A. 1989. The Carboxyl Terminus of the Chicken A3 Chain of Collagen VI is a Unique Mosaic Structure with Glycoprotein Ib-Like, Fibronectin Type III, and Kunitz Modules. *Journal of Biological Chemistry*. 264(34): 20235-20239.
Karousou, E., D'Angelo, M. L., Kouvidi, K., Vigetti, D., Viola, M., Nikitovic, D., De Luca, G., & Passi, A. 2014. Collagen VI and Hyaluronan: The Common Role in Breast Cancer. *BioMed Research International.* 2014
[22] Chen, P., Cescon, M., & Bonaldo, P. 2013. Collagen VI in Cancer and Its Biological Mechanisms. *Trends in Molecular Medicine*. 19(7): 410-417.
[23] Hewitt, R. E., Powe, D. G., Morrell, K., Balley, E., Leach, I. H., Ellis, I. O. & Turner, D. R. 1997. Laminin and Collagen IV Subunit Distribution in Normal and Neoplastic Tissues of Colorectum and Breast. *British Journal of Cancer*. 75(2): 221-229
[24] Nakano, S., Iyama, K., Ogawa, M., Yoshioka, H., Sado, Y., Oohashi, T. & Ninomiya Y. 1999. Differential Tissular Expression and Localization of Type IV Collagen Alpha1(IV), Alpha2(IV), Alpha5(IV) and Alpha6(IV) Chains and Their mRNA in Normal Breast and in Benign and Malignant Breast Tumors. *Laboratory investigation; a Journal of Technical Methods and Pathology*. *79*(3): 281-292.
[25] Reed, G. F., Lynn, F., & Meade, B. D. 2002. Use of Coefficient Ofvariation in Assessing Variability of Quantitative Assays. *Clinical & Diagnostic Laboratory Immunology*. 9:1235-1239.
[26] Ikeda, K., Iyama, K., Ishikawa, N., Egami, H., Nakao, M., Sado, Y., Ninomiya, Y. & Baba, H. 2006. Loss of Expression of Type IV Collagen α5 and α6 Chains in Colorectal Cancer Associated with the Hypermathethylation of Their Promoter Regions. *The American Journal of Pathology*. 168: 856-865.
[27] Monteagudo, C., Merino, M. J., San-Juan, J., Liotta, L. A. & Stetler-Stevenson, W. G. 1990. Immunohistochemical Distribution of Type IV Collagenase in Normal, Benign and Malignat Breast Tissue. *The American Journal of Pathology*. 136(3): 585-592.
[28] Zhang, K., Corsa, C. A., Ponik, S. M., Prior, J. L., Piwnica-Worms, D., Eliceiri, K. W., Keely, P. J. & Longmore, G. D. 2013. The Collagen Receptor Discoidin Domain Receptor 2 Stabilize SNAIL1 to Facilitate Breast Cancer Metastasis. *Nature Cell Biology.* 15(6): 677-687.
[29] Egeblad, M., Rasch, M. G. & Weaver, V. M. 2010. Dynamic Interplay between the Collagen Scaffold and Tumor Evolution. *Current Opinion in Cell Biology*. 22: 697-706.
[30] Ajeti, V., Nadiarnykh, O., Ponik, S. M., Keely, P. J., Eliceiri K. W. &Campagnola, P J. 2011. Structural Changes in Mix COL I/COL V Collagen Gels Probed by SHG Microscopy: Implications for Probing Stromal Alterations in Human Breast Cancer. B*iomedical Optics Express*. 2(8): 2307-2316.