Volume : 3 | Issue : 4 | April 2014 • ISSN No 2277 - 8160

Research Paper | Medical Science

The Role of Methylation of Deoxy Rebonucleid Acid Receptor β_2 Promotor on Pathogenesis of Breast Cancer Through Epigenetic Process

Muliartha Biomedic Laboratory Medical Faculty Brawijaya University

ABSTRACT

The breast cancer is the most tumor malignant lead second cause of death among the female. Diagnostic and therapy of the breast cancer are developed. The aim of the research to prove that the pathogenesis of the breast cancer is the related epigenetic concept. Design of the research are descriptive survey and true experimental descriptive post test only control group design in vitro. This research use PCR technique, Immunohistochemistry, Chromogenic In Situ Hibridisasi. The result and conclusion that on the breast cancer shown lost of band on 1000 Bp until 1900 Bp. Both sample shown DNA metilation promotor RAR β2 on band 550 bp.

KEYWORDS : Epigenetic Process, Methylation DNA, Expressi RAR β2, breast cancer.

INTRODUCTION

BACKGROUND

Breast cancer is the second leading cause of death in women. Treatment and diagnosis of breast cancer to date progress has been rapid. Theories about the role of gene P 53 and PRb have revealed much, but the approach retinoic acid receptor $_2$ gene as a tumor suppressor gene has not been disclosed and also the lost of activity of retinoid acid receptor β_2 gene is caused by many factors including the loss of heterogenecity retinoid acid receptor β_2 also be referred as silence genes supresor. All of that are epigenetic modification theory. [1]

The research need aim cover the problem. To prove this, we refer to the research Gote et. al, 1998, which states support the claim that breast cancer occurs in the process of methylation of RAR β_2 gene, causing a slight decline of gene expression of RAR β_2-called epigenetic silencing process that occurs in the RAR β_2 gene promoter. [2,3]

The objective of this research is to prove that there in breast cancer RAR β_2 is decrease, also proves decreased RAR β_2 gene caused by the occurrence of methylation at RAR β_2 gene promoter.

Expected results of this paper will be able to provide the benefits of open opportunities prevention of breast cancer through epigenetic approach by observing the expression of RAR β_2 gene. This paper is directly generate a theory about patomekanisme breast cancer through a mechanism based on the theory of epigenetic silencing.

General concept of carcinogenesis

The process of carcinogenesis through several phases starting from the initiation, promotion, progression to invasive and metatasis occur. In normal circumstances the growth and cell differentiation regulated by protooncogene (growth promoting gens) that play a role in various aspects of cell proliferation and differention on including the ras gene, myc, c-ERB. On the other hand it's growth is strictly controlled by the anti-oncogenes (tumor suppressor gens), P53 and several other genes that function inhibits growth. In addition to control oncogenes and anti- protooncogene, a cell is controlled also by the mechanism of programmed cell death (apoptosis) that is aimed at getting rid of cells that had not desired.[4]

Research in molecular biology, many researchers have revealed that mutations or over-activity of oncogenes and or inactivation of suppressor genes and mutations can lead to uncontrolled cell proliferation of one or more DNA strands increased (amplification) or loss or translocation of reciprocal and non reciprocal so that DNA recombination occurs is wrong. Mutations of this gene cause cells to function abnormal.[5,6]

The process of carcinogenesis takes place through several stages, where early stage is the initiation process is a process that involves the target cells by stimuli that cause growth carsinogen gene that is irreversible. Cells that have not been initiated before the change can be detected phenotype. The second process is the promotion process, for example someone who is constantly smoking or chronic viral infection. Furthermore, the progression is the occurrence of mutations activating mutations of oncogenes or inactivation of tumor suppressor genes. while the tumor suppressor genes include the gene P 53, the gene PRb.

Retenoat

Retenoat is a group of materials that are structurally and functionally analogous to vitamin A. There are 3 forms of Vitamin A, one of which is still not widely studied is the retinoid acid. Retinoids are a mediator appropriate marker for embryonic morphogenesis, cell growth and differentiation. The use of retinoids as a tumor suppressor has been evaluated in several animal models of carcinogenesis. Models include skin cancer, breast cancer, cancer of the oral cavity, lung, liver, gastrointestinal tract, prostate and bladder. Clinical reports prove retenoid premalignant lesions can heal and inhibits tumor primer progression.[7]

Regulate growth and differentiation in normal cells, premalignan or malignant cells, retenoid stimulate, either directly or indirectly, a receptor found in the nucleus so that the resulting retenoid genes expression. Receptor in question, among others retenoat acid receptors (RARS) and Receptor x Retenoid (RXRS). Receptor is a transcription factor and is a member of the steroid hormone receptor superfamily. To work, these receptors must be activated by ligand. Retenoid receptor subtype is composed of three subtypes α, β, and χ. In activating the transcription function retenoat, must bind to the RAR / RAR heterodimers or RXR homodimer must bind to promoter regions of target genes in the region that responds retenoat acid. One target is the RAR β_2 receptor gene in chromosome 3p24 to mapping on (at most 45%) which is the area most delete heterozygosity (LOH), from primary breast tumors. [8,9]

In cell lines, breast tumors RAR β_2 can inhibit cell proliferation is concerned. In addition it has been reported that RAR β_2 can reduce or suppress the growth of some malignant tumors such as lung carcinoma, squamous cell carcinoma of the head and neck region and breast cancer. This discovery proves that the RAR β_2 have an important role in inhibiting the growth of several types of cells and eliminate regulatory activity tumorgenesis. To answer why the RAR β_2 can suppress and even eliminate tumors maglinan, then the effort has been made to identify possible changes that occur directly from occupational RAR β_2 promoter in addition to the factors that regulate the mechanisms. A recent report suggests that breast cancer showed methylation in the promoter region of RAR β_2 .[9,10]

Retinoids Acid Receptor β2 gene

Several studies have shown that the observation of the RAR β_2 gene is the RAR β_2 mRNA. Reduced expression of RAR β_2 was observed in several solid tumors such as lung carcinoma, squamous cell carcinoma and breast cancer. The evidence supports the hypothesis that RAR β_2 gene is a tumor suppressor gene but the mechanism of suppressor RAR β_2 on other targets have not been knowed.[11,12,13]

RAR β_2 gene promoter including the RARE motif can be activated by RAR / retinoic X receptor (RXR) heterodimers. In breast cancer cell line retinoic not able to induce gene expression of RAR β_2 . although the tumor cells are able activated β2 RARE. This fact supports the concept expressed endogenous receptors contain a mutation or polymorphism that description in function. Other studies have also found no mutations in the promoter region of hela cervical carcinoma cells that lack the RAR β_2 promoter region appear to be RAR β_2 is not a target of some cancer mutations that predicted the existence of another mechanism for suppression of RAR β_2 need to be considered. Gote et al, (1998) first said that in the Cell line colon cancer, methylation may be a response

mechanism due to lack of RAR β_2 gene expression. These data suggest that epigenetic silencing of genes RARβ_2 promoter. thought to be important in breast cancer incidence and mechanisms of epigenetic silencing associated with methylation of RAR β_2 gene promoter.[2,12,14]

The discovery of RAR β_2 and LOH (Lost of heterogeneity) in chromosomes 3p24 is common in cases of breast cancer. It is like the cancer of the esophagus showed a weak correlation between the expression of retinoic acid receptor β_2 and LOH located on chromosomes 3p24.

Research shows that the RAR β_2 promoter region methylation associated with gene silencing and epigenetic mechanisms of gene silencing caused biallele RAR β_2 inactivation can be eliminated by demetilasi agents. Knowledge of epigenetic changes at RAR β_2 have implications on cancer prevention and therapy. Business speculation demetilasi agents have a role in the prevention of cancer or someone who is at risk occurs RAR β_2 promoter methylation that is characteristic of early neoplastic changes. Thus, the RAR β_2 methylation status in primary breast cancer is useful to identify tumors that respond to retinoic acid Terapi.[17,18]

The purpose of this paper is to reveal the mechanism of action benefits of RAR β_2 in activated or reduce the function of RAR β_2. on the occurrence of breast cancer with the occurrence of methylation of RAR β_2 promoter region.

PROCEDURES AND METHODOLOGY

Methodology

To achieve goals and solve problems in this paper uses research design true experimental descriptive and observational in vitro.

The steps undertaken in the study were:

Cancer cells obtained from patients with breast cancer and control normal breast grown in tissue culture. In cancerous tissue culture testing is done about the loss of heterogeneity on chromosome 3P24 and the loss of RAR β_2 gene and also analyzed the presence of RAR β_2 methylation promoter.

Materials research and for management

Culture Human female breast cancer cell obtained from the Laboratory of Surgery Saiful Anwar General Hospital in Malang has been successfully collected. Two tumor samples breast cancer and two normal breast and also peripheral blood both was collected in biomedical laboratories in Faculty of Medicine Brawijaya University Malang.

The samples obtained are stored in a room-temperature -70°C. To confirm the diagnosis then the sample histologist examined by a pathologist. From the examination 90% of samples confirmed as suffering from a breast cancer.

The loss of RAR β2 on chromosome 3P24

To identify the loss or decrease of DNA on chromosome 3P24 RAR β_2 in the blood and tissues of breast cancer patients using the PCR technique using primer RAR β_2 , forward and reverse on chromosome 3P24 with a primary series of exon-5 with the sequence 5'-ATC GAT GCC AAT ACT GTC GA-3 'with position 358 034, followed by electrophoresis and confirmed by examination immunositokimia.[12,19]

Determination of DNA Methylation of the RAR β_2 promoter

To determine the presence of the RAR β_2 promoter methylation in blood and tissues of breast cancer patients using the PCR technique by assembling the DNA methylation-F primer RAR β2 promoter with the sequence 5'-TGC TCA ACG TGA GCC AGG A-3 'position with the primary methylation R 358 749 with sequence 5'-AGG GCT CTT CGG CCA ATC CA-3 'position 358 033 followed by electrophoresis technique reinforced by examination of in situ hybridization technique chromogenic using RAR promoter β_2 .[12,19]

The research results without performing Data Analysis

This paper reveals epigenetic theory on breast cancer. Therefore used a descriptive survey research design, purely descriptive and experimental post test only control group design in vitro without statistical analysis and without random sample without using a sample size, the expected results are only the presence or absence (loss) RAR β_2 in breast cancer and the occurrence methylation process.

RESULTS AND DISCUSSION

This paper describes the results achieved and that the results obtained from the blood and tissues of breast cancer patients get the results of the loss of RAR β_2 on chromosome 3P24. The results obtained by using PCR technique followed by electrophoresis on breast cancer cells. Shown in Figure 1 the results of electrophoresis on a thin line to 6 and 8 on the line to disappear, by using PCR primer 3P24 be thinning and loss of the tape on the blood and tissues of breast cancer on chromosome 3P24, at position 1000 Bp, Bp 1100, 1300 Bp and 1900 Bp (Fig. 1).

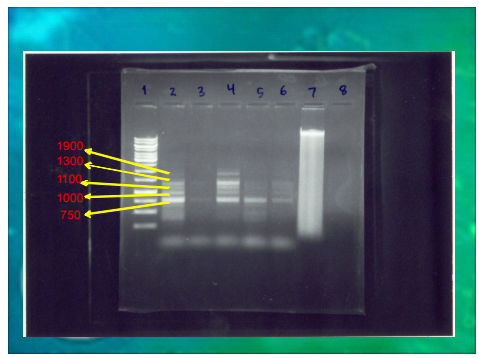


Figure 1: The decline and loss of RAR β_2 band DNA on chromosome 3p24 on line 2, 6 and 8.
Show result 1000 Bp, Bp 1100, 1300 Bp and 1900 Bp
Description:

1. Marker 1 Kb Ladder
2. PCR BN-A (normal control of blood)
3. BCA-R1 PCR (blood cancer)
4. PCR BN-D (control blood)
5. PCR TN (normal tissue control)
6. PCR TCA (cancerous tissue)
7. TN DNA (normal tissue control)
8. DNA TCA (cancerous tissue)

Reinforced by examination immunositokimia. Visible image of the RAR β_2 decrease expression of grade III (Figure 2) and compared with grade IV (Fig. 3) on cytology of breast cancer cells.

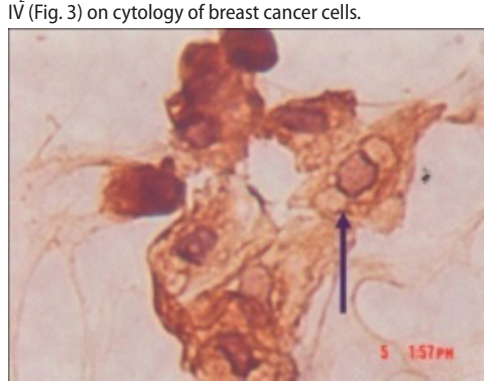


Figure 2: Decreased expression of RAR β_2 in breast cancer cell cytology
grade 3 looks brown color thinned with immunositokimia staining (p 900x)

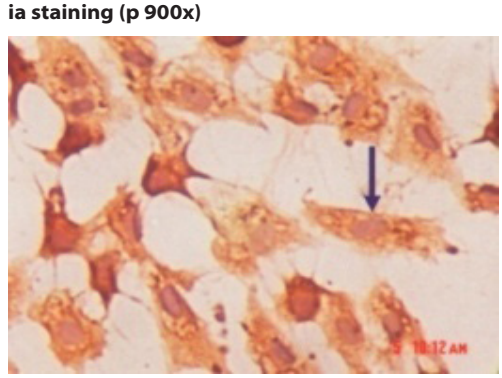


Figure 3: Decreased expression of RAR β_2 in breast cancer cell cytology

grade 4 appear thinner brown color with immunositokimia staining (p 900x)

At the next stage we get the expression of RAR β_2 promoter DNA methylation in blood and tissues of patients with breast cancer in line to the 2 to 3 with the band 550 kb in the blood and tissue and normal tissue located on line 4 and 5. These results are performed using primer PCR technique 3P24 at RAR β_2 promoter methylation, DNA methylation on expression seemed RAR β_2 promoter. Results of blood and tissue of breast cancer patients compared with blood and normal tissues (Figure 4).

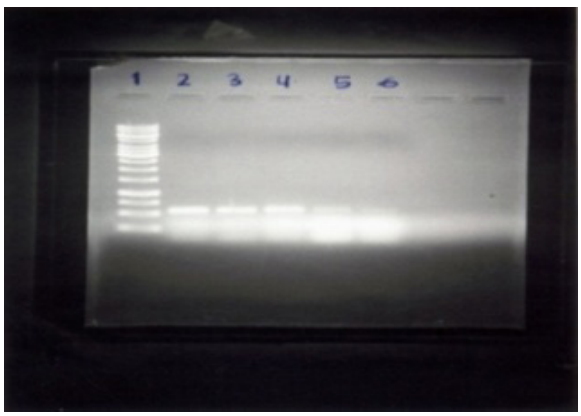


Figure 4 : The results of blood DNA methylation detection RAR β2 promoter gene by PCR followed by electrophoresis show the band 550 bp

Description:

1. Marker
2. DNA of breast cancer tissue
3. Lymphocyte blood cell DNA of breast cancer
4. Normal breast tissue DNA of normal / healthy
5. Blood lymphocyte DNA normal / healthy

These results were confirmed by examination with chromogenik situ hybridization technique using a primary antibody RAR β_2 promoter methylation in breast cancer cells. Expression results were obtained with DAB chromogen in brown on breast cancer cells that have RAR β_2 methylation, some cancer cells appear blue without RAR β_2 promoter methylation (Fig. 5).



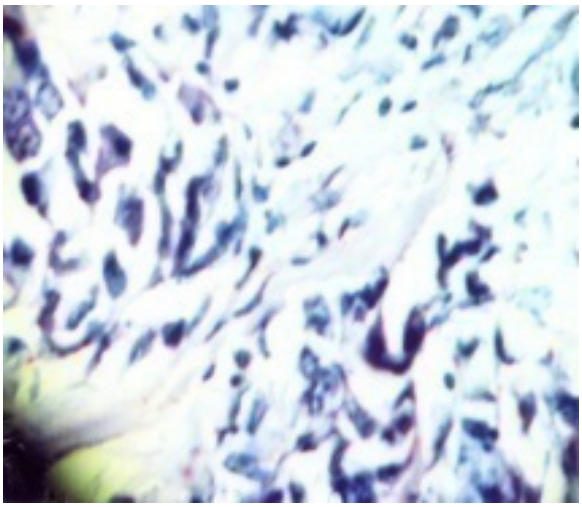


Figure 5 : Results of RAR β_2 promoter in breast cancer mammary infiltrating ductal carcinoma with CISH technique show expression of RAR β_2 methylation in brown colour of methylated RAR β_2 breast cancer (p 900x)

DISCUSSION

Results of research has been conducted on stage I get the results that there is a decrease in breast cancer as well as the disappearance of expression of retinoic acid receptor β_2 (RAR β2) on chromosome 3P24 in patients with breast cancer. The results are consistent with the findings of Hong, 1990 and P. Card et al.1999 who say there is a role of RAR β_2 on the growth of breast cancer, cancer cells squamus, blood cancer and neck.12,20 This study also obtain results that RAR β_2 in the mapped on chromosome 3p24 which is a region on chromosome locus showed the greatest role in the loss heterogenity of breast cancer.

Retinoic acid acts as a tumor suppressor that regulates cell growth and differentiation of pre-malignant and can directly stimulate the receptors in the nucleus genes associated with retinoid acid β type sub type that generally bind to the promoter region. The results showed in breast cancer cells, RAR β_2 disappeared or decreased from grade 3 compared with grade 4 (Figure 2 and 3).7

In the next stage of research, we also get the results that the blood and tissues of patients with breast cancer occur RAR β_2 promoter methylation process, it is evidenced by the expression of DNA methylation in 550Bp. It is similar to the results obtained by Keene et al. Widshwender and Muliartha et al, who said that the process of methylation of the promoter region of RAR β_2 causes in the activation of RAR β_2 gene, so that the function of tumor suppressor genes resulting in decreased, and also be proliferation process of carsinogenesis.[9,13,19]

CONCLUSION

The results show that in this descriptive survey study, found that in the early stages of breast cancer cells occur disappearance and declining RAR β_2 on chromosome 3P24 on the band's 1000 bp, 1100 bp, 1300 bp and 1900 bp as well as testing the technique immunohistochemistry be thinning the color brown on breast cancer cells compared with grade 3 grade 4.

In the next phase of this study also obtained results on two samples of breast cancer cells is a process of methylation RAR β_2 promoter with the expression of DNA methylation at 550 Bp.

ADVICE

For the completion of this study are expected to be continued by adding criteria sample criteria inclution and exclution, sample size and appropriate data analysis for this study.

ACKNOWLEDGEMENTS

The research team expressed his gratitude to the head of the project with an intensive program of basic 39/RD/Insentif/PPK/I/2007 numbers that have been approved and gratitude to the study.

REFERENCES

1. P. card E. Segun C monhaven N Rochette Eg. Ciat gobe boreilv J martinel Y Martinel N vignaud JM, 1999, Expression of retinoid receptor genes proteins in non small lung cancer C. Nati Cancer insr 91 059 – 1066 | 2. Gote S sinrett D Momparier RL. 1998, Demmethylation by 5-aza 2 deoxy cytidine at specific 5-methylcytosine s les in the promoter region ct the retinoid acid receptor beta gene in human colon carsinoma cell antitumor drugs 9 743 – 750 | 3. Mikko O. Hitunen, Yla-Hertuala S. 2003 DNA Methylation, Smooth Muscle Cells, and Atherogenesis, Atherioscler Thromb Vasc Bio. Publication American Heart Association Inc. P. 1950-1753. | 4. Cotran, Ramzis, Vinay Kumar, Tuccercoln. 1999. Breast Cancer, Pathologic Basic of Disease, WB Saunders, International Edition, Four Edition, Page 192-200. | 5. Havieb B Rochette Egiy C Bradey WE. 1998,Tumor suppressor effect of retinoic acib by human epidernoid in cancer cel Bros Natt Acad Sci USA, 90 985-989 | 6. Oregonstate. 2003. Dandelion (online) (http://oregonstate.Edu. Diakses 15 Desember 2004. | 7. Deng G. Luy Zionikov G. tor AD Smith AS. 1995. Loss of heterozygosity a normal tissue adjacert to breast carsinoma, science 1995 274 20572059 | 8. Evans TRJ. Kayes. B,1999, Retinoids present role and future BRJ Cancer, 80, 1 – 6. | 9. Kane MF, Loda M, Gaida GM Lipmann J, Mishra, Goldman – fassud JM Kolodner R, 1997,Methylation of the aMLH promoter correates with lack of expression of VLH a sporadic colon tumor and smatch rapa detective humar tumor cell cancer res 57 5556 – 5560 tanggal 15 Desember 2004) | 10. Sporn MB Goodman Chemoprevention of cancer with retinoid Fed Proc 1979 38 2528 – 2534. | 11. Bradshaw, Chad. 2004. Dandelion. (Online),(http:/www.Geocities.com/chadrx /dande.html,diakses tanggal 17 November 2004) | 12. Qi Feng, Young, Chiromori, Liangshon, Misanakamura, Nakamura Y, Utsunomia H, Yoshimura G, Sucumo T, Tamati T, Umemura T, Sakukae T, and Kenichi K. 2001. Bilalelic in Activation of Retinoid Acid Reseptor β2 Gen by Epigenetik Chante in Breast Cancer, American Journal of Pathology, Further Reproduction Publisher vol. 158, P.299-303 | 13. Widshwender M. Berger J Hermann M Mulier HM Amperger A Zeschn g M Widshwender A Abenstein B Zaimet AG Daxenbichler G marth C, 2000, Methylation and silencing of the retinoic acid receptor beta 2 bene in breast cancer J Natt Cancer Ir st 92 826 532 | 14. Doerfler. 2005. Denovo Methylation Long Term Promotor Silencing, Methylation Pattern in Human Gen and Concequences of Foreign DNA Insertion DNA Methylation, basic Mechanism. Springer Verleg Berlin Heidenberg P. 127-146. | 15. Heidifiegl, Milinger S., Goebell G,, Muller E., Heaznewr, Mark C., Petter W, Laird and Martin, Watchwenter. 2006.Breast Canceer DNA Methylation Profile in Cancer cell ant Tumor Stroma,Association with HER2/New Status in Primary Breast Cancer, Cancer-est,AmericanAssociation for Cancer Research, P. 29-32. | 16. Hong W. K Lippman SM tr LM, Karp DD, Lee JS. Byers RM Schuntz SS Kramer AM. Lotar Peters LL. Dimery TW. Brown BW Goeptert H, 1990, Prevention of second primary tumors with isotrenoid in squamus cell carcinoma of the head neck and NL. J. Med, 323 – 795. | 17. Ferguson AT, Nass SJ. 2001. DNA Methylation and Breast Cancer Current Genomic 2000, Benthar Science Publisher Ltd. P.48-53. | 18. Young X, Young L and Davidson NE. 2001. DNA Methylation in Breast Cancer Endocrine Related Cancer P.115-127. | 19. Muliartha. Wibi Riawan, Fitri Arrmenia, Asnah. 2009. Pengaruh ekstrak akar Taraxacum Officinale untuk menekan pertumbuhan kanker payudara. Laporan Akhir Penelitian Riset Dasar. Lemlit 1-20. | 20. Jones Lamen. 2002. Molecular Pathology an In Situ Hibridization in Histological Technic Theory and adireclty by John D. Bancroft Marlyn Gamble. Fifth Edition. Harcourt Publishers Limited 2002. Churchill Living Storm. P. 553-575.