International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Relationship of P-glycoprotein (Pgp) Expression and Estrogen Receptor (ER) Expression in Invasive Ductal Carcinoma of Breast Cancer

Christian B[a]*, Daniel Sampepajung[b], Prihantono[c], Berti Nelwan[d]

[a,b,c]*Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[d]*Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[a]*Email : Chris77@yahoo.com*

Abstract

Increased of estrogen receptor (ER) expression resulting in activation of downstream signaling pathways including cAMP/PKA, MAPK/ERK and PI3K/AKT. The Association of estrogen receptor expression and P-glycoprotein 1 (P-gp) expression is not clear yet. The aims of this study is to explore the influence of signaling cascades of estrogen receptors and P-gp expression. This is an observational study with cross sectional design. Total 55 samples of invasive ductal carcinoma acquired for Immunohistochemistry examination. Analysis of ER and P-gp using chi square. This study found proportion of ER expression were 35/ 55 (63.6 %), and P-gp expression were 10/55 (18.2%) in invasive ductal carcinoma of breast cancer. Co-expression of ER and P-gp expression were 17.1%. There was no significant association between expression of ER and P-gp with *p value*=0.076 (p>0.05), OR(95%CI) 1.2(0.21-5.99). This results suggest that ER does not play a significant role in the expression of P-gp in breast cancer.

Keywords: Immunohistochemistry; ER ; P-gp ; invasive ductal carcinoma; breast cancer.

---

* Corresponding author.

1. Introduction

The main problem in the treatment of breast cancer is the development of resistance to various chemotherapy Agents. P-glycoprotein 1 (permeability glycoprotein, abbreviated as P-gp or Pgp) also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B member 1 (ABCB1) or cluster of differentiation 243 (CD243) is an important protein of the cell membrane that pumps many foreign substances out of cells. More formally, it is an ATP-dependent efflux pump with broad substrate specificity. Although the role of multidrug resistance 1 (MDR1) gene in inducing drug resistance in cancer has been studied clinically but expression in breast cancer is unclear [1]. Mechanisms of chemotherapy resistance can occur intrinsically or acquired through multiple mechanisms [2]. Resistance to chemotherapy in breast cancer is involving of estrogen receptor (ER) expression and permeability glycoprotein (P-gp), causing the efflux of various chemotherapeutic agents [3]. Research on the contribution of ER in resistance to chemotherapy involving P-gp or MDR1 is still controversy [4,5]. The aims of this study is to explore the influence of signaling cascades of estrogen receptors to P-gp expression.

2. Materials and Method

2.1. Collection of Samples

This is an observational study with cross sectional methods. Study was conducted within a population of breast cancer patients who had been diagnosed through clinical and histopathology examination, which entered the Wahidin Sudiro Husodo Hospital in Makassar, South Sulawesi, Indonesia, starting from July 2015 to September 2016. Inclusion criteria are locally advanced breast cancer female patient and invasive ductal breast cancer. Exclusion criteria were inadequate tissue samples for immunohistochemistry and bilateral breast cancer. All samples who fulfilled inclusion and exclusion criteria and willing to participate in the study and signing informed consent recruited as study samples.

2.2. Laboratory Procedures

Acquired samples from incisional biopsy of breast cancer patients treated in Wahidin Sudirohusodo hospital for paraffin block and immunohistochemistry examination of Estrogen Receptors and P-gp. Each paraffin block was cut with a microtome size of 4 microns and placed on an object glass. Immunohistochemistry staining using standard techniques. From each paraffin blocks, subject were stained with hematoxylin eosin for histopathological examination, and staining for estrogen receptors and P-gp. Subject then examined to determine the type of cancer and determination ER and P-gp expression.

2.3. Immunohistochemistry Interpretation

Expresion of ER and P-gp is the accumulation of proteins in the membrane and cytoplasms of cells, detected by Immunohistochemistry methods were then expressed positive when using a light microscope, it would look brown on the location of the antigen to be detected. This expression is calculated using a scoring system based on the intensity of color and proportion of epithelial cells stained.

2.4. Data Analysis

Data analysis using the SPSS (Statistical Package for Social Science) version 22. Chi square analysis were used to assess the relationship of ER and P-gp expression.

2.5. Ethical Clearence

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Number; 581/H4.8.4.5.31/PP36-KOMETIK/2015.

3. Results

We recruited 55 patients of invasive ductal carcinoma during July 2015 to September 2016, originated from Wahidin Sudirohusodo Hospital.

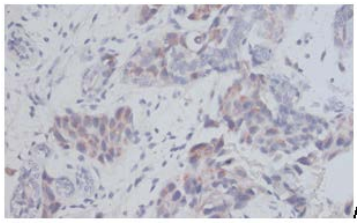

Figure 1: Microphotographs of ER immunostaining in invasive ductal carcinoma. Positive Results in 400 x

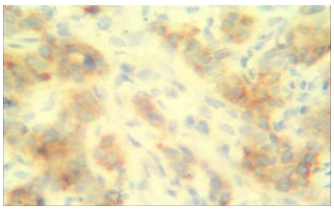

Figure2: Microphotographsof Pgp immunostaining in invasive ductal carcinoma. Positive Results in 400 x

Table 1: Characteristics of Samples

Characteristics		N	%
Age	< 50	33	60
	≥ 50	22	40
Grading	Low	9	16
	Moderate	32	58
	High	14	26
ER expression	Positive	35	63.6
	Negative	20	36.4
PR expression	Positive	24	44
	Negative	31	56
HER expression	Positive	19	35
	Negative	36	65
P-gp expression	Positive	10	18.2
	Negative	45	91.

Youngest age of subject was 29 years and the oldest was 75 years old, 60% in the age group < 50 years. Low grade 9(16%), Moderate grade 32(58%) and in High grade 26(26%). Results of immunohistochemistry examination; ER+ showed in 35 (63.6%) samples, PR + in 24 (44%), HER2 + in 19(35%) while P-gp (+) as much as 10 (18.2%) samples.

Table 2: Relationship of ER expression and PgP expression

ER expression	P-gp Expression		Total
	Positive (%)	Negative (%)	
Positive	6 (17.1)	29(82,9)	35 (100)
Negative	4(20)	16(80)	20(100)
Total	10(37.1)	45(62.9)	55(100)

p value=0.076 (p>0.05), OR(95%CI) 1.2(0.21-5.99)

In chi square analysis, there is no significant relationship between ER and Pgp expression with $p = 0.076$ ($p>0.05$). It means that ER expression is less associated with Pgp expression in invasive ductal carcinoma.

4. Discussion

Studies revealed that drug-resistant influenced by Multidrug Resistance gene 1 (MDR1) in association with over-expression of P-gp, which is a member of the ATP-binding cassette (ABC) transporter which serves as pumps out a variety of chemotherapy agents such as anthracyclines and taxanes [6]. The expression of P-gp encoded by the gene Multidrug-resistant (MDR) located at kromosum 7q2.1.1 7q2.1.1 [7], comprises 28 exons that encode a protein of 1280 amino acids containing two ATP-biding site and two transmembrane domains that work as energy dependent pump, P-gp is actively pumping out a particular group of cells chemotherapeutic agents [8,9]. comprises 28 exons that encode a protein of 1280 amino acids containing two ATP-biding site and two transmembrane domains that work as energy dependent pump, P-gp is actively pumping out a particular group of cells chemotherapeutic agents[10]. While other studies have shown a positive expression of P-glycoprotein prior chemotherapy by 52% [11]. Some P-gp expression research report that the percentage of P-gp expression increased post chemotherapy [12,13].

This study found the proportion of positive expression of P-gp by 6 (17.1%) in invasive ductal breast carcinoma expressing ER (+) 35 samples. This indicates that ER + contribute to the expression of P-gp.

Relations ER and P-gp / MDR1 proven through many research, ER activates transcription of MDR1 in MCF-7 breast cancer cells by binding to ERE 1/2 and interacting with AP-1 element CG-Rich in MDR1 promoter [15,

16]. The transcription factor AP-1 has been identified in the promoter region of the gene MDR1 [17, 18]. ER involvement on the expression of P-gp may also through the activation of signaling pathways PI3K / AKT indicating convergent point, the deviation of this pathway led to increased expression of P-gp / MDR1 and resistance to chemotherapy [19]. Similarly, activation of MAPK / ERK significantly increased the expression of P-gp / MDR1 (Tomiyasu and his colleagues 2013). In addition, non-genomic pathways may also be activated by a heat shock protein, Platelet-derived growth factor (FDGF) and Epidermal growth factor (EGF) [20].

Similarly, the expression of P-gp in breast carcinoma with expression of ER (-) indicates that the expression of P-gp not only depend on the ER (+), but the expression of P-gp also affected by overexpression of signal cascades such as stimulation factor of epidermal growth (EGF), overexpression of HRAS, c-Raf, MEK1 / 2, ERK1 / 2 also increased expression of P-gp [21].

P-gp expression in this study may reflect the MDR1 gene expression as an intrinsic resistance in invasive ductal carcinoma of the breast. MDR intrinsic (primary) mediated by overexpression of MDR1 / P-gp was MDR acquired (secondary) induced by a variety of cytotoxic agents, UV radasi, heat shock [22,23].

The limitations of this study were; Neoadjuvant chemotherapy is only done three cycles, which aims to downsizing until the tumor becomes resectable, so that in this study no cases were found to achieve pCR (pathological complete response). Measurement of dependent variable were using the manual method by using a caliper.

5. Conclusion

Results of this study suggest that ER does not play a significant role in the expression of P-gp in invasive ductal carcinoma of breast cancer. Based on this study, it can be suggested for further study of P-gp expression in breast cancer.

Acknowledgments

We give our gratitude for all breast cancer patients that have participated in this study.

6. Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

[1] Taheri, M. and F. Mahjoubi, MRP1 but not MDR1 is associated with response to neoadjuvant chemotherapy in breast cancer patients. Dis Markers, 2013. **34**(6): p. 387-93.

[2] Fan, W. and M. Sui, Roles and Mechanisms of Estrogen and Estrogen Receptors in Breast Cancer Resistant to Chemotherapy. 2011.

[3] Martin, H.L., Smith, L. & Tomlinson, D. , Multidrug-resistant breast cancer: current perspectives. 2014. .

[4] Zampieri, L., et al., Differential modulation by estradiol of P-glycoprotein drug resistance protein expression in cultured MCF7 and T47D breast cancer cells. Anticancer research, 2001. **22**(4): p. 2253-2259.

[5] Mutoh, K., et al., Estrogen-mediated post transcriptional down-regulation of P-glycoprotein in MDR1-transduced human breast cancer cells. Cancer science, 2006. **97**(11): p. 1198-1204.

[6] Hamidovic, A., Hahn, K. & Kolesar, J. , Clinical significance of ABCB1 genotyping in oncology. Journal of Oncology Pharmacy Practice, , 2010. . **16,** : p. 39-44.

[7] Fan, W.S., M. , Roles and Mechanisms of Estrogen and Estrogen Receptors in Breast Cancer Resistant to Chemotherapy. 2011. .

[8] Fairchild, C.R., et al., Isolation of amplified and overexpressed DNA sequences from adriamycin-resistant human breast cancer cells. Cancer research, 1987. **47**(19): p. 5141-5148.

[9] Singh, J.P., et al., Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer-a prospective clinical study. World journal of surgical oncology, 2005. **3**(1): p. 61.

[10] Gottesman, M.M., T. Fojo, and S.E. Bates, Multidrug resistance in cancer: role of ATP–dependent transporters. Nature Reviews Cancer, 2002. **2**(1): p. 48-58.

[11] Singh, J.P., Mittal, M. K., Saxena, S., Bansal, A., Bhatia, A. & Kulshreshtha, P. , Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer-a prospective clinical study. . World journal of surgical oncology, , 2005. . **3,**: p. 61.

[12] Chevillard, S., et al., Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. Cancer, 1996. **77**(2): p. 292-300.

[13] Chung, H.C., et al., P-glycoprotein: the intermediate end point of drug response to induction chemotherapy in locally advanced breast cancer. Breast cancer research and treatment, 1997. **42**(1): p. 65-72.

[14] Shi, J.F., et al., ERalpha directly activated the MDR1 transcription to increase paclitaxel-resistance of ERalpha-positive breast cancer cells in vitro and in vivo. Int J Biochem Cell Biol, 2014. **53**: p. 35-45.

[15] Mutoh, K., Tsukahara, S., Mitsuhashi, J., Katayama, K. & Sugimoto, Y. , Estrogen-mediated post

transcriptional down-regulation of P-glycoprotein in MDR1-transduced human breast cancer cells. . Cancer science, , 2006. . **97,** : p. 1198-1204.

[16] Chen, Q., Y. Bian, and S. Zeng, Involvement of AP-1 and NF-κB in the Up-regulation of P-gp in Vinblastine Resistant Caco-2 Cells. Drug metabolism and pharmacokinetics, 2013.

[17] Martin, H.L., L. Smith, and D. Tomlinson, Multidrug-resistant breast cancer: current perspectives. 2014.

[18] Tomiyasu, H., et al., Regulation of expression of ABCB1 and LRP genes by mitogen-activated protein kinase/extracellular signal-regulated kinase pathway and its role in generation of side population cells in canine lymphoma cell lines. Leukemia & lymphoma, 2013. **54**(6): p. 1309-1315.

[19] kYang JM, V.A., Hait WN, Activation of phospholipase C induces Raf-MAPK pathway. . Mol Pharmacol 2001 **60**: p. 674-680.

[20] Katayama, K., et al., Inhibition of the mitogen-activated protein kinase pathway results in the down-regulation of P-glycoprotein. Molecular cancer therapeutics, 2007. **6**(7): p. 2092-2102.

[21] Lage, H., Drug resistance in breast cancer. Cancer Therapy, 2003. **1**: p. 81-91.

[22] Zhang, Z., et al., Evaluating the response of neoadjuvant chemotherapy for treatment of breast cancer: are tumor biomarkers and dynamic contrast enhanced MR images useful predictive tools? Journal of thoracic disease, 2014. **6**(6): p. 785.

[23] Tanei, T., et al., Prognostic significance of Ki67 index after neoadjuvant chemotherapy in breast cancer. European Journal of Surgical Oncology (EJSO), 2011. **37**(2): p. 155-161.