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HER-2/NEU RECEPTOR GENE ISOLATION STUDY FROM INDONESIAN BREAST CANCER PATIENTS TISSUE

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ABSTRACT

Breast cancer is a deadly disease, occupied as first ranking among woman patients cancer in Indonesia (the Hospital Information System, 2007). 20-30% of total breast cancer patients having HER-2/neu overexpression. Unfortunately the problem of resistance to monoclonal antibody treatment, trastuzumab, was found. There are several theories that suspected as the cause of trastuzumab resistence such as polimorphisme, receptor dysfunction and so on. This research was conducted to study and to isolate of the HER-2/neu receptor gene in breast cancer patients Indonesia. The successful of the HER-2/neu receptor gene issolation is an important stage for the study and analysis of HER-2/neu receptor in Indonesia. Research methods were breast cancer tissue collection from Dharmais Cancer Hospital and M. Djamil Hospital Padang, RNA isolation, cDNA, PCR, sequencing. Of the total 110 samples, 20 samples of breast cancer patients identified as HER-2/neu+3 through immunohistochemistry (IHC) screening. Of the 20 samples,4 DNA fragments (2127bp) have successed isolated. This DNA fragment sequenced, then identified as a gene encoding HER-2/neu receptor using BLAST. Due to the variation of HER-2/neu overexpression in the tissue sample, while the part is taken as a source of RNA isolation is only a little of it, the possibility to use of non HER-2/neu overexpression part had a large, and other reason RNA low stability were also affected to the success of a gene encoding HER-2/neu receptor issolation.

KEY WORDS: **HER-2/neu Recepor gene, Breast Cancer, Trastuzumab, Resistance.**

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INTRODUCTION

Based on data from the Hospital Information System (SIRS) in 2007, breast cancer occupied as first ranking as a deadly disease for women cancer patients in Indonesia, followed by cervical cancer. 20-30% of the total breast cancer patients, having HER-2/neu over expression. HER-2/neu breast cancers are invasive, with a high malignancy rate and shortened survival. HER-2/neu protein is a member of the family of epidermal growth factor receptor (EGFR) consists of an extracellular binding domain, a transmembrane lipophilic segment, and a functional intercellular tyrosine kinase domain[1]. In normal conditions, the HER-2/neu protein together with other EGFR family is required to activate the metabolic trajectory through a process known as signal transduction that will effect to proliferation, survival, motility, adhesion, angiogenesis, migration/invasion and differentiation[2,3]. The tyrosine kinase domains are activated by both homodimerization and heterodimerization. HER-2/neu heterodimers are more stable and their signaling is more potent than receptor combinations without HER-2/neu. HER-2/neu gene amplification and or protein overexpression will be affected to the uncontrolled cell metabolism.

For the detection of HER-2/neu over expression can be done using several ways such as IHC, FISH, PCR and others[2]. IHC, is the method which widely used. There are four score level for HER-2/neu identification with IHC methods. Patients with a score of +3, is declared as the HER-2/neu breast cancer patients. For borderline/weakly positive score of +2 on IHC it is recomendded followed by a FISH assay[3]. One method of medication used for patient's HER-2/neu is a method using a monoclonal antibody, trastuzumab[4]. Trastuzumab targets the HER-2/neu receptor. Trastuzumab works by preventing dimerization, induces an immune response, inducing the process of endocytosis. Unfortunately the resistance problem was found on treatment with trastuzumab. There are several theories that suspected as the cause of trastuzumab resistence such as polimorphisme, receptor dysfunction and so on[5,6]. The research conducted aims to isolate HER-2/neu receptor gene from Indonesia patients withHER-2/neu . The successful isolation of genes encoding HER-2/neu receptor would strongly support further study of the HER-2/neu receptor analysis in Indonesia, especially of trastuzumab resistance problems.

MATERIALS AND METODHS

(i) Primer Design

Primer design was done by multiple alignmant, and analyzed using Vector NTI software from Invitrogen.

(ii) Breast Cancer Tissue Collection

110 breast cancer tissue were collected from years 2008-2012, Dharmais Cancer Hospital National Cancer Center in Jakarta, and M. Djamil hospital in Padang. Screening of tissue HER-2 +3 performed using immunohistochemistry method.

(iii) cDNA synthesis and PCR amplification

Total RNA was extracted using purelink RNA Mini Kit from Ambion catalog no 12183-018A. Then to perform cDNA synthesis and PCR amplification from total RNA was using Superscript III One-Step RT-PCR system with platinum Taq High Fidelity Cat no. 12574-030. PCR products were then confirmed in 1% agarose.

(iv) Sequencing

PCR product sequencing was conducted using ABI machine. Then sequencing results analyzed and identified by BLAST software.

RESULTS AND DISCUSSION

The research was conducted using ethical clearance issued by Ministry of Health Directorate General of Health Efforts Dharmais Cancer Hospital National Cancer

Center. We have managed to collect 110 breast cancer tissue collected (started from 2008), from two hospitals in Indonesia, Dharmais Cancer Hospital National Cancer Center in Jakarta, and M. Djamil hospital in Padang. The fresh tissue samples were stored at -80 ° C or liquid nitrogen. Of the total 110 samples, 20 samples from breast cancer patients identified through immunohistochemistry screening as HER-2/neu IHC +3which will then be used as a source of RNA isolation. For its primary design, processed by using multiple alignmant of HER-2/neu genes from humans and from other mammals. Areas selected for primer design was taken from the high conserve region. PCR products generated from this primer design results were 2127bp, consisting of half of HER-2/neu receptor and its epitop and also include fraction of transmembrane area.

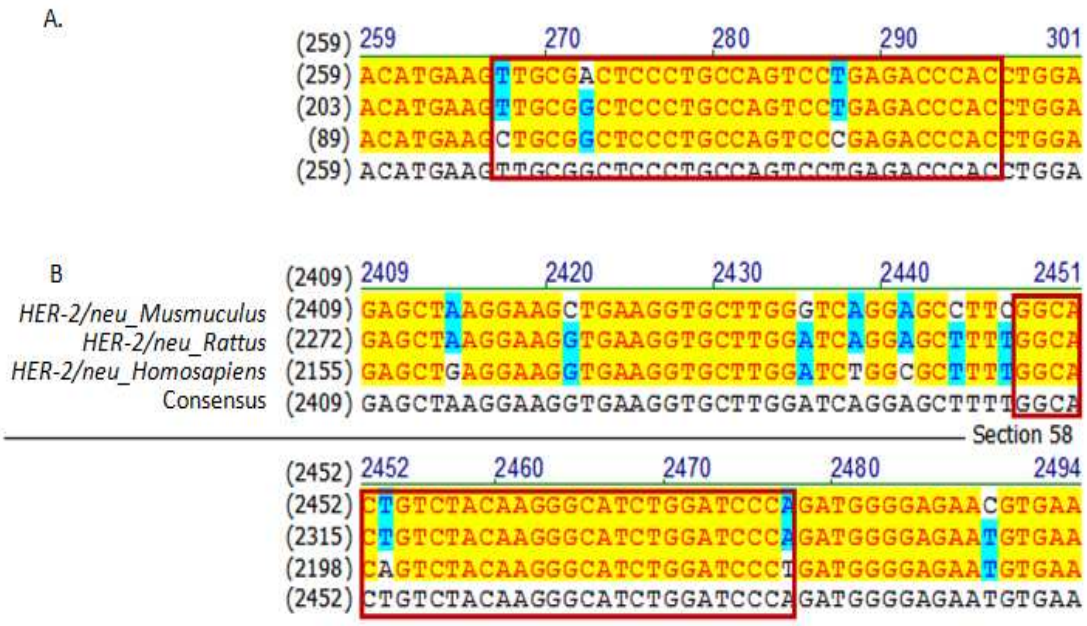

Figure 1

The results of multiple alignment of human HER-2/neu receptor gene with other mammals.A. Area for primer forward orientation. B. Area for primer reverse orientation.

Of the total 20 samples, PCR product with a size of 2127bp DNA bands have been successed isolated from four tissue sample. There were no PCR product generated from negative control, non HER-2/neu overexpression fresh tissue. Some of the isolated HER-2/neu gene receptors can be seen in the figure below.

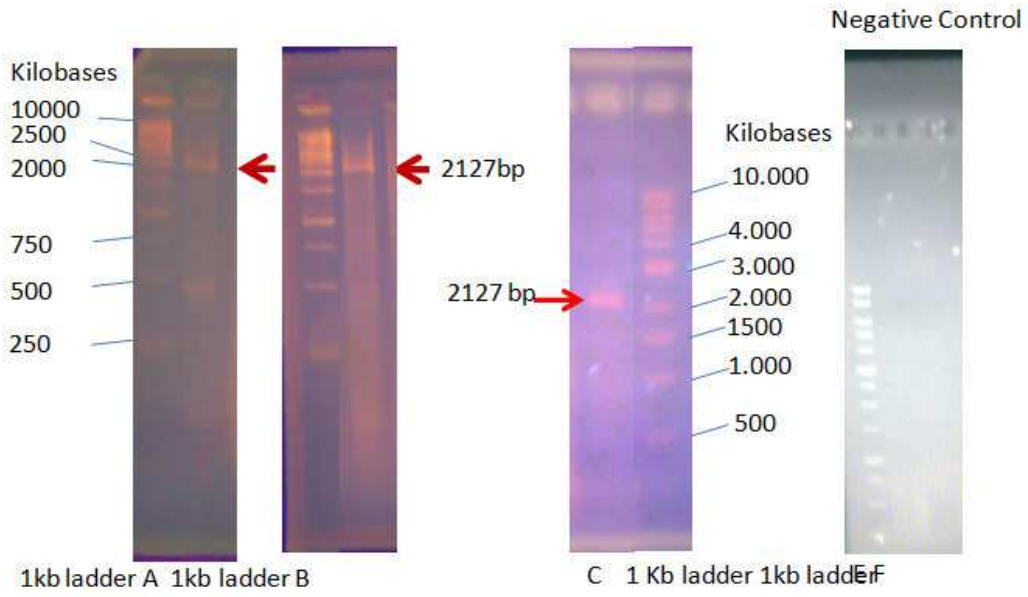

Figure 2
The results of the HER-2/neu receptor gene isolation, 1127bp.

Identification with BLAST, one of sequensed PCR product have showed that these bands are HER-2/neu receptor gene (data was not shown). Its mean isolation of HER-2/neu receptor gene have been successed. Although the genes encoding the HER2 neu receptor from all fresh tissue sample have not been successed isolated yet, but it can be stated that the method used for the isolation of genes has been quite good and has a high reproducibility. As long the breast tissue used as a source of RNA had HER-2/neu overexpression, the isolation of genes would work well, while on the tissue that are not HER2 overexpression, its gene isolation will not be successful as in the negative controls.

Due to the variation of HER-2/neu overexpression in the tissue sample, while the part is taken as a source of RNA isolation is only a little of it, the possibility to use of non HER-2/neu overexpression part had a large. According to Jacobs at al (2000), who studied about HER-2/neu protein expression in breast cancer evaluated by IHC, it is difficult to extrapolate the result of HER-2/neu IHC since of regional variation while the tissue sample to be evaluated were very small[7]. The successful isolation of genes encoding HER-2/neu receptor, will contribute to the successful its analysis from breast cancer HER-2/neu patients in Indonesia especially for trastuzumab resistance studies.

CONCLUSIONS

1. DNA fragments of HER-2/neu, 2127bp, has been isolated. The method used for the isolation of genes has been quite good and has a high reproducibility.
2. The success of the HER-2/neu receptor gene isolation will contribute to the analysis of HER-2/neu receptor in Indonesia.

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