Polymorphism C3435T of The MDR-1 Gene Predict Response to Preoperative Chemotherapy in Locally Advanced Breast Cancer with Her2/neu Expression

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ABSTRACT

Aim: To reveal the role of polymorphism C3435T of MDR-1 gene in the response to preoperative chemotherapy in locally advance breast cancer.

Methods: The analytical observational research in nineteen patients diagnosed between January and December 2005 with locally advanced breast cancer treated by preoperative Anthracycline chemotherapy to evaluate its predictive outcome was performed. On all samples Immunohistochemistry, PCR, and sequencing methodology of the MDR1 target gene were performed.

Results: The polymorphism of MDR1 gene at cDNA position 3435 located in exon 26 has been shown to be correlated with clinical response to Anthracycline chemotherapy in breast cancer patients, without being affected by the positive or negative Her-2 expression. Patient with T/T genotype developed clinical response, while patient with C/T genotype did not develop clinical response.

Conclusion: Breast cancer patients with positive Her-2 expression do not always respond to Anthracycline, it means that only patients with T/T genotype at position 3435 located in exon 26 of MDR1 gene have clinical response, while patients have C/T genotype do not show clinical response. MDR-1 polymorphism C3435T in exon 26 may co-determine resistance to chemotherapy and provide useful information to individual therapy.

Key words: polymorphism C3435T, MDR-1, Her-2, anthracycline, breast cancer.

INTRODUCTION

The MDR1 gene product P-glycoprotein (P-gp) is a member of the ATP-binding cassette transporter family. P-gp utilizes the energy derived from ATP hydrolysis to pump a wide range of compounds, including numerous clinically used drugs, out of cells; this activity has important pharmacokinetic and pharmacodynamic consequences. In addition to the roles of P-gp in absorption, distribution, and elimination, the over-expression of P-gp is implicated in the development of the multi-drug resistance (MDR) phenotype of some tumor cells. Understanding the functional and clinical consequences of MDR1 variants is important—if this variability could be assigned to a mutation in the MDR1 gene, patients could be screened and appropriate dose adjustments could be made on the basis of their MDR1 genotype. Recently, a number of papers have reported the discovery and initial characterization of MDR1 variants; to date, more than 20 mutations in the MDR1 gene have been identified. Until now, however, the functional and clinical consequences of only one common MDR1 variant, C3435T, have been investigated. A polymorphism of MDR1 gene has been shown to be correlated with intestinal P-glycoprotein (P-gp) expression and activity in vivo. This polymorphism consists of a C-to-T exchange at cDNA position 3435 located in exon 26 of the MDR1 gene. Although this base exchange does not affect the amino acid sequence of P-gp, the T allele appears to be associated with markedly lower MDR1 expression compared with the C allele. Because of its silence on the protein level and its location in a non-regulatory region of the MDR1 gene, it is conceivable that this particular polymorphism is not causative for differences in P-gp expression. It is rather likely that this polymorphism is linked to other, as yet unidentified, changes in regions of the MDR1 gene that control expression, e.g., in the promoter/enhancer region or in regions that are relevant for mRNA
processing. Nevertheless, the C3435T polymorphism appears to allow the differentiation of alleles with distinct MDR-1 expression and activity. Therefore, genotyping of the C3435T polymorphism may provide a basis for treating patients more effectively with agents that are substrates of the MDR-1 gene product, e.g., anticancer drugs such as vincristine and doxorubicin.\(^5\) The prevalence of breast cancer in Indonesia nowadays has been unknown definitely. Breast cancer cases each year have a tendency to increase and most of them come at an advanced stage.

This research tries to examine the mechanism of breast cancer resistance, which in the future can be used as the basis of anthracycline in breast cancer patients which expressed Her-2 and related with MDR-1 expression.

**METHODS**

**Patient samples.** Between January and December 2005, nineteen patients diagnosed with locally advanced breast cancer were treated by preoperative anthracycline-based chemotherapy (3-4 cycles). Mean age of the 19 patients that gave their informed consent to the study was 46.5 years (range 20-59) at the time of diagnosis. Tumour size was evaluated by physical examination, ultrasound and mammography at baseline before therapy, and after 3-4 courses of chemotherapy or prior surgery. According to histopathological examination, all tumours were infiltrating ductal carcinomas. Evaluation of response to treatment was based on the recommended response evaluation criteria in solid tumors (RECIST). Clinical tumor response to preoperative chemotherapy was graded as complete (cCR) partial (cPR), or no response (cNR). Patient, who clinically responsive (operable), were operated and patients having no response undergo core biopsy. After that, all samples are performed immunohistochemistry, PCR, and sequencing of MDR1 target gene.

**Immunohistochemical staining.** Nineteen specimens were obtained after chemotherapy. Immunohistochemical staining was performed using the avidin-biotin peroxidase kit (Vector Laboratories, Burlingame, CA). The sections were deparaffinized in xylene and rehydrated in serial ethanol. Endogenous peroxidase activity was blocked with methanol and 0.3% hydrogen peroxide. Slides were then incubated with 1.5% horse serum at 37\(^\circ\)C for 20 minutes, and then incubated with monoclonal primary antibodies at 37\(^\circ\)C for 30 minutes. Murine monoclonal antibody specific for p-glycoprotein, JSB-1 (Sanbio, Am Uden, Holland), was used as the primary antibody. Biotinylated secondary antibody was dropped on all slides, which were then incubated at 37\(^\circ\)C for 30 minutes and washed in phosphate buffered saline (PBS). The slides were then incubated with avidin-biotin complex at room temperature for 30 minutes and washed in PBS. Diaminobenzidine was used as a peroxidase substrate for color development. Multidrug resistant KB-8-5 cells and multidrug sensitive KB-3-1 cells were used as positive and negative controls, respectively. Expression of p-glycoprotein was found in both the cytoplasm and the cell membrane. In cases where there was extremely small number of cells with homogenous and weak cytoplasmic staining, the tumors were categorized as negative. Grades of p-gp expression were arbitrarily determined according to the percentage of positive cells against the total number of cancer cells (+; less than 25% cells, ++; 26-50%, +++; 51-75%, ++++; more than 75%). When p-gp was expressed + until ++, we defined it as negative expression criteria, and when p-gp was expressed ++++, we defined it as positive expression criteria.

**Genotyping.** Genomic DNA was isolated from patient’s tissue samples with a Qiagen DNA Blood Kit and a 106 bp fragment containing the C3435T polymorphism in exon 26 of the MDR-1 gene was amplified with the primer pair 5’-CCTATGGAGACAACAGCC-3’ / 5’-GAGAGACTTACATTAGGCAG-3’. The quality of PCR products was checked by agarose gel electrophoresis. Strands of the PCR product were separated by incubation of the beads with 50 µl 0.2 M NaOH for 1 min followed by two washes with 150µl 10 mM Tris acetate.

**RESULTS**

**Clinical response to preoperative chemotherapy.** Based on RECIST criteria, cCR was not observed but cPR in 14 (74%) resulting in an overall rate of clinical response to preoperative chemotherapy and 5(26%) cNC was observed in the other patients.

**Immunohistochemical Her2 and MDR1.** Based on immunohistochemical, Her2 expression was observed in 11(58%) of patients and 8(42%) of patients was negative Her2 expression.

**Genotype at the polymorphic site C3435T of the MDR-1 gene and response to therapy:** The genotypes of all 19 patients at position C3435 in exon 26 of MDR-1 gene were determined by PCR sequencing methodology. The C/T genotype occurred in 5(26%) of the subjects, the others were homozygous T/T (74%). Most of the patients (74%) with a T/T genotype responded clinically to preoperative chemotherapy, but in the group possessing C/T genotype did not responded
clinically to preoperative chemotherapy. Statistical analysis revealed an insignificant correlation (p=1.000) between clinical response and the T/T genotype.

**DISCUSSION**

The in vivo model of preoperative chemotherapy has proved to be useful to investigate novel therapeutic concepts and evaluate new predictive and prognostic factors. Bonadonna and Fisher et al, clearly demonstrated that response to preoperative chemotherapeutic treatment is an important indicator of both disease-free and overall survival. The identification of new predictor factors for response to preoperative chemotherapy may, therefore, help to define subgroups of patients that most likely benefit from this type of therapeutic approach.

Numerous drug resistance-associated genes have been described but their precise roles in the development of resistance to chemotherapy observed in the different types of human tumors still remain to be elucidated. This is true even for the most extensively studied MDR-1 gene that was first discovered as a factor mediating multi-drug resistance in cancer chemotherapy. Its gene product P-gp as found reduced bioavailability of various compounds at the cellular level. Conferring multi-drug resistance to several types of tumor cells, MDR-1 expression and P-gp function are considered to contribute to cellular even determine cancer patient’s resistance to chemotherapy.6

The initial study by Verelle et al and several other investigations point to an implication of the MDR-1 genes in the manifestation of resistant phenotypes in locally advanced breast cancer treated by preoperative chemotherapy that co-determines the clinical outcome. Furthermore, MDR-1 expression induced by
chemotherapy of locally advanced breast cancer was also found to be an indicator of a worse long-term prognosis. Chevillard et al. assessed MDR-1 expression sequentially during preoperative chemotherapy and also demonstrated a significant correlation with response to treatment. If we take a good look, some theory that says that mdr-1 expression is one of the factors that influence the chemotherapy resistance, is yet to be proven in this research. It is still unknown, whether this clinical response is caused by other factor, such as mdr-1 expression level that is encoded by MDR-1 gene, that might have an ability to determine which one is recognized matters or not and then being transported more effectively.

For the last 2 decades, neo-adjuvant or preoperative chemotherapy is developed to become one of the relatively new concepts in locally advanced breast cancer therapy. Many reports stated that the use of neo-adjuvant chemotherapy in locally advanced stage breast cancer caused primary tumor regression around 60%-80%. Usually, anthracycline group (epirubicin/doxorubicin) has good response, around 87%-91%.10-12 In one of the researches result, patients with positive Her-2 expression are more responsive to anthracycline compared with negative Her-2 expression and statistically is significantly different. It was approved that Her-2 expression could be used as prediction biomarker in choosing regimen of medication of Anthracycline group or others.12 Although anthracycline gives better survival and there are relations between Her-2 expression with anthracycline sensitivity in breast cancer patient, the mechanism of cell cancer sensitivity which expresses Her-2 to anthracycline if it is related with MDR-1(P-gp) expression is still unclear.

One of the factors which influences chemotherapy-response is the variation at some MDR-1 genome area. Apparently MDR-1 gene in located exon 26 is found to have several variations. A polymorphism of MDR1 gene has been shown to be correlated with intestinal P-glycoprotein (P-gp) expression and activity in vivo. This polymorphism consists of a C-to-T exchange at cDNA position 3435 located in exon 26 of the MDR1 gene. Although this base exchange does not affect the amino acid sequence of P-gp, the T allele (T/T genotype) appears to be associated with markedly lower MDR1 expression compared with the C allele (C/T genotype).4,5,13 Because of its silence on the protein level and its location in a non-regulatory region of the MDR1 gene, it is conceivable that this particular polymorphism is not causative for differences in P-gp expression. It is rather likely that this polymorphism is linked to other, as yet unidentified, changes in regions of the MDR1 gene that control expression, e.g., in the promoter/enhancer region or in regions that are relevant for mRNA processing. Nevertheless, the C3435T polymorphism appears to allow the differentiation of alleles with distinct MDR-1 expression and activity. Therefore, genotyping of the C3435T polymorphism may provide a basis for treating patients more effectively with agents that are substrates of the MDR-1 gene product, e.g., antitumor drugs such as vincristine and doxorubicin.5

In Kafka et al study6 rates of clinical and histopathological response to preoperative chemotherapy were comparable to the results of the other neoadjuvant trial. We determined the genotypes at position 3435 in located exon 26 of the MDR-1 gene in the study patients. Remarkably, statistical analysis revealed a significant correlation between the T/T genotype and clinical response of the patients to preoperative chemotherapy. This correlation may be explained by genotype-dependent expression of P-gp that was demonstrated recently by Hoffmeyer et al. Although it is not obvious how expression is affected by the C3435T polymorphism that is not causing any variability in amino acid sequence (because C3435T is a silent mutation), MDR-1 expression in intestinal biopsies (n=21) was found to be 2-fold lower in individuals with a T/T genotype compared to those possessing C/C genotype.3,6 A similar correlation between final clinical outcome and MDR-1 exon 26 variants was recently reported for patients suffering from acute myeloid leukemia.9

CONCLUSION

Our results showed the difference in clinical response of breast cancer patients was caused by variation of MDR-1 gene exon 26 on 3435 position, resulting in difference in clinical response not affected by Her-2 expression. Patients with T/T genotype have clinical response, while patients who do not show clinical response have C/T genotype. Our results and other reports point to a correlation of single nucleotide polymorphism in the MDR-1 gene and expression or function of P-gp contributes to the information on genetic background that may be relevant to predict individual outcome in therapeutic approaches with drugs that are affected by P-gp.

REFERENCES

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