# EXPRESSION OF TOPOISOMERASE IIα AND CYCLIN D1 PROTEINS IN VARIOUS DEGREES OF BREAST DUCTAL CARCINOMA

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## ABSTRACT

*Breast cancer is the most common malignancy and a major cause of death in women worldwide. Protooncogene cyclin D1 is to control the cell cycle and topoisomerase IIα has an important role in DNA replication as well as a specific target of some chemotherapeutic agents. Amplification of topoisomerase IIα is regarded to a poor prognosis, but it is a good predictor response to cytotoxic chemotherapy. The relationship between the histopathological degree of invasive ductal breast carcinoma and the expression of topoisomerase IIα and cyclin D1 is still unclear. This study aimed to determine the relationship between topoisomerase IIα and cyclin D1 with the histopathological degree of invasive ductal breast carcinoma. In this retrospective and cross-sectional study, researchers obtained the degree of histopathological data from medical records. Immunohistochemical examination of cyclin D1 and topoisomerase IIα was performed on paraffin block specimens from 35 patients with invasive ductal breast carcinoma at the Department of Pathology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital from January to December 2009. Based on the histopathological degree, found 5 cases (14.3%) degree of good, 12 cases (34.3%) moderate and 18 cases (51.4%) degrees bad. The expression of topoisomerase IIα were 62.9% (22 cases) and cyclin D1were were 54.3% (19 cases). It is known that the expression of topoisomerase IIα and cyclin D1 was not related to the histopathological degree of invasive ductal breast carcinoma (p = 0.091). As a conclusion, topoisomerase IIα and cyclin D1 does not play a role in differentiation, proliferation and carcinogenesis of tumor cells in invasive ductal breast carcinoma.*

***Keywords:*** *invasive ductal breast carcinoma, topoisomerase IIα, cyclin D1, histopathological degree*

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## INTRODUCTION

Breast cancer in Indonesia ranks second of all malignancies in women. The Department of Pathology, Airlangga University Faculty of Medicine/Dr. Soetomo Hospital in January-December 2009 recorded 123 cases of invasive ductal breast carcinoma (Kusumowardojo et al. 2004). Topoisomerase IIα is a specific target of some chemotherapeutic agents, including anthracycline. The drugs are bound to the Topo-DNA complex and inhibit religation process, so the double DNA chain is split into stable and is expected to stimulate apoptosis (Kellner et al. 2002). Overexpression of c-erbB2 (HER-2/neu) or topoisomerase IIα showed a shorter life expectancy, whereas patients with overexpression of both showed the worst disease outcome ($P < 0.0001$) (Fritzl et al. 2005), but the relationship between topoisomerase IIα with histopathological degree remains controversial, while cyclin D1 is a target of major derivative of the HER-2/neu and ER. Cyclin D1 is a proto-oncogene, which is an important regulator in the G1 phase into S phase in cell cycle (Alao 2007). After binding to CDK 4 and CDK 6 it will become an active complex which promotes phosphorylation and inactivation of retinoblastoma protein (Rb). Furthermore, we will increase the occurrence of G1-S phase progression in cell cycle (Alao 2007). Increased expression of cyclin D1 associated with an increase in cell proliferation (Holley et al. 2001). Where the relationship between expression of cyclin D1 with histopathological degree of breast ductal carcinoma has not been much studied.

## MATERIALS AND METHODS

All (35) paraffin blocks were obtained from all patients with invasive ductal breast carcinoma in the Department of Pathology, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, from January 1 to December 31, 2009, with the inclusion criteria: (1) histopathologic examination in the Pathology Laboratory of Anatomy Faculty of Medicine Airlangga University, Dr. Soetomo Hospital, (2) are microscopic in accordance with the criteria of invasive ductal breast

carcinoma, (3) neoplasms architecture can be evaluated clearly, no abundant necrosis or hemorrhage, (3) has not been subjected to chemotherapy and radiotherapy (no medical record data). Samples were taken by simple random sampling. The author then performed immunohistochemical examination with antibodies to topoisomerase IIα and cyclin D1 in all samples.

Each paraffin block was cut in 4 microns thickness, and then paraffinized with xylol and alcohol. Immunohistochemical staining used a standard streptavidin-biotin technique with monoclonal antibodies to topoisomerase IIα (Thermo) with a dilution 1/50 and cyclin D1 monoclonal antibody (Dako) with a dilution 1/100. Topoisomerase IIα expression and cyclin D1, seen as brown granules in the nucleus. Expression of topoisomerase IIα and cyclin D1 was counted in 400x magnification, with cut off 25% (Fritzl et al. 2005). Histopathological degree was determined based on 'Nottingham Modification of Bloom-Richardson System 6' classification. The existence of the relationship between expression of topoisomerase IIα and cyclin D1 with the degree of histopathological invasive ductal breast carcinoma was analyzed using non-parametric statistical Chi-square test.

## RESULTS

The results obtained by data collection at the Department Pathology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital, from January 1 to December 31, 2009, revealed 123 cases of invasive ductal breast carcinoma. Based on inclusion criteria and determined sample size, total sample size obtained was 35. From these samples, the age range of patients with invasive ductal breast carcinoma was between 30 to 87 years, with a mean of 51 years. Based on age groups, it was mostly in the age group 50 to 59 years, as many as 11 patients (31.4%) (Table 1). Based on histopathological degree, found 5 cases (14.3%) degree of good, 12 cases (34.3%) moderate and 18 cases (51.4%) degrees bad (Table 1). On immunohistochemical examination of topoisomerase IIα, there were 22 cases (62.9%) with positive topoisomerase IIα and 13 cases (37.1%) with negative expression of topoisomerase IIα (Table 1). Degree of good differentiation groups showed positive expression of topoisomerase IIα as much as 20%, while in group with moderately differentiated degree the increase was apparently 75%, and in the group of poor differentiation degree it reached 66.7% (Table 2). Chi square test to determine the expression of topoisomerase IIα significant correlation with the degree of histopathological breast invasive ductal carcinoma revealed that the result x2 = 4.803, contingency coefficient = 0.347, with p = 0.091 (p> 0.05), which means there was no significant correlation between the expression topoisomerase IIα with the degree of histopathology of invasive ductal breast carcinoma. From these results it appears that cyclin D1 was positive in most of the invasive ductal breast carcinoma of II degree (66.7%), then III (50%) and the degree I (40%). After statistical analysis using Chi Square test, the obtained value of x2 = 1.286 and coefficient of contingency = 0.526 (p <0.05) which means we did not find significant correlation between cyclin D1 expression with the degree of histopathology (Table 3).

Table 1. Characteristics of Research Subjects

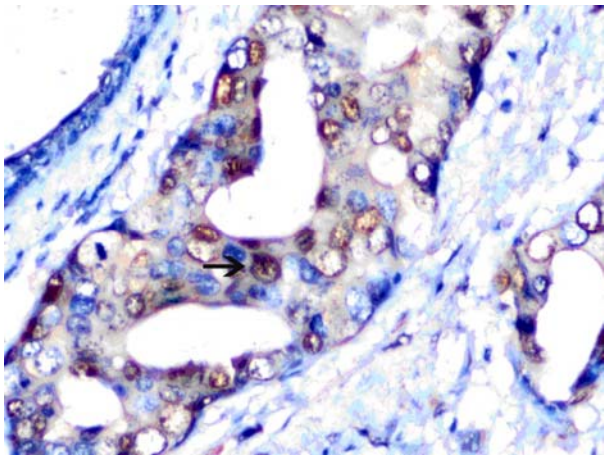
| Characteristics | Σ cases | % |
|---|---|---|
| Age (years) | | |
| 30-39 | 7 | 20 |
| 40-49 | 10 | 28.6 |
| 50-59 | 11 | 31.4 |
| 60-69 | 6 | 17.2 |
| 70-79 | 0 | 0.0 |
| 80-89 | 1 | 2.8 |
| Degree | | |
| Good (I) | 5 | 14.3 |
| Moderate (II) | 12 | 34.3 |
| Bad (III) | 18 | 51.4 |
| Expression | | |
| Topoisomerase IIα | | |
| Positive | 22 | 62.9 |
| Negative | 13 | 37.1 |
| Cyclin D1 | | |
| Positive | 19 | 54.3 |
| Negative | 16 | 45.7 |

Table 2. The distribution of topoisomerase IIα protein expression at various histopathological degrees of breast invasive ductal carcinoma

| Histopatho-logical degree | Expression of Topoisomerase IIα | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Good (I) | | | |
| Total | 1 | 4 | 5 |
| % | 20.0% | 80.0% | 100% |
| Moderate (II) | | | |
| Total | 9 | 3 | 12 |
| % | 75.0% | 25.0% | 100% |
| Bad (III) | | | |
| Total | 12 | 6 | 18 |
| % | 66.7 % | 33.3% | 100% |

Table 3. The distribution of topoisomerase IIα protein expression at various histopathological degrees of breast Invasive ductal carcinoma

| Histopatho-logical degree | Expression of Cyclin D1 | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Good (I) | | | |
| Total | 2 | 3 | 5 |
| % | 40.0% | 60.0% | 100% |
| Moderate (II) | | | |
| Total | 8 | 4 | 12 |
| % | 66.7% | 33.3% | 100% |
| Bad (III) | | | |
| Total | 9 | 9 | 18 |
| % | 50.0 % | 50.0% | 100% |



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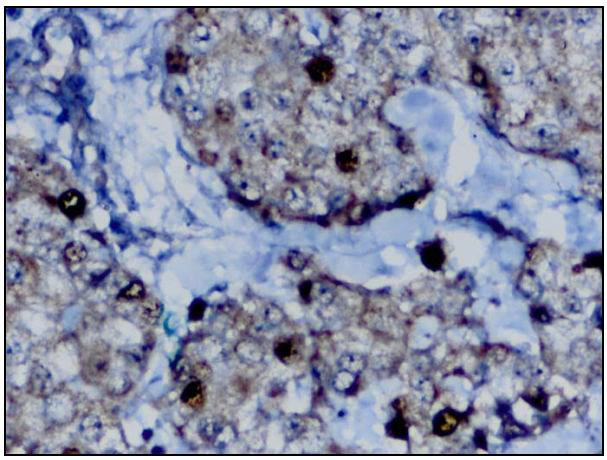

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Figure 1. A. Invasive ductal breast carcinoma with positive expression of cyclin D1 (magnification 400x). B. Topoisomerase IIα expression in the nucleus is stained brown (immunohistochemistry, 400x).

## DISCUSSION

Topoisomerase IIα plays an important role in DNA replication, chromatin regulation, maintaining genomic stability, DNA recombination, and is the target of various chemotherapeutic agents. In breast carcinoma, the expression of topoisomerase IIα is associated with cell proliferation, and overexpression of HER-2/neu (Depowski et al. 2000, Jarvinen et al. 1996). In this study, 62.9% of cases of invasive ductal breast carcinoma showing positive expression of topoisomerase IIα. Some literature mentions topoisomerase IIα expression in breast invasive ductal carcinoma varies between 25% and 39.4%. The difference was due to the lack of uniformity in the determination of cut-off value. Expression of topoisomerase IIα is also recognized as increasing in tumors with ER and PR negative. The location of topoisomerase IIα adjacent HER-2/neu oncogene on chromosome 17q12, leading to a suggestion that there is a mechanism that causes aberrant co-expression of these proteins. At the level of genomic DNA, both gene amplification and deletion of topoisomerase IIα is related with HER-2/neu amplification (Lee et al. 2007). From the literature it can be assumed that the topoisomerase IIα expression increases in tumors with HER-2/neu positive.

Javinen stated in his research, that topoisomerase IIα is associated with a high degree of histopathology, and DNA aneuploidy, but not associated with clinical variables, such as patient age, tumor size and nodal status (Lee et al. 2007). This research revealed that the expression of topoisomerase IIα lowest in degrees both (20%), and the highest at moderate (75%), whereas in severe degrees obtained positive in 62.9%, so there is no statistically significant correlation ($p = 0.091$). The same was reported by Depowski, that the expression of topoisomerase IIα are not related to tumor size, degree of histopathological, status of estrogen/progesterone receptor or recurrence of disease, but it associated with HER-2 overexpression (Jarvinen et al. 1996). This difference is theoretically possible since there are many interactions that control or stimulate cell growth, so increased expression of topoisomerase IIα does not automatically increase the histopathological degree. In the studied samples, positive cyclin D1 was found mostly in breast invasive ductal carcinoma with IIα degrees (66.7%), then the degree IIαI (50%) and the degree I (40%). After conducting statistical analysis using Chi Square test, we did not find significant correlation between cyclin D1 expression with the degree of histopathology ($p > 0.05$). This confirms previous studies that showed there was no significant correlation between cyclin D1 with proliferation

markers such as Ki-67 and S-phase fraction (Schnitt et al. 2005, Rudas et al. 2008). Leong et al. in their study also found no relationship between cyclin D1 with the histopathological degree. In vitro studies showed that cyclin D1 can shorten the G1 phase of the cell cycle. At first it was estimated that cyclin D1 expression was associated with tumor cell proliferation in humans. However, cell cycle regulation in vivo appears more complex than in vitro. Tumors that have a low degree of histopathology can show the overexpression of cyclin D1. This is due to the absence of consensus on the assessment methods of cyclin D1 expression, in which group with weak staining intensity should be assessed separately (Park et al. 2001). Another possible cause is the presence of multiple tumors with a low degree of differentiation that have a poor prognosis. But whether the poor prognosis has to do with the expression of cyclin D1 still needs further research, since all of these issues remain controversial. Some researchers say that the cyclin D1 is associated with poor prognosis, some say it has no relation with the prognosis, and several others mentioned the link between cyclin D1 with a good prognosis (Schnitt et al. 2005, Lee et al. 2007, Park et al. 2001). Another reason is because the determination of histopathological degree is a subjective, even though it has a standard examination, so that bias could occur among the examiners.

## CONCLUSION

The expression of topoisomerase IIα and cyclin D1 proteins is not in compliance with the increase of the degree of breast invasive ductal carcinoma. This shows that the topoisomerase IIα and cyclin D1 do not play a role in the differentiation and proliferation of tumor cells in invasive ductal breast carcinoma.

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