Citation: Roshdi A. El Tayeb and Ageeb M. Hasan. Immunohistochemical Expression of Cyclin D1 Gene in Invasive Ductal Carcinoma of the Breast. African Journal of Medical Sciences, 2018, 3 (4) ajmsc.info

Immunohistochemical Expression of Cyclin D1 Gene in Invasive Ductal Carcinoma of the Breast

Roshdi A. El Tayeb and Ageeb M. Hasan
Al Neelain University, Khartoum, Sudan.

Abstract

Background: Breast cancer is a major health problem in women. The molecular mechanisms of tumor growth and progression are complicated but likely involve the interaction of tumor suppressor genes, oncogenes, cell cycle regulatory proteins, and other factors. Recently some studies showed that cyclin D1 is a cell cycle regulatory gene emerging as a potentially significant oncogene in invasive breast cancer.

Objective: To evaluate the immunohistochemical expression of cyclin D1 gene in invasive ductal carcinoma of the breast among women population.

Materials and methods: This was a retrospective study. 46 formalin-fixed, paraffin-embedded tissues collected from women diagnosed with invasive ductal carcinoma of the breast were investigated. Tissue sections were prepared from all cases and stained with haemotoxylin and eosin (H&E) stain. Histopathological and immunohistochemical examination was then performed to detect the immunohistochemical expression of cyclin D1 gene.

Results: The age distribution of the patients investigated was ranging from 30-71 years (with a mean age of 50.5 years). Most positive tumor cases were aged 30-50 years. Histologically the tumor grades identified were: well differentiated (grade I), moderately differentiated (grade II), and poorly differentiated (grade III). 10 cases (21.73%) were found to have grade II tumors; and 36 cases (78.26%) were found to have grade III tumors. Positive Cyclin D1 expression was positive in 26 (56.52%) cases, and negative cyclin D1 expression was found in 43.47% of cases.

Conclusion: Cyclin D1gene expression was more prevalent in invasive ductal carcinoma. Cyclin D1gene is good marker of invasiveness in breast cancer, and associated with age and tumor grade.

Key words: Immunohistochemical expression, Cyclin D1 gene, Breast ductal carcinoma.

Introduction

Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers that are developing from the ducts are known as ductal carcinomas, while those are developing from lobules are known as lobular carcinomas. In addition, there are more than 18 other sub-types of breast cancer. Some cancers develop from pre-invasive lesions such as ductal carcinoma *in situ*. Signs of breast cancer may include a lump

in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin[1].
There are several well-established risk factors for breast cancer, e.g. early onset of menarche, a late age for a first complete pregnancy, a late age for menopause, the presence of atypical hyperplasia, a positive family history of breast cancer, and exposure to ionizing radiation.
Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (Cdk) enzymes, such as Cdk2, 4, 5, and 6. Most human cancers contain overactive CDK4/6-cyclin D, and CDK4/6-specific inhibitors are promising anti-cancer therapeutics[2].
Cyclin D1 gene is important for the development and progression of several cancers including those of the breast, esophagus, bladder and lung. Overexpression of cyclin D1 has also been linked to the development of endocrine resistance in breast cancer cells. An *in vitro* study performed by Li and his colleagues reported that cyclin D1 gene encodes the regulatory subunit of a holoenyzme that phosphorylates the retinoblastoma protein (pRb) and nuclear respiratory factor (NRF1) proteins. The abundance of cyclin D1 determines estrogen-dependent gene expression in the mammary gland of mice[3].

Materials and methods

This was a retrospective study investigating 46 women patients diagnosed as having invasive ductal carcinoma of the breast and attending different hospitals in Khartoum (Sudan) in 2017. This study was approved by the Ethical Committee of Al Neelain University. Confidentiality of information obtained from biopsies investigated was maintained. Permission to investigate the specimens was obtained from the different hospitals enrolled in the study. Demographic and clinical data were collected from all participants using a structured questionnaire.
Breast tissue sections were cut at 4 µm and placed on positively-charged slides. One section was stained with hematoxylin and eosin (H&E) and a second section was used for the detection of cyclin D1 by the immunohistochemistry technique (IHC). This technique was performed according to manufacturer's instructions (anti-cyclin D1 antibody 'ab16663' and rabbit specific antigen HRP/DAB 'ABC'). The IHC Kit used for detection was (ab64261)and interpreted as positive when >10% of the tumor cells expressed the marker with a moderate to strong intensity of staining.
Statistical analysis was performed by the SPSS (Statistical Package for Social Sciences) program, version 13.0 (SPSS inc. Chicago, IL). T-test and Fischer exact test were used for quantitative parameters such as age incidence. Chi-square test was used for qualitative parameters such as the histological grades.

Results

The age distribution patients investigated was ranging from 30-71 years with a mean age of 50.5 years. Majority of breast cancer cases was observed in the age group 41-55 years; and a minority was observed in the age group 56-70 years. There are no significant difference in age incidence

and frequency rate of cancer cases ((p > 0.05).
Histologically the tumor grades detected were: well differentiated (grade I), moderately differentiated (grade II), and poorly differentiated (grade III). 10 cases (21.73%) were found to have grade II tumors; and 36 cases (78.26%) were found to have grade III tumors. No case was detected in grade I. There are no significant correlation between the tumor grades and the frequency rate of cancer cases (p > 0.05).
In this study, the cyclin D1 gene expression was positive in 26 (56.52%) cases, while 20 (43.47%) cases were negative. Statistical analysis showed no significant correlation (p > 0.05) of the cyclin D1 gene expression with the frequency rate of invasive ductal carcinoma of the breast among the women population investigated (p = 3.52).
As shown in Table (1), the age incidence of patients with positive cyclin D1 gene expression was 30 to 71 years. 26 cases (out of 46) were in the age group 30-50 years; and were found to have a high frequency rate (15/57.69%) of cyclin D1 gene expression. This finding was statistically significant showing (p < 0.01). According to tumor grades statistical analysis revealed significant differences between expressions of cyclin D1 (p < 0.01).

Table (1): Immunohistochemistry expression of cyclin D1 according to age incidence and tumor grades

Variables	Positive	Negative	p - value
Age incidence: 30-50 years 51-60 years 63-71 years	 15 (57.69%) 9 (81.81%) 3 (3.33%)	 11(42.30%) 3 (27.27%) 6 (6.66%)	 p < 0.01
Tumor grades: Grade I Grade II Grade III	 0 (0.0%) 6 (60%) 19 (52.77%)	 0 (0.0%) 4 (40%) 17 (47.22%)	 p < 0.01

Discussion

The cyclin D1 proto-oncogene is a vital regulator of G1 to S phase progression in several different cell types. Together with its binding associates cyclin dependent kinase 4 and 6 (CDK4 and CDK6), cyclin D1 form active complexes that promote cell cycle progression by phosphorylating and inactivating the retinoblastoma protein (RB). Further studies have

demonstrated that cyclin D1 can function as transcriptional modulator by regulating the activity of several transcription factors and histone deacetylase (HDAC3). This activity is independent of CDK4 activity.

The current study had demonstrated that cyclin D1 was expressed in women with breast cancer. This finding was in agreement with findings of Alao (2007) who reported increased levels of cyclin D1 in several cancers[4].

Many of the studies in western literature had documented a positive expression of cyclin D1 in about 65-70% of breast carcinomas[5]. In the present study, cyclin D1 expression was seen in about 56.52% of IDC, which is in concordance with that reported in western studies. It is now known that cyclin D1 is necessary for the normal development of the breast and dysregulated expression stimulates aberrant mammary epithelial proliferation.

Liu and his co-workers[6] studied the expression of cyclin D1 in the histological model of progression of normal breast epithelium to proliferative breast disease with atypia, atypical ductal hyperplasia, DCIS and invasive carcinomas, to evolve a paradigm for the course of genetic alterations in the development of breast carcinomas. They found that the over-expression occurs in the early stages of breast oncogenesis and plays a crucial role in the further progression of the tumor.

Interestingly, a similar study[1] showed a high expression of cyclin D1 with smaller tumor size although the other factors failed to show a positive correlation. The study conducted by Wu and his co-authors showed no significant association was seen between these factors and their cyclin D1 expression finding was similar to the present study[7].

Detection of over-expression of cyclin D1 by immunohistochemistry had been reported in 35–81% of breast carcinomas; a finding similar to our results. In this context, over 56% of cases were cyclin D1 positive; and there was a positive correlation between cyclin D1 expression and tumor grade and age incidence; which confers an aggressive course of disease. Cyclin D1 plays a crucial role as a cell cycle regulator, promoting progression through G1-S phase[7]. However, expression of cyclin D1 was found associated with lower histological grades.

Also in this study, the findings showed significant differences between cyclin D1 expression and age groups. In general cancer is an age-associated disease, however, the mechanisms of age-associated increase in cancer incidence are not completely understood. According to the histological tumor grade, cyclin D1 expression was found higher in tumors of grade II.

Conclusion: Cyclin D1gene expression was more prevalent in invasive ductal carcinoma. Cyclin D1gene is good marker of invasiveness in breast cancer, and associated with age and tumor grade.

References

1. Mathilde B, Jean-Marc G, Elisabeth K, Virginie G, Ellen B, *et al*. Detection of Epstein - Barr virus in Invasive Breast Cancers. *Journal of the National Cancer Institute*. 1999; 91: 1367-1381.

2. Yamamoto M, Tamakawa S, Yoshie M, Yaginuma Y, Ogawa K Neoplastic hepatocyte growth associated with cyclin D1 redistribution from the cytoplasm to the nucleus in mouse hepato-carcinogenesis. *Mol Carcinog*. 2006; 45: 901-913.

3. Li Z, Chen K, Jiao X, Wang C, Willmarth NE, *et al.* Cyclin D1 integrates estrogen-mediated DNA damage repair signaling. *Cancer Res.* (2014); 74: 3959-3970.
4. Alao JP . The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer.* 2007; 6: 24.
5. Coqueret O. Linking cyclins to transcriptional control. *Gene.* 2002; 299: 35-55.
6, Liu Q, Boudot A, Ni J, Hennessey T, Beauparlant SL, *et al.* Cyclin D1 and C/EBPÎ² LAP1 operate in a common pathway to promote mammary epithelial cell differentiation. *Mol Cell Biol.* 2014;34: 3168-3179.
7. Wu Y, Kim J, Elshimali Y, Sarkissyan M, Vadgama JV. Activation of Akt1 accelerates carcinogen-induced tumorigenesis in mammary gland of virgin and post-lactating transgenic mice. *BMC Cancer.* 2014;14: 266.