JBUON 2017; 22(4): 947-952
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

The prognostic significance of cyclin D1 expression in patients with triple-negative breast cancer

Betul Bolat Kucukzeybek[1], Ibrahim Vedat Bayoglu[2], Yuksel Kucukzeybek[2], Ahmet Alacacioglu[2], Seyran Yigit[1], Aysegul Akder Sari[1], Murat Akyol[2], Mustafa Oktay Tarhan[3]

[1]Department of Pathology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir; [2]Department of Medical Oncology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir; [3]Department of Preventive Oncology, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey

Summary

Purpose: *Breast cancer (BC) is the most common cancer and the second leading cause of cancer death among women. While receptor-targeted therapies are used for other subtypes due to the presence of such receptors, studies are still continuing on receptor expression in order to identify new therapeutic targets as the triple-negative breast cancer (TNBC) lacks a target receptor and its prognosis is worse than the other subtypes. Cyclin D1 (CycD1) is a cell cycle regulator protein. It is stated that its overexpression plays a role in carcinogenesis. With the present study, we aimed to evaluate the prognostic significance of immunohistochemical expression of CycD1 in patients with TNBC.*

Methods: *The study included 56 operated patients with TNBC who were diagnosed between 2006 and 2011 at Izmir Katip Celebi University, Ataturk Research and Training Hospital, Department of Pathology. In tumor paraffin-embedded sections, CycD1 was immunohistochemically (IHC) studied. Demographic and survival data of the patients were obtained from the Department of Medical Oncology follow-up files. ROC curve analysis was used to calculate the cut-off value for CycD1 staining density. Patients were divided into two groups using 11.5 cutoff value for the expression of CycD1, obtained by ROC analysis. Kaplan-Meier analysis was utilized for survival analyses, and log rank test for comparisons between the two groups.*

Results: *Of the patients, 62.5% had CycD1 expression (37.5% had not). In the whole group, the 5-year disease-free survival (DFS) was 51%, and the 5-year overall survival (OS) was 65%. No difference in DFS between the two groups was noticed (p=0.37). The 5-year DFS was 47% in the group with CycD1 expression below 11.5, while it was 57% in the group above the 11.5 value. The difference in OS between the groups was statistically significant (p=0.044). The 5-year OS was 55% in the group with a CycD1 expression below 11.5, while it was 79% in the group above the 11.5 value (p=0.044).*

Conclusion: *OS differed significantly between the high and low-CycD1 expression. It was also demonstrated that CycD1 may have prognostic significance in TNBC. Further studies with larger populations are required to confirm the prognostic significance of CycD1.*

Key words: *breast carcinoma, cyclin D1, prognosis*

Introduction

BC is the most common cancer and the second leading cause of cancer death among women [1]. It comprises a heterogeneous group. Immunohistochemically BC is classified according to the estrogen receptor (ER), progesterone receptor (PR), and HER2/neu expression. Immunohistochemically, the subtype without staining and is categorized as TNBC. TNBC comprises 15-20% of all BC cases and exhibits a high risk of early relapse and poorer prognosis compared to other subtypes [2-4]. Following the early 2000s, BC has also started to be classified according to gene expressions. The

Correspondence to: Betul Bolat Kucukzeybek, MD. Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Pathology, 35360, Izmir, Turkey.
Tel: + 90 (232) 2434343-1373, E-mail: bbkzeybek@yahoo.com
Received: 12/12/2016; Accepted: 30/12/2016

basal-like breast cancer (BLBC) is another subtype in the classification performed based on gene expression profile. This subtype also expresses a poorer prognosis compared to others [5-8]. TNBC includes 70% of BLBC cases according to the immunohistochemical (IHC) classification. Moreover, 70% of the TNBCs consist of BLBCs [9]. Due to the presence of receptor expression in other subtypes, therapies targeting these receptors have been used, and new therapeutic agents for these receptors continue to be developed. Studies are still continuing on receptor expression in order to identify new therapeutic targets as the TNBC lacks a target receptor and its prognosis is worse than the other BC subtypes.

CycD1 is a cell cycle regulator protein. It is coded by the CCND1 gene located on chromosome 11q13 and it binds to the cyclin-dependent kinase 4/6 to control its activity. The resulting complex causes retinoblastoma gene product inactivation by phosphorylation. There are numerous gene activations that lead to G1/S transition through released E2F family transcription factors [10-12]. CCND1 gene amplification is detected in around 15-20% of BC cases [13,14]. Expression of CycD1 is approximately 50% in BC [15] and it is stated that its overexpression may play a role in carcinogenesis [16]. Although CCND1 gene amplification has been demonstrated to be associated with poor prognosis in patients with BC, conflicting reports are available regarding the prognostic significance of CycD1 expression. There are studies showing that it may be associated with either a good prognosis or with a poor prognosis [13,14,17-22].

In the present study, we planned to retrospectively evaluate the prognostic significance of IHC expression of CycD1 in patients with TNBC who were not administered neoadjuvant chemotherapy.

Methods

Patients and tissues

This study included operated patients with TNBC diagnosed between 2006 and 2011 at Izmir Katip Celebi University, Ataturk Research and Training Hospital, Department of Pathology, and who were followed up at the Medical Oncology Clinic. Patient demographic and survival data were obtained from the follow-up files of the Medical Oncology Outpatient Clinic. Clinical and pathological data including patient age at diagnosis, stage at diagnosis, tumor size, lymph node status, recurrence status, use of adjuvant chemotherapy, histological grade, and operation type were registered. DFS was defined as the time period from diagnosis until relapse or metastasis, and OS was defined as the time period from the date of diagnosis to the time of death due to any cause. Patient diagnostic tumor blocks at the Department of Pathology were used for IHC staining of CycD1. IHC staining density and staining intensity were assessed.

Immunohistochemical method

IHC staining for CycD1 was performed on formalin-fixed and paraffin-embedded tissue using the streptavidin-biotin-peroxidase method. Tissue blocks containing representative tumor areas were selected for IHC stains. The expression of CycD1 was evaluated using a Dako Autostainer (DAKO, Santa Clara, CA, USA) and the DAKO Envision staining method. Sections were stained using monoclonal rabbit anti human CycD1 antibody (Clone EP12 – DAKO, Pleasanton, CA, USA). Normal tonsil tissue was used as positive control for CycD1 staining. Two of the authors evaluated the staining intensity and staining density. Nuclear staining in tumor cells were considered positive. Besides CycD1, material concerning ER, PR, HER2 and Ki67 taken at the time of initial diagnosis was re-evaluated.

Statistics

Statistical analyses were performed using the SPSS software, version 20. ROC curve analysis was employed to calculate the cutoff value for CycD1 staining density, Kaplan-Meier analysis for survival analyses, and log rank test for survival comparisons between the two groups. The independent t-test, Spearman's rho correlation test and Pearson's correlation test were used to evaluate the relationship between CycD1 expression and other prognostic factors. $P<0.05$ was considered statistically significant. Patients were divided into two groups according to the cutoff value obtained by ROC analysis as part of the survival analyses. For the independent t-test, patients were analyzed immunohistochemically under two groups according to the presence of CycD1 expression.

Results

The clinical and histopathological characteristics of the patients included in the study are shown in Table 1. The study included 56 operated patients with TNBC. All patients were female with median age 49 years (range 27-85). Except one patient, all patients received postoperative adjuvant chemotherapy. Twenty-two (40%) patients developed recurrence/metastasis after a median follow-up of 57 months and 25 (44.6%) died during follow-up. Thirty-nine (69.6%) patients had invasive ductal carcinoma, 4 (7.1%) invasive lobular carcinoma, and 3 (5.4%) had mixed breast carcinoma histology. Six patients (10.7%) had grade I tumors, 30 (53.6%) grade II, and 20 (35.7%) grade III. Thirty (53.6%) patients had lymph node metastasis at diagnosis. Thirty (53.6%) patients were subjected to total mastectomy and 26 (46.4%) underwent breast-conserving surgery. Thirty (53.6%) patients had TNM stage II at diagnosis, 3 (5.4%)

Table 1. Patient and disease characteristics

Characteristics	*n (%)*
Age, years, median (range)	49 (27-85)
Menopausal status	
Premenopausal	29 (51.8)
Postmenopausal	27 (48.2)
Histological subtype	
Invasive ductal carcinoma	39 (69.6)
Invasive lobular carcinoma	4 (7.1)
Mixed type	3 (5.4)
Other	10 (17.9)
Histological grade	
Grade I	6 (10.7)
Grade II	30 (53.6)
Grade III	20 (35.7)
Surgical procedure	
Modified radical mastectomy	30 (53.6)
Breast-conserving surgery	26 (46.4)
Lymph node status	
Metastatic	30 (53.6)
Non-metastatic	26 (46.4)
Stage at diagnosis	
Stage I	3 (5.4)
Stage II	30 (53.6)
Stage III	23 (41)
Cyclin D1 expression	
Positive	35 (62.5)
Negative	21 (37.5)

had stage I, and 23 (41%) had stage III. Immunohistochemically, 37.5% of the patients did not exhibit CycD1 expression and 62.5% showed this expression (Figures 1 and 2).

In the whole group, the 5-year DFS was 55%, and the 5-year OS 65%. Patients were divided into two groups according to CycD1 expression using 11.5 cutoff value for the expression of CycD1, which was obtained from ROC analysis. The 5-year DFS in the low CycD1 expression group was 47%, while it was 57% for the high CycD1 expression group (p=0.37; Figure 3). The 5-year OS was 55% in the group with CycD1 expression below 11.5, while it was 79% in the group above the 11.5 value (p=0.044; Figure 4).

Regarding the relationship between prognostic indicators of patients and CycD1 expression, the results demonstrated that presence of multiple BC foci at diagnosis was not associated with CycD1 expression at diagnosis (p=0.576). Similarly, presence of lymph node metastasis and menopausal status were not associated with CycD1 expression (p=0.77 and p=0.765), respectively. No correlation was found between CycD1 expression and histological grade (p=0.431) or CycD1 expression and tumor size at diagnosis (p=0.46). Also, although

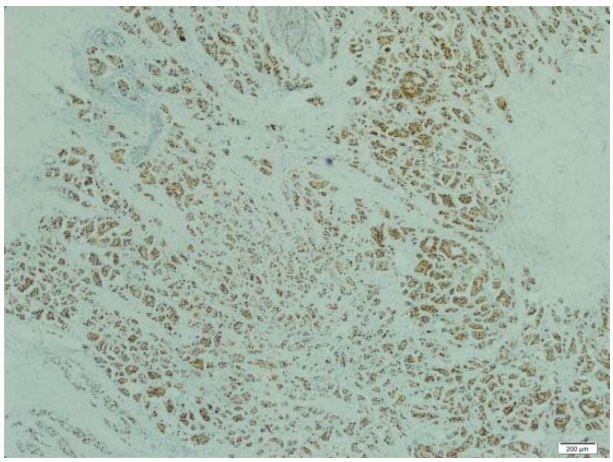

Figure 1. Diffuse and strong nuclear immunopositivity of the tumor cells for CycD1 (x40).

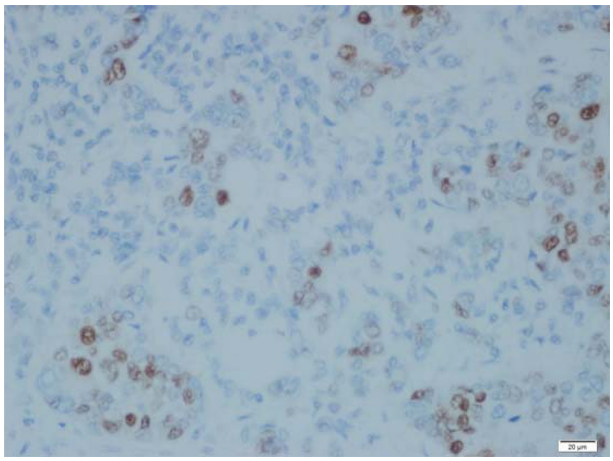

Figure 2. Focal nuclear immunopositivity of the tumor cells for CycD1 (x200).

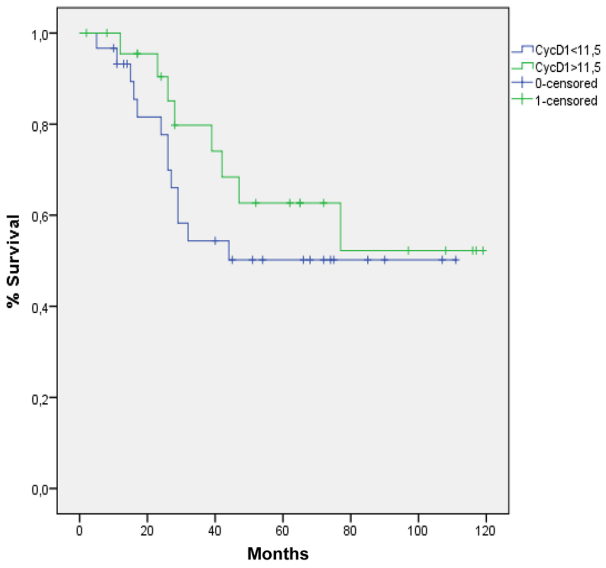

Figure 3. Disease free survival according to Cyclin D1 level (p=0.37).

there was no correlation between Ki67 proliferation index and CycD1 (p=0.66), CycD1 expression was negatively correlated with the stage at diagnosis (p=0.020) and the number of lymph nodes involved at diagnosis (p=0.044).

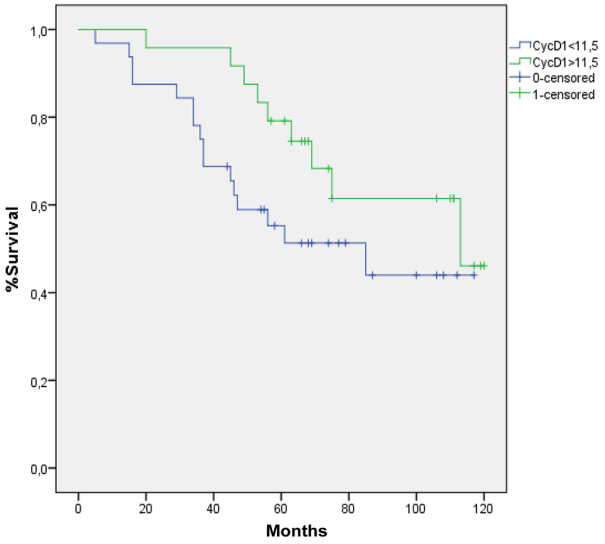

Figure 4. Overall survival according to Cyclin D1 level (p=0.044).

Discussion

BC is a heterogeneous disease. Multiple genetic modifications may play a role in its pathogenesis. TNBC is the group without an IHC receptor expression and has a worse prognosis compared with other subtypes. Due to the absence of receptor expression, the targeted therapies available for other subtypes cannot be employed in TNBC. Although 70% of TNBCs are included in the basal-like subgroup based on the gene expression profile, the TNBC is a heterogeneous group as well. A study conducted exclusively on TNBCs divided them into 6 subtypes based on the gene expression. Those subtypes differed in terms of survival as well [23]. Controversial results have been reported in studies evaluating the prognostic significance of CyclD1 in BC. The present study presents an IHC evaluation of CycD1 expression and its prognostic significance in patients with TNBC.

In our study, CycD1 expression was detected in 35 (62.5%) of 56 operated patients with TNBC (except one patient) who were administered postoperative adjuvant chemotherapy. The patients were categorized into two groups with ROC analysis based on CycD1 expression. Unexpectedly, due to the effect of CycD1 on G1/S transition in the cell cycle, no difference was detected between the two groups in terms DFS (p=0.37). OS in the low and high CycD1 expression groups was 55% and 79% at 5 years, respectively. The difference in OS between the two groups was statistically significant (p=0.044). A study conducted by Pelosio et al., immunohistochemically evaluated CycD1 expression in tissue samples of 180 operated BC patients with lymph node metastases who were treated with adjuvant chemotherapy. The authors detected nuclear CycD1 staining in 70% of the patients and demonstrated that it was associated with DFS in this group of patients [24]. Another study conducted by Chung et al. assessed 236 operated patients with BC. The group with increased CycD1 expression showed no difference in terms of DFS, while the disease-specific OS was higher in this group [25].

The study in which Gillett et al. immunohistochemically evaluated CycD1 expression in 345 operated patients with BC reported that increased CycD1 expression was associated with increased DFS and OS [20]. Bao et al. demonstrated in a study, in which they immunohistochemically evaluated 102 cancerous and 60 normal breast tissue samples, that tissue samples of patients with BC exhibited increased CycD1 expression compared to normal tissue samples, which confirmed the role of CycD1 in the pathogenesis of BC. The same study also found an association between increased CycD1 expression and increased OS [19]. As part of the present study, we evaluated CycD1 expression and its relationship with other prognostic factors. Despite the negative correlation between CyCd1 expression and histological grade (p=0.431) and tumor size at diagnosis (p=0.46), the difference was not significant. Also, increased CycD1 expression was negatively correlated with the stage at diagnosis (p=0.020) and the number of lymph nodes involved at diagnosis (p=0.044).

A study published by Van Diest et al. immunohistochemically evaluated the CycD1 expression in 148 patients with BC and found 60% positivity. The authors also found that CycD1 expression negatively correlated with the histological grade and mitotic index [26]. In the study conducted by Quintayo et al. on 1686 operated patients with BC, 13.6% of the patients presented CCND1 gene amplification and 80.1% of them exhibited IHC CycD1 expression. The gene amplification and expression of CycD1 were proved to be correlated. Although the gene amplification was found to be associated with poor DFS, high histological grade, increased lymph node involvement and increased tumor size, the CycD1 expression was associated with increased DFS, low tumor grade, and low proliferation index [17]. Another study published by Peurala et al. immunohistochemically evaluated the CycD1 expression in 102 operated patients with BC; the authors reported that increased CycD1 expression was associated with low Ki67 proliferation index and increased OS [27].

Lehn et al. studied MDA-MB-231 TNBC cell line and demonstrated that the loss of siRNA and CycD1 increased cell migration and they also reported a high upregulation of the differentiation inhibitor 1 (ID1) gene following the CycD1 loss. The authors also showed that ID1 overexpression

increased cancer cell migration with respect to the control group [28]. A study by Tobin et al., which included 1106 patients with BC, immunohistochemically evaluated CycD1 expression, ID1 expression, as well as CCND1 and the differentiation inhibitor gene expression in ZR75.1 and MDA-MB-231 TNBC cell line. The authors demonstrated that reduced CycD1 expression caused an increase in ID1 expression. The study further showed that reduced CycD1 expression caused an increase in cancer cell migration through ID1, CycD1 expression was associated with low histological grade, and increased ID1 expression negatively correlated with tumor grade and size [29].

CycD1 plays a role in the G1/S transition of the cell cycle. Binding to cyclin-dependent kinase 4/6, it forms a complex which then causes the phosphorylation and inactivation of retinoblastoma gene product [10-12]. CycD1 levels increase in the early G1 phase of the cell cycle, which continues until G1/s transition, and then sharply decrease. CycD1 degradation is required for DNA replication. Cell culture experiments conducted with fibroblasts showed that acute CycD1 overexpression blocked DNA replication, reduced cyclin-dependent kinase 2 activity by binding to cyclin-dependent kinase 2, and inhibited cell proliferation [30,31].

The CycD1 protein comes as a result of rs9344 polymorphism of CCND1 gene. CycD1a is known as the classical CycD1 [32]. Studies on BC cell cultures have demonstrated that CycD1b also inhibits the activity of cYCd1A and prevents tumor proliferation [33].

The inhibiting effect of P16 on cyclin-dependent kinase 4/6 is another control mechanism part of G1/S transition in the cell cycle. P16 inhibits cyclin-dependent kinase 4/6 and thereby causes the retinoblastoma gene product to remain in the hypophosphorylated form, prevents the release of E2F family transcription factors, and controls G1/S transition [34,35].

In conclusion, although the CCND1 gene amplification is found to be associated with poor prognosis in BC, the role of CycD1 in the prognosis of BC remains controversial. Similar to the findings of recent studies, we found CycD1 expression to be associated with low grade and low lymph node involvement at diagnosis, which is an indicator of increased OS and good prognosis. We also demonstrated that CycD1 might have prognostic significance in the TNBC, which is one of those subtypes exhibiting poor prognosis.

The association we found between increased CycD1 expression and prolonged survival and good prognostic factors might have been due to the small sample size of our study. Except one patient, all patients received adjuvant chemotherapy. The finding that the group with increased CycD1 expression had a longer OS can be explained by the fact that the same group benefited more from the adjuvant chemotherapy. On the other hand, the absence of sharp CycD1 decrease in G1/S transition, required for DNA replication as demonstrated in cell culture experiments, can be explained by its inhibitory effect on DNA replication. Coded as a result of the CCND1 gene polymorphism in BC cells, the CycD1b's inhibition of CycD1a's effect might be explained by its inhibiting effect on tumor proliferation. The elimination of CycD1 controlling effect on cells with low CycD1 expression might account for the high number of defects in those cells, which are related to P16, cyclin-dependent kinase 4/6, and retinoblastoma gene product, and which play a role in G1/S transition in those cells. The finding that other pathways were active in the carcinogenesis process in the cells with low CycD1 expression compared to those with a high expression might be explained by the association of low CycD1 expression and poor prognosis in those patients. The relationship between increased CycD1 expression and good prognosis in TNBC may be due to a possibly increased expression in the TNBC subtype that bears a better prognosis according to gene expression.

Further studies with larger populations are required to confirm the prognostic significance of CycD1, which will guide future studies on its predictive significance as well as attempts for new therapies targeting CycD1.

Conflict of interests

The authors declare no confict of interests.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
2. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California Cancer Registry. Cancer 2007;109:1721-8.

3. Dent R, Trudeau M, Pritchard KI et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13 (15 Pt 1):4429-34.
4. Haffty BG, Yang Q, Reiss M et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol 2006;24:5652-7.
5. Sørlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.
6. Sørlie T, Tibshirani R, Parker J et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-23.
7. van 't Veer LJ, Dai H, van de Vijver MJ et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-6.
8. Sotiriou C, Neo SY, McShane LM et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A 2003;100:10393-8.
9. Parker JS, Mullins M, Cheang MC et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160-7.
10. Kato J, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. Genes Dev 1993;7:331-42.
11. Lundberg AS, Weinberg RA. Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. Mol Cell Biol 1998;18:753-61.
12. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323-30.
13. Li Z, Cui J, Yu Q, Wu X, Pan A, Li L. Evaluation of CCND1 amplification and CyclinD1 expression: diffuse and strong staining of CyclinD1 could have same predictive roles as CCND1 amplification in ER positive breast cancers. Am J Transl Res 2016;8:142-53.
14. Schuuring E, Verhoeven E, van Tinteren H et al. Amplification of genes within the chromosome 11q13 region is indicative of poor prognosis in patients with operable breast cancer. Cancer Res 1992;52:5229-34.
15. Arnold A, Papanikolaou A. Cyclin D1 in breast cancer pathogenesis. J Clin Oncol 2005;23:4215-24.
16. Weinstat-Saslow D, Merino MJ, Manrow RE et al. Overexpression of cyclin D mRNA distinguishes invasive and in situ breast carcinomas from non-malignant lesions. Nat Med 1995;1:1257-60.
17. Quintayo MA, Munro AF, Thomas J et al. GSK3β and cyclin D1 expression predicts outcome in early breast cancer patients. Breast Cancer Res Treat 2012;136:161-8.
18. Huang W, Nie W, Zhang W, Wang Y, Zhu A, Guan X. The expression status of TRX, AR, and cyclin D1 correlates with clinicopathological characteristics and ER status in breast cancer. Onco Targets Ther 2016;9:4377-85.
19. Yang C, Nan K, Zhang Y, Chen Y, Qin S. High expression of cyclin D1 is correlated with the expression of estrogen receptor and good prognosis in breast cancer. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2016;32:84-7.
20. Gillett C, Smith P, Gregory W et al. Cyclin D1 and prognosis in human breast cancer. Int J Cancer 1996;69:92-9.
21. Tobin NP, Bergh J. Analysis of Cyclin D1 in Breast Cancer: A Call to Arms. Curr Breast Cancer Rep 2012;4:171-3.
22. Bilal E, Vassallo K, Toppmeyer D et al. Amplified loci on chromosomes 8 and 17 predict early relapse in ER-positive breast cancers. PLoS One 2012;7(6):e38575.
23. Lehmann BD, Bauer JA, Chen X et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750-67.
24. Pelosio P, Barbareschi M, Bonoldi E et al. Clinical significance of cyclin D1 expression in patients with node-positive breast carcinoma treated with adjuvant therapy. Ann Oncol 1996;7:695-703.
25. Chung J, Noh H, Park KH, Choi E, Han A. Longer survival in patients with breast cancer with cyclin d1 over-expression after tumor recurrence: longer, but occupied with disease. J Breast Cancer 2014;17:47-53.
26. van Diest PJ, Michalides RJ, Jannink L et al. Cyclin D1 expression in invasive breast cancer. Correlations and prognostic value. Am J Pathol 1997;150:705-11.
27. Peurala E, Koivunen P, Haapasaari KM, Bloigu R, Jukkola-Vuorinen A. The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer. Breast Cancer Res 2013;15:R5.
28. Lehn S, Tobin NP, Berglund P et al. Down-regulation of the oncogene cyclin D1 increases migratory capacity in breast cancer and is linked to unfavorable prognostic features. Am J Pathol 2010;177:2886-97.
29. Tobin NP, Sims AH, Lundgren KL, Lehn S, Landberg G. Cyclin D1, Id1 and EMT in breast cancer. BMC Cancer 2011;11:417.
30. Fukami-Kobayashi J, Mitsui Y. Cyclin D1 inhibits cell proliferation through binding to PCNA and cdk2. Exp Cell Res 1999;246:338-47.
31. Pagano M, Theodoras AM, Tam SW, Draetta GF. Cyclin D1-mediated inhibition of repair and replicative DNA synthesis in human fibroblasts. Genes Dev 1994;8:1627-39.
32. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995;11:1005-11.
33. Zhu J, Sen S, Wei C, Frazier ML. Cyclin D1b represses breast cancer cell growth by antagonizing the action of cyclin D1a on estrogen receptor alpha-mediated transcription. Int J Oncol 2010;36:39-48.
34. Li J, Poi MJ, Tsai MD. Regulatory mechanisms of tumor suppressor P16(INK4A) and their relevance to cancer. Biochemistry 2011;50:5566-82.
35. Witkiewicz AK, Knudsen KE, Dicker AP, Knudsen ES. The meaning of p16(ink4a) expression in tumors: functional significance, clinical associations and future developments. Cell Cycle 2011;10:2497-503