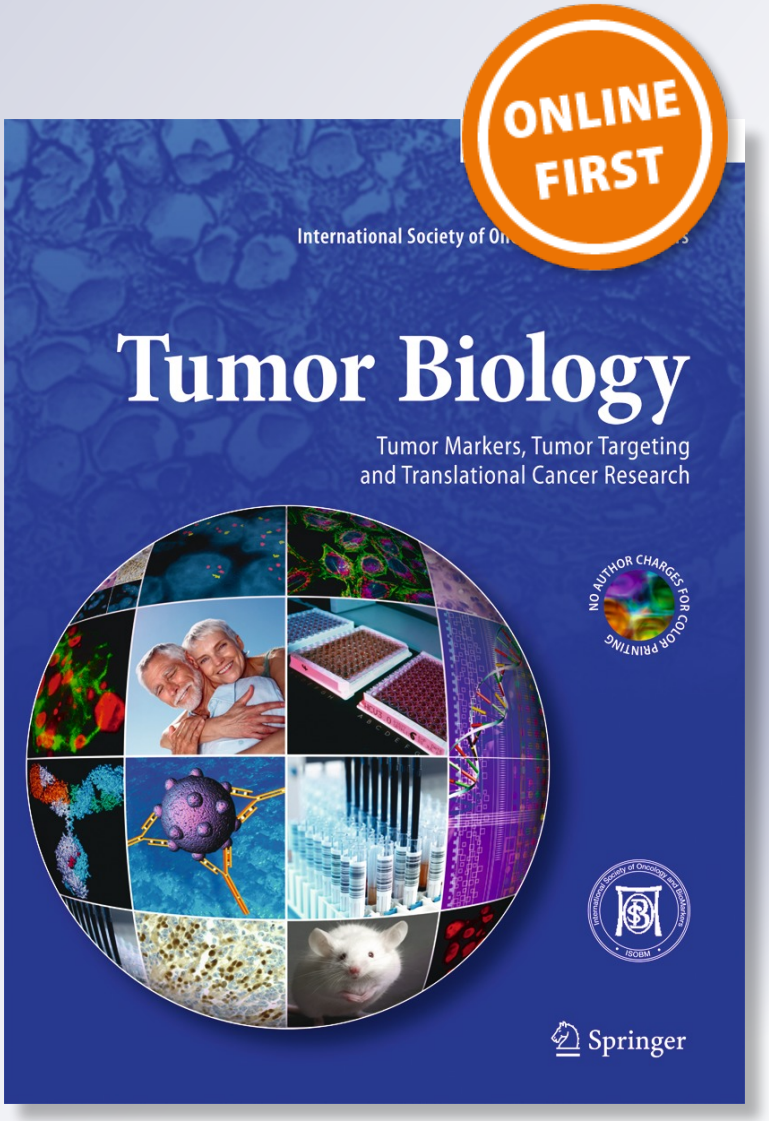
Downregulation of DYRK2 can be a predictor of recurrence in early stage breast cancer

Yasuko Enomoto, Shin-ichi Yamashita, Yasuteru Yoshinaga, Yasuyoshi Fukami, So Miyahara, Kazuki Nabeshima & Akinori Iwasaki

Tumor Biology
Tumor Markers, Tumor Targeting and Translational Cancer Research

ISSN 1010-4283

Tumor Biol.
DOI 10.1007/s13277-014-2413-z



Springer

Tumor Biol.
DOI 10.1007/s13277-014-2413-z

RESEARCH ARTICLE

Downregulation of DYRK2 can be a predictor of recurrence in early stage breast cancer

Yasuko Enomoto · Shin-ichi Yamashita · Yasuteru Yoshinaga · Yasuyoshi Fukami · So Miyahara · Kazuki Nabeshima · Akinori Iwasaki

Received: 18 June 2014 / Accepted: 28 July 2014

Abstract This study investigated the potential of DYRK2, a dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase gene, to predict disease-free survival for patients with early stage breast cancer. Two hundred and seventy-four patients with breast cancer underwent surgery from January 2000 to December 2009. All patients were in stage I or II. Immunohistochemical (IHC) analysis was used to determine the expression of DYRK2, which was examined for its association with clinicopathological factors or prognosis. A total of 85 of 274 cases (31 %) were DYRK2 positive. No correlation was found between DYRK2 expression by IHC and clinicopathological factors such as tumor size, histological grade, hormone receptor status, and HER2 status; however, lymph node involvement was closely associated with DYRK2 expression. Ten-year disease-free survival in the DYRK2-positive group without node metastasis (95.9 %) was significantly better than that in the DYRK2-negative group (87.3 %, p=0.015). These data show that DYRK2 expression is associated with lymph node involvement and is a possible predictive factor of breast cancer recurrence.

Keywords Breast cancer · DYRK2 · Prognostic factor

Introduction

Breast cancer is the most common malignancy in women worldwide. Approximately 25–30 % of breast cancer patients without lymph node metastases will develop distant metastases within 10 years of surgery [1]. Several genetic characteristics of breast cancer were reported for predicting the prognosis or for decision-making regarding adjuvant therapy for patients with breast cancer [2]. Gene profiling has been established as a tool for predicting clinical outcome [3]. In addition, we need to find an improved marker to stratify breast cancer patients into different risk groups more accurately, so that low-risk favorable patients can be spared with unnecessary treatment. Furthermore, it may be possible to separate out high-risk patients to apply more aggressive treatment modalities.

DYRK2 was identified as a member of the conserved family of dual-specificity tyrosine-phosphorylation-regulated kinases that autophosphorylate a tyrosine residue but act as serine/threonine kinases on their substrates [4]. Previous reports showed that DYRK2 regulates p53 to induce apoptosis in response to DNA damage via the phosphorylation of Ser 46 [5]. Pérez et al. reported that seven in absentia homolog (SIAH), which is a member of E3 ubiquitin ligases, interacts with DYRK2 and DYRK2 function was negatively regulated by SIAH-dependent degradation under hypoxic conditions [6]. On the other hand, Taira et al. showed that DYRK2 phosphorylate c-Jun and c-Myc to lead ubiquitin-mediated degradation and knockdown of DYRK2 increase cell proliferation in human cancer cells [7]. Furthermore, it is reported that DYRK2 phosphorylates telomerase reverse transcriptase (TERT), which is followed by ubiquitin-mediated degradation, and negatively regulates telomerase activity [8]. These findings suggest that DYRK2 inactivation may play a critical role in human cancer cell proliferation. Recently, we identified the potential of DYRK2 as a favorable prognostic marker in non-small cell lung cancer (NSCLC), and we found that bronchioloalveolar carcinoma, which shows better prognosis than the other adenocarcinoma subtypes, expressed this protein at a high level [9]. As recently reported, DYRK2 is also

Y. Enomoto · S.-i. Yamashita (✉) · Y. Yoshinaga · Y. Fukami · S. Miyahara · A. Iwasaki
Department of General Thoracic, Breast, and Pediatric Surgery, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonanku, Fukuoka 814-0180, Japan
e-mail: yamashi1@fukuoka-u.ac.jp

K. Nabeshima
Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

Published online: 06 August 2014

downregulated in ductal carcinoma of the breast [7]. They showed that breast cancer patients with low DYRK2 expression had significantly worse distant recurrence-free survival than those with high expression [10].

In this study, it was hypothesized that the alternative expression of DYRK2 is associated with breast carcinogenesis or tumor progression. Therefore, the expression of DYRK2 in breast cancer and the correlation between recurrence and DYRK2 expression were investigated. We report our evaluation of this molecule and its ability to predict worse survival.

Materials and methods

Patients and samples

Between January 2000 and December 2009, 274 samples from breast cancer patients who had undergone surgery were obtained from Fukuoka University Hospital (Fukuoka, Japan). Our institutional ethical committee approved the retrospective study and waived the need for patient consent. The samples were histologically diagnosed for primary adenocarcinoma of the breast by hematoxylin and eosin (H&E) staining. None of the patients had received radiation therapy or chemotherapy before surgery.

Immunohistochemical analysis

Four-micrometer sections were prepared for tissue slides. Antigen retrieval was performed at 121 °C for 10 min in an autoclave with citrate buffer (pH 6.0) after deparaffinization. Ten percent goat serum (Nichirei Tokyo, Japan) was used to block nonspecific binding. Staining with polyclonal anti-DYRK2 antibody (AP7534a; Abgent, San Diego, CA, USA) with diluents, 1:50, was performed overnight at 4 °C. After a reaction with 3 % hydrogen peroxide for 20 min at room temperature, polymer anti-rabbit (goat) antibody (K4002; Dako, Glostrup, Denmark) for DYRK2 was applied and incubated for 30 min at room temperature. Negative controls were incubated without the primary antibody.

Immunohistochemical (IHC) staining was evaluated as follows: 0, no staining or faint cytoplasmic staining in less than 10 % of tumor cells; 1+, faint cytoplasmic staining in more than 10 % of tumor cells; 2+, weak or moderate cytoplasmic staining in more than 10 % of tumor cells; and 3+, more than 10 % of tumor cells having strong cytoplasmic staining. Since we previously reported a DYRK2 IHC staining study, 0 or 1+ staining intensity was considered DYRK2 negative and 2+ or 3+ staining was considered positive [11]. For evaluation of the reliability, two independent assessors estimated the staining positivity of two serial sections. For Ki-67 staining, 4-μm sections were prepared and antigen retrieval was performed at 121 °C for 15 min in an autoclave with citrate buffer (pH 9.0) after deparaffinization. Ten percent goat serum (Nichirei Tokyo, Japan) was used to block nonspecific binding. Staining with polyclonal anti-Ki-67 antibody (Dako, Glostrup, Denmark) with diluents, 1:100, was performed overnight at 4 °C. After a reaction with 3 % hydrogen peroxide for 20 min at room temperature, polymer anti-mouse antibody (K4002; Dako, Glostrup, Denmark) for Ki-67 was applied and incubated for 30 min at room temperature.

Table 1 Patient characteristics

Characteristics	All patients		DYRK2-positive (%)	DYRK2-negative (%)	*p*
Menopause	Post	210	60 (29)	150 (71)	0.11
	Pre	64	25 (39)	39 (61)	
Tumor size	T1a	9	4 (44)	5 (56)	0.05
	T1b	27	14 (52)	13 (48)	
	T1c	104	30 (29)	74 (71)	
	T2	131	35 (27)	96 (73)	
	T3	3	2 (67)	1 (33)	
Nodal status	(+)	59	12 (20)	47 (80)	0.04
	(−)	215	73 (34)	142 (66)	
Histological subtype	IDC	245	78 (32)	167 (68)	0.12
	ILC	3	1 (33)	2 (67)	
	Others	15	1 (7)	14 (93)	
	Unknown	11	5 (45)	6 (55)	
Histological grade	1	118	39 (33)	79 (67)	0.84
	2	102	30 (29)	72 (71)	
	3	42	13 (31)	29 (69)	
	Unknown	12	3 (45)	9 (83)	
ER	(+)	212	68 (32)	144 (67)	0.49
	(−)	62	17 (27)	45 (73)	
PgR	(+)	181	59 (33)	122 (67)	0.67
	(−)	90	26 (30)	64 (70)	
	Unknown	3	–	3 (100)	
HER2	(+)	45	14 (31)	31 (69)	0.97
	(−)	223	70 (31)	153 (69)	
	Unknown	6	1 (17)	5 (83)	
Ki-67	(+)	96	28 (29)	68 (71)	0.78
	(−)	148	47 (32)	101 (68)	
	Unknown	30	10 (33)	20 (67)	
CT	(+)	94	19 (20)	75 (80)	0.01
	(−)	123	44 (36)	79 (64)	
	Unknown	57	22 (40)	35 (60)	
HT	(+)	188	61 (32)	127 (68)	0.21
	(−)	48	10 (21)	38 (79)	
	Unknown	38	14 (37)	24 (63)	

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *CT* chemotherapy, *HT* hormone therapy

Negative controls were incubated without the primary antibody. IHC staining (labeling index) was evaluated as previously described [12]. Ki-67 index higher than 15 % was defined as positive in this study. Conventionally, estrogen receptor and progesteron receptor status higher than 1 % was defined as positive. HER2 expression was evaluated by using HercepTest (Dako, Japan). HER2 status was defined as 0, 1+, 2+, or 3+ according to the HercepTest score and considered positive when 3+ membrane labeling was observed; 2+ was followed by fluorescence in situ hybridization (FISH) (Path Vision HER-2DNA Probe Kit; Abbott Japan Co., Ltd.), and amplification showed that HER2 and CEP17 signal ratio of ≥2.0 was defined as positive.

Statistical analysis

All statistical analyses were performed using SPSS 14.0 (SPSS Inc., Tokyo, Japan). Different variables of the tumors and normal tissues were analyzed with the chi-square test or Fisher's exact test. Disease-free and overall survival was analyzed using the Kaplan-Meier method and evaluated by the log-rank test. Significant differences were accepted at $p<0.05$. Confounding factors were evaluated by Cox regression hazard models.

Results

The relationship between clinicopathological characteristics and DYRK2 expression

We investigated the correlation between the clinicopathological characteristics of breast cancer and DYRK2 expression. A total of 85 out of 274 cases (31 %) were DYRK2 positive. As shown in Table 1, we found a significant correlation between DYRK2 expression and nodal involvement, but not other factors. The expression pattern of DYRK2 is shown in Fig. 1. Positive cases showed strong granular staining in the cytoplasm of breast cancer cells from the resected specimen of invasive ductal carcinoma. Furthermore, although normal stromal cells did not show any positive staining of DYRK2, ductal epithelial cells showed weak expression of DYRK2. The correlation between DYRK2 and the other markers, including HER2 and Ki-67, was investigated; however, no relationship was found between them.

Prognostic value of DYRK

We investigated the potential of this protein to predict prognosis. Figure 2 shows disease-free survival according to the stratification of DYRK2 expression. Median follow-up period

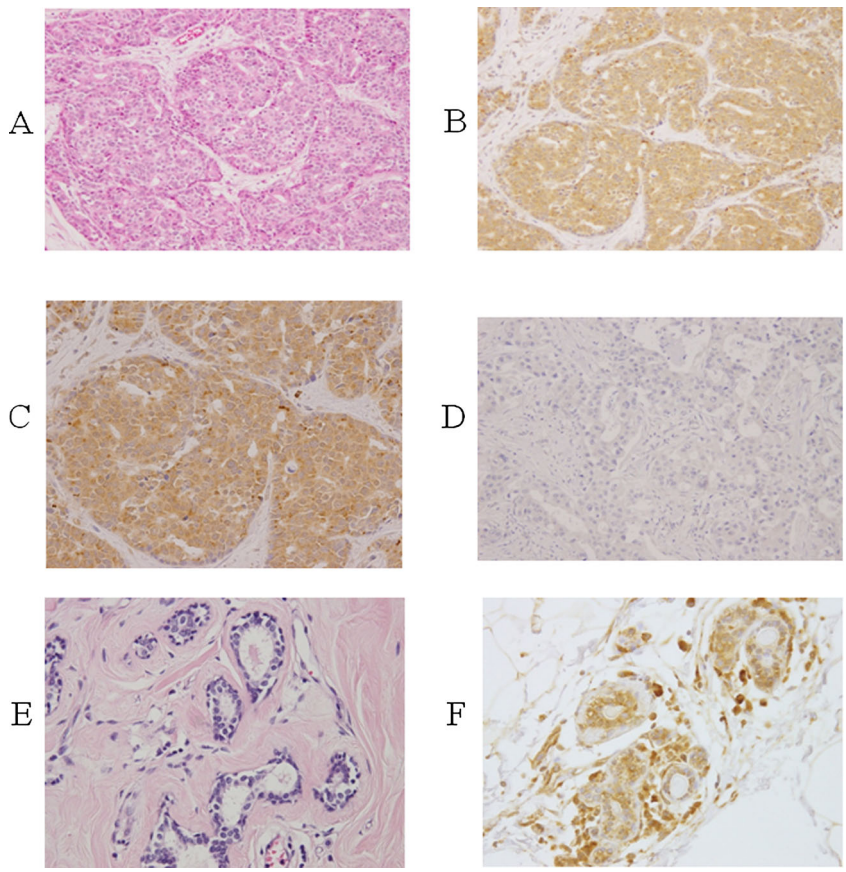


Fig. 1 Representative DYRK2 protein expression in breast cancer by immunohistochemical staining. Cytoplasm of tumor cells in invasive ductal cancer was strongly stained (**a** HE; **b** DYRK2, ×100; **c** DYRK2, ×200). Negative staining of invasive ductal cancer (**d** DYRK2, ×200). Weak staining was found in ductal epithelial cells (**e** HE; **f** DYRK2, ×200). *HE* hematoxylin-eosin staining

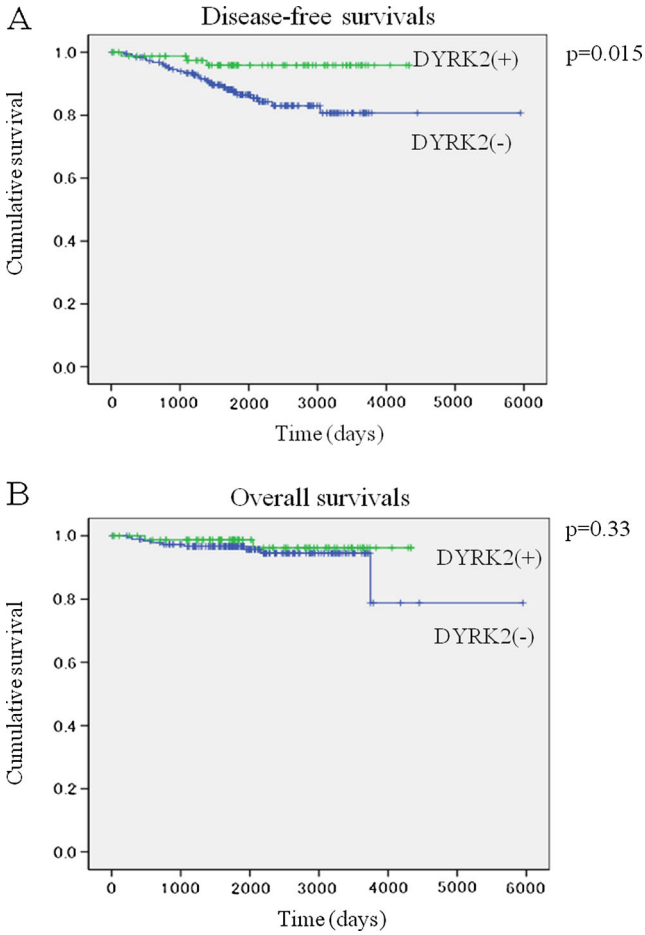


Fig. 2 Disease-free survival of patients without nodal involvement according to DYRK2 protein expression (**a**). Ten-year disease-free survival was 95.9 % in the DYRK2-positive group (n=73) and 87.3 % in the negative group (n=142, p=0.015). Overall survival was not significantly different between the two groups (**b**)

was 63 months. Ten-year disease-free survival in the DYRK2-positive group was significantly better than that in the negative group in all patients (p=0.016). Furthermore, it was found that

Table 2 Univariate Cox proportional hazard model

Characteristics	HR	95 % CI	p
Menopausal status (pre- vs. postmenopausal)	0.85	0.27–2.63	0.77
T factor (T1 vs. >T2)	2.32	0.86–6.23	0.095
Histological grade (G1 vs. >G2)	1.88	0.88–4.04	0.11
Estrogen receptor (negative vs. positive)	0.36	0.13–0.99	0.049
Progesterone receptor (negative vs. positive)	0.37	0.14–0.99	0.048
HER2 (negative vs. positive)	0.64	0.00–23.12	0.32
Ki-67 (negative vs. positive)	2.13	0.74–6.17	0.16
Adjuvant chemotherapy (without vs. with)	2.02	0.71–5.77	0.19
Adjuvant hormone therapy (without vs. with)	0.37	0.13–1.01	0.05
DYRK2 expression (negative vs. positive)	0.12	0.02–0.94	0.04

HR hazard ratio, *CI* confidence interval, *HER2* human epidermal growth factor receptor 2

Table 3 Multivariate Cox proportional hazard model

Characteristics	HR	95 % CI	p
Adjuvant hormone therapy (without vs. with)	1.62	0.54–48.8	0.78
Estrogen receptor (negative vs. positive)	0.46	0.02–12.4	0.64
Progesterone receptor (negative vs. positive)	0.47	0.13–1.71	0.25
DYRK2 expression (negative vs. positive)	0.13	0.02–1.02	0.05

HR hazard ratio, *CI* confidence interval

disease-free survival rates were significantly different between positive and negative groups of DYRK2 expression by IHC without node positivity (95.9 and 87.3 %, respectively, p= 0.015, Fig. 2a). However, 10-year overall survival did not show a significant difference between the two groups because of the low incidence of death in the follow-up period (p=0.33, Fig. 2b). We found that estrogen receptor status, progesterone receptor status, adjuvant hormone therapy, and DYRK2 expression showed prognostic significance in node-negative cases by univariate analysis (Table 2); however, only DYRK2 expression showed a tendency for the potential to have a prognostic impact by multivariate Cox regression analysis (Table 3).

Discussion

In this study, we focused on the assessment of DYRK2 as a new prognostic marker of breast cancer. DYRK2 is a dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase gene that phosphorylates both Ser/Thr and Tyr [4]. The DYRK family is involved in regulating processes such as cell proliferation, cytokinesis, and cell differentiation [13]. In addition to these activities, DYRK2 is an effector kinase for Ser46 of p53, which leads to apoptosis in the response to severe DNA damage [3]. Since the knockout of DYRK2 function attenuates adriamycin (ADR)-induced apoptosis, DYRK2 is a key protein in p53-induced apoptosis [5]. Furthermore, DYRK2 can induce apoptosis in a p53-independent manner [5].

As was recently reported, DYRK2 is downregulated in breast, colon, and prostate cancer [7]. Furthermore, downregulated DYRK2 was shown to contribute to breast cancer invasion by increased Snail protein, which is a zinc finger protein, and suppresses E-cadherin promoter, via epithelial-mesenchymal transition (EMT) [10]. Since tumors with EMT lead to metastasis, early recurrence may occur in patients with low expression of DYRK2. Although we have not evaluated the expression of Snail protein or E-cadherin, our result that DYRK2 is closely associated with lymph node metastasis may support this possible mechanism. In addition, these findings suggest that DYRK2 is a possible predictive marker of early recurrence regardless of the case of node negative.

Another report showed that the downregulation of DYRK2 stabilizes c-Jun and c-Myc, which is a transcriptional factor for cell proliferation, to accelerate tumor progression and invasion in breast cancer [7, 10]. We investigated the correlation between the proliferative activity, represented by the Ki-67 index, and DYRK2 expression. Since our results revealed that the low expression of DYRK2 was not related to the expression of Ki-67, DYRK2 may have another mechanism of involvement in tumor progression. In our study, tumor cells consisting of the intraductal component showed positive expression of DYRK2 and decreased in invasive carcinoma of the breast. Taking these findings together, it is speculated that the lack of DYRK2 induces cell proliferation and invasion through the basement membrane.

The aim of this study is not only to investigate the DYRK2 expression in breast cancer but also possibly to identify an early recurrence marker of early stage breast cancer. Therefore, we evaluated the relationship between recurrence-free survival and DYRK2 expression in patients without nodal involvement. Downregulation of DYRK2 is closely associated with a high recurrence rate and worse recurrence-free survival. These results may suggest that DYRK2 can be a promising marker of recurrence for early stage breast cancer. Although previous report showed that the DYRK2 low expression group had significantly worse distant recurrence-free survival than high expression group, these findings were analyzed from the results including patients with variable stages [10]. Therefore, we evaluated prognostic significance in the early stage breast cancer without node metastasis in our study. Although DYRK2 expression by multivariate analysis showed a prognostic tendency for disease-free survival, a study on more patients without nodal involvement may show a significant impact because of a lack of power here due to the low incidence of recurrence.

In conclusion, this study demonstrated that patients with DYRK2-negative breast cancer had a higher frequency of lymph node metastasis than patients with DYRK2-positive tumor. DYRK2 might be a powerful tool to stratify worse candidate groups in breast cancer patients. Further investigation of DYRK2 might offer new insight into this possibility.

Acknowledgments The authors appreciate the technical support of Ms. Yoko Miyanari.

Conflicts of interest None.

References

1. Fisher B, Bauer M, Wickerham DL, Redmond CK, Fisher ER, Cruz AB, et al. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. Cancer. 1983;52:1551–7.
2. Mulligan JM, Hill LA, Deharo S, I Irwin G, Boyle D, Keating KE, Raji OY, McDyer FA, O'Brien E, Bylesjo M, Quinn JE, Lindor NM, Mullan PB, James CR, Walker SM, Kerr P, James J, Davison TS, Proutski V, Salto-Tellez M, Johnston PG, Couch FJ, Paul Harkin D, Kennedy RD. Identification and validation of an anthracycline/cyclophosphamide-based chemotherapy response assay in breast cancer. J Natl Cancer Inst. 2014 Jan;106(1)
3. Kim C, Paik S. Gene-expression-based prognostic assays for breast cancer. Nat Rev Clin Oncol. 2010;7:340–7.
4. Becker W, Weber Y, Wetzel K, Eirmbter K, Tejedor FJ, Joost HG. Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases. J Biol Chem. 1998;273:25893–902.
5. Taira N, Nihira K, Yamaguchi T, Miki Y, Yoshida K. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. Mol Cell. 2007;25:794–6.
6. Pérez M, García-Limones C, Zapico I, Marina A, Schmitz ML, Muñoz E, et al. Mutual regulation between SIAH2 and DYRK2 controls hypoxic and genotoxic signaling pathways. J Mol Cell Biol. 2012;4:316–30.
7. Taira N, Mimoto R, Kurata M, Yamaguchi T, Kitagawa M, Miki Y, et al. DYRK2 priming phosphorylation of c-Jun and c-Myc modulates cell cycle progression in human cancer cells. J Clin Invest. 2012;122:859–72.
8. Jung HY, Wang X, Jun S, Park JI. Dyrk2-associated EDD-DDB1-VprBP E3 ligase inhibits telomerase by TERT degradation. J Biol Chem. 2013;288:7252–62.
9. Yamashita S, Chujo M, Tokuishi K, Anami K, Miyawaki M, Yamamoto S, et al. Expression of dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) can be a favorable prognostic marker in pulmonary adenocarcinoma. J Thorac Cardiovasc Surg. 2009;138:1303–8.
10. Mimoto R, Taira N, Takahashi H, Yamaguchi T, Okabe M, Uchida K, et al. DYRK2 controls the epithelial-mesenchymal transition in breast cancer by degrading Snail. Cancer Lett. 2013;339:214–25.
11. Yamashita S, Chujo M, Moroga T, Anami K, Tokuishi K, Miyawaki M, et al. DYRK2 expression may be a predictive marker for chemotherapy in non-small cell lung cancer. Anticancer Res. 2009;29:2753–7.
12. Domagala W, Markiewski M, Harezga B, Dukowicz A, Osborn M. Prognostic significance of tumor cell proliferation rate as determined by the MIB-1 antibody in breast carcinoma: its relationship with vimentin and p53 protein. Clin Cancer Res. 1996;2:147–54.
13. Yoshida K. Role for DYRK family kinases on regulation of apoptosis. Biochem Pharmacol. 2008;76:1389–94.