

## DYRK2 Expression May be a Predictive Marker for Chemotherapy in Non-small Cell Lung Cancer

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**Abstract.** *Background:* The possibility of a dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase gene, DYRK2, predicting benefit from chemotherapy for patients with recurrent non-small cell lung cancer (NSCLC) was investigated. *Materials and Methods:* Forty patients with recurrent disease after surgery received several combinations of platinum-based chemotherapy. The chemotherapy effectiveness was evaluated according to RECIST (response evaluation criteria in solid tumors). Immunohistochemical (IHC) analysis was used to determine the expression of DYRK2. *Results:* Although the response rates between the two groups did not show statistical differences, the disease control rate of the DYRK2-positive group (15 out of 17, 88%) was significantly different from the DYRK2-negative group (8 out of 23, 35%,  $p=0.001$ ). The median time to the progression (TTP) of disease was significantly different between the two groups (310 days in the positive group, 120 days in the negative,  $HR=2.12$ , 95% CI=[1.1-4.09],  $p=0.024$ ). Multivariate analysis showed that negative DYRK2 expression was a strong predictor of disease progression ( $HR=3.01$ , 95% CI=[1.45-6.26],  $p=0.003$ ). *Conclusion:* Patients with high DYRK2 expression could be good candidates for chemotherapy.

Non-small cell lung cancer (NSCLC) represents approximately 80% of all lung carcinomas. Although early-stage (I and II) NSCLC patients are treated surgically with curative intent, 30% to 60% develop recurrence and die as a result of their disease (1, 2).

A number of anticancer agents have become available for the treatment of recurrent NSCLC, including the taxanes, gemcitabine and vinorelbine with platinum (3). The

combination of one or more of these agents has resulted in high response rates and prolonged survival at one year in an Eastern Cooperative Oncology Group (ECOG) study (4). Additionally, cisplatin-based chemotherapy for metastatic NSCLC has resulted in improved survival, as compared with supportive care alone (5). However, the absolute improvement in time to progression (TTP) was modest, suggesting that only a minority of patients may benefit from the administration of these drugs.

Recently, predictive markers which may influence and predict the outcome of treatment in terms of either response or survival benefit have been reported, such as the expression of DNA excision repair cross complementing-1 (ERCC1) in NSCLC (6), tau protein in gastric (7) and breast cancer (8), and p53 in breast cancer (9). Nevertheless, a predictive marker of chemotherapy efficacy remains undetermined.

DYRK2, a dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase gene, has been identified as the most frequently amplified and overexpressed gene in lung adenocarcinomas and esophageal carcinoma (10). DYRK genes have the potential to phosphorylate both Ser/Thr and Tyr (10) and DYRK family genes are involved in regulating processes such as cell proliferation, cytokinesis, and cell differentiation. It has also previously been reported that DYRK2 regulates p53 to induce apoptosis in response to DNA damage via phosphorylation of Ser 45 (11). As knocking down of the DYRK2 function attenuates ADR (adriamycin)-induced apoptosis, DYRK2 has a key role in p53-induced apoptosis. In addition, DYRK2 can induce apoptosis in a p53-independent manner (11). However, the functional significance of DYRK2 has not been reported and still remains unclear.

Herein an evaluation of this marker and the ability to predict benefit from chemotherapy for patients with recurrent NSCLC is reported.

### Materials and Methods

*Patients and samples.* Forty samples from surgically treated NSCLC patients were obtained at Oita University Hospital (Oita, Japan) between 2000 and 2007. All samples were

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formalin-fixed, paraffin-embedded and histologically diagnosed for primary non-small cell lung cancer by hematoxylin and eosin (H&E) staining.

**Chemotherapy regimens and evaluations.** Several regimens of chemotherapy for recurrent disease were used. 30 mg vinorelbine/m<sup>2</sup> with 80 mg/m<sup>2</sup> cisplatin were administered on day 1, of a four-week cycle; 175 mg/m<sup>2</sup> paclitaxel was administered on day 1, followed on the same day by carboplatin at a dose calculated to produce an area under the curve (AUC) of 5 mg/ml/min, in a three-week cycle, gemcitabine was given to the patients at 1,000 mg/m<sup>2</sup> on days 1, 8, and 15 with carboplatin AUC 5 on day 1. Other regimens included 5-fluorouracil (5-FU), tegafur plus 5-chloro-2,4-dihydroxypyridine and potassium oxonate (TS-1), tegafur uracil (UFT) and irinotecan (CPT 11) in variable combinations. Cisplatin was combined with vinorelbine in 14 patients; cisplatin with other agents in 10 patients; carboplatin with paclitaxel in 11 patients; carboplatin with gemcitabine in 4 patients and carboplatin with other agents in 1 patient.

Prior irradiation at the target sites was evaluable if the radiation therapy was completed before chemotherapy was initiated. Stable brain metastases after irradiation were evaluable. All the indicator sites were evaluated according to the RECIST (response evaluation criteria in solid tumors) (12) and targeted lesions were measured by CT scan. The disease control rate or clinical benefit was defined as the rate of the sum total of CR (complete response), PR (partial response) and SD (stable disease) (13). Metastatic sites included 20 at a single site (13 lymph nodes, 5 lung, 2 chest wall) and 20 in multiple organs (hilar or mediastinum lymph nodes, lung, chest wall, bone, brain and liver).

**Immunohistochemical (IHC) analysis.** Four-micrometer sections were prepared for tissue slides. Antigen retrieval was performed at 121°C for 10 minutes in an autoclave with citrate buffer (pH 6.0) after deparaffinization. Ten percent goat serum (Nichirei Tokyo, Japan) was used for blocking of nonspecific binding. Staining with anti-P53 antibody (M7001; Dako, Glostrup, Denmark) and with polyclonal anti-DYRK2 antibody (AP7534a; Abgent, San Diego, CA, USA) with diluent, 1:50 was performed overnight at 4°C. After reaction with 3% hydrogen peroxide for 20 minutes at room temperature, polymer anti-rabbit (goat) antibody (K4002; Dako) for DYRK2 and anti-mouse antibody (K4000; Dako) for p53 was applied and incubated for 30 minutes at room temperature. Negative controls were incubated without the primary antibody.

The IHC staining was evaluated as follows: 0, no staining or faint cytoplasmic staining in less than 10% of the tumor cells; 1+, faint cytoplasmic staining in more than 10% of the tumor cells; 2+, weak or moderate cytoplasmic staining in more than 10% of tumor cells; 3+, more than 10% of strong cytoplasmic staining. Since DYRK2 IHC staining study has not been reported previously, 0 or 1+ staining intensity was considered as DYRK2 negative and 2+ or 3+ staining was considered as positive according to a previous report referring to HER-2 (Human epidermal growth receptor-2) protein (14). p53 staining was evaluated as previously described (2). For reliability of evaluation, two independent assessors (M.C. and S. T. (pathologists)) estimated positivity by the staining of two serial sections.

**Statistical analyses.** All the statistical analyses were performed using the Stat View J5.0 (SAS Institute Inc Tokyo, Japan). The supplier detail different variables of the tumors and normal tissues

Table I. Characteristics of the patients.

Characteristics	All patients	DYRK2 (+)	DYRK2 (-)	p-Value
Age (years)				
<70	29	13	16	0.63
>70	11	4	7	
Gender				
male	30	13	17	0.85
female 10	4	6		
p-Stage				
I	11	4	7	0.25
II	7	5	2	
III	20	8	12	
IV	2	0	2	
Tumor				
T1	14	5	9	0.64
T2	19	9	10	
T3	3	2	1	
T4	4	1	3	
Nodal status				
(-)	21	10	11	0.49
(+)	19	7	12	
Histological type				
Ad	26	12	14	0.55
Sq	5	1	4	
Other	9	4	5	
Chemotherapy 1				0.76
CDDP	24	10	14	
CBDCA	15	7	8	
Chemotherapy 2				0.9
PTX	11	5	6	
VNR	14	6	8	
Other 15	6	9		
Time to recurrence (days, mean±SD)		1042±661	788±675	0.08

CDDP, cisplatin; CBDCA, carboplatin; PTX, paclitaxel; VNR, vinorelbine; Ad, adenocarcinoma; Ad, adenocarcinoma; Sq, squamous cell carcinoma.

were analyzed by Chi-square test or Fisher's exact tests. Time-to-progression (TTP) was analyzed using the Kaplan-Meier method and evaluated by the log-rank test. Statistical significant differences were accepted at  $p<0.05$ .

## Results

**Clinicopathological characteristics.** The relationship between the clinicopathological characteristics and DYRK2 expression was investigated and as shown in Table I, no correlation between age, sex, pathological stage, tumor size, nodal status, histological type, chemotherapy regimen (cisplatin compared with carboplatin or paclitaxel with vinorelbine) or time to recurrence and DYRK2 expression was found. The expression pattern of DYRK2 is shown in Figure 1. Positive cases showed strong granular staining in the cytoplasm of the cancer cells (Figure 1 A and B).

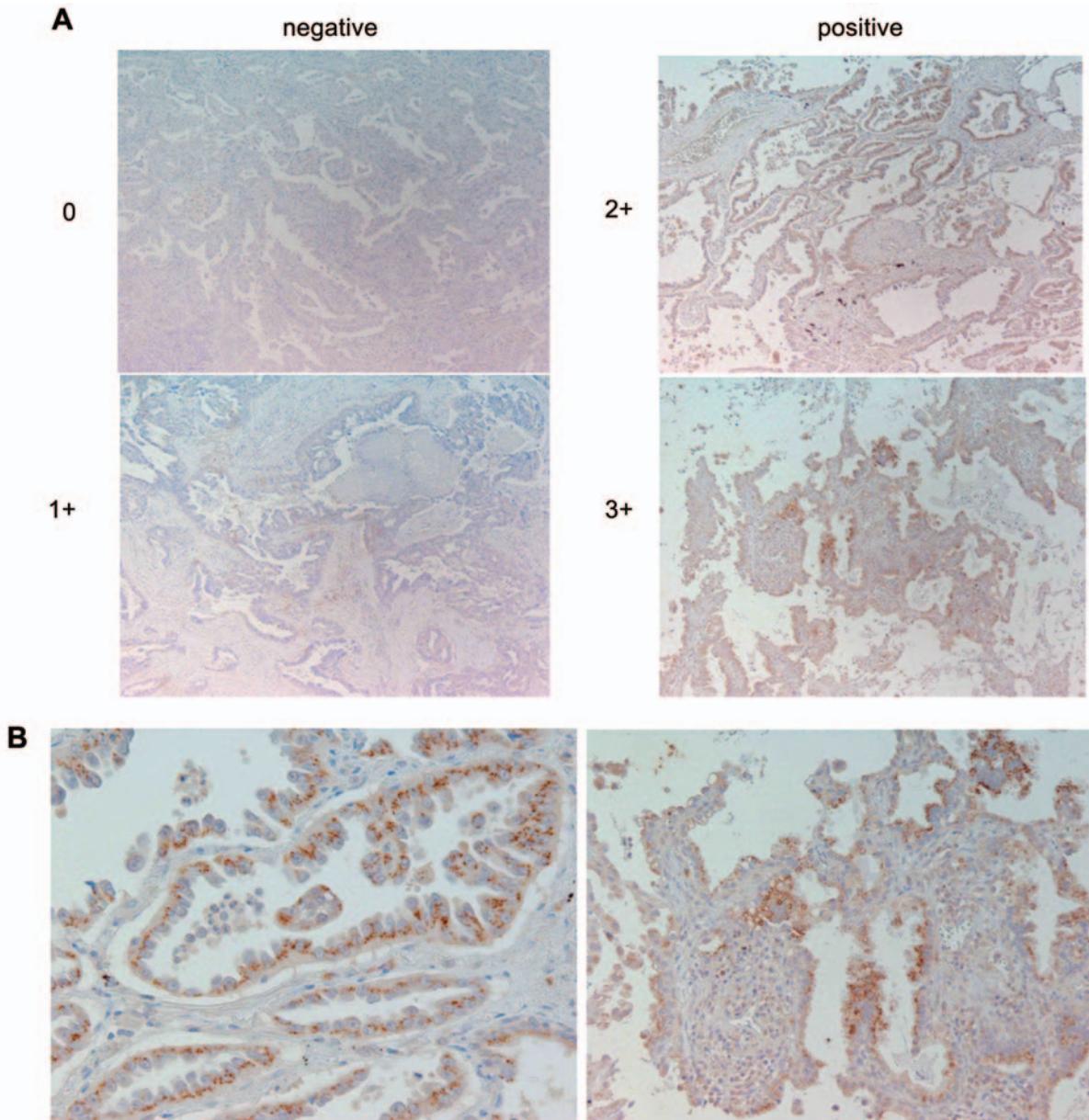


Figure 1. Representative DYRK2 protein expression in NSCLC by immunohistochemistry. (A) 0 or 1+ were considered negative and 2+ or 3+ were considered positive staining of adenocarcinoma, ( $\times 100$ ). (B) Cytoplasm of cancer cells was stained strongly in 3+ cases ( $\times 400$ ).

*Clinical benefit according to DYRK2 expression.* As the treatment regimen was basically the combination of one or more taxanes, vinorelbine, gemcitabine with a platinum compound, the effectiveness of a cisplatin-based regimen and a carboplatin-based regimen were analyzed together. Table II shows the relationship between the disease control rate (CR, PR and SD) and the DYRK2 expression. The overall response rate was 24% (4 out of 17) in the DYRK2-positive group compared with 4% (1 out of 23) in the negative group and the response rates

between the two groups did not show a statistical difference ( $p=0.14$ , Fisher's exact test). On the other hand, the disease control rate of the DYRK2-positive group (15 out of 17, 88%) was significantly different from that of the DYRK2-negative group (8 out of 23, 35%,  $p=0.001$ , Fisher's exact test).

*Progression-free survival.* Figure 2 shows the progression-free survival according to the DYRK2 expression. The median TTP of disease was 310 days in the DYRK2-positive

Table II. Disease control rates and DYRK2 expression.

Clinical response	All patients	DYRK2 (+) (%)	DYRK2 (-) (%)	p-Value
CR	-	-	-	
PR	5	4	1	
SD	18	11	7	
PR+SD	23	15 (88)	8 (35)	p=0.001
PD	17	2 (12)	15 (65)	
Total	40	17	23	

CR, Complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table III. Multivariate Cox proportional hazard model.

Characteristics	HR	95% CI	p-Value
Age [<70 vs. 70≥]	1.36	0.62-2.97	0.44
Stage [I vs. II-IV]	1.51	0.67-3.43	0.32
Histology [adenocarcinoma vs. non-adenocarcinoma]	1.32	0.61-2.84	0.49
DYRK2 [positive vs. negative]	3.01	1.45-6.26	0.003

HR, Hazard ratio; CI, confidence interval.

group, as compared with 120 days in the DYRK2-negative group ( $HR=2.12$ , 95% CI=[1.1-4.09],  $p=0.024$ ). In the multivariate Cox regression analysis, negative DYRK2 expression was a strong predictor of disease progression ( $HR=3.01$ , 95% CI=[1.45-6.26],  $p=0.003$ , Table III).

## Discussion

In the present study, 42.5% of DYRK2-positive cases were found which was higher than in previous reports (10). This difference might have been due to the staining methods, the use of a different antibody or the evaluation of IHC. As in a previous report (10), no correlation between the clinicopathological factors and DYRK2 expression was found. More number of patients should be investigated to determine this relationship.

As only five patients showed a partial response, the overall response rate was quite low and DYRK2 expression was not a good tool to stratify between the good and poor responders for chemotherapy. However, SD is a benefit of chemotherapy for patients in the clinical setting, and the disease control rate (including CR, PR, and SD) according to the expression of

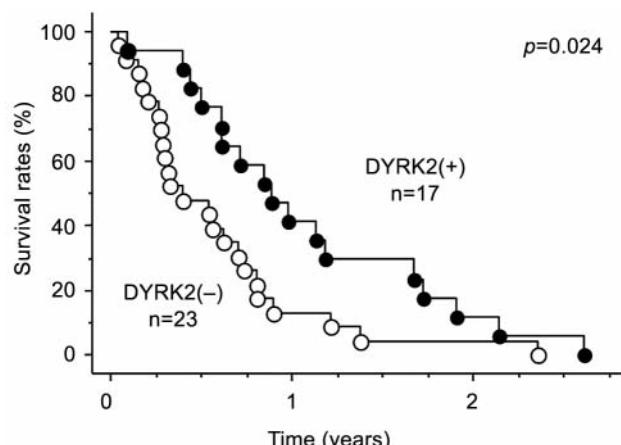


Figure 2. Time to progression according to DYRK2 expression. Median time to progression, 310 days in the DYRK2-positive group and 120 in the negative group.

DYRK2, showed a positive correlation with clinical benefit. Additionally, the DYRK2-positive group had longer TTP than the negative group and multivariate analysis showed that DYRK2 was a strong predictor of disease progression. However, as several combinations of anticancer agents, such as taxanes, gemcitabine, and vinorelbine with platinum were used, bias due to the power of these drugs cannot be ruled out. More patients treated with the same anticancer agents will be needed to clarify this possibility. When the relationship between the effectiveness of preoperative induction chemotherapy and DYRK2 expression was investigated in 16 patients, no significant correlation was found (data not shown). It is possible that DYRK2-positive cells underwent apoptosis induced by the induction chemotherapy but negative cells survived because of resistance. Accordingly, the discrepancy between clinical effectiveness and DYRK2 expression may be due to the modification of chemotherapy.

The p53 positivity was quite low (37.5%, 15 out of 40 cases) and no relationships between p53 expression and the clinical effectiveness of chemotherapy was found, which may have been due to the antibody or the scoring methods used. Although the power of this study was low because of the limited sample size, a tendency toward clinical benefit for chemotherapy in combination with p53-negative and DYRK2-positive tumors was shown (data not shown). If p53 negativity can exert an effect on normal function, DYRK2 which is upstream of the p53 gene has a critical role for inducing apoptosis with chemotherapy. However, as it has been reported that p53 mutation and protein overexpression are not significant prognostic or predictive factors in NCSLC (15), DYRK2 may induce apoptosis in a p53-independent manner or another effector of apoptosis located downstream

of DYRK2 may persist. Taken together, it is quite difficult to define the DYRK2 effect because of the complexity concerning p53 status. Further investigation will be needed. Whereas *DYRK2* is a promising single gene marker of sensitivity to chemotherapy, it is also clear that many tumors are not fully sensitive to treatment, suggesting additional pathways of resistance. Multigene predictors from several distinct molecular pathways of resistance will be more powerful than any single gene.

In conclusion, although the sample size was small, patients with DYRK2-positive tumors in recurrent NSCLC had a substantial benefit from chemotherapy, as compared with patients with DYRK2-negative tumors. DYRK2 might be a powerful tool for stratifying favorable candidate groups in lung cancer patients and further investigation might offer new insight into this possibility.

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