



# Cyclin-dependent kinase 4 upregulation mediates acquired resistance of dabrafenib plus trametinib in *BRAF* V600E-mutated lung cancer

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**Background:** Combination therapy with the B-Raf inhibitor, dabrafenib, and the MEK inhibitor, trametinib (DT) is commonly used to treat patients with B-Raf proto-oncogene, serine/threonine kinase V600E (*BRAF* V600E)-mutated non-small cell lung cancer (NSCLC). However, the mechanisms through which cancer develops DT resistance are unclear. Here, we investigated new mechanisms underlying acquired DT-resistant NSCLC with the *BRAF* V600E mutation.

**Methods:** We compared genomic signatures before and after DT treatment in patients with NSCLC.

**Results:** Two of four patients treated with DT developed carcinomatous pleuritis within 3 months. Target DNA sequencing and quantitative polymerase chain reaction (PCR) analyses revealed the increased expression level of cyclin-dependent kinase 4 (*CDK4*). We also found prominent protein expression of *CDK4* after DT treatment. Induction of *CDK4* expression in a cell line derived from a patient with the *BRAF* V600E mutation resulted in partial resistance to dabrafenib.

**Conclusions:** Our findings suggest a possible relationship between *CDK4* upregulation and acquired resistance to DT therapy.

**Keywords:** B-Raf proto-oncogene, serine/threonine kinase V600E (*BRAF* V600E); cell cycle; cyclin-dependent kinase 4 (*CDK4*); dabrafenib; trametinib

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## Introduction

Since the discovery of the oncogenic mutation in the B-Raf proto-oncogene, serine/threonine kinase V600E (*BRAF* V600E), combination therapy with the B-Raf inhibitor,

dabrafenib, and the MEK inhibitor, trametinib (DT) has been used for treating *BRAF* V600E-mutated non-small cell lung cancer (NSCLC) (1). Although mutations in the mitogen-activated protein kinase (MAPK) or phosphatidylinositol

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3-kinase (PI3K)/AKT pathways have been reported, the mechanisms of resistance to DT therapy are unclear (2-4).

Cyclin-dependent kinase 4 (*CDK4*) is involved in regulating cell proliferation during the G1 phase. *CDK4* forms a complex with cyclin D, which is regulated by MAPK activity, thereby mediating retinoblastoma (Rb) phosphorylation and resulting in cell cycle progression (5). *CDK4/6* inhibitors have been used as anticancer agents in hormone receptor-positive breast cancer and *KRAS*-mutated NSCLC (6). Moreover, combined inhibition of *RAF* and *CDK4/6* may have synergistic effects on tumors with *RAS* or *RAF* mutations (7). Additionally, *CD4/6* inhibitors can overcome resistance to *BRAF* inhibitors caused by cyclin D1 upregulation in *BRAF* V600E-mutated melanoma (8). Thus, targeting CDK may overcome resistance to *RAS*- and *RAF*-mutant cancers. *CDK4* may be implicated in the development of resistance in *BRAF* V600E-mutant NSCLC; however, the involvement of *CDK4* in acquired resistance is unclear.

Here, we established clinically resistant cell lines derived from pleural effusion samples of patients with NSCLC. We report two cases of clinically DT-resistant *BRAF* V600E-mutated NSCLC and compared the expression levels of cell cycle-related genes, including *CDK4*, in DT-naïve and DT-resistant cases. We present the following article in accordance with the STROBE reporting checklist (available at <https://dx.doi.org/10.21037/tlcr-21-415>).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All study participants provided informed consent and this study was approved by the Institutional Review Board of Asahikawa Medical University (No.: 18185/19081). Written consent was obtained from all patients whose samples were analyzed, including pleural effusions.

### Patients and treatments

Four patients were treated with DT, two of whom developed carcinomatous pleuritis after treatment. Pleural fluid from these two patients (LC-1, LC-6) was used for post-DT samples; lung tissue from corresponding patients collected before DT treatment was used for DT-naïve samples. The *BRAF* V600E mutant (IG: pleural effusion; ON: transtracheal lymph node biopsy) and the *BRAF* K601E mutant (MY: pleural effusion) were also subjected to the Oncomine Dx target test (Thermo Fisher Scientific, Waltham, MA, USA),

and cancer cells derived from DT-naïve patients harboring these genetic mutations were used as a reference.

### Establishment of cell line and expression constructs

Lymph node biopsies of patients with the *BRAF*\_V600E mutation (ON) were performed, and the excess specimens were used. The isolated lung cancer cells were cultured in RPMI 1640 medium (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin and 1 mmol/L sodium pyruvate as the primary culture medium. After confirming that the tumor cells were proliferating, single cells were cultured in 96-well plates, and only the proliferating lung cancer cells were passaged as cell lines.

The *CDK4* cDNA vector was a gift from Sander van den Heuvel (Addgene, plasmid #1876, Watertown, MA, USA). The *CDK4* cDNA vector or empty vector was transfected by electroporation (Neon system, Thermo Fisher Scientific, Waltham, MA, USA). Dissociated ON cells were resuspended in 8  $\mu$ L of Neon Resuspension Buffer R for every one million cells. For each electroporation, cells and 2  $\mu$ L of vector were aliquoted into a sterile microcentrifuge tube. A Neon Tip was inserted into the Neon Pipette and the cell-DNA mixture was aspirated into the tip avoiding air bubbles. The Neon Pipette was then inserted into the Neon Tube containing 3 mL of Neon Electrolytic Buffer E in the Neon Pipette Station. Cells were pulsed twice with a voltage of 1,400 and a width of 20. After the pulse, cells were quickly transferred into a RPMI1640 medium without antibiotics.

### Cell proliferation and growth assays

Dabrafenib and erlotinib were purchased from Selleckchem (Boston, MA, USA) and inhibition of growth was assessed by MTS assay according to previously established methods (9). All experimental points were set up in 6 to 12 wells and all experiments were repeated 2 times.

### Genetic analysis

*BRAF* mutations were identified by the droplet digital polymerase chain reaction (PCR) or OncomineDx Target Test (Thermo Fisher Scientific, Waltham, MA, USA) in 12 of 233 patients with NSCLC over a 5-year period (2015–2020) in Asahikawa Medical University Hospital. DNA was extracted from bronchial lavage fluid (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany). Digital

PCR was performed using a *BRAF* V600E-specific probe (Taqman cfDNA assay *BRAF* V600E, A44177 Thermo Fisher Scientific, Waltham, MA, USA) according to the QuantStudio3D (Thermo Fisher Scientific, Waltham, MA, USA) protocol. Genomic DNA mutations and copy number variations (CNVs) were assessed by a comprehensive cancer panel (160 genes, GeneRead DNaseq Targeted HC panel v2; QIAGEN, Hilden, Germany) on an Illumina MiSeq platform (Appendix 1).

### *mRNA expression analysis*

mRNA was extracted from formalin-fixed paraffin-embedded samples of LC-1 and LC-6 samples using RNeasy Mini Kit (QIAGEN, Hilden, Germany). mRNA expression was assessed by the real-time PCR using a TaqMan Array human anticancer drug panel (RAPRJ2Y, Thermo Fisher Scientific, Waltham, MA, USA).

### *Antibodies and Western blotting*

Cell lysis, Western blotting and immunohistochemistry (IHC) was done as previously described (9,10). The following antibodies were used for immunostaining and Western blotting: anti-CDK4 (D9G3E), anti-cyclin D1 (92G2) and anti- $\beta$ -actin (13E5) (Cell Signaling Technology, Danvers, MA, USA). The receptor tyrosine kinase (RTK) array (Proteome Profiler Human Phospho-RTK Array Kit, R&D Systems, Minneapolis, MN, USA) was purchased and used according to the manufacturer's recommended conditions as previously described (9). List of 49 RTKs measuring relative phosphorylation status on antibody arrays were shown in Appendix 2.

### *Statistical analysis*

In all cell growth assays, the mean and standard deviation were calculated from 6 wells of sample and 12 wells of control. The statistical analysis was carried out with two-sample unpaired Student *t*-test performed using R (version 3.4.1; The R Foundation, Vienna, Austria) and 95% CI of the mean difference was plotted.

## **Results**

### *Case LC-1*

A 61-year-old man was diagnosed with lung adenocarcinoma

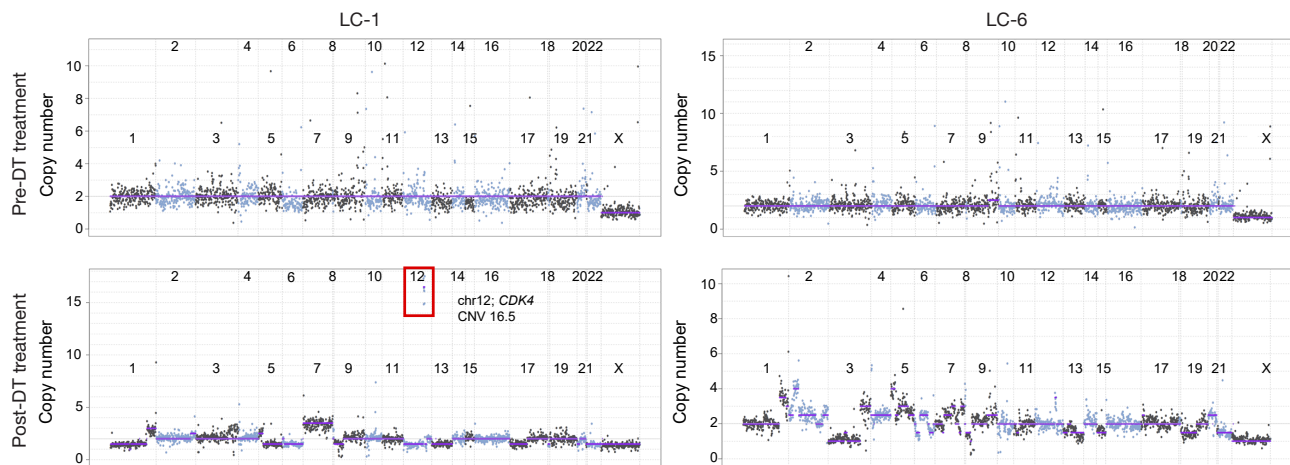
and underwent surgical resection of the right middle lobe (pathological stage 1A). Four years later, the adenocarcinoma recurred in the right upper lobe and metastasized to multiple lymph nodes, bone and the adrenal glands. The patient received four cycles of cisplatin/pemetrexed/bevacizumab, followed by maintenance therapy with pemetrexed/bevacizumab. After five cycles of maintenance therapy, new metastases were found in the cervical lymph nodes. Following a cycle of pembrolizumab, the patient developed fever and hypoxemia owing to drug-induced pneumonia. The drug was discontinued and the patient was treated with oral steroids for 3 months. The *BRAF* V600E mutation was detected in the surgical specimen (LC-1 pre); thus, DT was administered as a third-line therapy. DT caused a temporary reduction in tumor size; however, the disease progressed to brain metastasis, meningitis and pleural effusion (LC-1 post) within 3 months. Whole-brain irradiation was performed, but the patient died 1 month after discontinuing DT.

### *Case LC-6*

A 62-year-old man was diagnosed with lung adenocarcinoma by transbronchial lymph node biopsy with bronchoscopy. Because the patient had clinical stage 3B disease, four cycles of cisplatin/pemetrexed/bevacizumab were administered, followed by maintenance therapy with pemetrexed/bevacizumab. After seven cycles of maintenance therapy, the patient was switched to nivolumab as second-line therapy owing to right lung progression. After 19 cycles of nivolumab, the patient was switched to docetaxel/ramucirumab owing to right lung progression and lymph node metastasis. After 10 cycles of docetaxel/ramucirumab, the disease progressed with carcinomatous lymphangitis and bone metastasis. *BRAF* V600E was detected in a re-biopsy specimen of the mediastinal lymph nodes (LC-6 pre); thus, DT was started as a fourth-line therapy. After 11 months of DT, the disease progressed with pleural effusion (LC-6 post). The patient died 4 months after discontinuing DT.

### *Genomic analysis and protein expression*

Next-generation sequencing was performed for LC-1 and LC-6 pre/post to evaluate acquired DT resistance (Figure 1, Table 1, Table S1). In LC-1, *BRAF* V600E was identified before and after DT treatment. The copy number variation (CNV) analysis showed that *CDK4* on chromosome (chr) 12 was amplified after DT treatment. In LC-6, *BRAF* V600E was confirmed by SNV analysis before and after treatment,

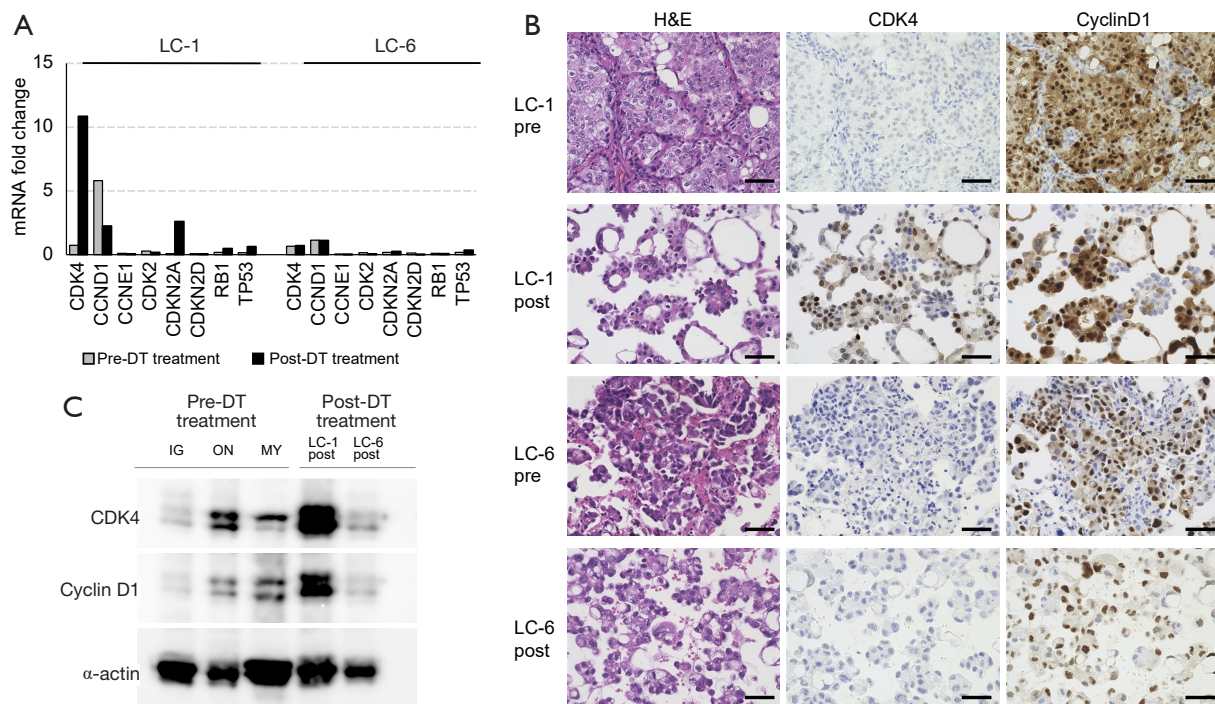


**Figure 1** Changes in DNA copy number before and after treatment with DT in patients with *BRAF* V600E-mutated lung adenocarcinoma. Left panel: focal amplification (CNV =16.5) of the gene locus encoding *CDK4* at chr12 in specimens collected after the acquisition of resistance to DT in patient LC-1. Right panel: four copies of the gene on chrs 2 and 5, and copy number loss on chr 3 were observed in specimens collected after the acquisition of resistance in patient LC-6. DT, dabrafenib and trametinib; chr, chromosome; *CDK4*, cyclin-dependent kinase 4; *BRAF* V600E, B-Raf proto-oncogene, serine/threonine kinase V600E.

**Table 1** CNV in patients LC-1 and LC-6

Cases	Chr	Gene name	Variant type	CNV change	Pre-DT treatment (copy number)	Post-DT treatment (copy number)
LC-1	Chr12	<i>CDK4</i>	CNV	Gain	2	16.5
	Chr7	<i>CARD11</i> <i>PMS2</i> <i>RAC1</i> <i>IKZF1</i> <i>EGFR</i> <i>MET</i> <i>SMO</i> <i>BRAF</i> <i>EZH2</i>	CNV	Gain	2	3.5
LC-6	Chr1	<i>MUTYH</i>	CNV	Loss	2	1.0
	Chr2	<i>ALK</i>	CNV	Gain	2	4.0
	Chr5	<i>TERT</i> <i>IL7R</i>	CNV	Gain	2	4.0
	Chr1	<i>DDR2</i> <i>CDC73</i>	CNV	Gain	2	3.5
	Chr3	<i>FANCD2</i> <i>VHL</i> <i>XPC</i> <i>MLH1</i> <i>SETD2</i> <i>BAP1</i> <i>PBRM1</i>	CNV	Loss	2	1

CNV, copy number variation; chr, chromosome; DT, dabrafenib and trametinib.



**Figure 2** Investigation of mRNA expression and protein expression (using IHC and Western blotting) in samples from patients LC-1 and LC-6 before and after DT treatment. (A) Transcript levels of cell cycle-related genes determined using quantitative real-time PCR. (B) Microscopy-based images of H&E, anti-CDK4 and anti-cyclin D1 staining before and after treatment with DT in samples from patients LC-1 and LC-6 (scale bar: 50  $\mu$ m). (C) Protein expression in pleural effusion-derived cells from *BRAF* mutation-positive patients before and after DT treatment (IG: pleural effusion; ON: transtracheal lymph node biopsy; MY: pleural effusion). *CDK4*, cyclin-dependent kinase 4; H&E, hematoxylin and eosin; DT, dabrafenib and trametinib; IHC, immunohistochemistry; PCR, polymerase chain reaction; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase.

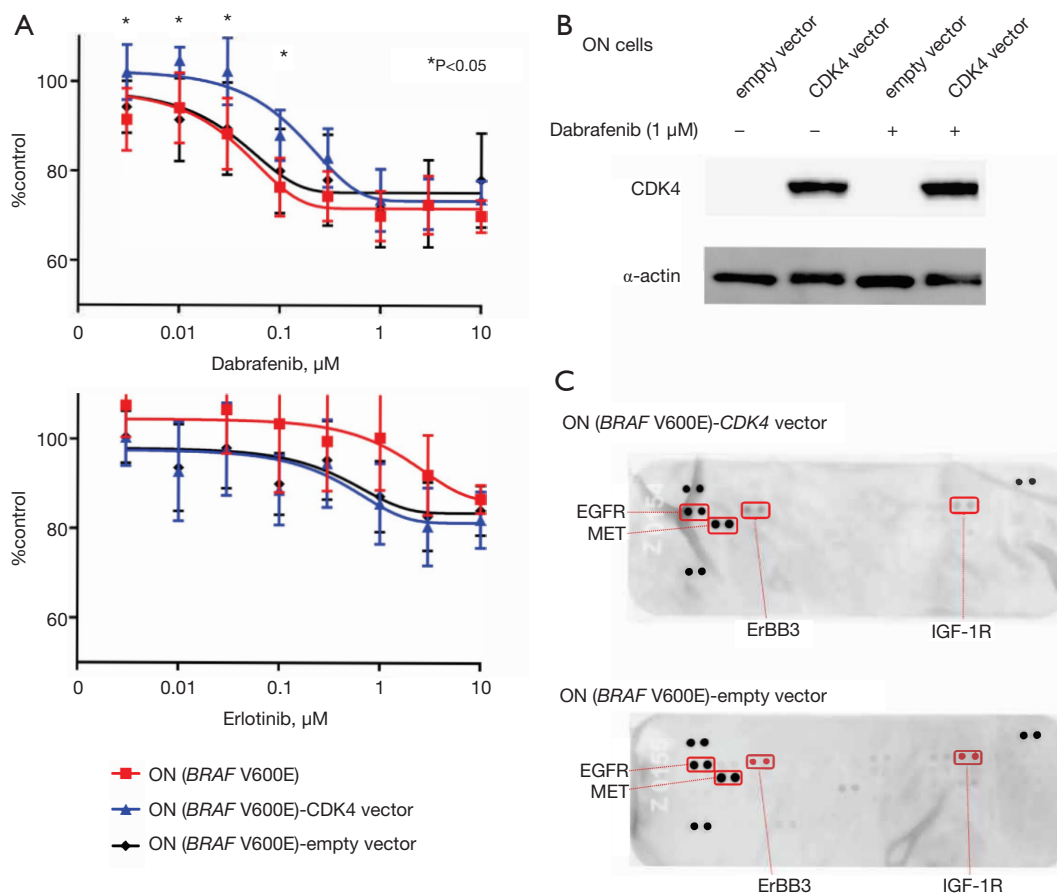
and missense S2264L and nonsense S2269\* mutations in the *AT-rich interaction domain 1A (ARID1A)* were detected on chr 1 in the post-DT sample. In both LC-1 and LC-6, other copy number aberrations were detected; however, there was no focal amplification directly associated with resistance.

Next, we performed quantitative PCR and IHC analyses on LC-1 and LC-6 pre/post samples (Figure 2A,2B). In the LC-1 post sample, *CDK4* mRNA was upregulated, as were the tumor suppressors *CDKN2A* (encoding p16), *TP53*, and *Rb1*. The *CDK4* protein was upregulated after DT therapy in LC-1, whereas *CCND1* mRNA (encoding cyclin D1) was downregulated. However, cyclin D1 expression was observed before and after treatment, and cell cycle signatures were not markedly altered in LC-6 (Figure 2A,2B). Western blotting results (Figure 2C) showed that *CDK4* was expressed abundantly in LC-1, but only marginally in the other samples. Finally, we tested *in vitro* experiments to determine whether cells in the *BRAF* V600E

cell line expressing the *CDK4* protein are resistant to *BRAF* inhibitors. ON cells were transfected with a vector encoding *CDK4* and treated with dabrafenib; at a concentration of 100 nM dabrafenib, ON cells transfected with the *CDK4* gene were more resistant than controls (Figure 3A). On the other hand, cells treated with erlotinib, an *EGFR* inhibitor, did not show any suppression of proliferation. The expression of *CDK4* protein was increased in ON cells transfected with a vector encoding *CDK4* (Figure 3B). We further examined whether overexpression of *CDK4* causes a bypass pathway for *BRAF* inhibitor resistance by increasing the phosphorylation of RTKs. The results showed that the phosphorylation of RTKs was not altered with or without forced expression of *CDK4* protein (Figure 3C).

## Discussion

Increased *CDK4* DNA copy numbers followed by increased



**Figure 3** ON cell growth assays and RTK phosphorylation. (A) ON cells were treated with dabrafenib or erlotinib and cell proliferation was assessed by MTS assay (ON: transtracheal lymph node biopsy). *CDK4* protein expressing cells were statistically resistant to dabrafenib at the concentration of 3–100 nM compared to control cells. (B) Protein expression of *CDK4* was confirmed by Western blotting. (C) Changes in RTK phosphorylation status in ON cells expressing *CDK4*. Phosphorylation of *EGFR*, *MET*, *ErBB3* and *IGF-1R* was also observed in control and *CDK4* expressing cells. We did not observe any RTKs altered by *CDK4* expression. \*,  $P < 0.05$ . *CDK4*, cyclin-dependent kinase 4; *BRAF V600E*, B-Raf proto-oncogene, serine/threonine kinase V600E; *EGFR*, epidermal growth factor receptor; *MET*, MET proto-oncogene, receptor tyrosine kinase; *ErBB3*, erb-b2 receptor tyrosine kinase 3; *IGF-1R*, insulin like growth factor 1 receptor; RTK, receptor tyrosine kinase.

mRNA and protein levels may be associated with the early acquisition of DT resistance in *BRAF V600E*-mutated lung adenocarcinoma. Data from clinical trials show that the median duration of response to the DT combination DT is 9.8 months in previously treated patients with *BRAF V600E*-mutated NSCLC, and the clinical course of LC-6 was similar. In contrast, LC-1 showed a short response period of about 3 months and rapidly worsened, exhibiting carcinomatous pleurisy, brain metastasis, and meningitis at relapse. Thus, there may be an association with an unknown resistance mechanism involving *CDK4*.

Rudin *et al.* first reported resistance to dabrafenib in

clinical specimens in *BRAF V600E*-mutated adenocarcinoma in 2013 (4), *KRAS G12D* mutations and *CDKN2A* nonsense mutations were the main causes of resistance. Previous case studies reporting acquired resistance to DT therapy in *BRAF V600E*-mutated adenocarcinoma have detected *KRAS G12V* and *NRAS Q61K* mutations. Facchinetti *et al.* (3) performed a genetic analysis of eight *BRAF V600E*-mutated lung cancers resistant to trametinib or dabrafenib and found mutations in *MEK1*, *NRAS*, *KRAS* and *PTEN* in four cases. Additionally, comparative genomic hybridization arrays were performed in the four cases in which no novel genetic mutations were identified by targeted next-generation

sequencing, and deletions of *CDKN2A/B* were found in two of these cases. Thus, the mechanisms of resistance to *BRAF* or *BRAF/MEK* inhibition include reactivation of the RAS/ MAPK pathway, supplementing signaling through other RAF family members, and activation of the PI3K/AKT pathway by *PTEN* deficiency. *CDKN2* deletion or nonsense mutations are common in many cancers, although the significance of these mutations in the acquisition of DT resistance is unclear. Reactivation of the MAPK pathway and suppression of p16ink4 lead to positive regulation of the cell cycle; however, abnormalities in *RAS* and *CDKN2* were not detected in our resistant cases.

Cyclin D-dependent kinases 4 and 6 are core proteins in the cell cycle machinery. In response to mitogenic signals, the cyclin D *CDK4/CDK6/Cip/Kip* complex is formed, resulting in the isolation of the Cip/Kip proteins from cyclin E-CDK2. Cyclin D- and E-dependent kinases phosphorylate Rb protein, releasing E2F, which is required for the transcription of genes necessary for S phase progression, including cyclin E itself, forming a positive feedback loop at the G1-S boundary. In malignant cells, alterations in the expression of CDK and its regulators, such as cyclin overexpression, regulate CDK activity and selectively promote proliferation (5). *CDK4/6* inhibitors inhibit cell cycle progression and shows anti-tumor effects (6). Smalley *et al.* reported *CDK4* mutations or *CCND1* amplification in *BRAF* V600E-mutated melanomas and evaluated their roles in resistance to *BRAF* inhibition (11). They found that *CCND1* amplification contributes to resistance to *BRAF* inhibition, particularly in the presence of *CDK4* mutations or overexpression. However, because LC-1 harbored only *CDK4* amplification, it is unclear whether the same resistance mechanism occurs in lung cancer.

The present *in vitro* study suggests that induction of *CDK4* protein in *BRAF* V600E mutant cells results in dabrafenib resistance. The mechanism of resistance in lung cancer includes protein mutation due to the acquisition of gene mutation of therapeutic target and expression of bypass pathway mainly by RTKs. In these cells, the absence of secondary resistance mutations in *BRAF* has been confirmed by the results of comprehensive genetic analysis. Therefore, we examined the phosphorylation of RTKs with an antibody array and did not find any specific changes. A weakness of this *in vitro* study is that ON cells are partially resistant to *BRAF* inhibitors; the inhibition of cell proliferation by dabrafenib above 100 nM was about 70%, suggesting that this is a naturally acquired function

during cell line establishment.

Taken together, these findings in LC-1 suggested that tumor cells originally harboring *BRAF* V600E mutations, and *de novo* or early acquired *CDK4* amplification, selectively survived under simultaneous *BRAF/MEK* inhibition. We are currently investigating how *CDK4* contributes to the mechanism of resistance. Unfortunately, it was not possible to establish cell lines with pre-DT-treated samples. In IHC, cell blocks were used for post-DT samples because tissue biopsy was clinically difficult in these cases. Immunostaining of cell blocks may overestimate the intensity of staining. However, changes in *CDK4* staining of LC-1 were evident.

In summary, *CDK4* may be involved in the early acquisition of resistance and a new target for overcoming such resistance.

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### Footnote

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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All study participants provided informed consent and this study was approved by the Institutional Review Board of Asahikawa Medical University (No: 18185/19081). Written consent was obtained from all patients whose samples were analyzed, including pleural effusions.

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## References

1. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol* 2016;17:984-93.
2. Abravanel DL, Nishino M, Sholl LM, et al. An Acquired NRAS Q61K Mutation in BRAF V600E-Mutant Lung Adenocarcinoma Resistant to Dabrafenib Plus Trametinib. *J Thorac Oncol* 2018;13:e131-3.
3. Facchinetti F, Lacroix L, Mezquita L, et al. Molecular mechanisms of resistance to BRAF and MEK inhibitors in BRAFV600E non-small cell lung cancer. *Eur J Cancer* 2020;132:211-23.
4. Rudin CM, Hong K, Streit M. Molecular characterization of acquired resistance to the BRAF inhibitor dabrafenib in a patient with BRAF-mutant non-small-cell lung cancer. *J Thorac Oncol* 2013;8:e41-2.
5. Cole AM, Myant K, Reed KR, et al. Cyclin D2-cyclin-dependent kinase 4/6 is required for efficient proliferation and tumorigenesis following Apc loss. *Cancer Res* 2010;70:8149-58.
6. Patnaik A, Rosen LS, Tolaney SM, et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. *Cancer Discov* 2016;6:740-53.
7. Chen SH, Gong X, Zhang Y, et al. RAF inhibitor LY3009120 sensitizes RAS or BRAF mutant cancer to CDK4/6 inhibition by abemaciclib via superior inhibition of phospho-RB and suppression of cyclin D1. *Oncogene* 2018;37:821-32.
8. Yadav V, Burke TF, Huber L, et al. The CDK4/6 inhibitor LY2835219 overcomes vemurafenib resistance resulting from MAPK reactivation and cyclin D1 upregulation. *Mol Cancer Ther* 2014;13:2253-63.
9. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Res* 2011;71:6051-60.
10. Hirai N, Sasaki T, Okumura S, et al. Novel ALK-specific mRNA in situ hybridization assay for non-small-cell lung carcinoma. *Transl Lung Cancer Res* 2020;9:257-68.
11. Smalley KS, Lioni M, Dalla Palma M, et al. Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol Cancer Ther* 2008;7:2876-83.

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