Nucleolar casein kinase 2 alpha as a prognostic factor in patients with surgically resected early-stage lung adenocarcinoma

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Received May 21, 2024; Accepted October 17, 2024

DOI: 10.3892/or.2024.8837

Abstract. Lung cancer remains a leading cause of global cancer-related deaths, therefore the identification of prognostic factors for lung cancer is critical. Casein kinase 2 alpha (CK2α) is one of the driver kinases in various cancers, and it was previously demonstrated that CK2α localization was associated with a poor prognosis in invasive breast cancer. In the present study, the importance of CK2α in the nucleolus was explored as a potential prognostic marker for surgically resected early-stage lung adenocarcinoma. The present study included 118 patients who underwent pulmonary lobectomy between 2014 and 2018 in Fukushima Medical University Hospital (Fukushima, Japan), and in whom CK2α localization in tumor samples was assessed by immunohistochemistry. Patient and tumor characteristics, including pathological stage, histological type and histological grade, were analyzed. Recurrence-free survival (RFS) and overall survival were evaluated in relation to nucleolar CK2α staining. CK2α staining in the nucleoli was observed in 50.8% of lung adenocarcinoma tumors. Positive nucleolar CK2α staining was independent of pathological stage, histological type and histological grade. Patients with positive nucleolar CK2α staining exhibited significantly worse RFS compared with patients with negative staining. Multivariate analysis identified nucleolar CK2α staining and lymph node metastasis as independent poor prognostic factors. The results of the present study suggested that nucleolar CK2α staining is a novel and independent prognostic factor in surgically resected early-stage lung adenocarcinoma. These findings indicated the potential of nucleolar CK2α as a predictive biomarker for future recurrence, and a guide to treatment decisions. Further research is required, particularly in understanding the molecular mechanisms linking nucleolar CK2α to recurrence.

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Abbreviations: ALK, anaplastic lymphoma kinase; CK2α, casein kinase 2 alpha; EGFR, epidermal growth factor receptor; Keap1, Kelch-like ECH associated protein 1; Nrf2, NF-E2-related factor 2; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TPS, tumor proportion score

Key words: non-small cell lung cancer, lung adenocarcinoma, nucleus, prognostic factor, protein kinase CK2, cancer recurrence

Introduction

Lung cancer is currently the leading cause of cancer-related deaths. The GLOBOCAN 2020 estimates of cancer incidence and mortality prepared by the International Agency for Research on Cancer reported an estimated 1.8 million deaths from lung cancer, representing 18% of all cancer-related deaths, in 2020 worldwide (1). Lung cancer staging is currently performed using the 8th edition of the tumor-node-metastasis (TNM) classification (2). The World Health Organization (WHO) classification of tumors was revised in 2021 (3) prior to the 9th edition of the TNM classification.

Lung adenocarcinoma, a form of non-small cell lung cancer (NSCLC), one of the main subtypes of lung cancer, consists of adenocarcinoma *in situ*, minimally invasive adenocarcinoma (MIA), invasive mucinous adenocarcinoma (IMA) and invasive non-mucinous adenocarcinoma (INMA). INMA is classified into three histological grades. Various agents have been developed for the treatment of NSCLC (4-8). For patients with very early-stage NSCLC, reduced surgery such as segmentectomy or partial resection is performed (9-11). However, recurrence is a challenge in these patients, and treatment of cases with recurrence is limited. Therefore, the identification of prognostic markers to predict recurrence is critical.

Several biomarkers for predicting therapeutic efficacy in patients with lung cancer have been identified, such as driver gene mutations for various molecular-targeted drugs and programmed death-ligand 1 (PD-L1) and tumor proportion score (TPS) for programmed death-1 (PD-1) antibody therapy. Loss of function alterations in *RB1*, *TP53* and *STK11/LKB1* have also previously attracted attention as prognostic biomarkers for drug therapy (12,13). However,

the development of biomarkers for recurrence in surgically resected NSCLC has not progressed. The most accurate prognostic factor for surgically resected early-stage NSCLC is the TNM classification. The International Association for the Study of Lung Cancer Pathology Committee proposed histological grade as a prognostic factor in INMA. The combination of predominant and worst histological patterns significantly improved patient outcome prediction in early-stage resected lung adenocarcinomas, and it was superior to mitotic count, nuclear grade, cytological grade, tumor spread through air spaces and necrosis (14). While in breast cancer, for example, genomic assays are used to predict recurrence and determine the administration of postoperative adjuvant therapy (15), treatment decisions in lung cancer depend on the TNM classification.

Casein kinase 2 (CK2) is a serine/threonine kinase that is essential for eukaryote cell survival. CK2α is the catalytic subunit of CK2. The first study linking CK2 and malignancy was in CK2α transgenic mice, which were reported to develop T lymphomas (16). CK2 is considered one of the driver kinases of carcinogenesis, and overexpression of CK2α has been reported in various types of cancer (17-19). Elevated nuclear CK2α protein levels were observed in squamous cell carcinoma of the head and neck (20) and breast cancer (21), and its high expression was associated with poor clinical outcomes. CK2 regulates various hallmarks of cancer (22), particularly via its association with nuclear transcription factors and its involvement in ribosomal gene transcription during rRNA synthesis (23,24). Therefore, its localization in the nucleus, especially in the nucleolus, is considered to be important for its function (23,24). It was previously found that the nucleolar localization of CK2α was a potential poor prognostic factor in invasive breast carcinoma (25). Nucleolar CK2α is involved in inflammatory pathways (26). Cancers are also characterized by an increased inflammatory burden (27,28). Thus, studying nucleolar CK2α in lung cancer is pertinent in the present study. CK2 is known to play a critical role in both innate and adaptive immune cells (29). CK2 is involved in i) activating PI3K/AKT/mTOR pathway by phosphorylating AKT at S129 to induce proliferation and cancer metastasis (30,31); ii) activating the NF-kB signaling pathway by phosphorylating p65 at S529 (32); and iii) activating the JAK/STAT pathway by phosphorylating JAK, to induce inflammation and immune response (33-35). CK2 inhibition by using low molecular weight inhibitor demonstrated potent antitumor effects in combination with immunotherapy. The inhibitor resulted in a decrease of tumor-associated macrophages and polymorphonuclear myeloid-derived suppressor cells in the tumor microenvironment (36). In pan-cancer analysis, CK2 alpha protein 1 expression had positive correlations with M1-macrophages and fibroblasts, and negative correlations with $CD8^+$ T cells and NK cells (37).

In the present study, the relationship between CK2α nucleolar localization and patient prognosis was examined. The subcellular localization of CK2α in surgically resected lung adenocarcinomas was determined by immunohistochemistry. Focus was addressed on adenocarcinoma, which is the same histological type as the breast carcinoma in our previous study (25) and the most frequent type of NSCLC.

Materials and methods

Antibody generation. For the production of recombinant human protein kinase CK2α (gene name *CSKN2A1*), the cDNA was subcloned into the pGEX-4T plasmid (Amersham; Cytiva). The GST fusion protein was expressed in *Escherichia coli* strain BL21(DE3) and then purified as a GST tag-free protein to the single band level (24). A total of four BALB/c BDF1 female mice (6 weeks old) which were housed (20˚C, auto-fresh ventilation of 14-15 times/h, 12/12-h light/dark cycle) at Immuno-Biological Laboratories Co., Ltd., according to the Guideline and the Law for the Human Treatment and Management of Animals, were immunized with 50 μg of full-length CK2α five times in weekly intervals. Sequential screening of mouse hybridoma clones was performed by enzyme-linked immunosorbent assay coated with serial dilution of recombinant full-length human CK2α or CK2α', and then western blotting by using 20 μg of cultured 293 cell cytosolic lysates with or without exogenously expressed human CK2α, which were solubilized by the lysis buffer containing 10 mM Hepes (pH 7.4), 20 mM NaCl, 25 mM β-glycerophosphate, 1.5 mM $MgCl_2$, 0.5 mM Na_3VO_4, 1 μg/ml of aprotinin, 0.5 mM PMSF, separated by 10% SDS-PAGE gels and transferred to PVDF membrane. Briefly, the detection of antigen, CK2α, was evaluated as follows: PVDF membrane was blocked with 5% BSA in Tris-HCl (pH 7.4) containing 150 mM NaCl for 30 min at room temperature; then primary anti-CK2α monoclonal antibody as purified IgG was used at the concentration of 0.1 μg/ml for 1 h at room temperature, followed by incubation with secondary anti-mouse IgG-peroxidase conjugated antibody (1:2,000; cat. no. 6789; Abcam) for 30 min, and detected by Chemiluminescent Detection Kit (cat. no. 32209; Thermo Fisher Scientific, Inc.) as previously described (24). A total of >6 clones with high affinity and specificity to CK2α both *in vitro* and *in vivo* that did not cross-react with CK2α' were selected (Fig. S1). The protein A-purified IgG fraction derived from the hybridoma clone 6A3, subclass mouse IgG2b κ, was used in the present study.

Patients. A total of 118 patients (64 males and 54 females) with lung adenocarcinoma who had undergone pulmonary lobectomy as complete resection between January 2014 and December 2018 at Fukushima Medical University Hospital (Fukushima, Japan) were enrolled. Median age was 69.5 (range; 40-86) years old. The patients did not receive neoadjuvant chemotherapy, radiotherapy, or immunotherapy before surgery. Pathological staging was evaluated using the International Staging System for Lung Tumors, 8th edition (2,38). Up to 2016, patients were re-diagnosed by pathologists using the 8th edition of the TNM classification. All patients were pathologically reclassified by pathologists following the WHO Classification of Tumors: Thoracic Tumors 5th Edition (3). For INMA, histological grade was evaluated and categorized by pathologists as follows: Grade 1, well-differentiated; grade 2, moderately differentiated; and grade 3, poorly differentiated (3,14). The present study was conducted according to the guidelines of the Declaration of Helsinki and was approved (approval no. 30113; August 30, 2022) by the institutional Ethics Committee of Fukushima

Medical University (Fukushima, Japan). Verbal informed consent was obtained from all subjects involved in the study.

Immunohistochemistry. Paraffin-embedded tumor specimens were cut into 4-μm thick sections. For rehydration, Tissue-Tek Prisma® Plus was used (Sakura FineTek Japan Co. Ltd.) following the manufacturer's protocol. Briefly, by descending concentration of ethanol from 99.5, 95, to 80% in every 3 min. After rehydration and antigen retrieval, the sections were autoclaved at 121˚C for 10 min in 10 mM citrate-Na buffer at pH 8.0. Following incubation with 1:200 normal serum (Vector Laboratories, Inc.) for 30 min at room temperature, the sections were incubated at 4˚C with primary monoclonal antibody against CK2α (6A3) overnight at a concentration of 0.1 μg/ml in phosphate-buffered saline containing 1% bovine serum albumin (cat. no. A8531; MilliporeSigma). The primary antibody was detected using the avidin-biotin-peroxidase complex method. Goat anti-mouse biotinylated IgG (H + L; 1:250; cat. no. BA-9200; Vector Laboratories, Inc.) was incubated at room temperature for 30 min, followed by VECTASTAIN ABC-HRP Kit (cat. no. PK-6100; Vector Laboratories Inc.) according to the manufacturer's protocol. The sections were not counterstained with hematoxylin to avoid false positive staining of nucleoli. After incubation with diaminobenzidine (Dojindo Laboratories, Inc.) for 40-80 sec, the sections were mounted on glass slides. The immunoreactivity of each specimen was scored independently by two pathologists using a light microscope based on the random selection of at least three tumor areas. CK2 staining of each specimen was evaluated as follows (25): I, nuclear staining was not visible, but cell bodies were stained; II, nuclear staining was more obvious compared with cytosolic staining; III, nuclear staining was more intense than in category II; IV, positive nucleolar staining was evident and nuclear staining was observed; and V, staining was mostly confined to nucleoli, but without intense staining of the nucleoplasm.

In some analyses, patients were categorized into two groups: Patients with nucleolar CK2α staining (categories IV and V) and those without nucleolar CK2α staining (categories I-III).

The EML4-ALK fusion protein was evaluated in 68 patients using the Nichirei Histofine ALK iAEP Kit (Nichirei Biosciences Inc.) (39). PD-L1 TPS was evaluated in 43 patients using a PD-L1 IHC 22C3 pharmDx immunohistochemistry assay on the Dako Autostainer Link 48 at SRL, Inc. PD-L1 TPS was defined as the percentage of viable tumor cells with partial or complete membrane staining for PD-L1 (40). *EGFR* mutations were evaluated in surgically resected tissue from 82 patients using the cobas EGFR Mutation Test v2 at SRL, Inc (41). These 68, 43 and 82 patients were randomly selected from the 118 patients.

Statistical analysis. The associations between nucleolar CK2α expression and pathological parameters (pathological stage, histological type and histological grade) were examined. Survival curves were created using the Kaplan-Meier method and analyzed in patients with and without nucleolar CK2α staining using the log-rank test which was performed using GraphPad Prism software v8.4.3 (GraphPad Software, Inc.; Dotmatics). Recurrence-free survival (RFS) and overall survival (OS) were defined as the time from surgery to relapse and the time from surgery to death from any cause, respectively. The Cox proportional regression model using the forward stepwise likelihood ratio method was performed to identify prognostic factors of survival using SPSS software v29 (IBM Corp.). The JMP Pro v17.0 platform (JMP Statistical Discovery LLC) was used to examine the relationship between

Table I. Patient characteristics (N=118).

Characteristics	Value
Median age, years (range)	69.5 (40-86)
Sex (male/female)	64/54
Smoking status	
Never	50
Former or current	68
Pathological stage (8th)	
0	15
IA1	35
IA2	21
IA3	14
IB	13
IIA	2
IIB	10
IIIA	8
Histological type	
Adenocarcinoma *in situ*	15
Minimally invasive adenocarcinoma	16
Invasive mucinous adenocarcinoma	5
Invasive non-mucinous adenocarcinoma	82
Histological grade	
1	11
2	42
3	29
Epidermal growth factor receptor mutation	
Ex 19 del	18
Ex 21 L858R	20
Ex 19 del + T790M	1
Ex 21 L858R + T790M	1
Ex 21 L861Q	1
Ex 21 insertion	1
ND	40
NA	36
Anaplastic lymphoma kinase rearrangement	
Positive	2
ND	66
NA	50
Programmed death-ligand 1 tumor proportion score	
<1%	16
1-49%	18
≥50%	9
NA	75

Ex, exon; ND, not detected; NA, not assessed.

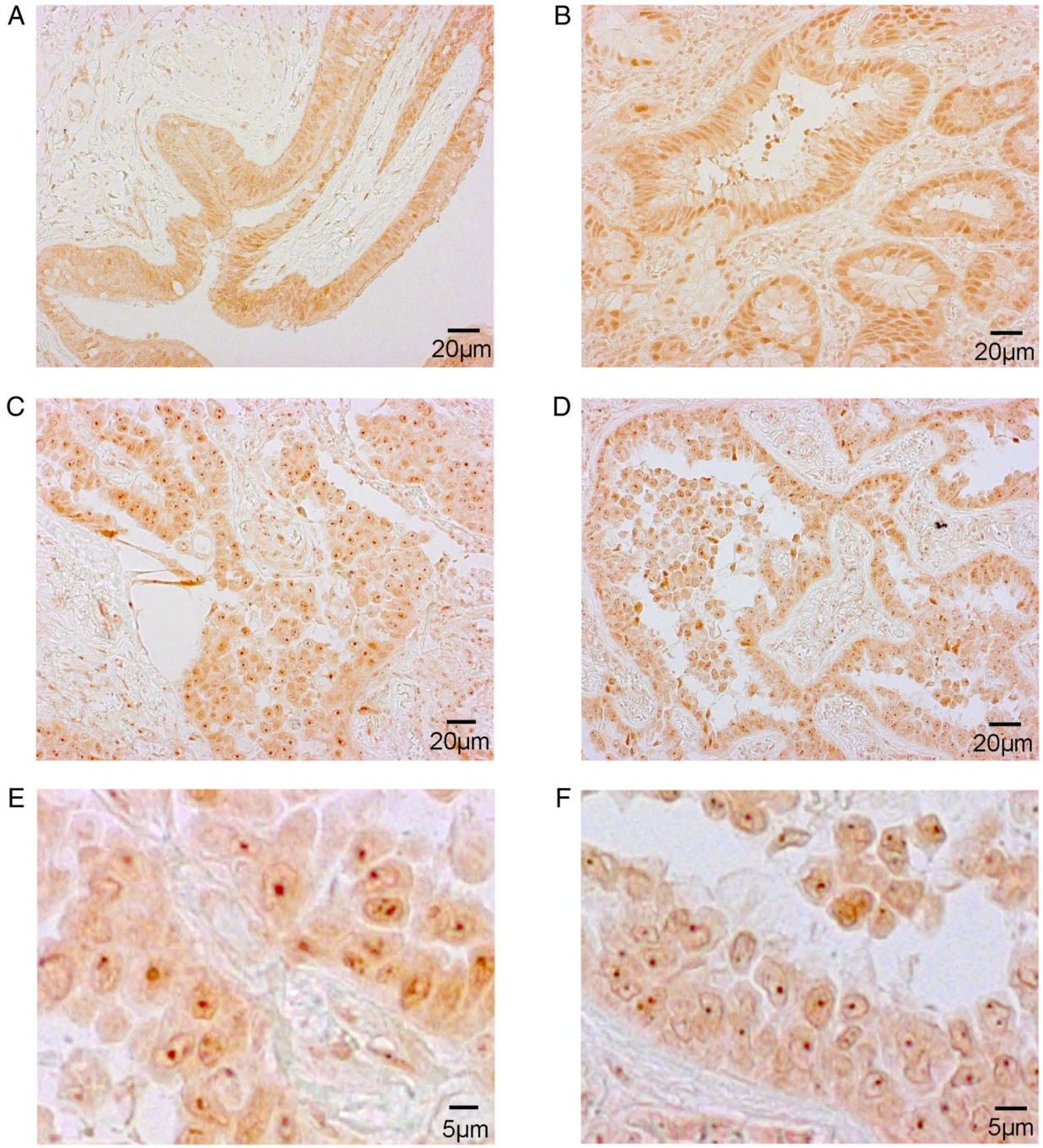

Figure 1. Representative images of immunohistochemical analysis of nuclear protein kinase CK2α in lung adenocarcinomas. (A-F) Representative results of CK2α staining categories (A) II, (B) III, (C) IV and (D) V are shown, with enlarged images for categories (E) IV and (F) V. There were no category I cases in the patient group in the present study.

nucleolar CK2α expression and recurrence in early-stage patients.

Results

CK2 localization in nuclei of cancer cells in lung adenocarcinoma. The characteristics of the 118 lung adenocarcinoma patients included in the present study are included in Table I. The intracellular localization of CK2 in tumor samples was categorized as aforementioned. Representative images of the five categories of CK2α expression are shown in Fig. 1.

CK2α staining was localized to the nucleoli of cancer cells (category IV and V) in 60 of 118 lung adenocarcinoma tumors (50.8%; Table SI). There were no category I specimens in the patient group. The relationship between CK2α staining status, nucleolar CK2α status and histopathological diagnosis is summarized in Table II. There were no apparent associations between CK2α staining status or nucleolar CK2α status and pathological stage, histological type and histological grade in INMA, the main subtype of lung adenocarcinoma.

Nucleolar CK2 is associated with poor prognosis in surgically resected early-stage lung adenocarcinoma. Nucleolar CK2α

Table II. Relationship between CK2α staining status and nucleolar CK2α status with histopathological diagnosis.

	Pathological stage				Histological type					
	0	I	II	III	AIS	MIA	IMA	INMA		
								Histological grade		
								1	2	3
CK2α staining										
1	0	0	0	0	0	0	0	0	0	0
2	1	6	0	1	1	1	1	1	1	3
3	7	37	2	4	7	7	3	5	16	12
4	5	28	5	1	5	6	0	2	18	8
5	2	12	5	2	2	2	1	3	7	6
Nucleolar CK2α										
–	8	43	2	5	8	8	4	6	17	15
+	7	40	10	3	7	8	1	5	25	14

CK2α, casein kinase 2 alpha; AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; IMA, invasive mucinous adenocarcinoma; INMA, invasive non-mucinous adenocarcinoma.

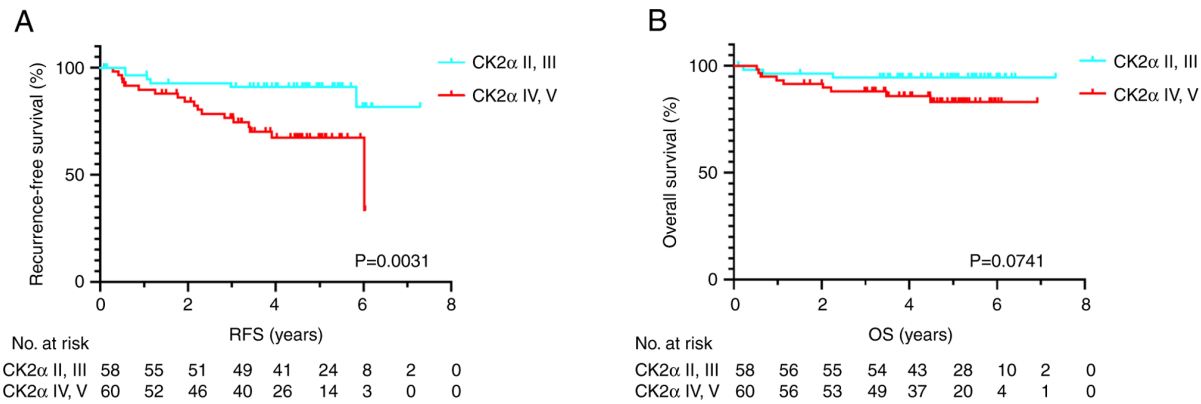

Figure 2. Nucleolar CK2α staining is associated with poor prognosis in patients with surgically resected early-stage lung adenocarcinoma. (A) RFS and (B) OS were stratified by nucleolar CK2α positive (categories IV and V) or negative expression (categories II and III). Differences between categories were analyzed by the log-rank test. CK2α, casein kinase 2 alpha; RFS, recurrence-free survival; OS, overall survival.

staining in relation to RFS and OS was next investigated. Among the 118 patients, 24 (20.3%) experienced lung cancer recurrence and 12 (10.2%) patients succumbed to any cause. The RFS time was significantly shorter in the positive nucleolar CK2α staining group compared with the negative group according to the log-rank test (P=0.0031; Fig. 2A). The OS time tended to be shorter in the positive nucleolar CK2α staining group than the negative group but without statistical significance (P=0.0741; Fig. 2B). The median RFS and OS were not reached in all groups.

The sites of first recurrence in the 24 recurrent cases were the lung (n=11), bone (n=8), mediastinal lymph nodes (n=4), and hilar lymph node, pleural dissemination, brain, and kidney (n=1 each). The sites of first recurrence in the CK2α-positive cases were the lung (n=9), bone (n=5), mediastinal lymph nodes (n=4), and hilar lymph node, pleural dissemination, and brain (n=1 each).

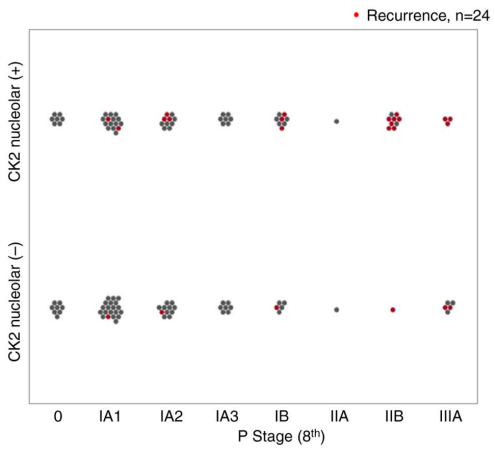

Figure 3. The association between nucleolar CK2α, pathological stage, and recurrence in patients with lung adenocarcinoma (N=118). Each dot represents one patient. CK2α, casein kinase 2 alpha.

Table III. Univariate and Cox regression multivariable stepwise procedure of recurrence-free survival in all patients (N=118).

		Univariate analysis			Multivariate analysis		
Characteristics	No.	HR	95% CI	P-value	HR	95% CI	P-value
Age (≥70 years)	59	1.004	0.469-2.324	0.916			
Sex (male)	64	1.732	0.737-4.072	0.208			
Lymph node metastasis (positive)	14	13.382	5.724-31.284	<0.001	11.448	4.890-26.803	<0.001
p-stage (II–III)	20	10.745	4.565-25.292	<0.001			
Lymphatic invasion (positive)	18	7.470	3.287-16.977	<0.001			
Microscopic vascular invasion (positive)	25	4.148	1.829-9.411	0.001			
Histological type (invasive mucinous adenocarcinoma)	5	1.718	0.231-12.789	0.597			
Epidermal growth factor receptor gene mutation (Neg and NE)	76	0.617	0.276–1.382	0.241			
Anaplastic lymphoma kinase gene translocation (Neg and NE)	116	20.746	<0.001-1000<	0.649			
Nucleolar casein kinase 2 alpha (positive)	60	3.745	1.470-9.541	0.006	3.038	1.178-7.836	0.022

HR, hazard ratio; CI, confidence interval; Neg, negative; NE, not evaluated.

Table IV. Univariate and Cox regression multivariable stepwise procedure of overall survival in all patients (N=118).

		Univariate analysis			Multivariate analysis		
Characteristics	No.	HR	95% CI	P-value	HR	95% CI	P-value
Age (≥70)	59	2.105	0.634-6.991	0.224			
Sex (male)	64	4.455	0.976-20.340	0.054			
Lymph node metastasis (positive)	14	5.787	1.835-18.250	0.003			
p-stage (II-III)	20	5.217	1.681-16.188	0.004			
Lymphatic invasion (positive)	18	6.240	2.009-19.378	0.002	6.240	2.009-19.378	0.002
Microscopic vascular invasion (positive)	25	2.874	0.910-9.071	0.072			
Histological type (invasive mucinous adenocarcinoma)	5	2.706	0.348-21.021	0.341			
Epidermal growth factor receptor gene mutation (Neg and NE)	76	2.747	0.601-12.555	0.192			
Anaplastic lymphoma kinase gene translocation (Neg and NE)	116	20.653	<0.001-1000<	0.754			
Nucleolar casein kinase 2 alpha (positive)	60	3.097	0.838-11.449	0.090			

HR, hazard ratio; CI, confidence interval; Neg, negative; NE, not evaluated.

Multivariate analysis revealed that lymph node metastasis and positive nucleolar CK2α staining were poor prognostic factors for RFS (Table III). Lymphatic invasion was the only poor prognostic factor for OS (Table IV).

Patients with adenocarcinoma *in situ* and MIA have a favorable prognosis, and patients with IMA have a worse prognosis relative to patients with INMA (2,3). Thus, focus was next addressed on invasive non-mucinous patients. Multivariate analysis of RFS showed that among patients with INMA, lymph node metastasis and nucleolar CK2α staining positivity were independent poor prognostic factors (Table SII). Age ≥70 years, lymph node metastasis and lymphatic invasion were poor prognostic factors for OS (Table SIII).

The relationship between nucleolar CK2α staining and recurrence by stage in all cases is demonstrated in Fig. 3. Recurrence was more frequent in patients with positive nucleolar CK2α staining, regardless of pathological stage. The percentages of recurrent cases positive and negative for nucleolar CK2α staining were 20% (8/40) and 7% (3/43) in stage I, and 77% (10/13) and 43% (3/7) in stage II-III, respectively.

Recurrence in stage I was more frequent among nucleolar CK2α-positive cases. Positive nucleolar CK2α staining tended

to be a poor prognostic factor for RFS even in patients in stage IA1 to IA2 (Fig. S2).

Discussion

Cancer is characterized by the accumulation of heterogeneous genetic mutations as it proliferates, and treatment is generally more difficult after recurrence as the tumors continue to grow as a non-monoclonal cancer cell population. Therefore, it is critical to identify patients at risk of recurrence as early as possible to administer treatment to prevent future recurrence.

The present findings identified that CK2α in the nucleolus of cancer cells in patients with early-stage lung adenocarcinoma was associated with poor prognosis. Patients with positive CK2α staining in nucleoli had significantly worse RFS after surgical resection compared with patients with negative staining (P=0.0031). The positive staining of CK2α in nucleoli was independent of pathological stage, histological type and histological grade (Table II). Multivariate analysis revealed that positive CK2α staining in nucleoli was an independent poor prognostic factor of RFS (Table III). This finding indicates that positive CK2α staining in cancer cell nucleoli is a novel poor prognostic factor in patients with early-stage lung adenocarcinoma. Moreover, CK2α positive staining in the nucleolus may be a useful marker for predicting future recurrence even in patients with stage I lung adenocarcinoma, as shown in Fig. 3. Nucleolus-positive staining associated with recurrence. Positive CK2α staining in the nucleolus may be a potential marker that can be identified in 2D histopathological images in cases in which there are extremely small lymphatic or venous invasions that are difficult to determine on pathological sections.

In INMA of the lung (3,14), nucleolus CK2α staining may improve the prediction of recurrence combined with histological grade. In a previous study of invasive breast carcinoma, positive CK2α staining in the nucleolus was independent of luminal type, human epidermal growth factor receptor 2, or the triple negative type and a poor prognostic factor (25). The absence of significant differences in the OS of patients with and without CK2α nucleolar staining in the present study may be because of the small number of events. A longer observation period may also be necessary to compare OS in patients with surgically resected early-stage NSCLC because of the influence of treatment after recurrence.

The present findings suggest the potential value of CK2α nucleolar staining to predict prognosis in surgically resected early-stage NSCLC. Currently, there are no clear prognostic markers in NSCLC other than TNM. While the International Association for the Study of Lung Cancer Pathology Committee proposed histological grade as a prognostic factor in surgically resected early-stage INMA (14), the results of the present study showed that CK2α staining in nucleoli is a prognostic factor independent of this histological grade. In recent years, limited resection approaches such as segmentectomy or partial resection for very early-stage NSCLC have become a standard treatment (9-11). CK2α staining of the nucleoli may be worth considering as a biomarker in such patients with very early-stage NSCLC to determine whether limited resection or lobectomy should be performed. Rapid immunostaining can be useful to make this decision intraoperatively (42). In breast cancer, the biological type determined from genetic analysis is used to predict prognosis and determine the indication for adjuvant therapy (43-45). In the present study, adjuvant chemotherapy was administered to eligible patients, making it difficult to consider the indication for this on the basis of CK2α nucleolar staining. Nevertheless, CK2α nucleolar staining could be used to identify those patients likely to benefit from treatment with adjuvant chemotherapy, including patients with early-stage non-small lung cancer.

Previous studies reported that CK2 is associated with lung cancer metastasis (46), and that chemical inhibitors of CK2 improve drug resistance (47-49). The relationship between CK2 and tumor immunity is also gaining attention (50,51). A previous study reported that CK2 activated NF-E2-related factor 2 (Nrf2) by degrading Kelch-like ECH associated protein 1 (Keap1) and activating AMP-activated protein kinase in human cancer cells (52). Mutations in the Keap1-Nrf2 pathway are common in NSCLC and have been associated with poor prognosis (53). Some clinical trials of CX-4945, a low-molecular weight inhibitor of CK2α, for various cancers are now underway. The current study included patients with NSCLC who underwent surgical resection, and future studies should be conducted in patients who have received drug therapy. Studies examining the efficacy of CK2 inhibitors in adjuvant therapy for surgically resected early-stage NSCLC patients are also required.

A couple of limitations of the present study are that it was a single-center, retrospective study, and future validation at multiple centers is needed. Additionally, future studies should investigate whether CK2α staining in nucleoli is related to the efficacy of drug therapy in NCSLC, including adjuvant therapy; these findings would indicate whether CK2α staining in nucleoli could be developed into a useful biomarker for treatment selection in addition to its utility as a prognostic factor. In normal cells, CK2 is mostly localized in the cytoplasm. The current results showed CK2 accumulation in the nucleolus in human cancer tissues. Whether this accumulation of CK2 in the nucleolus is predictive biomarker of a future recurrence should be confirmed in future studies. The CK2 complex in MCF-7 breast cancer cells is associated with protein synthesis (25), and it was previously reported that CK2 interacts with chromatin in the cell nucleus to enhance gene expression and is involved in rRNA synthesis (24). The molecular mechanisms underlying the association of nucleolar CK2α with recurrence in lung adenocarcinoma are yet to be determined.

In summary, the current findings indicated that CK2α staining in nucleoli may be a useful marker for poor prognosis in patients with surgically resected early-stage lung adenocarcinoma. Positive staining of CK2α in nucleoli was independent of pathological stage, histological type and histological grade. Combining CK2α with TNM and histological grade may more accurately predict recurrence in surgically resected early-stage lung adenocarcinoma. Rapid evaluation by immunostaining of CK2α in nucleoli could also be used to identify patients with early-stage lung adenocarcinoma in whom limited surgery may be appropriate. In patients with surgically resected nucleolar CK2α-positive lung adenocarcinoma, CK2 inhibitors may reduce the risk of recurrence when administered as adjuvant therapy after surgery. With the global clinical development of CK2α inhibitors now underway, CK2α is also a promising

therapeutic target in lung adenocarcinoma, including advanced disease, and should be studied in squamous cell lung cancer.

In conclusion, surgically resected early-stage lung adenocarcinoma patients with positive nucleolar CK2α staining had significantly worse RFS compared with patients with negative staining. Positive staining of CK2α in the nucleoli was independent of pathological stage, histological type and histological grade, and was an independent poor prognostic factor in the multivariate analysis of RFS. The findings of the present study indicated that nucleolar CK2α may be a prognostic factor and promising therapeutic target for lung adenocarcinoma. These results require validation in a multicenter setting with a larger number of patients.

Acknowledgements

The authors are grateful to Ms Yukiko Kikuta, Ms Moe Muramatsu and Ms Junko Yamaki for technical support. The authors would like to thank Dr Gabrielle White Wolf for editing a draft of this manuscript.

Funding

This present study was supported by Japan Agency for Medical Research and Development to MKH (AMED; grant nos. 20lm0203006j0004 and 22ym00126808j0001).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SM, MKH, YH and HS conceptualized the study. SM, YK and MKH conducted investigation. SM, YK and MKH confirm the authenticity of all the raw data. SM, MKH, YO, MW, NO and KH acquired data. SM, YK and MKH analyzed and validated data. SM and MKH prepared the original draft of the manuscript. MKH and HS wrote, reviewed and edited the manuscript. SM visualized data. YH and HS supervised the study. SM and MKH performed project administration. MKH acquired funding. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted according to the guidelines of the Declaration of Helsinki and was approved (approval no. 30113; August 30, 2022) by the institutional Ethics Committee of Fukushima Medical University (Fukushima, Japan). Verbal informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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