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From Cell Biology to Tissue Engineering

High expression of DEK is associated with poor prognosis in hepatocellular carcinoma

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Summary. DEK is an oncogene that has been identified as part of the DEK-CAN fusion gene. DEK plays a role in carcinogenesis through WNT signaling and induces cell proliferation through cyclin-dependent kinase signaling. DEK overexpression has been reported in HCC, but the clinical significance is unclear. This study enrolled 221 cases of HCC. The expression of DEK protein was evaluated by immunohistochemical staining. Cdk4, cyclin D1, Wnt10b, E-cadherin, and β-catenin were also immunohistochemically stained and analyzed for correlation. The association of clinicopathologic factors with DEK expression was analyzed. DEK expression was observed in 44.8% (99/221) of cases. DEK expression showed a statistical association with clinicopathologic factors, including Edmondson-Steiner grade, presence of vascular emboli, and multiplicity ($p<0.05$). Among the other IHC markers, the expression of cdk4 was correlated with DEK expression ($p<0.05$). Patients with high DEK expression showed a significantly lower overall survival rate ($p=0.006$). However, the disease-free survival rate did not differ significantly. In addition, in a Cox regression model analysis, DEK expression was an independent prognostic factor. In summary, high expression of DEK was observed in HCC and was associated with poor prognostic marker expression and poor prognosis.

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Introduction

Primary liver cell carcinoma was the second most common cause of cancer death worldwide in 2012 (Ferlay et al., 2015). Hepatocellular carcinoma (HCC) accounts for the largest portion of primary liver cancer. Even after receiving curative resection, patients with HCC have a 5-year survival rate of about 50%, and the 5-year recurrence rate is about 70% (Dimitroulis et al., 2017). Because of its poor prognosis, the need for novel therapeutic targets and treatments for HCC is high.

The DEK gene was first discovered as a fusion partner with the NUP214 gene in the (6;9) (p23;q34) chromosomal translocation in acute myeloid leukemia, forming the DEK-NUP214 fusion gene (von Lindern et al., 1992). DEK is a chromatin binding protein involved in various nuclear processes, including DNA damage repair (Kavanaugh et al., 2011), DNA replication (Alexiadis et al., 2000), and RNA splicing (Riveiro-Falkenbach and Soengas, 2010). Therefore, DEK plays a role in cell proliferation, cell differentiation, and cell death (Ageberg et al., 2006; Wise-Draper et al., 2009; Riveiro-Falkenbach and Soengas, 2010; Privette Vinnedge et al., 2015). DEK is expressed in most human cells and is overexpressed in many kinds of cancer from different origins, including the breast, stomach, colon, and lung (Lin et al., 2013; Piao et al., 2014; Ying and Wu, 2015; Liu et al., 2016). High DEK expression has been associated with poor prognosis in various types of

carcinoma (Lin et al., 2013; Lin and Chen, 2013; Piao et al., 2014; Ying and Wu, 2015; Liu et al., 2016, 2017). However, the exact mechanism of the DEK gene is not yet clear. Privette Vinnedge et al. (2015) reported that in Ron receptor-positive breast cancers, the DEK gene promotes cellular proliferation through Wnt/β-catenin signaling. DEK expression also plays a role in the metastasis of breast cancer cells. The invasive potential of breast cancer is associated with DEK expression via the β-catenin signaling pathway. Yu, L., et al. (Yu et al., 2016) demonstrated that DEK induced cell proliferation by upregulating cell cycle-related cyclin-dependent kinase (CDK) signaling. DEK also promotes cell migration through the downregulation of E-cadherin and the activation of β-catenin signaling. When β-catenin is released to translocate from the cytoplasm to the nucleus by activation of the Wnt pathway, it forms a complex with other transcription factors and stimulates cyclin D1 gene transcription (Klein and Assoian, 2008). High DEK expression has been reported in HCC (Lin and Chen, 2013), but its clinical significance is unclear.

In this study, we examined DEK expression using immunohistochemical staining to analyze its association with clinicopathologic features. Moreover, several markers associated with cell cycle related CDK signaling and Wnt/β-catenin signaling were selected, immunohistochemically stained, and analyzed to investigate the relationship between DEK expression and the clinicopathologic features of HCC.

Materials and methods

Samples

We examined surgical specimens from 264 patients who were diagnosed with HCC and underwent liver resection at Korea University Guro Hospital from 2000 to 2013. Histologic type and tumor stage were classified according to the World Health Organization blue book (Bosman et al., 2010) and the American Joint Committee on Cancer (AJCC) 8th edition (Amin et al., 2017). The following clinicopathologic features of patients were obtained: age at diagnosis, sex, tumor size, multiplicity, Edmondson-Steiner grade, presence of cirrhosis, model for end-stage liver disease (MELD) score, etiology of HCC, nodal metastasis, vascular cancer emboli, metastasis status, serum alpha-fetoprotein (AFP) level before surgery, pathologic stage, other primary malignancy history, recurrence of HCC, treatment after surgery, type of surgery, status of margin, disease-free survival, and overall survival data. Multiplicity is defined as the presence of more than two different tumor nodules in the liver, including satellite nodules and excluding vascular invasion. The MELD score is calculated using the mathematical formula (MELD $=9.57 \times \ln(\text{creatinine}) + 3.78 \times \ln(\text{total bilirubin}) + 11.2 \times \ln(\text{International Normalized Ratio}) + 6.43$) (Kamath et al., 2007). Thirteen cases were excluded due to liver transplantation. Twenty-seven cases were excluded because of the presence of another primary cancer. Two cases were excluded due to death within 48 hours after surgery. One case was excluded due to the presence of tumor tissue at the resection margin visible to the naked eye (R2 resection). In the end, 234 cases were selected for this study.

This study was approved by the Institutional Review Board of Korea University Guro Hospital (KUGH17239) before data collection.

Tissue microarray (TMA) construction

Representative tumor areas from all specimens (n=234) were selected and used for TMA construction. Non-tumor areas from 55 patients were also selected to analyze the protein expression in non-tumor liver cells. Tissue cores 2.0 mm in diameter were obtained from donor blocks and moved to recipient blocks to construct TMA blocks.

Immunohistochemical (IHC) staining

IHC staining was performed as follows for DEK, Wnt10b, E-cadherin, β-catenin, cyclin D1, and cdk4. Paraffin-embedded TMA blocks were cut into 4 μm sections. Deparaffinization with xylene was performed first. After that, sections were rehydrated via a graded alcohol series and treated with 3% hydrogen peroxide for 20 minutes to block endogenous peroxidase. Antigen retrieval was then performed for 20 minutes using 10 mM citrate buffer (pH 6.0). A bond-max autostainer (Leica Microsystems, Wetzlar, Germany) was used for the IHC studies, along with the following antibodies: DEK (1:100, clone 2/DEK, BD Biosciences, San Jose, CA, USA), Wnt10b (1:800, clone #793116, R&D Systems, Minneapolis, MN, USA), E-cadherin (1:400, clone 4A2C7, Thermo Fisher Scientific, Rockford, IL, USA), β-catenin (1:800, clone 6F9, Thermo Fisher Scientific), cdk4 (1:100, clone EP180, Epitomics, Burlingame, CA, USA), and cyclin D1 (1:100, clone EP12, Dako, Glostrup, Denmark). We stained the positive and negative control blocks in the same autostainer run and examined the adequacy of the staining. The control block for the positive and negative controls consisted of multiple tumor and normal tissues from the stomach, colon, and placenta.

Evaluation of IHC staining

IHC staining for DEK was interpreted as a percentage of positive cells, as used in other studies (Lin and Chen, 2013). A nuclear staining pattern was considered positive staining. Staining intensity was not considered in the interpretation of DEK staining because all cases showed similar staining intensity in the positive cells. Staining was scored as follows: no positive cells, 0; <5% positive cells, 1; ≥5% and <50% positive cells, 2; and ≥50% positive cells, 3. Scores of 2 and 3 were included in the high expression group. IHC staining for

cdk4 and cyclin D1 were interpreted as positive when a nuclear staining pattern was present. The intensity of staining was categorized into four categories: no staining, 0; weak staining, 1; moderate staining, 2; strong staining, 3. Again, scores of 2 and 3 were included in the high expression groups. IHC staining for Wnt-10b was interpreted by the cytoplasmic staining pattern. Staining intensity was categorized as scores of 0 to 3. Cases with an intensity score 3 were included in the high expression group. IHC staining for E-cadherin showed a membranous staining pattern, and that for β-catenin showed nuclear or cytoplasmic patterns. Staining of E-cadherin and β-catenin were interpreted as the presence of an aberrant staining pattern. Cases in which more than 50% of tumor cells showed the loss of membranous staining for E-cadherin were considered to have aberrant expression. Cases in which more than 50% of tumor cells showed nuclear staining or cytoplasmic staining for β-catenin were considered to have aberrant expression.

All TMAs were interpreted by one pathologist (Soo Yeon Lee) at first, and then another pathologist (Baek-hui Kim) did a second review. In the case of differences, the two pathologists reached consensus through consultation. The pathologists were blinded to the clinical patient data during interpretation.

Statistical analysis

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The relationship between DEK expression and the clinicopathologic features of HCC were analyzed by the chi square test or Fisher's exact test. The correlations between DEK and other biomarkers were tested by the chi square test or Fisher's exact test. Correlation with the survival rate was examined by the Kaplan-Meier method, and differences in survival curves were examined with the log rank test. The independent prognostic value of DEK expression was analyzed using a Cox regression test. $P<0.05$ was considered statistically significant.

Results

Clinicopathologic features of patients

The mean age of patients was 55.6 years (range 26 to 80), and the male to female ratio was 4.70:1. The etiology of HCC was hepatitis B in 214 cases (91.45%), hepatitis C in 13 cases (5.55%), and alcohol in 7 cases (2.99%). The mean MELD score was 7.54 (range 6.43 to 24.98). The MELD score exceeded 20 in only 3 cases (1.28%). Two hundred and thirteen cases were classified as low stage (stage I and II) (91.02%), and 21 cases were classified as high stage (stage III and IV) (8.97%). At the time of surgery, regional lymph node dissection was performed in 11 (4.70%) patients, and no case had lymph node metastasis. Four cases (1.70%) had at least one distant metastasis at the time of surgery. There were 38 cases of laparoscopic surgery (16.2%), and 12 cases showed tumor cells microscopically at the resection margin (R1 resection) (5.12%). The mean follow-up period was 1,855 days (range 9 to 5904). During the 5 years after surgery, HCC recurred in 121 patients (52.77%). Treatment after recurrence was categorized as local treatment [transarterial chemoembolization (TACE) or radiofrequency ablation(RFA)], operation, systemic treatment (chemotherapy), and end of treatment (hospice or denial of treatment). Ninety-two cases received local treatment (76.03%), 14 cases underwent surgery (11.57%), 10 cases received systemic treatment (8.26%), and 2 cases stopped treatment (1.65%).

Relationship between high DEK expression and clinicopathologic features

Among 234 cases, 221 cases were analyzed. Thirteen cases could not be interpreted due to the loss of TMA cores during the staining process. Of the 221

Table 1. Correlation between high DEK expression and clinicopathologic features.

Clinicopathologic features	DEK expression			
	N (%)	Low (%)	High (%)	P value
Sex				0.493
Male (%)	185 (83.7%)	104 (47.1%)	81 (36.7%)	
Female (%)	36 (16.3%)	18 (8.1%)	18 (8.1%)	
Age (years)				0.054
<60 (%)	148 (67.0%)	75 (33.9%)	73 (33.0%)	
≥60 (%)	73 (33.0%)	47 (21.3%)	26 (11.8%)	
Tumor size				0.987
<5 cm (%)	172 (77.8%)	95 (43.0%)	77 (34.8%)	
≥5 cm (%)	49 (22.2%)	27 (12.2%)	22 (10.0%)	
Cirrhosis				0.873
Present (%)	117 (52.9%)	64 (29.0%)	53 (24.0%)	
Absent (%)	104 (47.1%)	58 (26.2%)	46 (20.8%)	
MELD score				0.336
<10 (%)	211 (95.5%)	115 (52.0%)	96 (43.4%)	
≥10 (%)	10 (4.5%)	7 (3.2%)	3 (1.4%)	
Multiplicity				0.029
Present (%)	32 (14.5%)	12 (5.4%)	20 (9.0%)	
Absent (%)	189 (85.5%)	110 (49.8%)	79 (35.7%)	
Vascular emboli				0.017
Present (%)	73 (33.0%)	32 (14.5%)	41 (18.6%)	
Absent (%)	148 (67.0%)	90 (40.7%)	58 (26.2%)	
Edmondson-Steiner grade				0.018
1 and 2 (%)	93 (42.1%)	60 (27.1%)	33 (14.9%)	
3 and 4 (%)	128 (57.9%)	62 (28.1%)	66 (29.9%)	
TNM stage				0.097
I and II (%)	200 (90.5%)	114 (51.6%)	86 (38.9%)	
III and IV (%)	21 (9.5%)	8 (3.6%)	13 (5.9%)	
Serum AFP level				0.977
≤400 ng/mL (%)	181 (81.9%)	100 (45.2%)	81 (36.7%)	
>400 ng/mL (%)	40 (18.1%)	22 (10.0%)	18 (8.1%)	

AFP, alpha-fetoprotein.

cases, 99 cases (44.8%) showed high DEK expression (Fig. 1). Tumors with vascular emboli or multiple tumors showed higher DEK expression (p=0.017 and 0.029) than other cases. Tumors with a high Edmondson-Steiner grade were also correlated with high DEK expression (p=0.018). Patient sex, age, serum AFP level before surgery, presence of cirrhosis in the background liver, MELD score, T stage, and TNM stage by the AJCC 8th edition showed no statistical correlation with DEK expression (Table 1).

Relationship between DEK expression and other biomarkers

Some TMA cores were lost during the staining process for each marker. DEK and Wnt-10b were stained in 221 cores, cdk4 was stained in 216 cores, cyclin D1 was stained in 215 cores, E-cadherin was stained in 214 cores, and β-catenin was stained in 215 cores (Fig.2). The high DEK expression group showed a correlation with cdk4 expression (p=0.004). Wnt-10b, E-cadherin, β-catenin, and cyclin D1 expression showed no statistical relationship with DEK expression. High Wnt-10b expression was associated with high cdk4 expression (p=0.021) but not with E-cadherin, β-catenin, or cyclin D1. High cdk4 expression correlated with cyclin D1 expression (p<0.001) (Table 2).

Correlation of DEK expression with 5-year survival rates of patients with HCC

Among 222 cases of HCC, 5-year survival rates were analyzed by the Kaplan-Meier method. The results show that the high DEK expression group had a lower 5-

Table 2. Correlation between DEK expression and other markers.

		DEK		p	Wnt-10b		p	Cdk4		p
		Low (%)	High (%)		Low (%)	High (%)		Low (%)	High (%)	
Wnt-10b	Low (%)	117 (52.9%)	94 (42.5%)	0.735						
	High (%)	5 (2.3%)	5 (2.3%)							
Cdk4	Low (%)	81 (37.5%)	47 (21.8%)	0.004	126 (58.3%)	2 (0.9%)	0.021			
	High (%)	38 (17.6%)	50 (23.1%)		81 (37.5%)	7 (3.2%)				
E-cadherin	Non-aberrant expression (%)	84 (39.3%)	69 (32.2%)	0.912	146 (68.2%)	7 (3.3%)	0.670	93 (43.5%)	60 (28.0%)	0.370
	Aberrant expression (%)	34 (15.9%)	27 (12.6%)		59 (27.6%)	2 (0.9%)		33 (15.4%)	28 (13.1%)	
β-catenin	Non-aberrant expression (%)	108 (50.2%)	90 (41.9%)	0.734	191 (88. 8%)	7 (3.3%)	0.104	119 (55.6%)	78 (36.4%)	0.122
	Aberrant expression (%)	10 (4.7%)	7 (3.3%)		15 (7.0%)	2 (0.9%)		7 (3.3%)	10 (4.7%)	
Cyclin D1	Low (%)	78 (36.3%)	54 (25.1%)	0.118	129 (60.0%)	3 (1.4%)	0.077	94 (43.9%)	38 (17.8%)	<0.001
	High (%)	40 (18.6%)	43 (20.0%)		77 (35.8%)	6 (2.8%)		32 (15.0%)	50 (23.4%)	

Table 3. Cox proportional hazard regression model survival analysis of multiple factors.

	Univariate analysis		Multivariate analysis	
Clinicopathologic features	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.134 (0.501-2.567)	0.763		
Sex	0.597 (0.179-1.994)	0.402		
Serum AFP level	2.656 (1.173-6.013)	0.019	1.690 (0.718-3.977)	0.229
Tumor size	2.920 (1.311-6.503)	0.006	2.565 (1.055-6.239)	0.038
Multiplicity	0.571 (0.135-2.421)	0.410		
Edmonson-Steiner grade	2.390 (0.954-5.985)	0.063		
MELD score	4.842 (1.661-14.112)	0.004	6.194 (1.941-19.768)	0.002
Cirrhosis	1.354 (0.608-3.014)	0.458		
Vascular emboli	2.517 (1.147-5.520)	0.021	1.966 (0.836-4.621)	0.121
TNM stage	4.000 (1.494-10.714)	0.006	1.325 (0.439-3.997)	0.618
Margin status	4.563 (1.561-13.336)	0.006	4.252 (1.412-12.804)	0.010
Type of surgery	1.634 (0.489-5.460)	0.425		
DEK	3.177 (1.327-7.608)	0.009	3.004 (1.214-7.431)	0.017
Wnt-10b	2.921 (0.874-9.762)	0.082		
E-cadherin	1.964 (0.872-4.423)	0.103		
β-catenin	1.880 (0.561-6.303)	0.307		
Cyclin D1	0.749 (0.320-1.749)	0.504		
Cdk4	2.060 (0.915-4.639)	0.081		

HR, hazard ratio; CI, confidence interval.

year survival rate (p=0.006) than the low DEK expression group. The disease-free survival rates of 222 patients were also examined, but there was no statistically significant difference between the high and low DEK expression groups (Fig. 3). In the survival analysis of other clinicopathologic data, vascular emboli, large tumor size (≥5 cm), high TNM stage (III or IV), high serum AFP level (>400 ng/mL), margin status, and MELD score were associated with poor patient survival (p=0.017, 0.006, 0.003, 0.015, 0.002, and 0.002, respectively).

DEK was an independent prognostic factor in a Cox proportional hazard regression model

A univariate survival analysis using a Cox proportional hazard regression model showed that the high DEK expression group had a lower 5-year survival rate than the low DEK expression group (p=0.009). Vascular emboli, large tumor size (≥5 cm), high TNM stage (III or IV), high serum AFP level (>400 ng/mL), marginal status, and MELD score also correlated with poor patient survival (p=0.021, 0.006, 0.006, 0.019

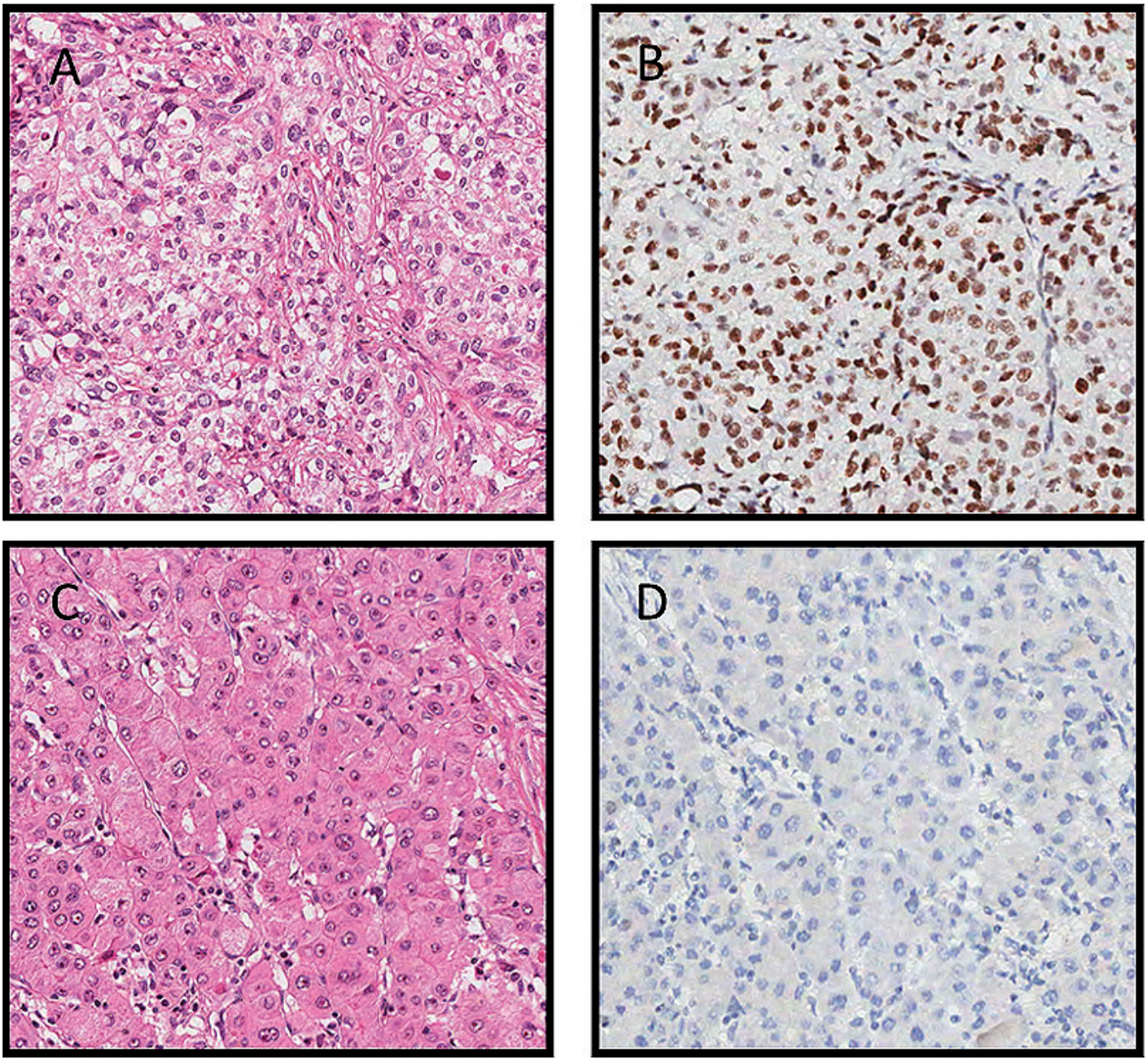

Fig. 1. Representative microscopic photographs showing immunohistochemical staining of DEK. **A, B.** Case with high DEK expression. **C, D.** Case with low DEK expression. A, C, hematoxylin and eosin stain; B, D, immunohistochemical staining of DEK. x 200.

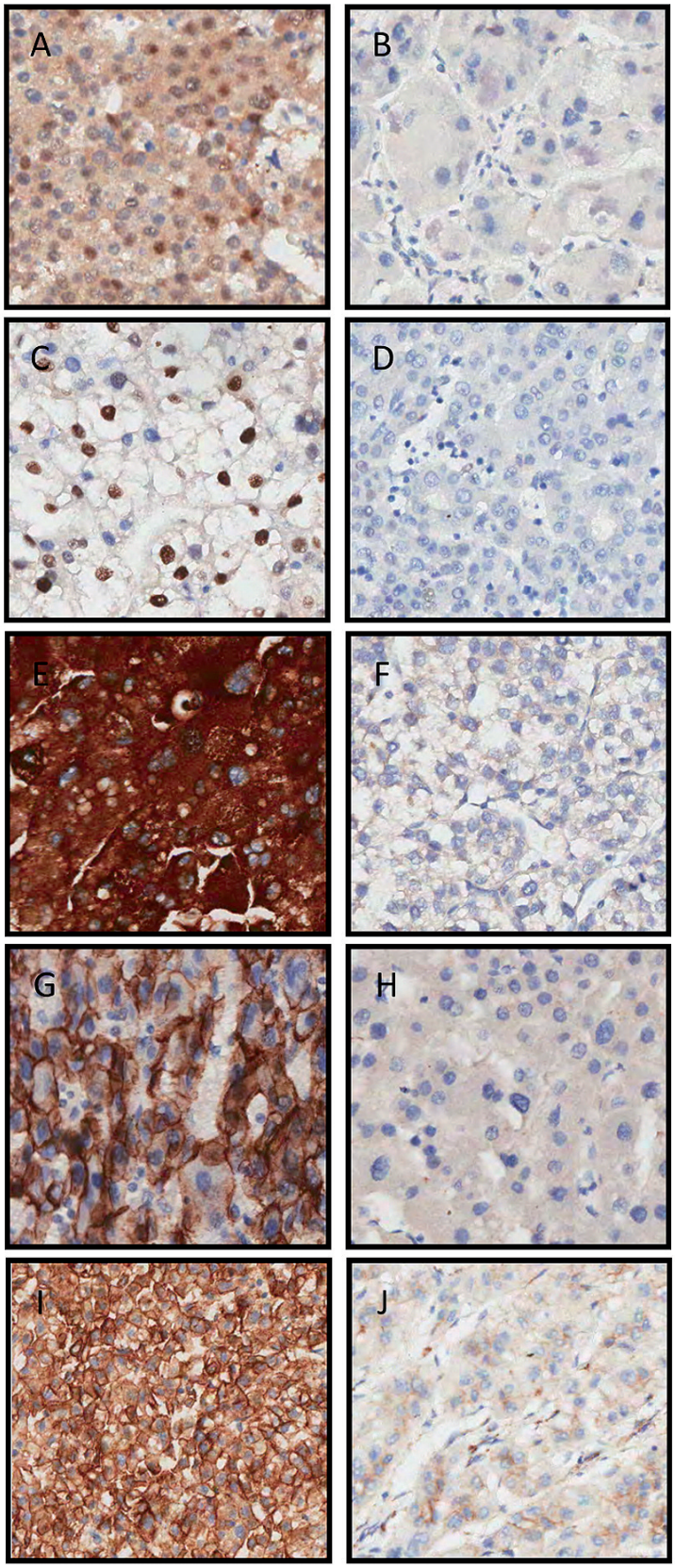

Fig. 2. Representative microscopic photographs of immunohistochemical staining of each marker. **A.** High cdk4 expression. **B.** Low cdk4 expression. **C.** High cyclin D1 expression. **D.** Low cyclin D1 expression. **E.** High Wnt 10b expression. **F.** Low Wnt 10b expression. **G.** Aberrant E-cadherin expression. **H.** Non-aberrant E-cadherin expression. **I.** Aberrant β-catenin expression. **J.** Non-aberrant β-catenin expression. x 200.

0.006, and 0.004, respectively). Age, sex, cirrhosis, multiplicity, Edmondson-Steiner grade, type of surgery, and the other IHC markers did not correlate with patient survival. A multivariate survival analysis was performed on factors that were statistically significant in the univariate Cox hazard regression analysis. DEK was identified as an independent prognostic factor for 5-year survival rates in HCC (HR, 2.766, 95% CI, 1.139 - 6.719; p=0.025). MELD score, margin status, and tumor size were also statistically independent prognostic

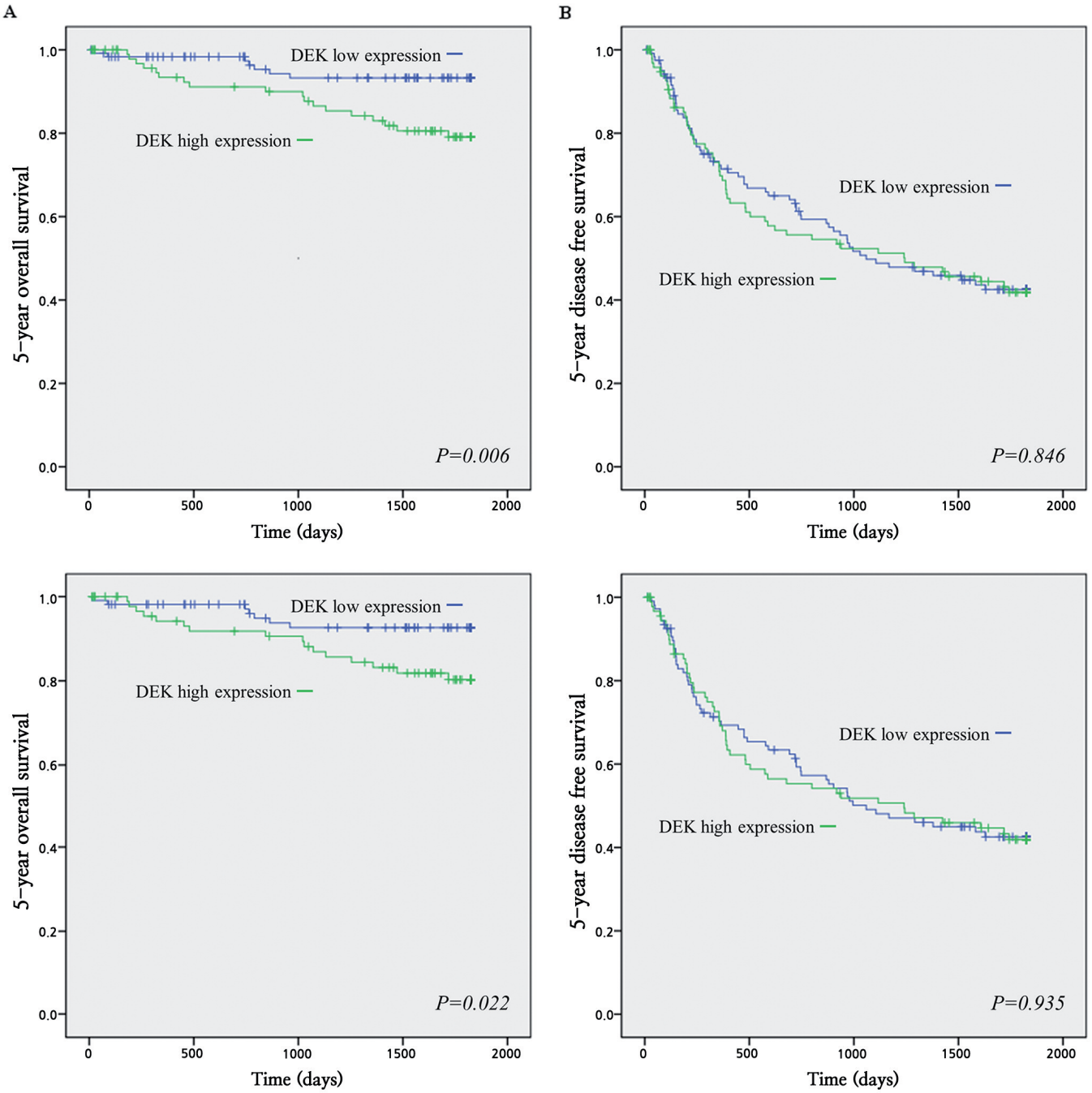

Fig. 3. A. The high DEK expression group showed a lower 5-year overall survival rate than the low DEK expression group (p=0.006). **B.** However, DEK expression showed no statistical correlation with 5-year disease free survival. **C.** In the HBV subgroup, high DEK expression also correlated with a lower 5-year overall survival rate. **D.** In the HBV subgroup, DEK expression also did not correlate with the 5-year disease free survival rate.

factors (Table 3).

DEK was identified as an independent prognostic factor in subgroup analyses

In addition, a subgroup analysis was performed to analyze differences according to etiology. In cases with hepatitis B virus (HBV), high DEK expression correlated with a poor 5-year overall survival rate when analyzed by the Kaplan-Meier method (p=0.022). In the univariate survival analysis using a Cox proportional hazard regression model, the high DEK expression group had a lower 5-year survival rate than the low DEK expression group (p=0.028). We performed a multivariate survival analysis on the following variables: serum AFP level, tumor size, MELD score, vascular emboli, TNM stage, margin status, and DEK. DEK was identified as an independent prognostic factor for 5-year survival rate in the subgroup of HBV patients (HR, 2.984, 95% CI, 1.157 - 7.695; p=0.024). Tumor size, MELD score, and margin status also showed statistical significance as independent prognostic factors in this subgroup (p=0.028, 0.002, and 0.007). DEK was not related to the 5-year disease free survival rate as determined by the Kaplan-Meier method (p=0.935) (Fig. 3). The hepatitis C virus (HCV) group and alcohol group showed no statistical relationship between DEK expression and 5-year overall survival as determined by the log rank test (p=0.248 and p=0.157, respectively).

We also did a subgroup analysis in R0 patients to eliminate the bias that can occur according to margin status. High DEK expression correlated with a poor 5-year overall survival rate in the analysis using the Kaplan-Meier method (p=0.003). In the univariate Cox hazard regression survival analysis, the high DEK expression group showed poorer 5-year survival than the low DEK expression group (p=0.007). The multivariate Cox hazard regression survival analysis showed that DEK can be used as an independent prognostic factor in HCC patients with an R0 resection as well (HR, 4.148, 95% CI, 1.458 - 11.802; p=0.008). Tumor size and MELD score were also identified as independent prognostic factors in this subgroup (p=0.010 and 0.001). DEK did not correlate with the 5-year disease free survival rate in the Kaplan-Meier analysis (p=0.790).

The patients who experienced recurrence after their first surgery were classified into 4 types according to the treatment method: local treatment, operation, systemic chemotherapy, and end of treatment. The patients who decided to discontinue treatment were excluded from this subgroup analysis. In the local treatment group, DEK expression correlated with a poor 5-year survival rate in both the log rank test and the univariate Cox regression test (p=0.006 and p=0.009, respectively). In the multivariate Cox regression test, DEK was identified as an independent factor in this subgroup (HR, 3.111, 95% CI, 1.241 - 7.797; p=0.015). In the operation and systemic chemotherapy groups, DEK had no statistical correlation with the 5-year overall survival rate (p=0.134 and p=0.170, respectively).

Discussion

DEK, located on chromosome 6p22.3, was first known as a fusion partner gene in the (6;9) (p23;q34) chromosomal translocation of acute myeloid leukemia. Since then, several studies have shown high DEK expression in various human solid tumors. DEK is reported to be highly expressed in 61.94% of breast cancers (Ying and Wu, 2015), 60.5% of gastric cancers (Piao et al., 2014), and 48.62% of colorectal cancers (Lin et al., 2013). In addition, 48.3% of HCC cases have been reported to have high DEK expression (Lin and Chen, 2013). In this study, we found high DEK expression in 45% of cases (100/222), a ratio similar to that in previous HCC studies.

High DEK expression correlated with Edmondson-Steiner grade, vascular emboli, and multiplicity in this study. In another study of HCC, DEK correlated with larger tumors, high histologic grade, and late stage (III and IV) (Lin and Chen, 2013). In our study, high DEK expression did not show a statistically significant correlation with TNM stage, possibly because our cohort contained too few patients with late stage HCC. DEK has multiple actions in cells, including cell proliferation, cell differentiation, regulation of cell death, and epithelial mesenchymal transition (EMT) (Ageberg et al., 2006; Wise-Draper et al., 2009; Riveiro-Falkenbach and Soengas, 2010; Privette Vinnedge et al., 2015). EMT might be correlated with vascular emboli and multiplicity. The Edmondson-Steiner grade is a grading method scored by tumor cell differentiation. DEK might have affected the clinicopathologic features by acting on cell differentiation and EMT.

The exact pathway for DEK is not clearly understood. In Ron receptor-positive breast cancer, high DEK expression stimulated the production of Wnt ligands and activated the Wnt/β-catenin pathway. As a result, high DEK expression contributed to breast cancer progression by means of cell proliferation, alterations in cell polarity, and invasion (Privette Vinnedge et al., 2015). We assessed whether DEK expression correlates with the Wnt/β-catenin pathway through an IHC study of Wnt10b, E-cadherin, and β-catenin. We found no statistically significant correlation between DEK and Wnt10b, E-cadherin, or β-catenin. However, we studied only the correlation, not causality, between groups divided by DEK grade and Wnt10b expression. Moreover, Wnt/β-catenin signaling can be activated by variable Wnt ligands that are not regulated only by DEK. Therefore, even if DEK affects the Wnt/β-catenin pathway, the result might not show statistical correlation.

DEK expression can promote cell proliferation by upregulating several cell cycle-related genes, including cdk4 (Yu et al., 2016). As in other studies, our results show a correlation between high DEK expression and high cdk4 expression, which supports the possibility that DEK plays a role in HCC tumorigenesis by means of

cell proliferation.

High DEK expression was associated with a lower overall survival rate (p=0.006), but it showed no statistical correlation with recurrence (p=0.846). Vascular emboli, large tumor size, high TNM stage, high serum AFP level, margin status, and MELD score were also associated with poor patient survival (p=0.017, 0.006, 0.003, 0.015, 0.002, and 0.002, respectively). In the univariate Cox regression analysis, patients with HCC and high DEK expression had significantly worse 5-year survival than patients with low DEK expression (p=0.009). Other significant variables in the Kaplan-Meier test (vascular emboli, large tumor size, higher TNM stage, high serum AFP level, margin status, and MELD score) were associated with 5-year survival rate. In another study, age, tumor size, histologic grade, lymph node status, portal vein tumor thrombus, stage, and DEK were associated with 5-year survival rate (Lin and Chen, 2013). The features associated with 5-year survival rate were thus similar in the two studies.

In this study, we performed several subgroup analyses. First, the subgroup analysis of the etiology found a relationship between DEK and prognosis in the HBV group but not in the HCV and alcohol groups. It is thought that the HCV and alcohol groups were too small (n=13 and n=7, respectively) to provide a meaningful statistical analysis. In South Korea, 70-80% of HCC patients have HBV (Song and Kim, 2009). In the subgroup analysis according to treatment after recurrence, DEK was statistically identified as an independent poor prognostic factor in the local treatment (TACE or RFA) group but not in the other groups. That result was probably also because the number of cases in each group (operation group n=14 and systemic therapy group n=10) was insufficient to yield statistically significant results.

In the multivariate Cox regression analysis using the clinicopathologic features listed above, high DEK expression was an independent poor prognostic factor in HCC (HR: 2.652, 95% CI: 1.097 - 6.471, p=0.032). Lin et al. studied the association of DEK with clinicopathologic features and the association between DEK and the survival rate for 178 cases (Lin and Chen, 2013). They concluded that DEK is an independent prognostic factor, as in our study. We analyzed 221 cases for DEK expression, the largest number of cases among studies of DEK expression in HCC. In addition, we analyzed whether DEK correlates with IHC staining of other biomarkers in an effort to clarify the mechanism of DEK expression. We found that DEK might affect prognosis due to its involvement in cell proliferation through CDK signaling. Because this study is not a functional study, we have a limited ability to confirm a causative relationship. However, IHC staining can easily be applied to a future clinical setting. IHC staining also has the advantage of correlating tumor morphology with biomarker expression.

This study was conducted using TMA. In TMA, one core represents the entire tumor, so there could be a limit to its representativeness. To overcome that limitation, we reviewed all hematoxylin and eosin slides from each surgical specimen and selected the most representative regions.

Because our study was conducted in a single institution, various factors, such as etiology, might not be representative of the overall population being analyzed. We attempted to overcome that limitation through our subgroup analyses (etiology and treatment method after recurrence). However, some subgroups did not produce statistically significant results due to an inadequate number of cases, so studies across a larger area are needed in the future.

In summary, high DEK expression is associated with factors that are mostly well-known as poor prognostic markers. Furthermore, high DEK expression is itself a statistically significant independent marker of a poor prognosis. This suggests that high DEK expression has an adverse effect on patient prognosis through cell proliferation and EMT. Although high DEK expression is not yet completely understood, IHC staining for DEK could be a useful prognostic marker in HCC.

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