ALPK2 is Crucial for Luminal Apoptosis and DNA Repair-related Gene Expression in a Three-dimensional Colonic-crypt Model

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Abstract. *Background: Oncogenic KRAS signaling is dysregulated in a three-dimensional (3D)-specific manner in human colorectal cancer (CRC) HCT116 cells. However, the identity of the crucial genes which are down-regulated through oncogenic KRAS in 3D cultures remains unclear. Materials and Methods: We established a specific anti-alpha-kinase 2 (ALPK2) antibody and addressed the ALPK2 function in HKe3 cells, which are HCT116 cells with a disruption in oncogenic KRAS, in a 3D colonic-crypt model. Results: In HKe3 cells grown in 3D culture, ALPK2 siRNA inhibited luminal apoptosis and reduced the expression of cleaved caspase-3. Furthermore, ALPK2 siRNA reduced the expression of DNA repair genes. Reduced expression of ALPK2 mRNA was found to be correlated with clinical colorectal adenomas in a public dataset of gene expression analyses. Conclusion: ALPK2, down-regulated by oncogenic KRAS, is crucial for luminal apoptosis and expression of DNA repair-related genes, possibly in the transition of normal colonic crypt to adenoma.*

Colorectal tumorigenesis in humans is associated with multiple genetic alterations. *v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) mutations are frequently observed during the early stages of colorectal cancer (CRC) development, and even in adenomas (1-3), suggesting that oncogenic *KRAS* plays several crucial roles in the adenoma–carcinoma sequence (4). We previously established HKe3 cells which are human CRC HCT116 cells with a disruption in oncogenic *KRAS* (5), and analyses using those cells have contributed to the understanding of tumor development through *in vitro* and *in vivo* oncogenic *KRAS* signaling (5-10).

We previously investigated the behavior of HKe3 cells in three-dimensional (3D) cultures and reported that the cells form an organized structure resembling to a colonic crypt (9). In this model, oncogenic *KRAS* was found to inhibit luminal apoptosis, affect cell polarity and down-regulate DNA repair genes (including *TP53*) in a 3D-specific manner (5, 9). These results suggested that this model could mimic the growth of the colonic epithelium *in vivo*, which indicates its use in determining the critical genes involved in CRC development, through oncogenic *KRAS*-mediated signals *in vivo*.

We previously identified alpha-kinase 2 *(ALPK2)* as being one of the differentially expressed genes between HCT116 and HKe3 cells in this model (9). The *ALPK2* gene was initially identified to have a domain with a strong similarity to the elongation factor 2 kinase catalytic domain (11), and is mapped to 18q21.31, the distal end of a minimal region of loss of heterozygosity frequently observed in colonic adenomas, as well as in colon cancer (12, 13). Furthermore, recent comprehensive sequence-based analyses of somatic mutations in potentially oncogenic kinases, suggest that *ALPK2* is one of the genes mutated in ovarian cancer (14, 15). However, as yet, no functional studies have been reported on ALPK2. In the present study, we established a specific anti-ALPK2 antibody and addressed the function of ALPK2 in the colonic-crypt model using HKe3 cells.

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Materials and Methods

Antibodies and reagents. Anti-ALPK2 (accession number NM052947.3) polyclonal antibodies were raised in rabbits using recombinant human ALPK2 (amino acids 601–843, N-terminal region), as described previously (16). Anti-Ki-67 antibody (SP6) was obtained from Thermo Scientific (Rockford, IL, USA). Anti-cleaved caspase-3 (5A1) was obtained from Cell Signaling Technology (Beverly, MA, USA). Anti-actin antibody (A2066) and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Cell culture. Human CRC HCT116, HCT15, DLD-1, SW48, LS180 and Colo201 cells were obtained from the American Type Culture Collection (Frederick, MD, USA). Two-dimensional (2D) cultures of CRC, HKe3, DKO-4 and e3-MKRAS#14 cells were managed as described previously (5, 9, 17). For the 3D culture, 8×10^3 cells were cultured using Matrigel reconstituted basement membrane (Growth Factor-reduced Matrigel; BD Bioscience, San Jose, CA, USA), as described previously (9, 18). Cells were seeded in medium containing 2% (*v/v*) Matrigel, and half of the medium was replaced every three days. All the cell lines used were confirmed to be mycoplasma-free, as determined using the MycoAlert system (Lonza, Verviers, Belgium). Cell morphology was checked regularly to ensure that the cell lines were not cross-contaminated.

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Real-time qRT-PCR was performed using the Perfect Real-Time Support System (Takara Bio Inc., Shiga, Japan) for *ALPK2* (HA104425), Fanconi anemia complementation group A (*FANCA*; HA076029), *FANCE* (HA129751), *FANCG* (HA112784), Rad 51 homolog (*RAD51*; HA130916), Bloom syndrome (BLM; HA031653) and β-actin (*ACTB*; HA067803), as described previously (9). The expression of genes was determined in relation to that of the control cells (1.0), as relative expression unit (REU).

Plasmid and transfection. Hemagglutinin (HA)-tagged human *ALPK2* (ALPK2-HA) cDNA was inserted into pCI-neo vector (Promega, Madison, WI, USA) at multi-cloning sites to generate a mammalian expression plasmid pCI-neo-ALPK2-HA, which was then transfected into HCT116 cells with Lipofectamine LTX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

siRNA. HKe3 cells were transiently transfected with siRNAs using Lipofectamine LTX according to the manufacturer's protocol (Invitrogen). The siRNA duplexes used were: *ALPK2* siRNA #1 top, 5'-AAA GCA ACC CAU UUG CAA UUU CUU C-3' and *ALPK2* siRNA #1 bottom, 5'-GAA GAA AUU GCA AAU GGG UUG CUU U-3'; scrambled siRNA #1 top, 5'-AAA UCC GCA AAC CUU UAC GUU AUU C-3' and scrambled siRNA #1 bottom, 5'-GAA UAA CGU AAA GGU UUG CGG AUU U-3'; *ALPK2* siRNA #2 top, 5'-AGG AGA UGA AGU ACA AGG GAA CCU G-3' and *ALPK2* siRNA #2 bottom, 5'-CAG GUU CCC UUG UAC UUC AUC UCC U-3'; scrambled siRNA #2 top, 5'-AGG ACG AGU AGA ACA UGA AAG GCU G-3' and scrambled siRNA #2 bottom, 5'-CAG CCU UUC AUG UUC UAC UCG UCC U-3'.

Cell proliferation assay. After transfection of *ALPK2* siRNA #1 or scrambled siRNA #1 into the HKe3 cells, 10^4 cells were cultured in 100 μl of medium per well. Cell proliferation was measured by using a [3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) assay-based Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to the manufacturer's instructions.

Immunofluorescence labeling and confocal microscopy. Immunofluorescence experiments were performed as described previously (9, 18). To examine 3D structures, a TCS-SP5 Laser Scanning Confocal Microscope (Leica, Wetzlar, Germany) was used.

Quantification of proliferative cells grown in 3D culture. HKe3 cells treated with *ALPK2* siRNA #1 or scrambled siRNA #1 were cultured for three days in a 3D culture. The ratio of Ki-67-positive cells in the total cells contacting Matrigel in the cross-section of 3D structures at the maximum diameter was calculated. Thirty 3D structures were evaluated for each condition.

Quantification of apoptosis in 3D structures. HKe3 cells treated with *ALPK2* siRNAs or scrambled siRNAs were cultured for four days in a 3D culture. Cleaved caspase-3-positive cells in 3D structures were counted by confocal microscopy in the serial cross-sections of the 3D structure. Three-dimensional structures containing more than two positively stained cells were defined as apoptotic structures. Sixty of the 3D structures from three different wells were counted. The 3D structures of HKe3 cells treated with *ALPK2* siRNAs or scrambled siRNAs were analyzed in three independent experiments, and the mean ratio (no. of apoptotic structures/60 3D structures) was calculated.

Immunoprecipitation and western blotting. HKe3 cells treated with *ALPK2* siRNAs or with scrambled siRNAs were harvested on day 4 of the 3D culture. Proteins were extracted using NDSB buffer [50 mM Tris-HCl (pH 7.5), 140 mM NaCl, 0.5% NP40, 0.25 M non-detergent sulfobetaine (NDSB), protease inhibitor cocktail] and immunoprecipitated with anti-cleaved caspase-3 antibodies, conjugated to protein G–Sepharose (GE Healthcare BioSciences, Piscataway, NJ, USA). Protein G–Sepharose complexes were boiled for 5 min and the supernatants were run on a 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. Western blotting was performed as described previously (9, 16). The intensity of actin was used as a control in the western blot analyses for cleaved caspase-3, and the relative intensity of the signal (cleaved caspase-3/actin) was normalized to the signal intensity in HKe3 cells treated with scrambled siRNAs, which was set as 1.0.

Microarray expression analysis. Gene expression arrays were performed using the Human Genome 1.0 ST Array GeneChips (Affymetrix, Santa Clara, CA, USA) and were analyzed using the GeneSpring v7.3 software program (Agilent Technologies, Santa Clara, CA, USA), as described previously (19, 20).

Dataset sources. The Sabates–Bellver dataset, which comprises of the microarray profiles of human colorectal adenoma and normal mucosa specimens from 16 patients (21), was obtained from the Gene Expression Omnibus (Series GSE8671) using the import module of GenePattern software (22). The differential expression of miRNAs between the two classes was ranked according to a signal-to-noise metric using GenePattern (22, 23). The statistical significance of the differentially expressed genes was determined by the comparative marker selection module of GenePattern (22). The gene expression value of *ALPK2* was normalized to that of β-actin.

Statistical analyses. The data are presented as the means±standard deviation. Statistical analyses were performed using the unpaired two-tailed Student's *t*-test. Differences at $p<0.05$ were considered to be statistically significant.

Results

ALPK2 is down-regulated by oncogenic KRAS in 2D and 3D cultures. To confirm the oncogenic *KRAS*-mediated down-regulation of *ALPK2*, qRT-PCR was performed on HCT116, HKe3 and e3-MKRAS#14 cells, re-expressing oncogenic *KRAS*. In 2D culture, the expression levels of *ALPK2* in HCT116 and e3-MKRAS#14 cells were lower by 2364- and 55-fold ($p<0.05$), respectively, in comparison to that of HKe3 cells (Figure 1A). In 3D culture, the expression of *ALPK2* in HCT116 and e3-MKRAS#14 was lower by 1485- and 4-fold ($p<0.05$), respectively, in comparison to that of HKe3 cells (Figure 1A).

Establishment of a specific anti-ALPK2 antibody. To confirm the exact expression of the ALPK2 protein, a polyclonal anti-ALPK2 antibody was generated. It was found to detect a protein product with a molecular weight of approximately 220 kDa by western blotting (Figure 1B). The expression level of the 220-kDa product in HCT116 and e3-MKRAS#14 cells was much lower compared to that in HKe3 cells, in both 2D and 3D cultures (Figure 1B), and the 220-kDa band was strongly detected in cells transiently expressing HA-tagged ALPK2 (ALPK2-HA) (Figure 1C, left). Furthermore, treatment of HKe3 cells with *ALPK2* siRNA #1 or *ALPK2* siRNA #2, resulted in a significant decrease in the 220-kDa product compared with those in the control cells transfected with scrambled siRNA (Figure 1C, right). These results suggest that the anti-ALPK2 antibody specifically recognizes the ALPK2 protein, and that oncogenic *KRAS* down-regulates *ALPK2* mRNA and ALPK2 protein in both 2D and 3D cultures.

Inhibition of apoptosis by ALPK2 siRNAs in HKe3 cells grown in 3D culture. To address whether ALPK2 influences cell proliferation and apoptosis, we first examined the time course-dependent proliferation of cells in 2D culture. No significant differences were observed in the growth rates between HKe3 cells treated with *ALPK2* siRNA #1 and those treated with scrambled siRNA #1 (Figure 2A). Furthermore, the proliferation rate detected by Ki-67 staining in 3D culture on day 3 was not different between HKe3 cells treated with *ALPK2* siRNA #1 and those treated with scrambled siRNA #1 (Figure 2B). Taken together, these results suggest that ALPK2 expression does not affect cell proliferation in 2D or 3D culture.

The ratio of the 3D structures containing apoptotic cells for HKe3 cells, transfected with *ALPK2* siRNAs #1 or #2 was significantly lower, compared to that for HKe3 cells transfected with scrambled siRNA #1 or #2 (Figure 2C), suggesting that ALPK2 is critically involved in luminal apoptosis in 3D culture. Furthermore, western blotting revealed that the signal intensity for cleaved caspase-3 of HKe3 cells transfected with *ALPK2* siRNAs #1 or #2 was significantly reduced by approximately 2-fold compared with that of HKe3 cells transfected with scrambled siRNAs (Figure 2D). Taken together, these results suggest that ALPK2 induces luminal apoptosis in 3D culture.

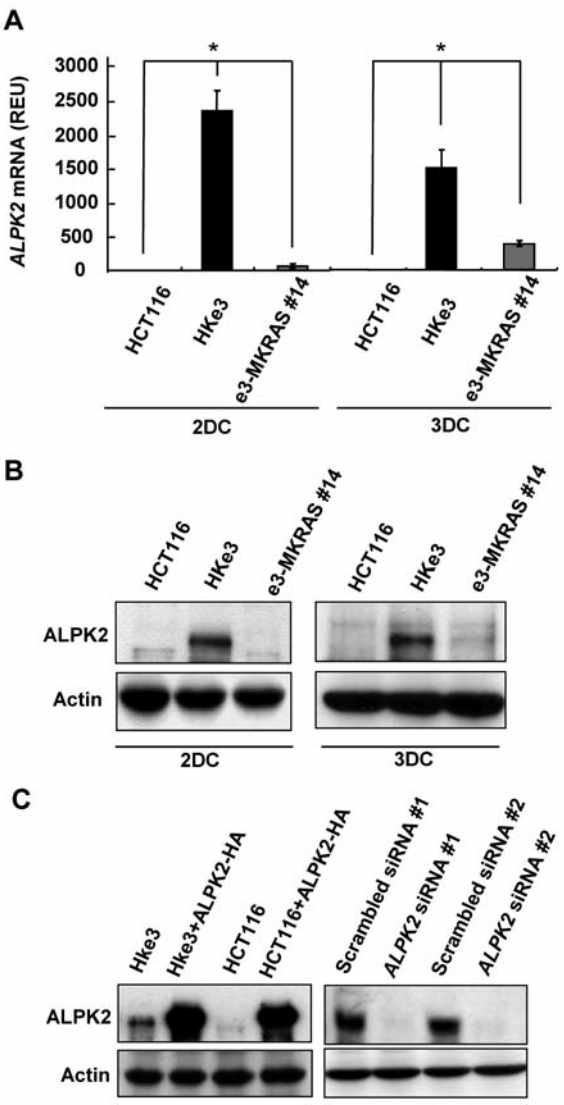

Figure 1. *Alpha-kinase 2 (ALPK2) is down-regulated by oncogenic KRAS in two- and three-dimensional cultures (3DC and 2DC, respectively). A: qRT-PCR analysis of ALPK2 expression in HCT116, HKe3 and e3-MKRAS#14 cells grown in 2DC or 3DC. p<0.05. B: Western blot analyses of ALPK2 in HCT116, HKe3and e3-MKRAS#14 cells grown in 2DC or 3DC. C: Western blot analyses of ALPK2 in HKe3 cells, HKe3 cells transfected with pCI-ALPK2-HA, in HCT116 cells and HCT116 cells transfected with pCI-ALPK2-HA in 2DC (left). Western blot analyses of ALPK2 in HKe3 cells treated with scrambled ALPK2 siRNA #1, ALPK2 siRNA #1, scrambled ALPK2 siRNA #2 or ALPK2 siRNA #2 in 2DC (right).*

ALPK2 is involved in the expression of DNA repair-related genes in 3D culture. To determine the type of genes that are regulated by ALPK2 in 3D cultures, microarray gene expression analyses were performed on HKe3 cells transfected with *ALPK2* siRNA #1 and those with scrambled *ALPK2* siRNA #1, and also of HKe3 cells transfected with *ALPK2* siRNA #2 and those with scrambled *ALPK2* siRNA #2. These analyses revealed that 291 genes were commonly down-regulated (fold-change>1.25) by *ALPK2* siRNAs in HKe3 cells, grown in 3D culture. Gene ontology analyses were performed on these 291 down-regulated genes through the GeneSpring software. These analyses showed that the DNA repair genes were enriched, *i.e.* 10.5% of the total DNA repair genes (21/200) were down-regulated by *ALPK2* siRNAs in HKe3 cells (Table I). These findings collectively suggest that ALPK2 might play a critical role in the up-regulation of DNA repair genes in the normal colonic epithelium.

Out of the 21 detected genes (Table I), we focused our attention on five: Bloom syndrome (*BLM*), Rad 51 homolog (*RAD51*), Fanconi anemia complementation group A, E and G (*FANCA*, *FANCE* and *FANCG*, respectively). These genes are critically associated with DNA repair and apoptosis. Quantitative RT-PCR revealed that the mRNA expression levels of all five genes were significantly lower in HKe3 cells transfected with *ALPK2* siRNA #1 in comparison to those in cells transfected with scrambled siRNA #1 (Figure 3). This finding suggests that these DNA repair genes are positively regulated by ALPK2.

Table I. *List of 21 down-regulated genes classified as DNA-repair genes by Gene Ontology terms*.

Gene name	Fold-change†	
	C1 *vs.* S1	C2 *vs.* S2
RAD51AP1	1.433	1.336
BLM	1.423	1.351
RAD51	1.407	1.609
POLD3	1.406	1.303
TOP2A	1.405	1.519
POLD1	1.390	1.369
CLSPN	1.379	1.351
LIG1	1.356	1.458
FANCE	1.355	1.374
RAD54L	1.346	1.419
POLE|LOC100128843	1.336	1.250
MUS81	1.326	1.467
H2AFX	1.320	1.350
HMGB2	1.312	1.561
MSH6	1.310	1.327
FANCG|VCP	1.292	1.312
RECQL4	1.289	1.370
CHAF1B	1.283	1.319
FANCA	1.281	1.264
CHEK1	1.275	1.250
SSRP1	1.261	1.320

†The relative expression levels of each gene in cells with *ALPK2* siRNA (S) in comparison to that in control cells (C). C1, HKe3 cells transfected with scrambled *ALPK2* siRNA #1; S1, HKe3 cells transfected with *ALPK2* siRNA #1; C2, HKe3 cells transfected with scrambled *ALPK2* siRNA #2; S2, HKe3 cells transfected with *ALPK2* siRNA #2.

Reduced ALPK2 expression and human CRC cell lines. To determine whether oncogenic *KRAS* regulates the mRNA expression of *ALPK2* in other human CRC cell lines, quantitative RT-PCR was performed on the human CRC DLD-1 cell line, on DKO-4 and DLD-1 cells, with a disruption in oncogenic *KRAS* (5). In 2D culture, no significant difference was observed in *ALPK2* mRNA expression between the two cell lines, whereas *ALPK2* mRNA expression in DLD-1 cells was reduced by 2.86-fold ($p<0.05$) in comparison to that in DKO-4 cells in 3D culture (Figure 4A). This founding suggested that oncogenic *KRAS* down-regulates ALPK2 expression in DLD-1 cells in a 3D-specific manner.

To evaluate the correlation between ALPK2 expression and *KRAS* mutation in human CRC cell lines, we performed qRT-PCR analyses. We found that *ALPK2* mRNA expression in human CRC cell lines, including HCT15 (24), DLD-1, HCT116, SW48 (25), LS180 (26) and Colo201 (27) cells were reduced in comparison to that in the normal colonic epithelium (Figure 4B). However, SW48 (25) and Colo201 cells (27) carried no mutation of the *KRAS* gene (Figure 4B). Taken together, these results suggest that reduced expression of ALPK2 might have a crucial role in the development of CRC. They also show that oncogenic *KRAS* mutations are not essential for the down-regulation of ALPK2 expression, and it appears that other specific factors also affect the de-regulated expression of ALPK2.

ALPK2 expression in clinical colorectal adenoma specimens. To examine the correlation between ALPK2 expression and clinical CRC, we analyzed public datasets of the microarray-based gene expression analyses of human clinical samples from 16 patients (21). We found that the *ALPK2* mRNA expression was significantly down-regulated in adenomas compared to the corresponding normal colonic mucosa specimen from the same individual (Figure 4C). Taken together, these results suggest that de-regulated ALPK2 expression might be critically involved in CRC development, and that our 3D model seems to reflect early molecular and morphological changes in CRC development.

Discussion

To our knowledge, this is the first report focusing on the functions of ALPK2 in cellular biology and cancer biology. We demonstrate novel findings regarding ALPK2 function in a 3D colonic-crypt model and a critical role for ALPK2 in CRC progression *in vivo*.

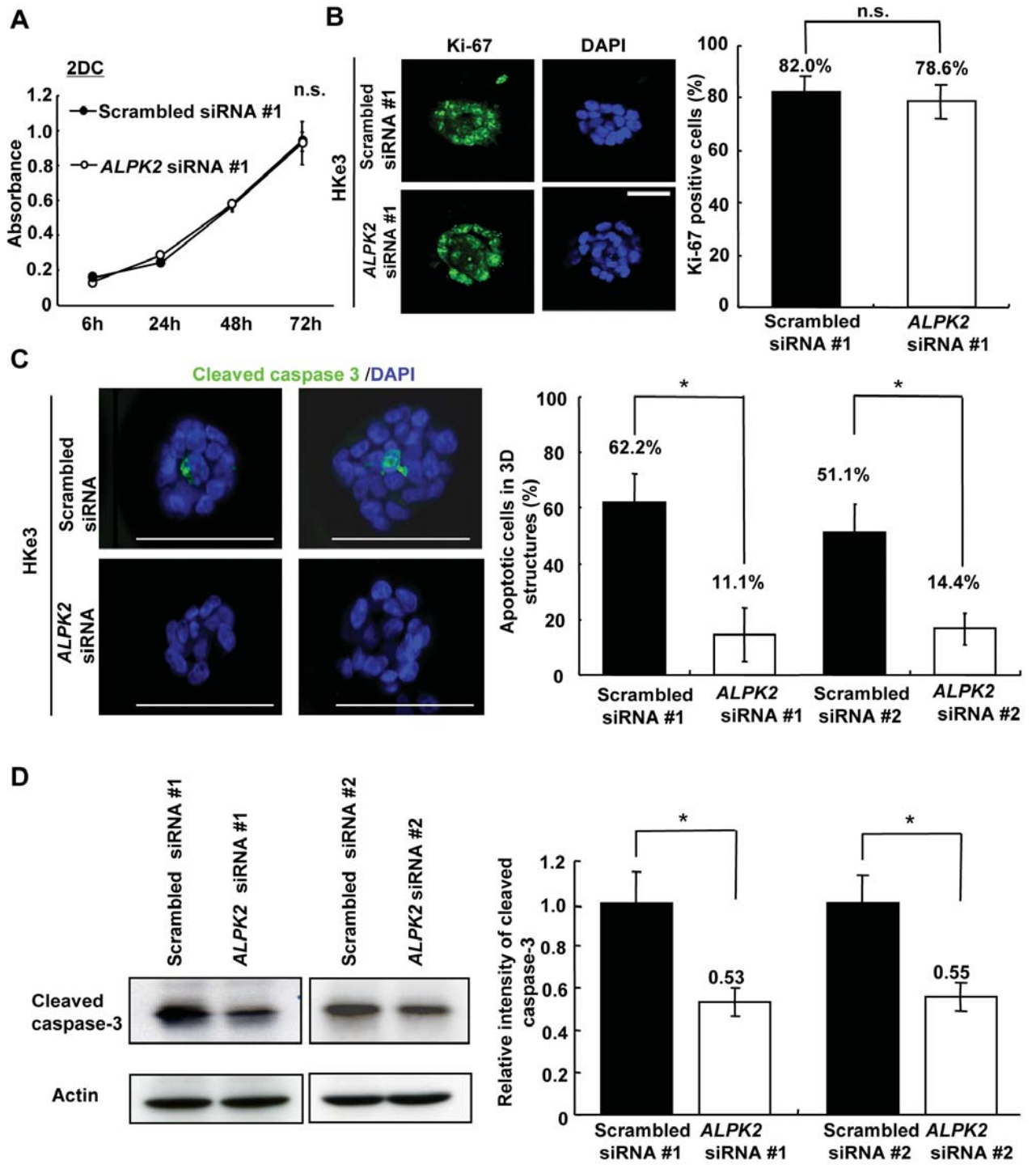

Figure 2. *Inhibition of apoptosis by alpha-kinase 2 (ALPK2) siRNAs in HKe3 cells grown in 2D (2DC) and 3D (3DC) cultures. A: Cell proliferation assay of HKe3 cells grown in 2DC. The data represent the relative growth rates at each time point compared with that at 6 h. n.s., Not significant. B: Signals for Ki-67 staining in HKe3 cells treated with ALPK2 siRNA #1 or scrambled siRNA #1 for three days in 3DC are shown. Ki-67, green; nuclear DNA (DAPI), blue (left). Scale bar=50 μm. The ratios of Ki-67-positive cells in the 3D structures are shown (right). n.s., Not significant. C: The signals for cleaved caspase-3 in HKe3 cells treated with ALPK2 siRNA #1 or scrambled siRNA #1 on day 4 of 3DC (left) are shown. Cleaved caspase-3, green; DAPI, blue; scale bar=50 μm. The ratio of 3D structures containing apoptotic cells (right) to all 3D structures. $p<0.005$. D: Immunoprecipitation of cleaved caspase-3. Western blot analyses of cleaved caspase-3 immunoprecipitated from HKe3 cells treated with ALPK2 siRNAs or scrambled siRNAs in 3DC (left). Quantitative analyses for the levels of cleaved caspase-3 immunoprecipitated from HKe3 cells treated with ALPK2 siRNAs or scrambled siRNAs on day 4 of 3DC are shown (right). $p<0.05$.*

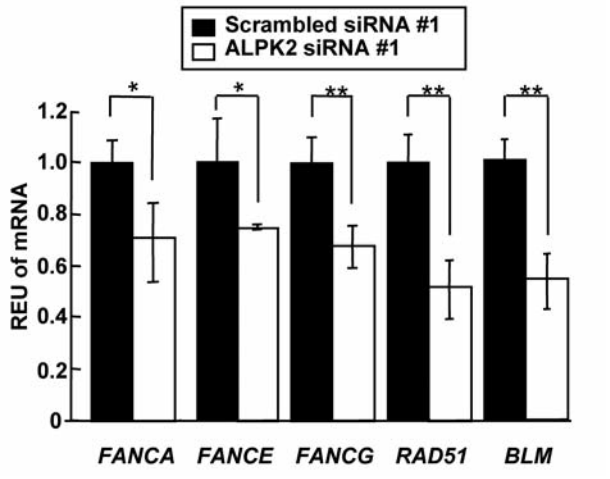

Figure 3. *mRNA expression levels for DNA repair- and apoptosis-related genes in 3D culture. A quantitative RT-PCR analysis for the genes Fanconi anemia complementation group A (FANCA), FANCE, FANCG, Rad 51 homolog (RAD51) and Bloom syndrome (BLM) in HKe3 cells, treated with scrambled siRNA #1 or alpha-kinase 2 (ALPK2) siRNA #1, grown in 3D culture. p<0.05; p<0.01.*

ALPK2 was down-regulated by oncogenic *KRAS* in human colon cancer HCT116 cells (Figure 1). Furthermore, *ALPK2* mRNA expression in DKO-4 cells (human colon cancer DLD-1-derived oncogenic *KRAS*-disrupted cells) was also reduced compared to that of parental DLD-1 cells in 3D culture (Figure 4A). These findings suggest a critical role for oncogenic *KRAS* in the regulation of ALPK2 expression in particular CRC cell lines. However, human colon cancer cell lines without a *KRAS* mutation (25, 27) also exhibited a reduction in *ALPK2* mRNA expression (Figure 4B), suggesting that other specific factors besides *KRAS* mutations also affect ALPK2 expression.

Reduction in ALPK2 expression by *ALPK2*-specific siRNA inhibited apoptosis of HKe3 cells in 3D culture (Figure 2). Inhibition of apoptosis and genetic instability have been reported in pre-cancerous adenomas and during the early stage of CRC development (28, 29). The analysis of the public datasets of the gene expression in human clinical samples (21) revealed down-regulation of ALPK2 expression in colorectal adenomas compared to normal colonic mucosa specimens (Figure 4C), suggesting that reduced ALPK2 expression is involved in the development and progression of CRC *in vivo*.

Another interesting finding was the correlation between ALPK2 expression and DNA repair-related gene expression in 3D culture (Figure 3). The five DNA repair-related genes (*BLM, RAD51, FANCA, FANCE* and *FANCG*) were down-regulated by the reduction of ALPK2 expression (Figure 3C). BLM is associated with p53-mediated apoptosis and has important multiple roles in DNA replication and repair (30). In addition,

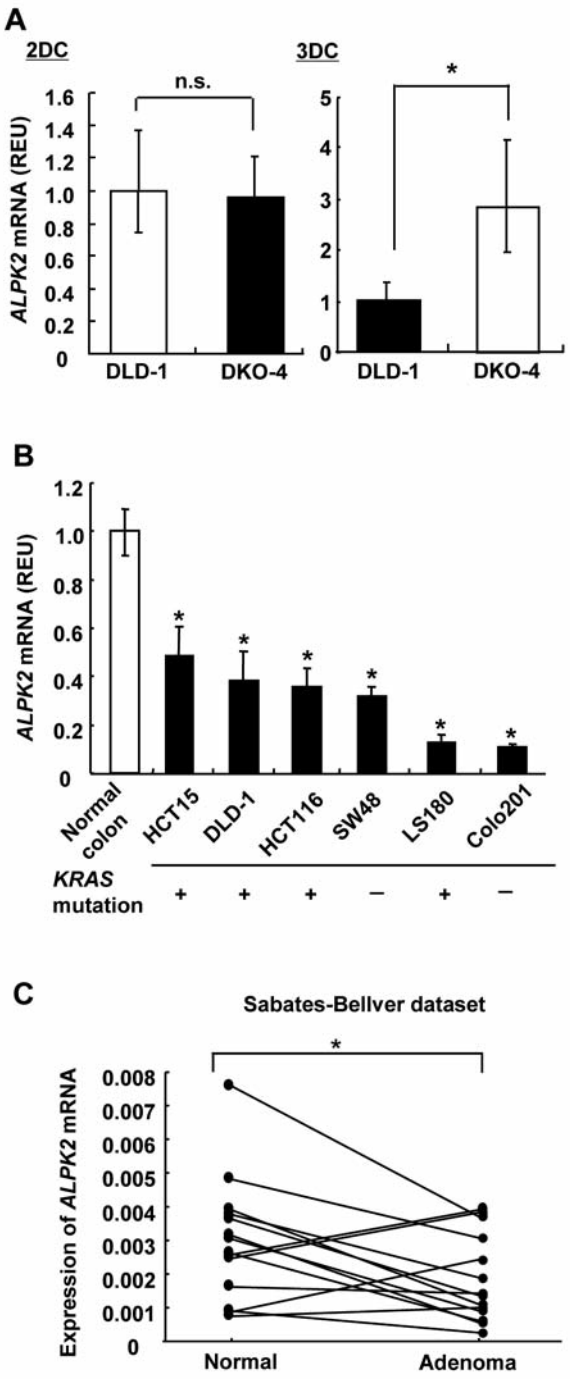

Figure 4. *Alpha-kinase 2 (ALPK2) expression in colorectal cancer cells and clinical colorectal adenoma samples. A: A quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of ALPK2 mRNA expression in DLD-1 cells and DKO-4 cells grown in 2D (2DC) (left) or 3D (3DC) culture (right). B: A quantitative RT-PCR analysis of ALPK2 mRNA expression in normal colon and colon cancer cell lines grown in 2DC. p<0.05. C: ALPK2 is down-regulated in clinical adenomas compared with the normal colonic mucosa from the same individuals as determined using the public dataset for gene expression analyses of 16 patients (GEO; Series GSE8671). The black circles connected by lines represent paired samples. p<0.05.*

the carriers of *BLM* mutations have an increased risk for CRC (31). BLM co-localizes with several proteins, including RAD51, which is essential for DNA repair by homologous recombination (32). Furthermore, the *RAD51* gene is an independent factor for the risk of CRC (33). The Fanconi anemia genes, including *FANCA, FANCE* and *FANCG,* are associated with apoptosis, and the inactivation of such genes is involved in tumorigenesis (34, 35). Fanconi anemia-related molecules, including breast cancer susceptibility gene 1 *(BRCA1), BRCA2* and *BLM,* coordinately affect the development of CRC (9, 31, 36, 37). All these facts collectively support the hypothesis that our 3D model mimics CRC progression through the suppression of DNA repair-related genes, as reported previously (9), and also suggest that reduced ALPK2 expression might play an important role in the genetic instability of CRC.

As reported in the recent comprehensive sequence-based analyses of somatic mutations in kinases in cancer, ALPK2 may have a crucial role in cancer progression (14, 15). *ALPK2* mRNA expression was significantly down-regulated in adenomas compared with the corresponding normal colonic mucosa specimen, and the present study indicates that ALPK2 is critical for the maintenance of a normal phenotype in the colonic epithelium. Taken together, these results suggest that de-regulated ALPK2 expression is critically involved in CRC development *in vivo*.

In summary, reduction in ALPK2 expression leads to inhibition of luminal apoptosis and to suppression of the expression of DNA repair-related genes in human colon cancer cells in 3D culture. These findings suggest that the loss of ALPK2 function is crucial in the accumulation of genetic alterations in the human colorectal epithelium in a 3D microenvironment. Further elucidation of the precise molecular mechanisms of ALPK2 function may provide a better understanding of the development and progression of CRC.

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