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Upregulation of δ-catenin is associated with poor prognosis and enhances transcriptional activity through Kaiso in non-small-cell lung cancer

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δ-Catenin is the only member of the p120 catenin (p120ctn) subfamily that its primary expression is restricted to the brain. Since δ-catenin is upregulated in human lung cancer, the effects of δ-catenin overexpression in lung cancer still need to be clarified. Immunohistochemistry was performed to investigate the expression of δ-catenin and Kaiso, a δ-catenin-binding transcription factor, in 151 lung cancer specimens. A correlation between cytoplasmic δ-catenin and Kaiso expression was also associated with high TNM stage, lymph node metastases and poor prognosis. Co-immunoprecipitation assay confirmed the interactions of δ-catenin and Kaiso in lung cancer cells. In addition, gene transfection and RNAi technology were used to demonstrate that increased δ-catenin expression was promoted, whereas its knockdown suppressed its lung cancer invasive ability. In addition, methylation-specific PCR and ChIP assay demonstrated that δ-catenin could regulate MTA2 via Kaiso in a methylation-dependent manner, while it could regulate cyclin D1 and MMP7 expression through Kaiso in a sequence-specific manner. In conclusion, a δ-catenin/Kaiso pathway exists in lung cancer cells. Increased δ-catenin expression is critical for maintenance of the malignant phenotype of lung cancer, making δ-catenin a candidate target protein for future cancer therapeutics. (*Cancer Sci* 2011; 102: 95–103)

p120 catenin (p120ctn) plays an important role in tumor progression and metastasis of non-small-cell lung cancer (NSCLC).[1,2] It is an Armadillo protein, which was first identified as a tyrosine kinase substrate implicated in cell transformation by Src.[3] It can bind to the juxtamembrane domain (JMD) of E-cadherin[4,5] where it modulates cell–cell adhesion by regulating cadherin turnover and stability at the cell surface.[6–8] In addition, p120ctn can bind directly with Kaiso, a transcription factor,[9] implicating a role for p120ctn in the regulation of transcriptional activity in addition to its cell–cell adhesion function.

As a binding factor of p120ctn, Kaiso is a member of the BTB/POZ (Broad complex, Tramtrak, Bric à brac/Pox virus and zinc finger) subfamily of zinc finger proteins. It has the characteristic POZ domain at its amino-terminus where it facilitates Kaiso homodimerization and heterodimerization with diverse proteins,[10] while the zinc finger domain at the carboxyl terminal of Kaiso is responsible for DNA association.[11] Unlike any of the previously characterized POZ proteins, Kaiso could recognize both sequence-specific DNA consensus (KBS, TCCTGCNA) and methylated CpG-dinucleotides.[12,13] As a transcription repressor,[14] the majority of candidate Kaiso target genes identified thus far, that is, CDH1 (E-cadherin), MMP7, MTA2 and Wnt11, have been linked with development and/or cancer.[15]

δ-Catenin is an adhesive junction associated protein,[16,17] which is the only member of the p120ctn subfamily and its primary expression is restricted to the brain. Initially, it was widely accepted that δ-catenin is the only member of the p120ctn family that is expressed specifically in neurons, where it binds to PDZ domain proteins in the post-synaptic compartment.[18–20] Latterly, the critical role of δ-catenin in the modulation of cellular morphogenesis was found.[21–24] Similar to p120ctn, δ-catenin could bind to the junxtamembrane domain (JMD) of E-cadherin[16,25] and regulate cadherin–cytoskeletal connections indirectly through functional interactions with Rho GTPases.[26]

It has become clear that δ-catenin is expressed in a variety of cancer tissues, including prostate, breast and esophageal tumors.[25,27] Recently, it was established that δ-catenin is involved in regulating small GTPases,[28] which are important mediators of cytoskeletal dynamics, cell migration, adhesion and growth.[29] Since p120ctn could also regulate transcriptional activity in cancer, the goal of the present study is to investigate the hypothesis that δ-catenin regulates transcriptional activity in lung cancer cells in addition to its established functions.

Materials and Methods

Tissue microarray development. Expression of δ-catenin and Kaiso in NSCLC were investigated using immunohistochemical analysis on tissue microarrays composed of 151 independent tumor samples. Among the 151 cases, 70 cases had complete follow-up records. All patients for this validation group were presented to the Hunan Province People's Hospital (Changsha, Hunan Province) between 2000 and 2005 with newly diagnosed, previously untreated NSCLC.

Tumor specimens from 50 patients with NSCLC were obtained following surgical resection at the First Affiliated Hospital of China Medical University. Tumor and the paired non-tumor portion of the same case were quickly frozen in liquid nitrogen and maintained at −70°C.

The tumors were classified according to the TNM stage revised by the International Union Against Cancer (UICC) in 2007.[30] All specimens were re-evaluated for diagnosis following the criteria for classification of lung cancer by the World Health Organization (WHO). This study was conducted under the regulations of the Institutional Review Board of China Medical University. Informed consent was obtained prior to surgery from all enrolled patients.

Immunohistochemical staining and evaluation. Immunohistochemistry was done using the ultrasensitive avidin–biotin–peroxidase complex method (Maixin Biotechnology, Fuzhou,

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Fujian, China) according to the manufacturer's instructions. The antibodies used were Kaiso monoclonal antibody (clone 6F, 1:400; Upstate, Lake Placid, NY, USA), monoclonal antibody δ-catenin I (ab54578, 1:100; Abcam, Cambridge, UK) and δ-catenin II (40.1, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Negative controls were prepared by non-immune rabbit IgG at the same dilution as the primary antibody.

The scoring criteria of δ-catenin described by Lu *et al.*[27] was used. We counted 200 tumor cells and calculated the percentage of positively stained cells. The proportion of cells exhibiting δ-catenin expression was categorized as follows: 0, absent; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, more than 75%. The staining intensity was categorized as follows: 1, weak; 2, moderate; 3, strong. The proportion and intensity scores were then multiplied to obtain a total score. A score <2 was considered negative. For evaluation of the staining of Kaiso, the scoring criteria used in our previous study was adopted.[31] Cases were scored nuclear positive when ≥5% of cells reacted with the anti-δ-catenin or Kaiso antibodies.

Table 1. Primer sequence, amplification sizes and annealing temperatures used in RT-PCR

Gene	Sequence	Length (bp)	Tm
δ-Catenin	5′-TACTCCGCAAGACGACTGACC-3′ 5′-CCATCACACTCTCTCATCCTTCTG-3′	283	58
Kaiso	5′-TGCCTATTATAACAGAGTCTTT -3′ 5′-AGTAGGTGTGATATTTGTTAAAG -3′	248	55
MTA2	5′-TGTACCGGGTGGGAGATTAC -3′ 5′-TGAGGCTACTAGAAATGTCCCTG -3′	153	53
β-actin	5′-AGAGCTACGAGCTGCCTGAC-3′ 5′-AGTACTTGCGCTCAGGAGGA-3′	318	55

Tm, annealing temperature (0°C).

Cell culture and drug treatment. Human lung adenocarcinoma cell line A549 was obtained from the American Type Culture Collection (Manassas, VA, USA) and SPC-A-1(SPC) was purchased from the Cell Bank of Chinese Academy of Science (Shanghai, China). The cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal calf serum (Invitrogen), 100 IU/mL penicillin and 100 IU/mL streptomycin (Sigma, St Louis, MO, USA).

Leptomycin B (LMB; Sigma, 50 nM) was added to the cell medium for inhibiting CRM-1-mediated nuclear export. For experiments with 5-Aza-dC, cells were incubated with media containing 5 μM 5-Aza-dC for 48 h.

Plasmid construction and transfection. δ-catenin expression plasmid pCMV5-FLAG/δ-catenin was a gift from Dr Nakamura (Kobe University, Kobe, Japan) and confirmed by sequencing (Fig. S1). Three pairs of δ-catenin siRNA sequences were designed and synthesized by TaKaRa (Shiga, Japan). Considering the relative effectiveness and stability, the following siRNA sequence was selected by comparison with our pilot experiments: 5-CUACGUUGACUUCUACUCAUU-3, 5-UGAGUAGAAGUCAA CGUAGUU-3. A nonsilencing siRNA sequence was used as a negative control (5-UUCUCCGAACUUGUCACAUUU-3, 5-AUGUGACAAGUUCGGAGAAUU-3). The pcDNA3-Kaiso expression plasmids are as previously described.[9] The RNAi-Kaiso plasmids, which had been used in our previous study,[31] were used again in this study.

RNA extraction, RT-PCR and methylation-specific PCR (MS-PCR). RNA extraction and RT-PCR were performed as previously described.[31,32] The pair PCR primers for δ-catenin, Kaiso, MTA2, MMP7 and cyclin D1 are listed in Table 1. The primers for MS-PCR were performed with a 5′ primer for unmethylated (AATTTGGTGAAGTGTTAAGGTGTGTGAG) or methylated (AATTTGGCGAA GCGTTAAGGCGCGCGA) and a common 3′ primer (AAAACAAAACCCA CTACTCAACTCAAATC).

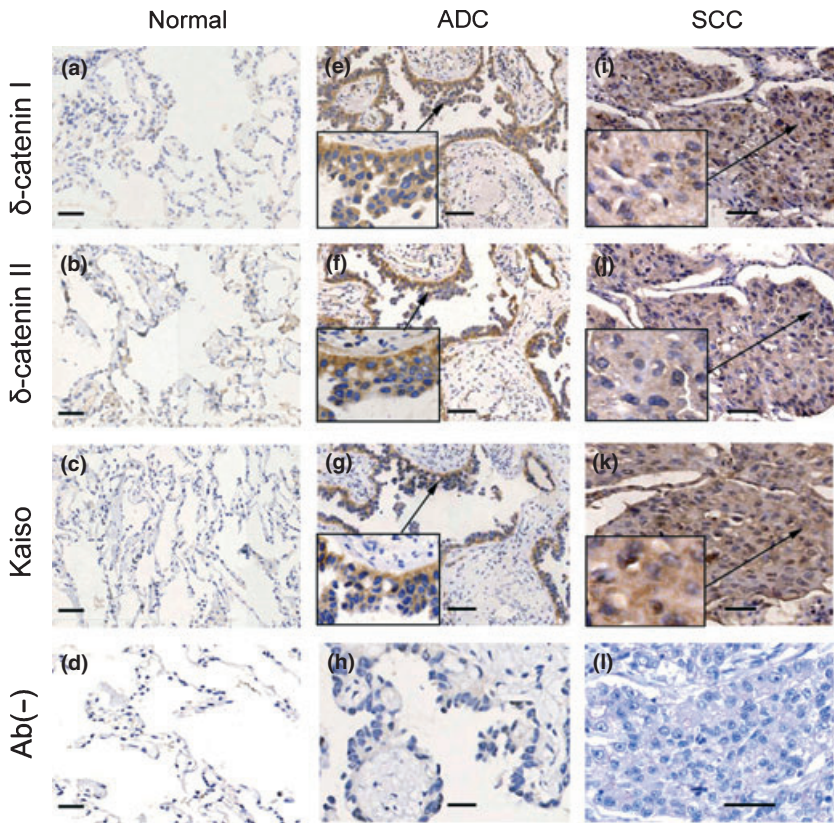

Fig. 1. Immunohistochemical staining of δ-catenin and Kaiso in normal lung tissues and lung cancer tissues. Compared with normal lung tissue (a,b,c), δ-catenin and Kaiso expression was significantly increased in lung cancer tissues (δ-catenin I: e,f; δ-catenin II: i,j; Kaiso: g,k). Insets show high magnification of subcellular localization indicated by arrows. δ-Catenin and Kaiso expression patterns in a similar region of the same sample were similar, mainly as a cytoplasmic staining. As a negative control, the primary antibody was replaced by non-immune rabbit IgG (d,h,l). Ab(–), negative control; ADC, adenocarcinoma; Normal, normal lung tissue; SCC, squamous cell cancer. Original magnification, ×200. Bar, 50 μm.

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Chromatin immunoprecipitation (ChIP) assays. ChIP assays were performed as previously described.[33] The following primers specific for a region flanking the two Kaiso binding sites within the human MTA2 promoter were used for PCR: 5′-CTCCTCCCTCGACGTTTGAT-3′ and 5′-GATCGGGAATGTCTCGAAGT-3′. The MMP7 promoter-specific primers are: 5′-CTCCTCCCTCGACGTTTGAT-3′ and 5′-GATCGGGAATGTCT CGAA GT-3′. The cyclin D1 promoter-specific primers are: 5′-CCCTCTCATGT AACCACGAA-3′ and 5′- TGGTTTTGTTGGGGGTGTAG-3′.

Immunoprecipitation and western blot. Lysates of cells or tissues were quantified by Bradford assay and equal amounts of total protein were used for immunoprecipitation with the anti-Kaiso mAb (6F; Upstate) or anti-δ-catenin mAb (ab54578; Abcam). The immunocomplexes were captured by protein G beads (Miltenyi Biotec, Bergisch Gladbach, Germany). The immunocomplexes were then subjected to SDS-PAGE.

All samples were loaded at constant protein concentrations for separation by SDS-PAGE (12%), and electrotransferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The relative protein levels were calculated based on β-actin protein as a loading control.

Immunofluorescent staining. Cells were fixed with 4% paraformaldehyde and incubated with δ-catenin and Kaiso antibody (all 1:100 dilutions) overnight at 4°C. The secondary antibody conjugated to rhodamine/fluorescein isothiocyanate (FITC)-labeled IgG was used. The nuclei were counterstained with Hoechst 33258.

Matrigel invasive assay. The cells' invasive abilities were examined using a 24-well Transwell with 8-μm pore polycarbonate membrane inserts (Corning Inc., Corning, NY, USA) according to the manufacturer's protocol. Cells that appeared on the lower surface of the filter were counted in five random ×200 fields using an inverted microscope (Olympus IX51; Olympus America Inc., Melville, NY, USA).

Statistical analysis. SPSS v13.0 software for Windows (SPSS, Inc. Chicago, IL, USA) was used for statistical analysis. A *P* value of <0.05 was considered statistically significant. All tests and *P* values were bilateral.

Results

δ-catenin and Kaiso overexpressed in NSCLC and associated with malignancy of lung cancer and poor prognosis. As shown in Figure 1, δ-catenin and Kaiso were expressed weakly in the cytoplasm of normal lung tissue. According to our evaluation criteria, they were judged as negative expression. δ-Catenin was primarily localized in the cytoplasm of lung cancer cells, and the cytoplasmic expression rate of δ-catenin was 59.60% (90/151), which was significantly higher than that in normal lung tissue. The expression rate of δ-catenin in stages III–IV was also higher than that in stages I–II (68.92% *vs* 50.65%, $P = 0.022$). In addition, the cytoplasmic δ-catenin expression rate in cases with lymph node metastases was higher than that in cases without (67.44% *vs* 49.23%, $P = 0.024$). Also, there was no significant correlation between cytoplasmic δ-catenin expression and gender, age or differentiation (Table 2). Because nuclear positive δ-catenin was seldom detected in lung cancer tissue, nuclear positive δ-catenin was not specially analyzed in the present study.

The positive rate of cytoplasmic Kaiso expression in lung cancer tissue was 57.62% (87/151), which was significantly higher than that in normal bronchial epithelial cells. Cytoplasmic Kaiso expression occurred more frequently in samples with advanced tumor stage and lymph node metastasis ($P = 0.015$ and 0.032, respectively), while no significant other association was found (Table 2). This result was consistent with our previous reports.[31,32]

δ-Catenin and Kaiso mRNA and protein levels were obviously higher in lung cancer in comparison with corresponding normal lung tissue (Tables S1,S2 and Fig. S2). Their mRNA expressions were positively correlated with lymph node metastasis ($P = 0.025$ and 0.033, respectively). In addition, δ-catenin and Kaiso protein was expressed more frequently in samples with advanced TNM and lymph node metastasis.

The immunohistochemical staining results of consecutive sections showed that cytoplasmic δ-catenin expression was frequently accompanied by cytoplasmic Kaiso expression, regardless of the clinical and histological features. Statistical analysis confirmed a significant relationship between cytoplasmic Kaiso expression and cytoplasmic δ-catenin expression ($P = 0.006$; Table 3).

Kaiso forms a complex with δ-catenin in lung cancer cells. In 18 cases of matched specimens, the complex of δ-catenin and Kaiso was not found in only one case of normal lung tissue (Fig. 2). Besides, δ-catenin was detected in anti-Kaiso immunoprecipitates from A549 and SPC cells. The specificity of this experiment was verified by probing the same δ-catenin immunoprecipitates with IgG, which yielded no signal.

Table 2. Clinical and histological features in 151 patients with lung cancer

Variables	All patients	Cytoplasmic δ-catenin expression	*P**	Cytoplasmic Kaiso expression	*P**
Total	151	90		87	
Age (years)					
≤55	67	43	0.306	35	0.232
>55	84	47		52	
Gender					
Male	68	38	0.399	39	0.553
Female	83	52		48	
Stage					
I/II	77	39	**0.022**	37	**0.015**
III/IV	74	51		50	
Histology					
Squamous cell carcinoma	71	37	0.077	42	0.537
Adenocarcinoma	80	53		45	
Grade					
Well, Moderate	85	53	0.434	47	0.512
Poor	66	37		40	
Lymph node metastasis					
No	65	32	**0.024**	31	**0.032**
Yes	86	58		56	

**P* values were obtained from the χ^2 test (two-sided). Bold underlines denote **P* values <0.05.

Table 3. Correlation between cytoplasmic Kaiso and δ-catenin in 151 cases of primary lung cancer

Antigen	Cytoplasmic δ-catenin-positive	Cytoplasmic δ-catenin-negative	Total	Kappa	*P**
Cytoplasmic Kaiso-positive	60	27	87	0.222	0.006
Cytoplasmic Kaiso-negative	30	34	64		
Total	90	61	151		

**P* values were obtained from the χ^2 test (two-sided).

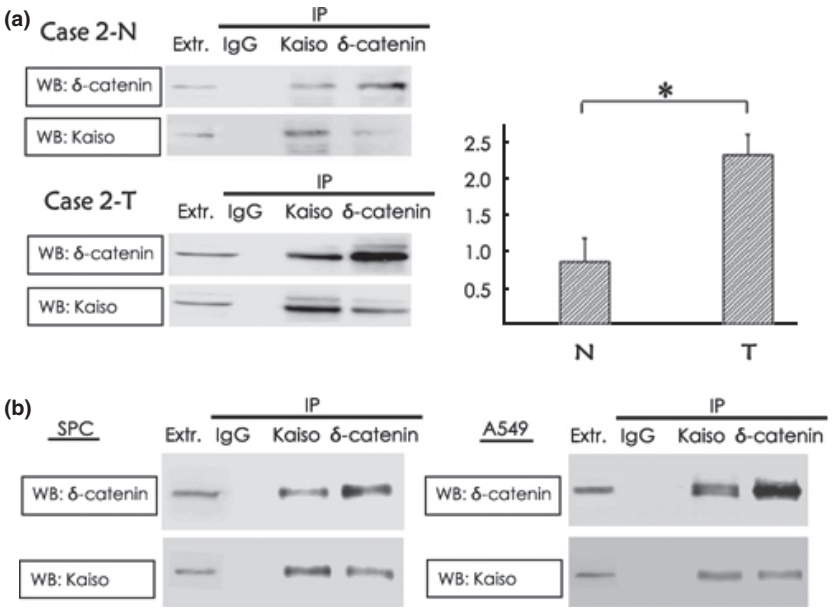

Fig. 2. δ-Catenin interaction with Kaiso *in vivo*. (a) Left panel: the representative result of immunoprecipitation was shown here to demonstrate that δ-catenin was co-precipitated by Kaiso mAb from both normal (N) and lung cancer (T) tissues. Right panel: the ratio between the optical density of bands (immunoprecipitation [IP] by Kaiso mAb and western blot [WB] with δ-catenin pAb) and extracts (Extr.) bands of the same patient was calculated and expressed graphically. *$P < 0.05$. (b) δ-Catenin was co-precipitated by Kaiso mAb from both A549 and SPC cells.

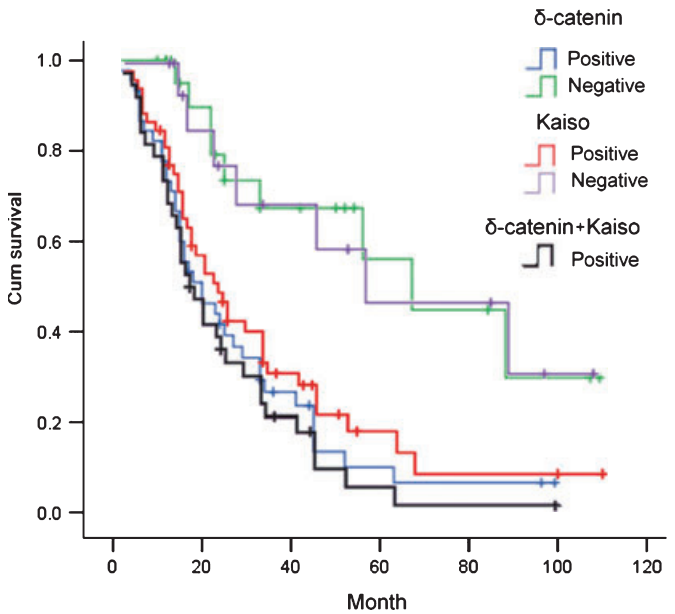

Fig. 3. Survival curves for cytoplasmic expression of δ-catenin and Kaiso. Kaplan–Meier curves from the analysis of 70 patients with non-small-cell lung cancer.

Table 4. Cox regression model for prediction of survival for 70 patients with lung cancer

Factor	Risk	95% CI	*P* value
Age	0.632	0.315–1.034	0.177
Gender	0.650	0.413–1.203	0.392
Histology	1.521	0.633–1.882	0.412
Differentiation	2.532	2.361–3.314	0.021
TNM stage	3.221	2.278–4.836	0.007
Lymphatic metastasis	4.111	3.551–7.031	0.003
Cytoplasmic δ-catenin expression	0.915	0.467–1.523	0.087
Cytoplasmic Kaiso expression	0.411	0.154–0.756	0.074

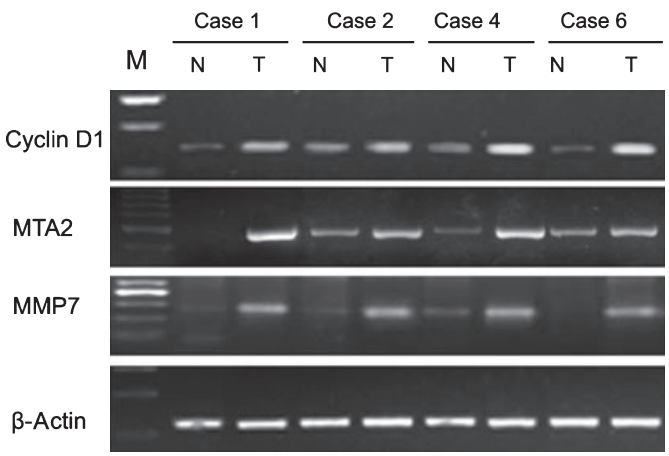

Fig. 4. Transcriptional levels of cyclin D1, MTA2 and MMP7 in lung cancer tissues and normal lung tissues. mRNA expression of cyclin D1, MTA2 and MMP7 in lung cancer tissues was significantly higher than that in normal lung tissues ($n = 50$, all $P < 0.05$).

Expression of δ-catenin, Kaiso and survival time. As shown in Figure 3, with regard to the expression of cytoplasmic δ-catenin, the lung-cancer-related 5-year survival rates were 12.2% for the positive expression and 67.4% for the negative expression ($P = 0.000$). With regard to cytoplasmic Kaiso, the survival rate was 59.3% for the negative expression and 21.8% for the positive expression ($P = 0.011$). Significantly, the group with both δ-catenin and Kaiso positive had a worse prognosis than those with positive expression ($P = 0.000$). A multivariate Cox regression analysis showed that cytoplasmic expression of δ-catenin and Kaiso was not an independent prognostic factor (Table 4).

mRNA levels of MTA2, MMP7 and cyclin D1 are significantly upregulated in lung cancer tissue. MTA2, MMP7 and cyclin D1 mRNA levels were significantly increased in lung cancer in comparison with corresponding normal lung tissue (Fig. 4 and Table S3). In addition, MMP7 mRNA expression was significantly higher in squamous cell carcinomas than in adenocarcinomas ($P = 0.044$), and MTA2 positively correlated with lymph node metastasis ($P = 0.017$).

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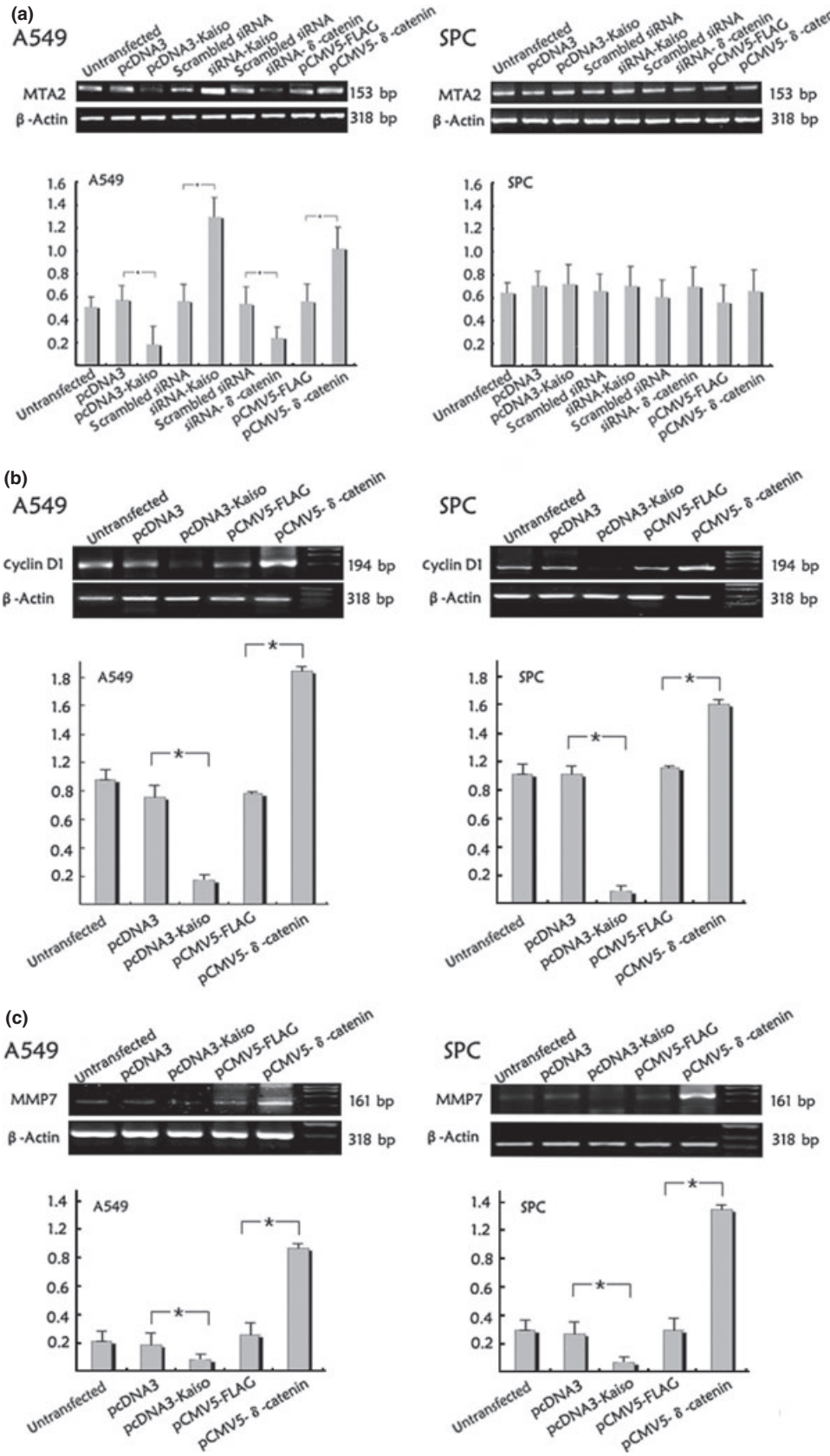

Fig. 5. Overexpression of δ-catenin could increase MTA2, cyclin D1 and MMP7 expression through transcription factor Kaiso. The ratio between the optical density of specific bands and β-actin of the same specimen was calculated and expressed graphically. (a) RT-PCR revealed MTA2 expression could be regulated by δ-catenin and Kaiso in A549 cells, but not in SPC cells. Cyclin D1 (b) and MMP7 (c) expression could be regulated by δ-catenin and Kaiso in A549 and SPC cells. β-Actin served as an internal control. *$P < 0.05$.

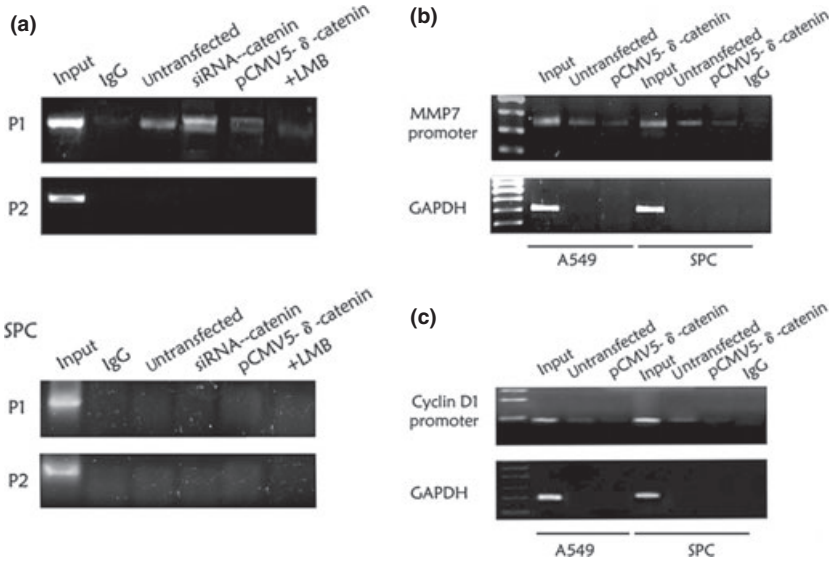

Fig. 6. The effects of δ-catenin and Leptomycin B (LMB) on the binding of Kaiso to the MTA2, MMP7 and cyclin D1 promoter. (a) ChIP assays showed that Kaiso only binded to the methylated MTA2 promoter in A549 cells. The association of Kaiso was detected in the promoter region (P1) but not in the coding region (P2). (b,c) δ-Catenin overexpression could significantly decrease the binding between Kaiso and MMP7 and cyclin D1 promoters.

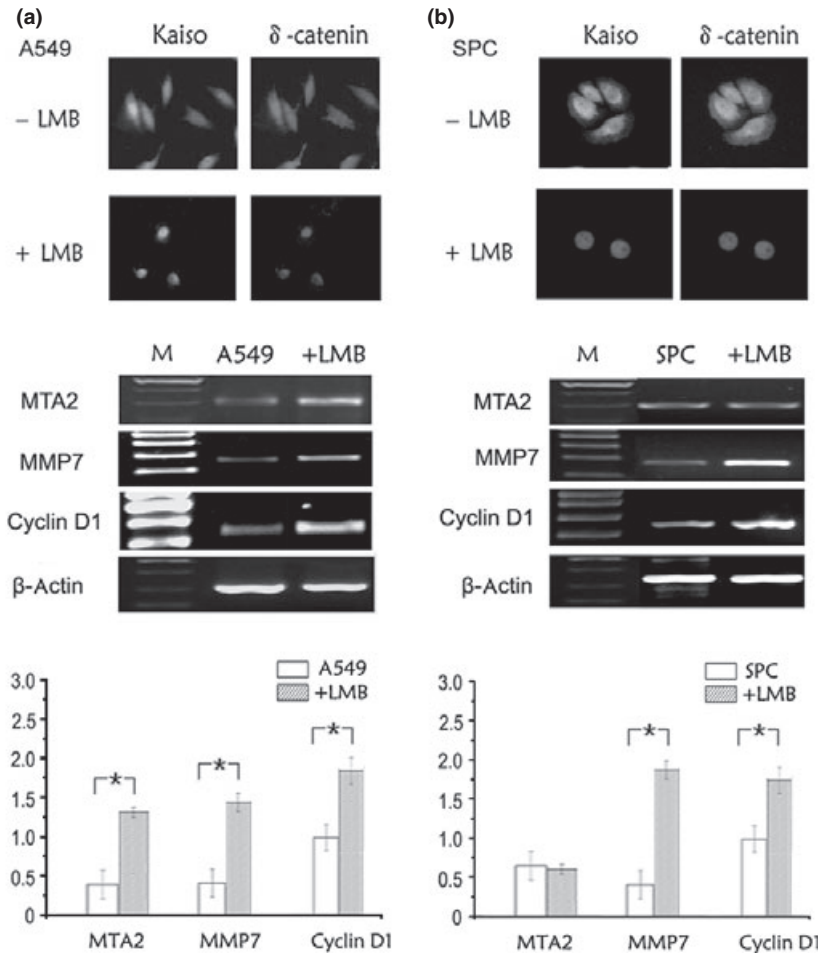

Fig. 7. δ-Catenin and Kaiso undergo rapid nucleocytoplasmic shuttling; they are both sensitive to Leptomycin B (LMB) in A549 and SPC cells. The LMB treatment significantly induced more nuclear localization of δ-catenin and Kaiso in A549 and SPC cells, with upregulated expression of MMP7 and cyclin D1. However, elevated MTA2 was only detected in A549 cells (a), but not in SPC cells (b). $*P < 0.05$.

Kaiso could regulate MTA2, MMP7 and cyclin D1 transcription in a methylation-dependent manner or KBS-dependent manner. Because KBS did not exist in the MTA2 promoter, we ruled out the possibility that Kaiso binds with the MTA2 promoter by KBS. However, at least three methylated CpG islands, which lie in the regions of 91–207, 791–917 and 1089–1203 bp upstream to the translation start site, were predicted in the promoter of the MTA2 gene by MethPrimer software. Methylation-specific PCR showed that CpG sequences in the MTA2 promoter is methylated in A549 cells, but not in SPC cells (Fig. S3).

Because siRNA-Kaiso led to significant reduction of Kaiso (Fig. S4), expression of the MTA2 gene was significantly elevated in A549 cells, but not in SPC cells (Fig. 5a). After transfection with Kaiso cDNA (Fig. S4), MTA2 expression was repressed

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obviously in A549 cells but not in SPC cells (Fig. 5a). In addition, MMP7 and cyclin D1 mRNA were significantly reduced in both A549 and SPC cells overexpressing Kaiso (Fig. 5b,c).

δ-Catenin could regulate MTA2, MMP7 and cyclin D1 expression through the transcription factor Kaiso. δ-Catenin was transfected and depleted in A549 and SPC cells (Fig. S4). δ-Catenin overexpression significantly upregulated MTA2 expression in A549 cells, but not in SPC cells (Fig. 5a). In addition, MMP7 and cyclin D1 expression were significantly enhanced in all δ-catenin transefected cells (Fig. 5b,c).

Kaiso binds with the promoter region of MTA2 in A549 cells, but not SPC cells (Fig. 6a). The association of Kaiso with MTA2 promoter was markedly reduced in A549 cells after δ-catenin overexpression, while such association was increased in δ-catenin depleted A549 cells. Obviously, no change was found between the association of Kaiso and MTA2 promoter in SPC cell groups. The specific Kaiso binding sequence (KBS) lies in the regions of −1059 to −1066 bp of the cyclin D1 promoter, while the KBS is located in the regions of −160 to −167 bp of the MMP7 promoter. The binding of Kaiso with promoters of MMP7 and cyclin D1 were confirmed by ChIP assays in the present study. In accordance with expectation, the association of Kaiso with MMP7 and cyclin D1 promoters were significantly reduced in all δ-catenin overexpression cells (Fig. 6b,c).

To test whether δ-catenin and Kaiso undergo rapid nucleocytoplasmic shuttling, we treated the A549 and SPC cells with Leptomycin B (LMB). The LMB treatment led to significantly increased nuclear localization of δ-catenin and Kaiso (Fig. 7). Correspondingly, the LMB treatment led to the enhanced transcription of MTA2 in A549 cells (Fig. 7a) but not in SPC cells (Fig. 7b). Besides, the association of Kaiso with MTA2 promoter was reduced in LMB-treated A549 cells. However, association of Kaiso with MTA2 promoter was still not found in SPC cells. As for MMP7 and cyclin D1, the transcriptional levels were significantly upregulated in LMB-treated A549 and SPC cells.

Exogenous expression of δ-catenin enhances the invasive ability of lung cancer cells. To examine the biological function of δ-catenin in the invasion of lung cancer cells, we performed a Matrigel Invasion assay in δ-catenin overexpression/knockdown cells. As shown in Figure 8, overexpression of δ-catenin significantly promotes while its knockdown suppresses lung cancer cells that invaded onto the lower surfaces of the Transwell filters ($P < 0.05$).

Discussion

The current study presents novel evidence that δ-catenin in epithelial cancer cells has an additional function that suppressed Kaiso-dependent transcription. This conclusion is at least based on three key observations: (i) both δ-catenin and Kaiso could shuttle between the cytoplasm and nucleus of lung cancer cells;

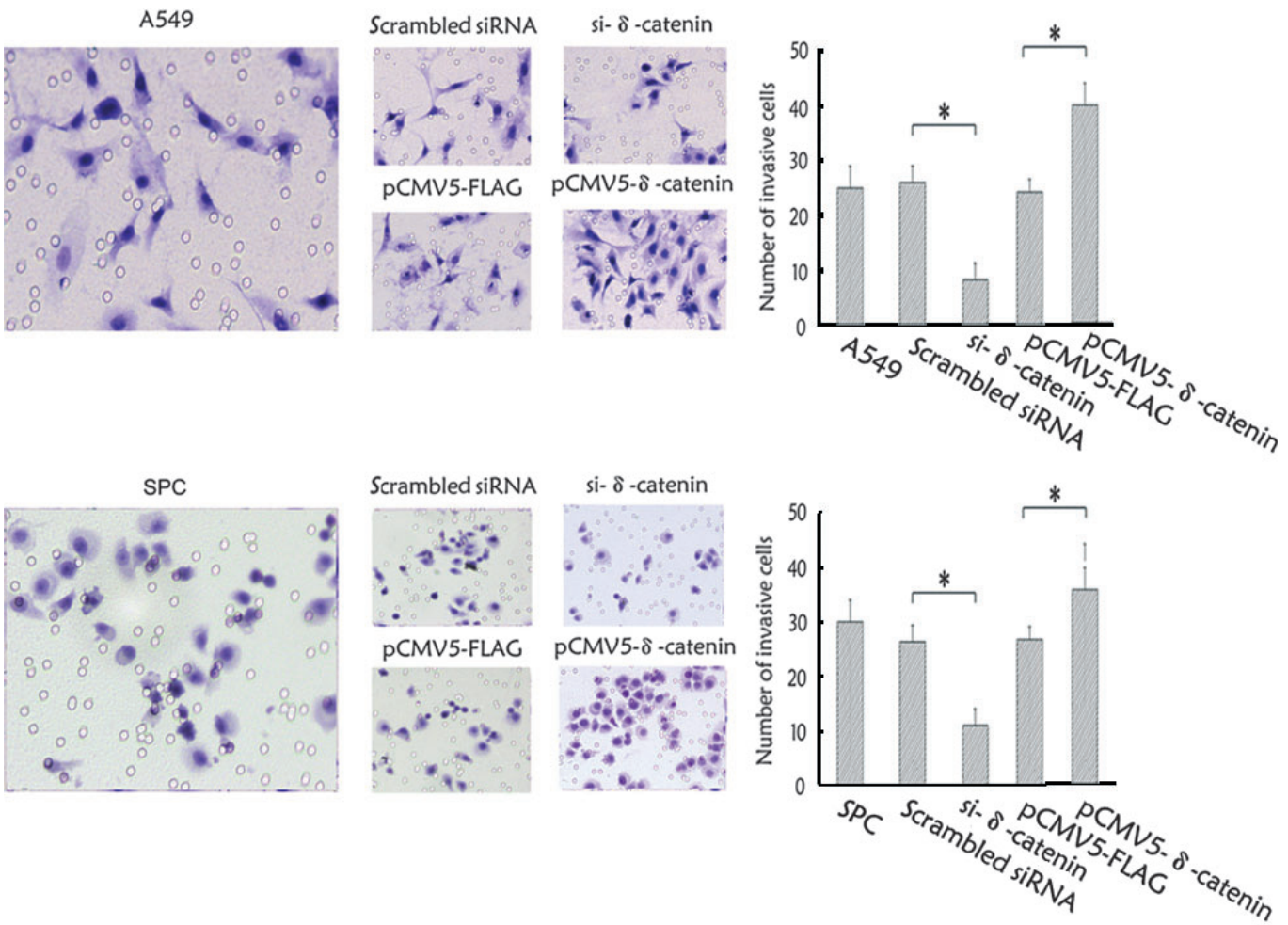

Fig. 8. δ-Catenin overexpression enhances while δ-catenin depletion suppresses the invasive ability of lung cancer cells. Invasion assays of A549 and SPC cells, which were transfected with δ-catenin plasmids or siRNA sequences. The number of cells invading the lower surface of the filter is shown. *$P < 0.05$.

(ii) δ-catenin and Kaiso formed a complex in lung cancer cells; and (iii) exogenous δ-catenin elevated MMP7, MTA2 and cyclin D1 expression in a methylation-dependent manner or KBS-dependent manner. MMP7 and MTA2 are known for their importance in tumor proliferation and malignant invasion.[34–37] Cyclin D1 is a key regulator of cell proliferation and its overexpression has important roles on tumor development and malignant transformation.[38] The findings support the notion that overexpression of δ-catenin enhances the δ-catenin-mediated transcriptional events. In addition, the pathological significance of δ-catenin/Kaiso interaction in lung cancer is revealed in the present study; patients with an aggressive phenotype and poor prognosis have been associated with increased δ-catenin and Kaiso.

At present, we found both δ-catenin and Kaiso were predominantly co-distributed in the cytoplasm of lung cancer cells. However, it is hard to detect nuclear Kaiso and δ-catenin in lung cancer tissues. The absence of nuclear Kaiso in lung cancer tissues might reflect a requirement for the expression of certain Kaiso-repressible genes, such as MMP7, MTA2 and cyclin D1. However, treatment of lung cancer cells with LMB resulted in nuclear accumulation of Kaiso and δ-catenin. These observations suggest that perhaps the failure to detect nuclear δ-catenin in tissue was because it was rapidly and efficiently exported from the nucleus, and that the nuclear localization of δ-catenin was a dynamic and tightly regulated process. Because Kaiso only have a nuclear localization signal (NLS) and did not have a nuclear export signal (NES),[39] while δ-catenin has both NLS and NES, it is conceivable that Kaiso is excluded from the nucleus by virtue of its interaction with δ-catenin. It is interesting to find that forcing nuclear localization of δ-catenin by LMB led to enhanced expression of MTA2 with less association of Kaiso with MTA2 promoter. Because the δ-catenin-binding site encompasses the Kaiso zinc finger domain, which was also used to bind DNA, it was conceivable that the DNA was ineffective in competing with nuclear δ-catenin for binding on Kaiso.

To date, there is no information regarding the interaction of δ-catenin and Kaiso in epithelial cells; we confirmed their interaction in lung cancer cells. In addition, we also found that the number of δ-catenin–Kaiso complexes in lung cancer cells is significantly more than that in normal lung cells. However, we still noticed that the number of δ-catenin–Kaiso complexes is almost the same as that in normal lung cells in 5 out of 18 cases we detected (data not shown). It is interesting to analyze the results of these five cases. In fact, to be a multiple factor, Kaiso not only interacts with δ-catenin as we reported here, but also can interact and form a complex with several other proteins, such as p120ctn[9,32] and CTCF.[40] In addition, the function of Kaiso in normal and cancer conditions might not be the same.[15,41] Therefore, Kaiso, which does not bind to δ-catenin, might interact with other binding partners, but not just as a free protein and function as a transcriptional factor.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Sequencing result of plasmid pCMV5-FLAG/δ-catenin.

Fig. S2. Overexpression of δ-catenin and Kaiso in lung cancer tissues.

Fig. S3. The methylated status of MTA2 promoter in A549 and SPC cells.

Fig. S4. Expression of Kaiso and δ-catenin after transfection of their cDNA or siRNA.

Table S1. Correlations between δ-catenin, Kaiso mRNA and clinicopathological factors.

Table S2. Correlations between δ-catenin, Kaiso protein and clinicopathological factors.

Table S3. Correlations between MMP7, cyclin D1 and MTA2 mRNA and clinicopathological factors.

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