玖、附表

Table 1. List of antibodies used in the present study.

Protein	Final conc.	Source
β-catenin[1]	1:150	BD Biosciences, San Jose, CA, USA
ICAT[2]	1:100	R&D Systems, Minneapolis, MN, USA
HBP1[2]	1:200	Santa Cruz Biotechnology, Santa Cruz, CA, USA

[1] Mouse monoclonal antibody. [2] Goat polyclonal antibodies.

Table 2. List of primer sequences used in the present study.

Gene	Primer	5'→3' sequences	PCR size (bp)	Tm (^{o}C)	Cycle number
ICAT-cDNA	Forward	GAGCACCTGTTTGCCTGAAG	321	65	32
	Reverse	GCCCTTCAACAGCATCCAGG			
HBP1-cDNA	Forward	CTTTGTCTTGTGGAGGACCTG	396	60	35
	Reverse	GTGAGCCTGAATTGGTTCTTTT			
MMP7-cDNA	Forward	TACAGTGGGAACAGGCTCAGG	181	60	30
	Reverse	GGCACTCCACATCTGGGCT			
GAPDH	Forward	AATCCCATCACCATCTTCCA	588	55	30
	Reverse	CCTGCTTCACCACCTTCTTG			
ICAT- methyl-M	Forward	CGACGTAGGAAGATTACGT	114	55	40
	Reverse	CGAAAACAACAAAACGAA		58	35
ICAT- methyl-U	Forward	AGTTTAATGATGTAGGAAGATTATG	125	57	40
	Reverse	AAAACAAAAACAACAAAACAAA			
HBP1-methyl-M	Forward	TTTTTCGAGACGGATTACGG	222	65	20
	Reverse	GCGAATTACTACTCCCGCGAC		70	30
HBP1-methyl-U	Forward	TTTTTTGAGATGGATTATGG	223	55	35
	Reverse	CACAAATTACTACTCCCACAAC			

Table 3. Alterations of *ICAT* gene in relative to clinicopathological parameters of resected primary lung tumors[a].

		Protein expression				mRNA expression				Promoter methylation			
Characteristics		Total	+	--(%)[c]	*P* value	Total	+	--(%)[d]	*P* value	Total	--	+(%)[e]	*P* value
Overall [b]		95	70	25(26.0)		95	59	36(37.9)		95	60	35(36.8)	
Age	<67	34	23	11(32.4)	0.318	34	17	17(50.0)	0.069	34	21	13(38.2)	0.834
	>67	61	47	14(23.0)		61	42	19(31.1)		61	39	22(36.1)	
Gender	Female	20	15	5(25.0)	0.880	20	13	7(35.0)	0.764	20	9	11(55.0)	**0.058**
	Male	75	55	20(26.7)		75	46	29(38.7)		75	51	24(32.0)	
Smoking habit	Nonsmoker	12	8	4(33.3)	0.860	12	9	3(25.0)	0.324	12	8	4(33.3)	0.944
	Smoker	65	45	20(30.8)		65	39	26(40.0)		65	44	21(32.3)	
Tumor type	AD	54	39	15(27.8)	0.893	54	36	18(33.3)	0.198	54	34	20(37.0)	0.910
	SQ	34	25	9(26.5)		34	18	16(47.1)		34	21	13(38.2)	
Tumor stage	Early	61	46	15(24.6)	0.609	61	40	21(34.4)	0.351	61	39	22(36.1)	0.834
	Late	34	24	10(29.4)		34	19	15(44.1)		34	21	13(38.2)	

[a] These results were analyzed by Pearson χ^2 test.

[b] Total number of sample in some categories is less than the overall number analyzed because clinical data was not available for these samples.

[c] ICAT protein (–) was defined as less than 40%of nuclear staining of the ICAT protein.

[d] *ICAT* RNA (–) was defined as less than 50% of the expression level in tumor sample compared to its normal counterpart.

[e] *ICAT* promoter methylation (+) was defined as tumor cell showed more methylation product than unmethylation product at their promoter CpG sites.

Table 4. Alterations of *HBP1* gene in relative to clinicopathological parameters of resected primary lung tumors[a].

		Protein expression				mRNA expression				Promoter methylation			
Characteristics		Total	+	--(%)[c]	*P* value	Total	+	--(%)[d]	*P* value	Total	--	+(%)[e]	*P* value
Overall[b]		95	65	30(31.6)		95	63	32(33.7)		95	52	43(45.3)	
Age	<67	34	22	12(35.3)	0.561	34	23	11(32.4)	0.838	34	19	15(44.1)	0.867
	>67	61	43	18(29.5)		61	40	21(34.4)		61	33	28(45.9)	
Gender	Female	20	13	7(35.0)	0.711	20	15	5(25.0)	0.355	20	14	6(30.)	0.123
	Male	75	52	23(30.7)		75	48	27(36.0)		75	38	37(49.3)	
Smoking habit	Nonsmoker	12	10	2(16.7)	0.320	12	11	1(8.3)	**0.035**	12	7	5(41.7)	0.630
	Smoker	65	45	20(30.8)		65	39	26(40.0)		65	33	32(49.2)	
Tumor type	AD	54	41	13(24.1)	**0.049**	54	40	14(25.9)	**0.021**	54	31	23(42.6)	0.681
	SQ	34	19	15(44.1)		34	17	17(50.0)		34	18	16(47.1)	
Tumor stage	Early	61	38	23(37.7)	0.085	61	41	20(32.8)	0.804	61	32	29(47.5)	0.550
	Late	34	27	7(20.6)		34	22	12(35.3)		34	20	14(41.2)	

[a] These results were analyzed by Pearson χ^2 test.

[b] Total number of sample in some categories is less than the overall number analyzed because clinical data was not available for these samples.

[c] HBP1 protein (–) was defined as less than 40%of nuclear staining of the HBP1 protein.

[d] *HBP1* RNA (–) was defined as less than 50% of the expression level in tumor sample compared to its normal counterpart.

[e] *HBP1* promoter methylation (+) was defined as tumor cell showed more methylation product than unmethylation product at their promoter CpG sites.

Table 5. Alterations of β-catenin protein in relative to clinicopathological parameters of resected primary lung tumors.

Characteristics		Total	β-catenin protein expression +(%)	--	*P* value
Overall		95	40(42.1)	55	
Clinicalpathological parameters					
Age	<67	34	15(44.1)	19	0.767
	>67	61	25(41)	36	
Gender	Female	20	8(40)	12	0.830
	Male	75	32(42.7)	43	
Smoking habit	Nonsmoker	12	4(33.3)	8	0.468
	Smoker	65	29(44.6)	36	
Tumor type	AD	54	23(42.6)	31	0.496
	SQ	34	12(35.3)	22	
Tumor stage	Early	61	26(42.6)	35	0.891
	Late	34	14(41.2)	20	

Table 6. The correlation of HBP1 protein, and *MMP7* gene in 26 patients with β-catenin accumulation[a].

	***MMP7* mRNA**	Over expression	Expression	*P*-value
HBP1 protein	Low expression	8	2	**0.069**
	Expression	7	9	

[a] These results were analyzed by Pearson χ^2 test.

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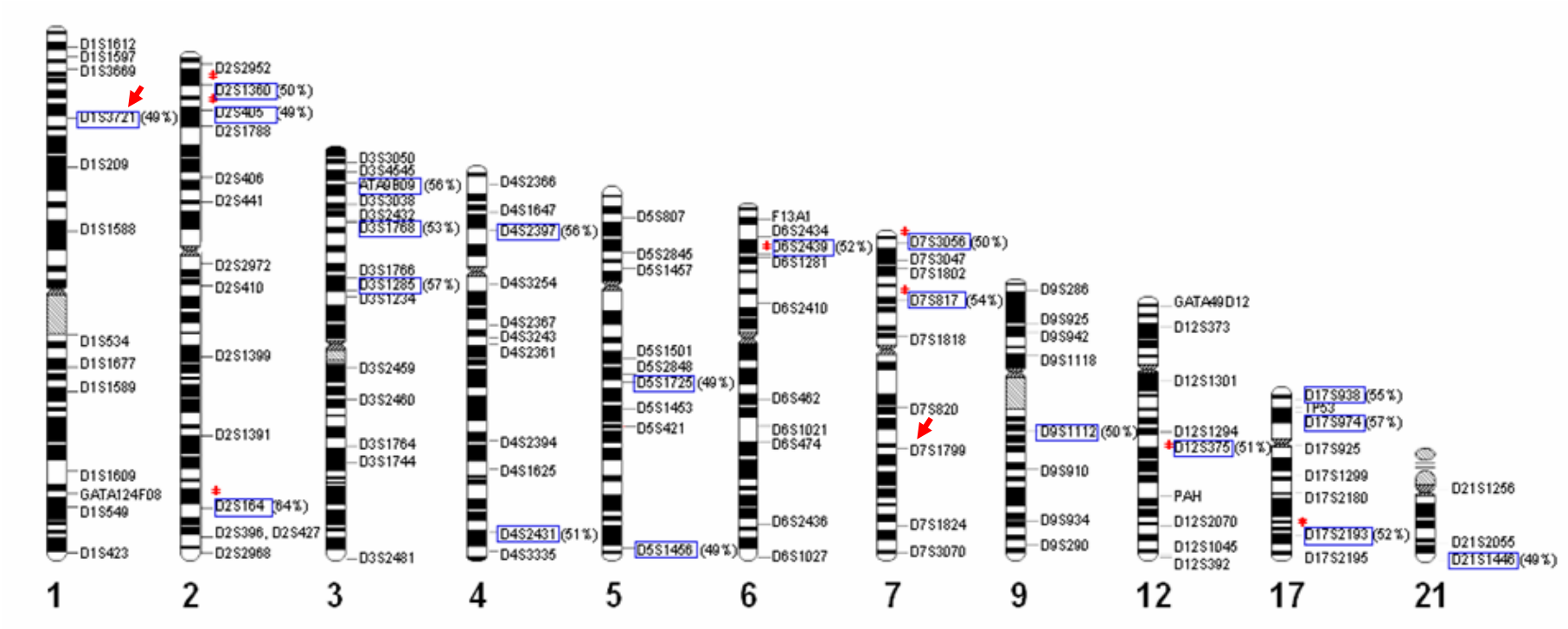

Fig. 1 Frequent chromosomal deletion regions in tumors from lung cancer patients.
Chromosomal location of 20 markers (squared) showing the high percentage of LOH (>48%) along with the markers analyzed at the respective chromosome. The frequency of LOH is noted on the side of the marker and the novel sites of frequent LOH is indicated by a symbol *. The genes studied including ICAT and HBP1 are located at 1p36.2 and 7q31 regions, respectively and are indicated by arrows. Figure was taken from ref. (37).

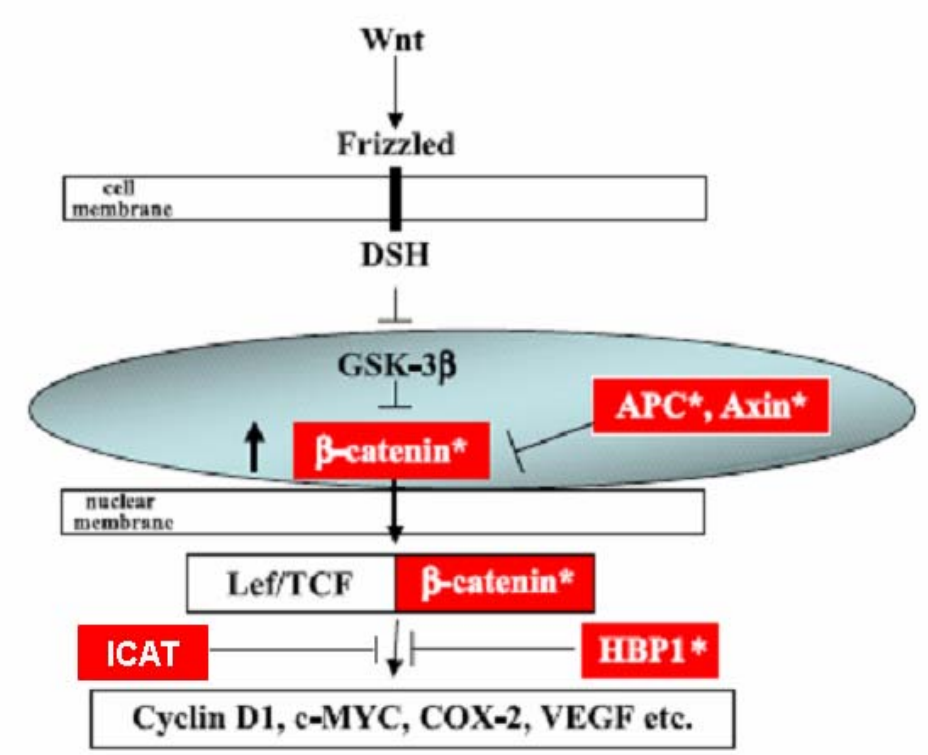

Fig. 2 Schematic of Wnt/β-catenin signaling pathway.

This is a schematic diagram of the Wnt/β-catenin signaling pathway, which is depicted with the relevant cancer-associated mutation (*). The suppression of Wnt/β-catenin signaling by HBP1 and ICAT is shown. Figure and description was modified from ref. (45)

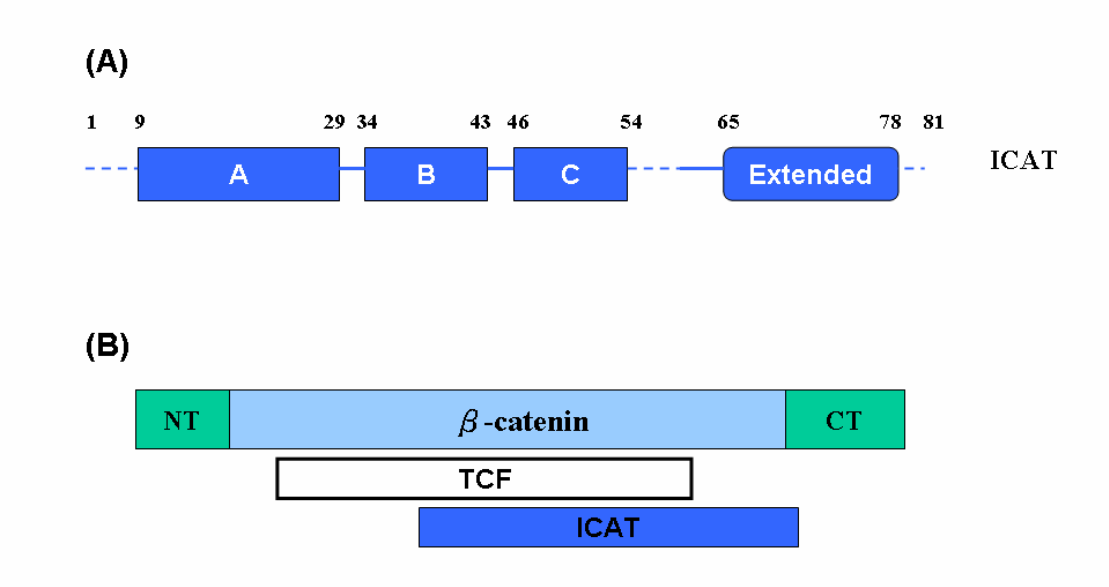

Fig. 3 Schematic representation of functional domain of ICAT and its interaction domains with TCF and β-catenin.

(A) The residues corresponding to the three helices of ICAT, termed A, B, and C, and the extended region are shown. The number above each domain are the amino acid sequences. (B) Schematic diagram of β-catenin and the binding regions for TCF and ICAT. The N- and C-terminal domains of β-catenin are indicated as NT and CT, respectively. Figures and description were modified from ref. (17)

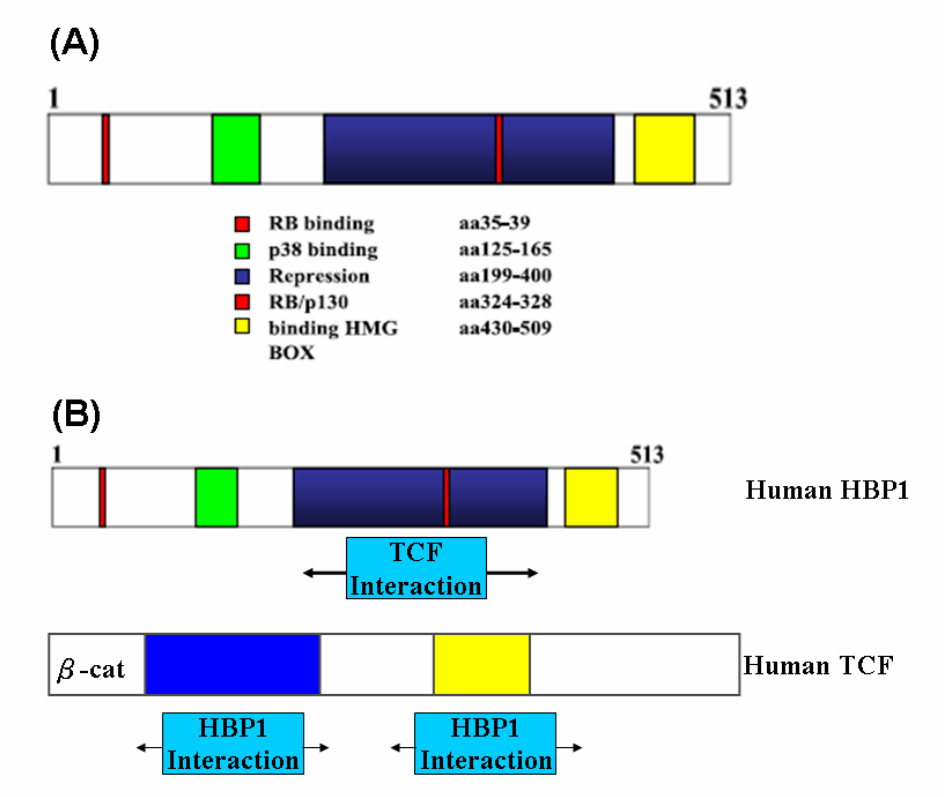

Fig. 4 Schematic representation of functional domain of HBP1 and its interaction domains with TCF.

(A) The N terminus contains a RB binding site (amino acids 35-39). Amino acids 125-165 are indicated as p38 binding site. Repression domain spans amino acids 199-400 which can confer repression to a heterologous DNA binding domain. Amino acids 324-328 are indicated as RB/p130 binding site. The DNA binding domain of HBP1 is a sequence-specific HMG motif (amino acids 430-509). (B) Summary of interactions on both TCF and HBP1. Figures and description were modified from ref. (35, 45)

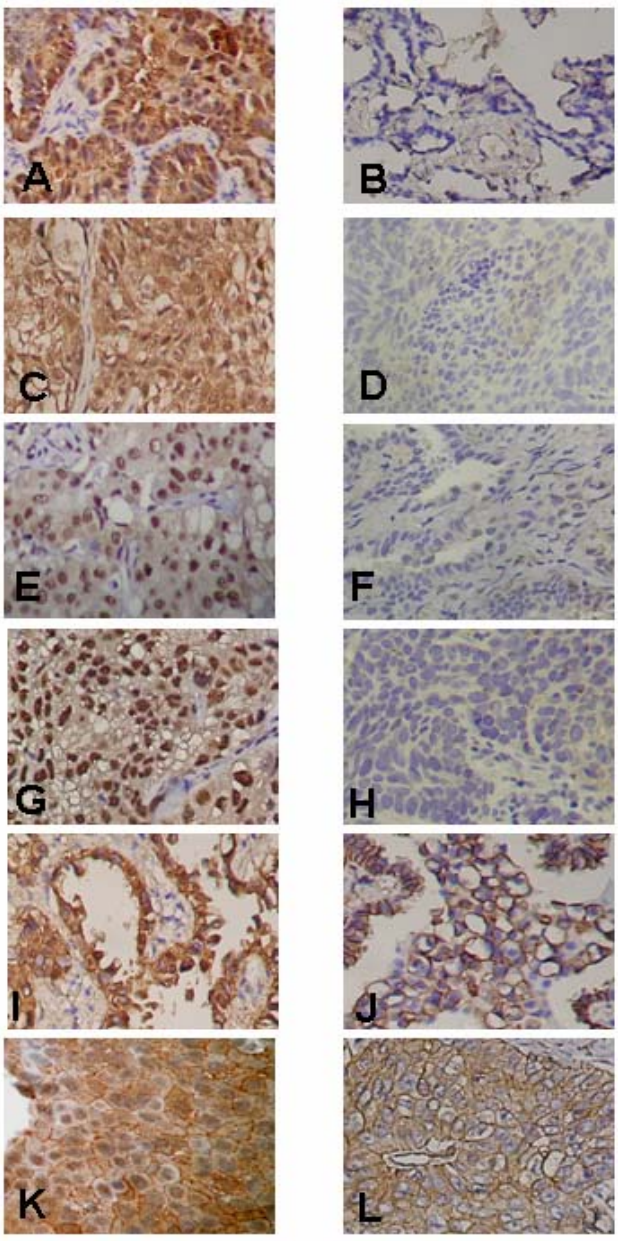

Fig. 5 Representative figures of immunohistochemistry analysis of ICAT (A-D), HBP1 (E-H) and β-catenin (I-L) in paraffin sections of lung tumors.

ICAT nuclear immunoreactivity and negative immunoreactivity were found in AD (A, B) and SQ patients (C, D). HBP1 nuclear immunoreactivity and negative immunoreactivity were found in AD (E, F) and SQ patients (G, H). β-catenin nuclear immunoreactivity and negative immunoreactivity were found in AD (I, J) and SQ patients (K, L). (Original magnification: 200X)

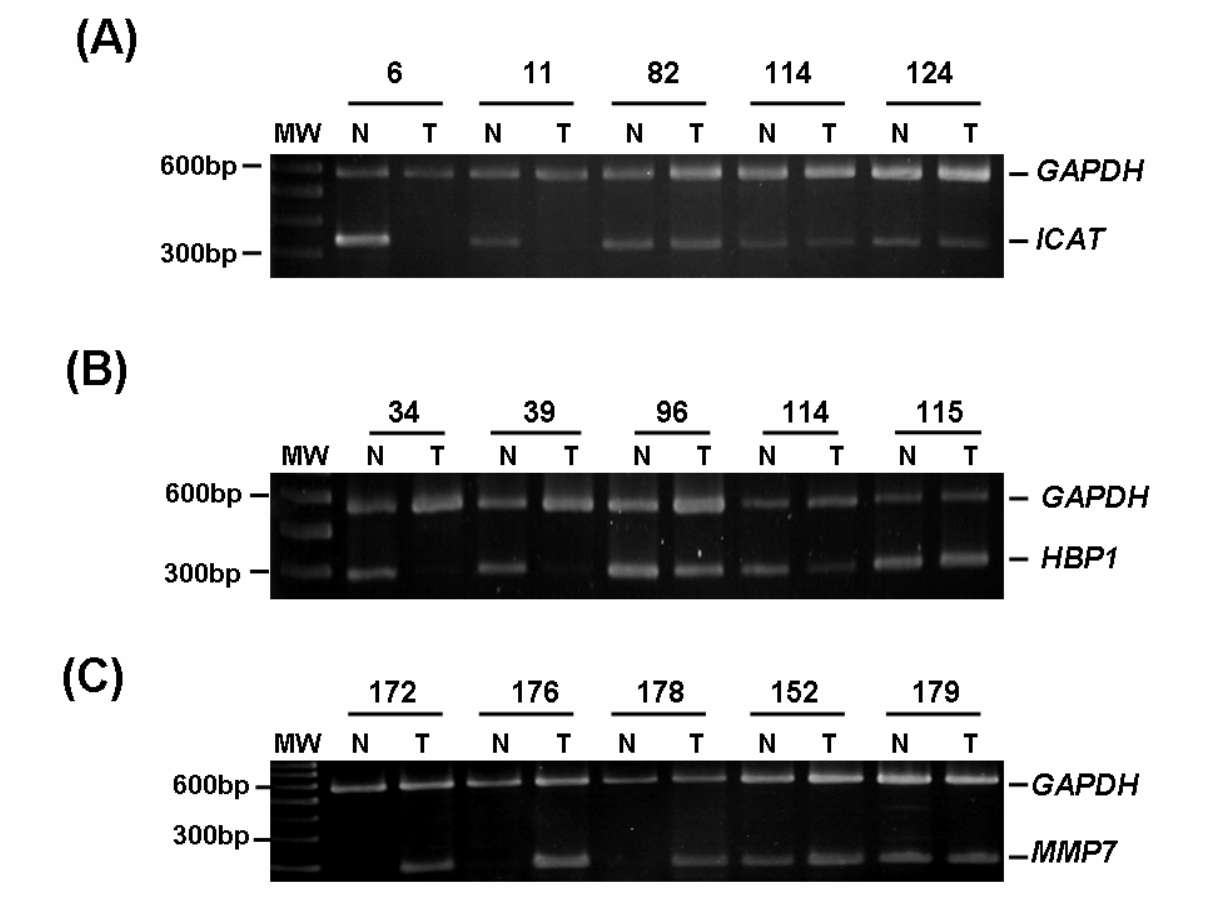

Fig. 6 Representative figure of *ICAT* (A), *HBP1* (B), and *MMP7* (C) mRNA expression analysis in lung cancer.

N: the normal lung tissue; T: the tumor lung tissue. Patients 82, 114 and 124 were positive for *ICAT* mRNA expression. Patients 6 and 11 had low *ICAT* mRNA expression. Patients 96, 114 and 115 were positive for *HBP1* mRNA expression. Patients 34 and 39 had low *HBP1* mRNA expression. Patients 172, 176 and 178 had over expression of *MMP7* mRNA. *GAPDH* was used as internal control for the analysis.

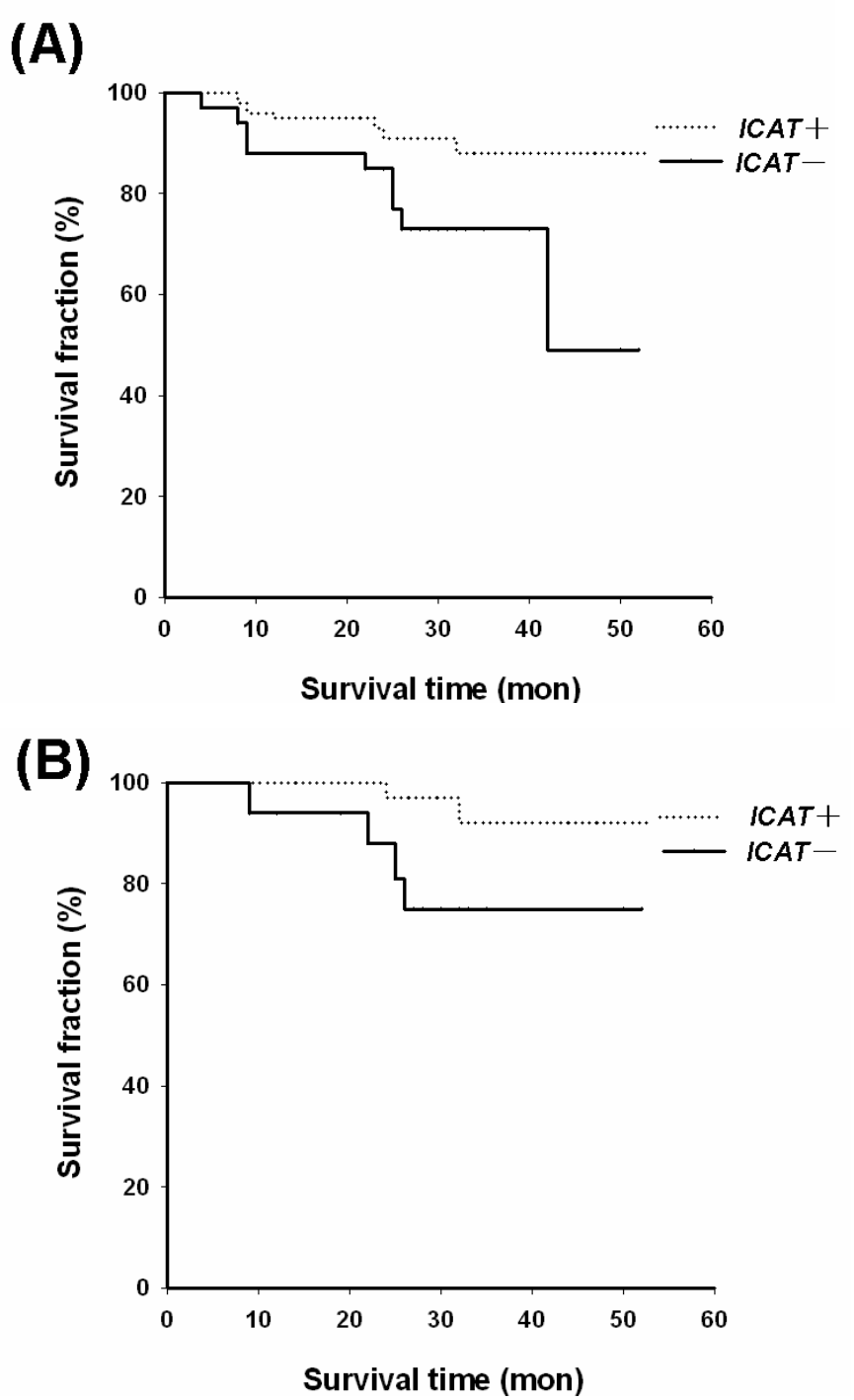

Fig.7 The Kaplan Meier survival curves with respect to *ICAT* mRNA expression.

"*ICAT* +" and "*ICAT* –" indicates positive and negative *ICAT* mRNA expression, respectively. (A) The *ICAT*-negative group had poor prognoses than the *ICAT*-positive group among 95 patients (P=0.025). (B) The *ICAT*-negative group had poor prognoses than the *ICAT*-positive group among 54 AD patients (P=0.048).

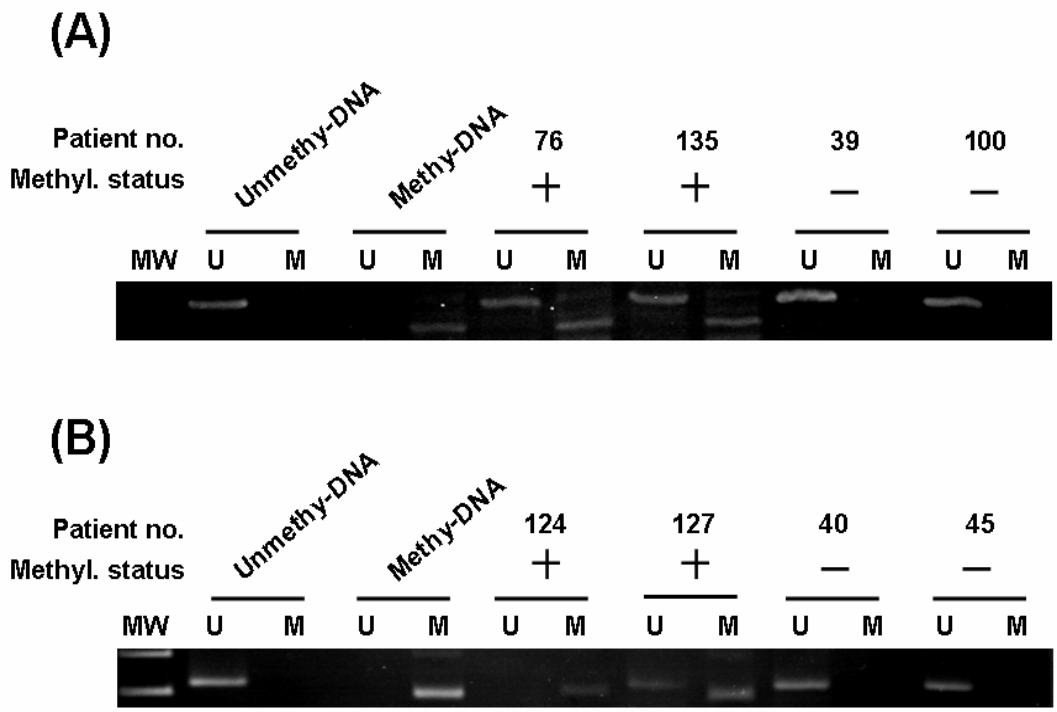

Fig. 8 Representative figure of MSP assay of the *ICAT* (A) and *HBP1* (B) methylation status in lung cancer patients.

M: methylated DNA; U: unmethylated DNA. Patients 76 and 135 showed high methylation at promoter region of *ICAT.* Patients 39 and 100 had low methylation at promoter region of *ICAT*. Patients 124 and 127 showed high methylation at promoter region of *HBP1.* Patients 40 and 45 had low methylation at promoter region of *HBP1*.

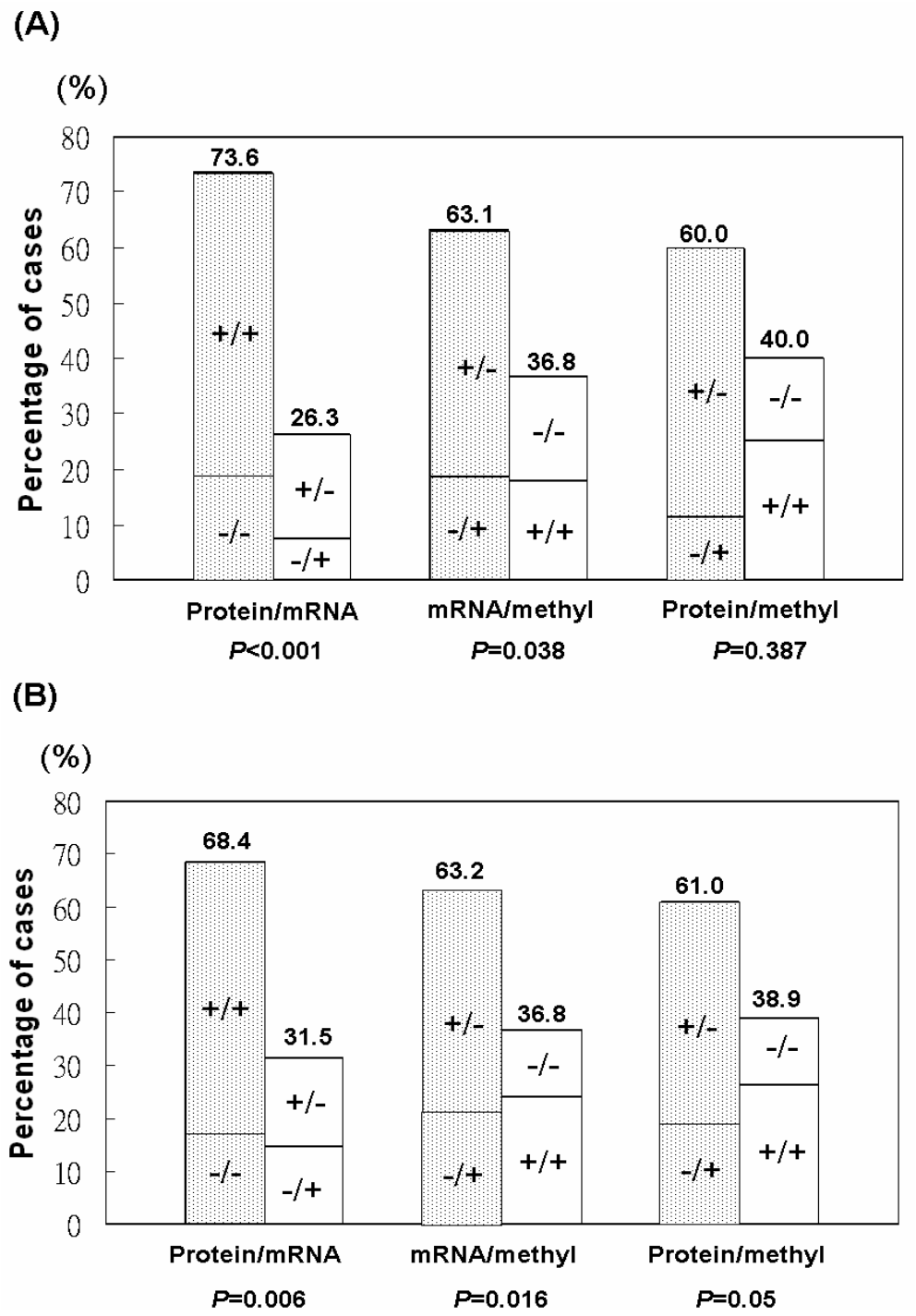

Fig. 9 Concordance analysis with protein expression, mRNA expression, promoter methylation of the *ICAT* (A) and *HBP1* (B) genes.

The percentage of cases is indicated in the X-axis, whereas the type of comparison is plotted as the Y-axis. "+" indicates positive protein expression, positive mRNA expression, and hypermethylation of promoter, as opposed to "–". The percentage of concordant group and non-concordant group is indicated above. *P* values for association between protein expression, mRNA expression, and promoter methylation are also showed at the bottom.

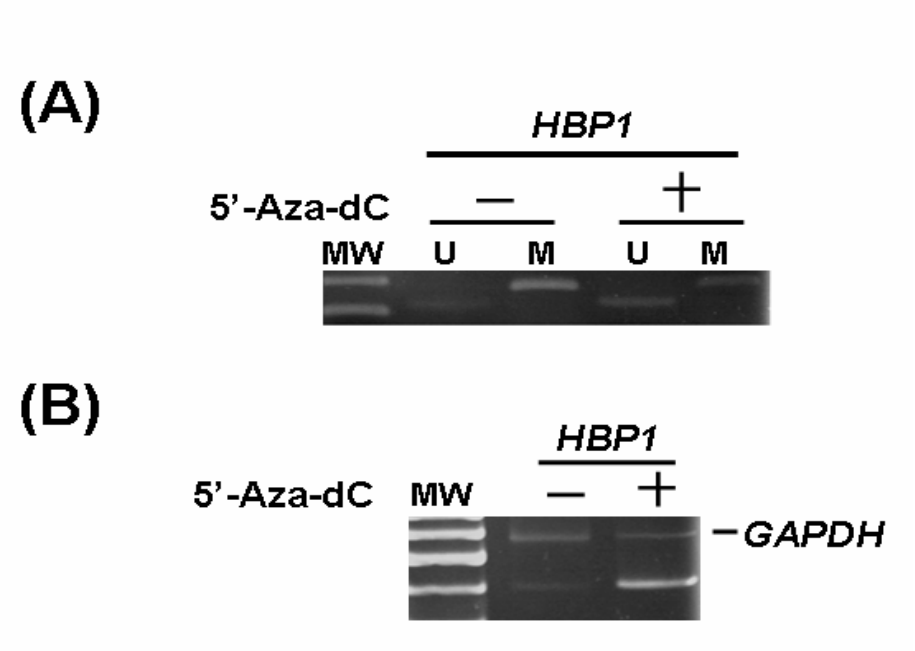

Fig. 10 *HBP1* promoter methylation status (A), mRNA expression level (B), in the CL1-5 Cell line treated with 5'-Aza-2'-deoxycytidin (5'-Aza-2'-dC) and untreated control.

(A) *HBP1* promoter is originally hypermethylated in the CL1-5 cell line, and is de-methylation after 5'-Aza-2'-dC treatment. (B) Low levels of *HBP1* mRNA were seen in the CL1-5 cell line before treatment. Expression was restored in the CL1-5 cell line after treatment with 5'-Aza-2'-dC.