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Significant Association of *Caveolin-1* Genotypes with Bladder Cancer Susceptibility in Taiwan

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Abstract

Many articles have reported the *caveolin-1* gene to be down-regulated thus suggesting that it might be a candidate tumor suppressor gene in many tumors. However, its involvement in bladder cancer is not clear and may be depending on pathological grade. In this case-control study, the association of *Cav-1* polymorphisms with bladder cancer risk in a central Taiwanese population was investigated. Three hundred and seventy-five patients with bladder cancer and the same number of age- and gender-matched healthy controls were genotyped. There were significant differences between bladder cancer and control groups in the distributions of their genotypes ($P = 1.0 \times 10^{-12}$ and 0.299) and allelic frequencies ($P = 1.4 \times 10^{-14}$ and 6.2×10^{-3}) in the *Cav-1* G14713A (rs3807987) and T29107A (rs7804372) polymorphisms, respectively. As for haplotype analysis, subjects who had GG/AT or GG/AA at *Cav-1* G14713A/T29107A showed a decreased risk of bladder cancer compared to subjects with GG/TT, while those of any other combinations were of increased risk. There were joint effects of *Cav-1* G14713A and T29107A genotypes with smoking status on individual bladder cancer susceptibility. This is the first report providing evidence that *Cav-1* was involved in bladder cancer in that the A allele of the *Cav-1* G14713A is risky, the A allele of the *Cav-1* T29107A is protective, and AA/TT on these two polymorphisms may be the most risky haplotype for the development of bladder cancer and may be novel useful genomic markers for early detection of bladder cancer.

Key Words: caveolin-1, polymorphism, bladder cancer, carcinogenesis

Introduction

Bladder cancer is the most serious urinary neoplasm worldwide and a public threat (23). In the western world, bladder cancer has become the fourth most common cancer among men, account for 7% of total malignancies (12). In Taiwan, the incidence and mortality of bladder cancer rank seventh among the common carcinomas (28). Bladder carcinogenesis is a complex, multistep and multifactorial process resulting from interactions between environmental and genetic factors. The environmental risk factors for bladder cancer include cigarette smoking, exposure to carcinogenic aromatic amines and the use of harmful drugs such as phenacetine, chlornaphrazine and cyclophosphamide (8, 27).

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In the recent years, investigators have become interested in caveolae to define how these lipid domains participate in the pathogenesis of human cancers and possible utility for the detection and treatment (4). Caveolae are vesicular invaginations of the plasma membrane which has been thought to play a critical role in transcytosis, communication between cell surface membrane receptors and intracellular signaling protein cascades such as apoptosis and tumorigenesis (18, 26). Caveolins are the major structural proteins of caveolae and this family contains three members in mammals, caveolin-1 (cav-1), caveolin-2 and caveolin-3 (13, 18), in which caveolin-1 is the principal structural protein. It has been demonstrated that cav-1 is down-regulated in sarcoma, lung carcinoma and ovarian carcinoma (3, 29, 30). However, elevated expression of cav-1 has been associated with the metastasis of esophageal squamous cell carcinoma and prostate cancer and negatively correlated with patient survival (19, 32, 33). These findings indicate that the role of cav-1 may vary considerably, depending on the tissue involved.

Most of the studies investigating the genetic role of cav-1 in carcinogenesis provided the evidence of sporadic mutations in breast (7, 16, 21), cervical (5) and oral (15) cancers, and few proposed the polymorphic hot sites. Most of the polymorphic studies of *Cav-1* are not closely related to cancer, such as Heshmati's group has reported that a novel polymorphic purine complex stretching approximately 150 bp of genomic DNA in the 1.5 kb upstream region of the human *Cav-1* gene the alleles and genotypes of which are associated with sporadic late-onset Alzheimer's disease (17). In 2010, the polymorphic site rs4730751 of *Cav-1* was reported to be associated with allograft failure of kidney (22). In a colorectal cancer study, rs3840634, rs3807990 and rs6867 of *Cav-1* together with rs1799983 and rs2070744 of eNOS were considered to be associated with colorectal cancer susceptibility (9). In prostate cancer, rs1543293, rs3815412, rs1022436 or rs3757732 alone was not associated with, but the haplotypic *Cav-1* genotype was associated with prostate cancer susceptibility (14). The emerging evidence pointing to the role of *Cav-1* in carcinogenesis led us to study whether different alleles of this gene were associated with bladder cancer.

Since sequencing of exonic and promoter regions has not revealed any variants in *Cav-1* that might have been directly involved in disease risk, we selected intronic single-nucleotide polymorphiosms (SNPs) from the NCBI database in order to evaluate the association with bladder cancer risk. The aims of the current study were to determine the genotypic frequency of six polymorphisms of the *Cav-1* gene at C239A (rs1997623), G14713A (rs3807987), G21985A (12672038), T28608A (rs3757733), T29107A (rs7804372) and G32124A (rs3807992), and their association with bladder cancer susceptibility. To the best of our knowledge, this is the first study carried out to evaluate the contribution of *Cav-1* polymorphisms in bladder oncology.

Materials and Methods

Study Population and Sample Collection

The study population consisted of 375 bladder cancer patients and 375 cancer-free control volunteers. The patients, diagnosed with bladder cancer, were recruited at the outpatient clinics of general surgery between 2004 and 2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients, including their histological details, were all graded and defined by expert surgeons (Dr. Chang's team). All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-cancer healthy volunteers, as controls, were selected by matching for age, gender and some habits after initial random sampling, from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin and any familial or genetic diseases, and those whose genotypes could not be identified in our system. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all the participants.

Genotyping Conditions

Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous methods (1, 2, 6). Briefly, the following primers were used: for *Cav-1* C239A (rs1997623), 5'-GTGTCCGCTTCTGCTATCTG-3' and 5'-GCCAAGATGCAGAAGGAG TT-3'; for Cav-1 G14713A (rs3807987), 5'-CCTTCCAGTA AGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCA TAGT-3'; for *Cav-1* G21985A (12672038), 5'-GGT GTCAGCAAGGCTATGCT-3' and 5'-CCAGACA CTCAGAATGTGAC-3'; for *Cav-1* T28608A (rs3757733), 5'-GCTCAACCTCATCTGAGGCA-3' and 5'-GGCCTATTGTTGAGTGGATG-3'; for *Cav-1* T29107A (rs7804372), 5'-GCCTGAATTGCA ATCCTGTG-3' and 5'-ACGGTGTGAACACGGA CATT-3'; and for *Cav-1* G32124A (rs3807992), 5'-GGTGTCTTGCAGTTGAATG-3' and 5'-ACGGAG CTACTCAGTGCCAA-3'. The following cycling

Table 1. Frequency distributions of characteristics of bladder cancer patients and controls

Characteristics	Controls (n = 375)			Patients (n = 375)			P^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			62.3 (9.7)			61.4 (10.3)	0.73
Age group (years)							0.71
≦55	152	40.5		158	42.1		
>55	223	59.5		217	57.9		
Gender							0.55
Male	287	76.5		279	74.4		
Female	88	23.5		96	25.6		
Habits							
Cigarette smokers	186	49.6		201	53.6		0.31
Alcohol drinkers	176	46.9		189	50.4		0.38

[a]*P* value based on Chi-square test.

conditions were performed: one cycle at 94°C for 5 min; 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were studied after digestion with Avr II, Bfa I, Hae III, Tsp509 I, Sau3AI and Nla III restriction enzymes for *Cav-1* C239A (cut from 485-bp C type into 170+315-bp T type), *Cav-1* G14713A (cut from 268-bp A type into 66+202-bp G type), *Cav-1* G21985A (cut from 251+43-bp A type into 153+98+43-bp G type), *Cav-1* T28608A (cut from 298-bp T type into 100+198-bp A type), *Cav-1* T29107A (cut from 336 bp A type into 172+164 bp T type), and *Cav-1* G32124A (cut from 213+142+67-bp A type into 142+118+95+67-bp T type), respectively.

Statistical Analyses

Only those matches with all the SNP data (case/control = 375/375) were selected for the final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *Cav-1* SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *Cav-1* genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. The data were recognized as significant when the statistical *P*-value was less than 0.05. To evaluate effect modification by smoking, stratified analyses were conducted for chosen SNPs to compare the association across exposure categories of smoking status (never-smokers and smokers). All statistical tests were performed using SAS, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on two-sided probabilities.

Results

The frequency distributions of selected characteristics of the bladder cancer patients and the controls are shown in Table 1. These characteristics of patients and controls are all well matched since none of the comparisons between groups were statistically significant ($P > 0.05$) (Table 1). In Table 2, the frequencies of the genotypes for the *Cav-1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between the controls and the bladder cancer patients are presented. Genotype distribution of various genetic polymorphisms of *Cav-1* G14713A and T29107A was significantly different between bladder cancer and control groups ($P = 1.0 \times 10^{-12}$ and 0.0299, respectively), while those for *Cav-1* C239A, G21985A, T28608A and G32124A were not significant ($P > 0.05$) (Table 2). To sum up, the polymorphisms of *Cav-1* G14713A and T29107A are shown to be associated with bladder cancer risk and may be an early detection biomarker for bladder cancer. The representative PCR-based restriction analyses for the *Cav-1* G14713A and T29107A polymorphisms are shown in Fig. 1.

The frequencies of the alleles for the *Cav-1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between controls and bladder cancer patients are shown in Table 3. The two SNPs of *Cav-1* found to be associated with bladder cancer in Table 2, G14713A and T29107A, are also found to be associated with higher bladder cancer susceptibility in their allele frequency analysis ($P = 1.4 \times 10^{-14}$ and 6.2×10^{-3}, respectively). As for the other four SNPs, the distributions of their allele frequencies are not significantly different in the controls and the bladder

Table 2. Distribution of *Cav-1* genotypes in bladder cancer patients and controls

Genotype	Controls	%	Patients	%	P^a
C239A rs1997623					0.4195
CC	366	97.6	370	98.7	
AC	9	2.4	5	1.3	
AA	0	0.0	0	0.0	
G14713A rs3807987					1.0×10^{-12}
GG	245	65.3	144	38.4	
AG	96	25.6	160	42.7	
AA	34	9.1	71	18.9	
G21985A rs12672038					0.9254
GG	222	59.2	226	60.3	
AG	126	33.6	121	32.3	
AA	27	7.2	28	7.4	
T28608A rs3757733					0.8996
TT	222	59.2	217	57.9	
AT	122	32.5	124	33.1	
AA	31	8.3	34	9.0	
T29107A rs7804372					0.0299
TT	198	52.8	231	61.6	
AT	142	37.9	122	32.5	
AA	35	9.3	22	5.9	
G32124A rs3807992					0.8634
GG	185	49.3	178	47.5	
AG	149	39.8	153	40.8	
AA	41	10.9	44	11.7	

[a]*P* based on Chi-square test.

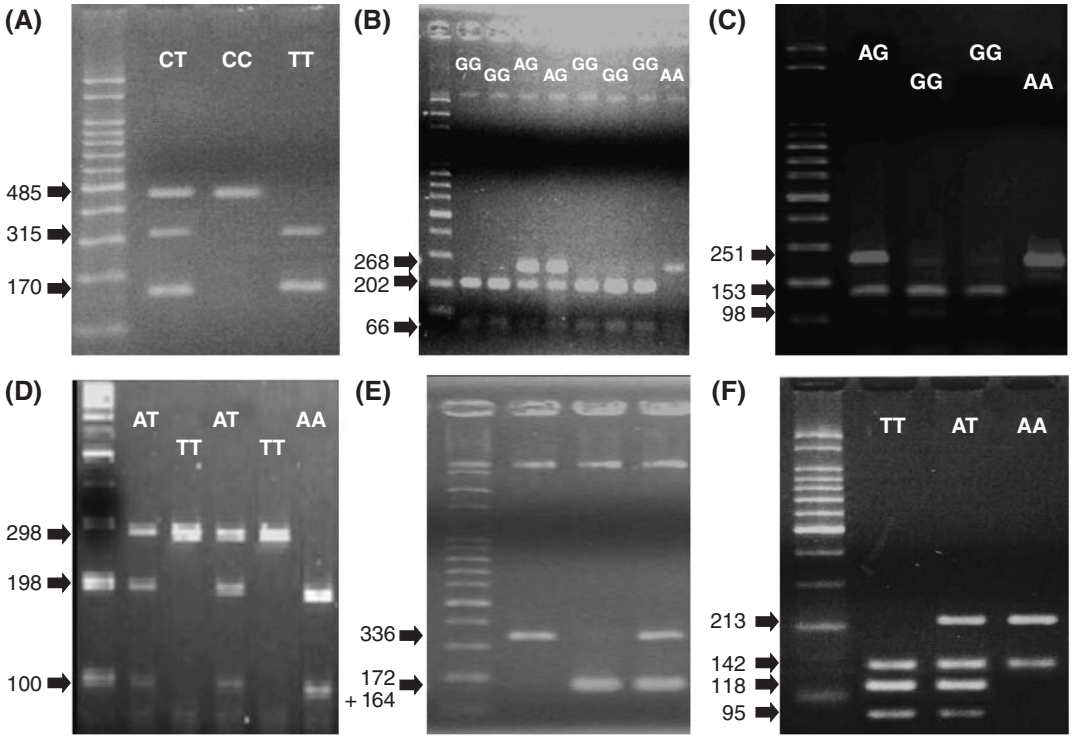

Fig. 1. PCR-based restriction analysis of the C239A rs1997623 (A), G14713A rs3807987 (B), G21985A rs12672038 (C), T28608A rs3757733 (D), T29107A rs7804372 (E) and G32124A rs3807992 (F) polymorphisms of the *Cav-1* gene displayed on 3% agarose electrophoresis. M, 100 bp DNA size marker.

Table 3. Distribution of *Cav-1* alleles in bladder cancer patients and controls

Allele	Controls	%	Patients	%	P^a
C239A rs1997623					0.4217
Allele C	741	98.8	745	99.3	
Allele A	9	1.2	5	0.7	
G14713A rs3807987					1.4×10^{-14}
Allele G	586	78.1	448	59.7	
Allele A	164	21.9	302	40.3	
G21985A rs12672038					0.8557
Allele G	570	76.0	573	76.4	
Allele A	180	24.0	177	23.6	
T28608A rs3757733					0.6336
Allele T	566	75.5	558	74.4	
Allele A	184	24.5	192	25.6	
T29107A rs7804372					6.2×10^{-3}
Allele T	538	71.7	584	77.9	
Allele A	212	28.3	166	22.1	
G32124A rs3807992					0.5782
Allele G	519	69.2	509	67.9	
Allele A	231	30.8	241	32.1	

[a]*P* based on Chi-square test.

Table 4. Distribution of *Cav-1* G14713A/T29107A haplotypes in bladder cancer patients and controls

G14713A/ T29107A haplotype	Controls	%	Patients	%	Odds Ratio (95% CI)[a]	Adjusted Odds Ratio (95% CI)[b]
GG/TT	129	34.4	89	23.7	1.00 (Reference)	1.00 (Reference)
GG/AT or GG/AA	116	30.9	55	14.7	0.69 (0.45-1.05)	0.68 (0.47-1.02)
AG/TT	51	13.6	99	26.4	2.81 (1.83-4.34)[c]	2.78 (2.04-4.22)[c]
AG/AT or AG/AA	45	12.0	61	16.3	1.96 (1.23-3.15)[c]	1.98 (1.25-2.94)[c]
AA/TT	18	4.8	43	11.5	3.46 (1.88-6.39)[c]	3.39 (1.76-6.13)[c]
AA/AT or AA/AA	16	4.3	28	7.4	2.54 (1.30-4.96)[c]	2.46 (1.29-4.73)[c]

[a]95% CI, 95% confidence interval. [b]95% CI, 95% confidence interval, and Date were calculated by unconditioned logistic regression and adjusted for age, gender, smoking, and alcohol drinking behaviors. [c]Statistically significant.

cancer patients (Table 3).

Considering potential interactions between the two significant SNPs of the *Cav-1* gene and bladder cancer susceptibility, the risk of bladder cancer related to haplotype distributions of *Cav-1* G14713A and T29107A were further analyzed (Table 4). Compared with the GG/TT haplotype of the *Cav-1* G14713A/T29107A combination, the GG/AT or GG/AA group has a lower risk of bladder cancer (OR = 0.68, 95% CI = 0.52-0.99). Other combinations of AG/TT, AG/AT or AG/AA, AA/TT, and AA/AT or AA/AA all showed increased bladder cancer risks compared to the GG/TT haplotype, conferring 2.78-fold (95% CI = 2.04-4.22), 1.98-fold (95% CI = 1.25-2.94), 3.39-fold (95% CI = 1.76-6.13) and 2.46-fold (95% CI = 1.29-4.73) increases, respectively (Table 4).

Since smoking habit is a predominant risk factor for bladder cancer, interactions between *Cav-1* genotypes and smoking habits were also analyzed by stratified individual smoking status (Table 5). We noticed that subjects with the hetero- or homozygous AA for *Cav-1* G14713A had higher risks of bladder cancer in both the smoker and non-smoker groups after adjustments for age, gender and the number of pack-year of smoking (Table 5, upper panel). In the case of *Cav-1* T29107A, the homozygous AA had lower risks of bladder cancer only in the smoker group. The heterozygous AT of *Cav-1* T29107A was of no protective effects in either non-smoker or smoker groups (Table 5, lower panel). Overall, there was an obvious interaction between smoking status and *Cav-1* genotypes in bladder cancer susceptibility.

Discussion

Although several investigations have shown that

Table 5. Distribution of the *Cav-1* G14713A and T29107A genotypes after stratification by smoking habit

	Overall			Never smokers			Ever smokers		
SNP/Genotype	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]
G14713A (rs3807987)									
GG	245 (65.3)	144 (38.4)	1.00 (Ref.[d])	116 (61.4)	67 (38.5)	1.00 (Ref.[d])	129 (69.4)	77 (38.3)	1.00 (Ref.[d])
AG	96 (25.6)	160 (42.7)	2.84 (2.05-3.93)	62 (32.8)	78 (44.8)	2.18 (1.39-3.41)	34 (18.3)	82 (40.8)	4.04 (2.48-6.59)
AA	34 (9.1)	71 (18.9)	3.55 (2.25-5.61)	11 (5.8)	29 (16.7)	4.56 (2.14-9.72)	23 (12.3)	42 (20.9)	3.06 (1.71-5.47)
T29107A (rs7804372)									
TT	198 (52.8)	231 (61.6)	1.00 (Ref.[d])	101 (54.0)	106 (60.9)	1.00 (Ref.[d])	97 (51.9)	125 (62.2)	1.00 (Ref.[d])
AT	142 (37.9)	122 (32.5)	0.74 (0.54-1.01)	71 (37.6)	56 (32.2)	0.76 (0.49-1.18)	71 (38.0)	66 (32.8)	0.72 (0.47-1.11)
AA	35 (9.3)	22 (5.9)	0.54 (0.31-0.95)	16 (8.4)	12 (6.9)	0.72 (0.33-1.60)	19 (10.1)	10 (5.0)	0.41 (0.18-0.92)

[a]Adjusted for age, gender and smoking (pack-years). [b]Adjusted for age and gender. [c]OR, odds ratio; CI, confidence interval. [d]Ref., reference.

Cav-1 plays a critical role in many tumors (3, 19, 29, 30, 32), few data are available that consider *Cav-1* for genetic predisposition to cancers (9, 15). In 2004, inactivation of *Cav-1* by mutation models or reduced expression was found to be involved in the pathogenesis of oral cancer (15). In that study, the sequences of exons 1 and 3 of *Cav-1* were investigated in 74 oral squamous cell carcinomas and 15 oral cancer cell lines, and the expression of *Cav-1* was also examined. It was reported that only five mutations (1 missense and 4 silent mutations) of *Cav-1* were identified in those cases, and mutations were all found in exon 3 (15). Since sequencing of exonic and promoter regions had not revealed any variants in *Cav-1* that might have been directly involved in cancer risk, it is reasonable for us to select intronic SNPs from the NCBI database and to evaluate the role of *Cav-1* polymorphisms, an approach that has never been reported in association with bladder cancer risk.

In ovarian, breast and colon human carcinomas, cav-1 was thought to be a suppressor of tumor growth and metastasis (11, 20, 29, 31). However, the function of cav-1 may be different in various tissues, and cav-1 could exert opposing functions resulting in promotion of tumor progression rather than inhibition. For instance, cav-1 expression is increased in tumor samples from the kidney, prostate and stomach with respect to the normal tissues, and re-expression is found in some advanced adenocarcinomas (29). Elevated expression of *Cav-1* is associated with progression in the prostate, colon, breast, lung carcinoma (29) and adult T-cell leukemia (25). Remarkably, activated expression of *Cav-1* is associated with higher grades of bladder cancer, while no statistically significant relationship was seen between *Cav-1* expression and tumor multiplicity, recurrence and tumor progression, or patient survival (24).

We postulated that induced *Cav-1* expression may alter the interactions between cell and extracellular matrix by lipid-raft internalization of adhesion molecules such as catenin and cadherin, and subsequently facilitates tumor metastasis as previously published (10). Thus, engagement of cav-1 as a tumor metastasis promoter, or as a tumor metastasis suppressor, is strongly determined by specific cellular context, and, at the molecular level, by signaling molecules interacting with cav-1 and by the signaling pathways affected and regulated by cav-1. We can only hypothesize here that the altered *Cav-1* expression may somehow lead to failure of homeostatic maintenance resulting in an increased frequency of bladder cancer.

Environmental factors such as cigarette smoking were reported to be closely related to bladder cancer carcinogenesis. In this study, the joint effects of *Cav-1* gene and smoking behaviors of individuals were analyzed, and significant genetic-environmental interactions were observed in both *Cav-1* G14713A (rs3807987) and T29107A (rs7804372) (Table 5). The sample size and similar trends of significant data after age- and behavior-adjustments strengthen the accuracy and reliability of our findings, and the frequencies of *Cav-1* polymorphisms variant alleles were similar to those reported in the NCBI website in other Asian population studies. For instance, the minor A allele frequency of *Cav-1* G14713A is 21.9% in our control group, close to those of 16.7% for a Beijing and 22.2% for a Tokyo population in the NCBI database, strongly suggesting no selection bias for the subject enrolments in terms of genotypes. Interestingly, different from that of a prostate cancer study which should be linked to the association between *Cav-1* and cancer by haplotypic analysis (14), we found that individual SNPs, *Cav-1* G14713A and T29107A, was associated with bladder cancer.

In conclusion, this is the first report to provide evidence that *Cav-1* G14713A and T29107A, but not C239A, G21985A, T28608A or G32124A, are asso-

ciated with higher susceptibility to bladder cancer. Both these SNPs have joint effects with smoking status on bladder cancer susceptibility. The G allele of *Cav-1* G14713A and A allele of *Cav-1* T29107A may be developed into potential biomarkers for early detection, prediction and integrative cancer therapy of bladder cancer.

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References

1. Bau, D.T., Tsai, M.H., Huang, C.Y., Lee, C.C., Tseng, H.C., Lo, Y.L., Tsai, Y. and Tsai, F.J. Relationship between polymorphisms of nucleotide excision repair genes and oral cancer risk in Taiwan: evidence for modification of smoking habit. *Chinese J. Physiol*. 50: 294-300, 2007.
2. Bau, D.T., Wang, H.C., Liu, C.S., Chang, C.L., Chiang, S.Y., Wang, R.F., Tsai, C.W., Lo, Y.L., Hsiung, C.A., Lin, C.C. and Huang, C.Y. Single-nucleotide polymorphism of the Exo1 gene: association with gastric cancer susceptibility and interaction with smoking in Taiwan. *Chinese J. Physiol*. 52: 411-418, 2009.
3. Belanger, M.M., Roussel, E. and Couet, J. Caveolin-1 is down-regulated in human lung carcinoma and acts as a candidate tumor suppressor gene. *Chest* 125: 106S, 2004.
4. Carver, L.A., Schnitzer, J.E., Anderson, R.G. and Mohla, S. Role of caveolae and lipid rafts in cancer: workshop summary and future needs. *Cancer Res*. 63: 6571-6574, 2003.
5. Chan, T.F., Su, T.H., Yeh, K.T., Chang, J.Y., Lin, T.H., Chen, J.C., Yuang, S.S. and Chang, J.G. Mutational, epigenetic and expressional analyses of caveolin-1 gene in cervical cancers. *Int. J. Oncol*. 23: 599-604, 2003.
6. Chang, C.H., Wang, R.F., Tsai, R.Y., Wu, H.C., Wang, C.H., Tsai, C.W., Chang, C.L., Tsou, Y.A., Liu, C.S. and Bau, D.T. Significant association of XPD codon 312 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. *Anticancer Res*. 29: 3903-3907, 2009.
7. Chen, S.T., Lin, S.Y., Yeh, K.T., Kuo, S.J., Chan, W.L., Chu, Y.P. and Chang, J.G. Mutational, epigenetic and expressional analyses of caveolin-1 gene in breast cancers. *Int. J. Mol. Med*. 14: 577-582, 2004.
8. Cohen, S.M., Shirai, T. and Steineck, G. Epidemiology and etiology of premalignant and malignant urothelial changes. *Scand. J. Urol. Nephrol.* 205: 105-115, 2000.
9. Conde, M.C., Ramirez-Lorca, R., Lopez-Jamar, J.M., Molero, E., Ramirez-Armengol, J.A., Moreno Nogueira, J.A., Pascual, M.H., Ruiz, A., Martin-Cordova, C.G., Real, L.M. and Royo, J.L. Genetic analysis of caveolin-1 and eNOS genes in colorectal cancer. *Oncol. Rep*. 16: 353-359, 2006.
10. del Pozo, M.A., Balasubramanian, N., Alderson, N.B., Kiosses, W.B., Grande-Garcia, A., Anderson, R.G. and Schwartz, M.A. Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. *Nat. Cell Biol*. 7: 901-908, 2005.
11. Engelman, J.A., Zhang, X.L., Razani, B., Pestell, R.G. and Lisanti, M.P. p42/44 MAP kinase-dependent and -independent signaling pathways regulate caveolin-1 gene expression. Activation of Ras-MAP kinase and protein kinase a signaling cascades transcriptionally down-regulates caveolin-1 promoter activity. *J. Biol. Chem*. 274: 32333-32341, 1999.
12. Franekova, M., Halasova, E., Bukovska, E., Luptak, J. and Dobrota, D. Gene polymorphisms in bladder cancer. *Urol. Oncol*. 26: 1-8, 2008.
13. Galbiati, F., Engelman, J.A., Volonte, D., Zhang, X.L., Minetti, C., Li, M., Hou, H., Jr., Kneitz, B., Edelmann, W. and Lisanti, M.P. Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and t-tubule abnormalities. *J. Biol. Chem*. 276: 21425-21433, 2001.
14. Haeusler, J., Hoegel, J., Bachmann, N., Herkommer, K., Paiss, T., Vogel, W. and Maier, C. Association of a CAV-1 haplotype to familial aggressive prostate cancer. *Prostate* 65: 171-177, 2005.
15. Han, S.E., Park, K.H., Lee, G., Huh, Y.J. and Min, B.M. Mutation and aberrant expression of Caveolin-1 in human oral squamous cell carcinomas and oral cancer cell lines. *Int. J. Oncol*. 24: 435-440, 2004.
16. Hayashi, K., Matsuda, S., Machida, K., Yamamoto, T., Fukuda, Y., Nimura, Y., Hayakawa, T. and Hamaguchi, M. Invasion activating caveolin-1 mutation in human scirrhous breast cancers. *Cancer Res*. 61: 2361-2364, 2001.
17. Heshmati, Y., Mirabzadeh, A., Feizzade, G., Gilanipour, M., Etminan, M.R., Khoram Khorshid, H.R., Kamali, K., Fakhri, M., Moghimi, N., Najmabadi, H. and Ohadi, M. A novel polymorphic purine complex at the 1.5 kb upstream region of the human caveolin-1 gene and risk of Alzheimer's disease; extra-short alleles and accumulated allele homozygosity. *Am. J. Med. Genet B Neuropsychiatr. Genet* 150B: 248-253, 2009.
18. Juhasz, M., Chen, J., Tulassay, Z., Malfertheiner, P. and Ebert, M.P. Expression of caveolin-1 in gastrointestinal and extraintestinal cancers. *J. Cancer Res. Clin. Oncol*. 129: 493-497, 2003.
19. Kato, K., Hida, Y., Miyamoto, M., Hashida, H., Shinohara, T., Itoh, T., Okushiba, S., Kondo, S. and Katoh, H. Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. *Cancer* 94: 929-933, 2002.
20. Lee, S.W., Reimer, C.L., Oh, P., Campbell, D.B. and Schnitzer, J.E. Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. *Oncogene* 16: 1391-1397, 1998.
21. Li, T., Sotgia, F., Vuolo, M.A., Li, M., Yang, W.C., Pestell, R.G., Sparano, J.A. and Lisanti, M.P. Caveolin-1 mutations in human breast cancer: functional association with estrogen receptor alpha-positive status. *Am. J. Pathol*. 168: 1998-2013, 2006.
22. Moore, J., McKnight, A.J., Simmonds, M.J., Courtney, A.E., Hanvesakul, R., Brand, O.J., Briggs, D., Ball, S., Cockwell, P., Patterson, C.C., Maxwell, A.P., Gough, S.C. and Borrows, R. Association of caveolin-1 gene polymorphism with kidney transplant fibrosis and allograft failure. *J. Am. Med. Assoc*. 303: 1282-1287, 2010.
23. Parkin, D.M. International variation. *Oncogene* 23: 6329-6340, 2004.
24. Rajjayabun, P.H., Garg, S., Durkan, G.C., Charlton, R., Robinson, M.C. and Mellon, J.K. Caveolin-1 expression is associated with high-grade bladder cancer. *Urology* 58: 811-814, 2001.
25. Sawada, S., Ishikawa, C., Tanji, H., Nakachi, S., Senba, M., Okudaira, T., Uchihara, J.N., Taira, N., Ohshiro, K., Yamada, Y., Tanaka, Y., Uezato, H., Ohshima, K., Sasai, K., Burgering, B.M., Duc Dodon, M., Fujii, M., Sunakawa, H. and Mori, N. Overexpression of caveolin-1 in adult T-cell leukemia. *Blood* 115: 2220-2230, 2010.
26. Smart, E.J., Graf, G.A., McNiven, M.A., Sessa, W.C., Engelman, J.A., Scherer, P.E., Okamoto, T. and Lisanti, M.P. Caveolins, liquid-ordered domains, and signal transduction. *Mol. Cell Biol*.

19: 7289-7304, 1999.
27. Steineck, G., Wiholm, B.E. and Gerhardsson de Verdier, M. Acetaminophen, some other drugs, some diseases and the risk of transitional cell carcinoma. A population-based case-control study. *Acta Oncol*. 34: 741-748, 1995.
28. Wang, Y.H., Lee, Y.H., Tseng, P.T., Shen, C.H. and Chiou, H.Y. Human NAD(P)H: quinone oxidoreductase 1 (NQO1) and sulfotransferase 1A1 (SULT1A1) polymorphisms and urothelial cancer risk in Taiwan. *J. Cancer Res. Clin. Oncol*. 134: 203-209, 2008.
29. Wiechen, K., Diatchenko, L., Agoulnik, A., Scharff, K.M., Schober, H., Arlt, K., Zhumabayeva, B., Siebert, P.D., Dietel, M., Schafer, R. and Sers, C. Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. *Am. J. Pathol*. 159: 1635-1643, 2001.
30. Wiechen, K., Sers, C., Agoulnik, A., Arlt, K., Dietel, M., Schlag, P.M. and Schneider, U. Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *Am. J. Pathol*. 158: 833-839, 2001.
31. Williams, T.M., Medina, F., Badano, I., Hazan, R.B., Hutchinson, J., Muller, W.J., Chopra, N.G., Scherer, P.E., Pestell, R.G. and Lisanti, M.P. Caveolin-1 gene disruption promotes mammary tumorigenesis and dramatically enhances lung metastasis *in vivo*. Role of *Cav-1* in cell invasiveness and matrix metalloproteinase (MMP-2/9) secretion. *J. Biol. Chem*. 279: 51630-51646, 2004.
32. Yang, G., Truong, L.D., Wheeler, T.M. and Thompson, T.C. Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. *Cancer Res*. 59: 5719-5723, 1999.
33. Yang, M.D., Hsu, Y.M., Kuo, Y.S., Chen, H.S., Chang, C.L., Wu, C.N., Chang, C.H., Liao, Y.M., Wang, H.C., Wang, M.F. and Bau, D.T. Significant association of Ku80 single nucleotide polymorphisms with colorectal cancer susceptibility in Central Taiwan. *Anticancer Res*. 29: 2239-2242, 2009.