Significant Association of *Caveolin-1* (Cav-1) Genotypes with Prostate Cancer Susceptibility in Taiwan

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Running title: Caveolin-1 genotypes in prostate cancer

Abstract

BACKGROUND. Multiple lines of evidence have implicated the CAV-1 gene in prostate cancer progression. CAV-1 is located within the prostate cancer aggressiveness locus at 7q31-33, and was identified as being over-expressed in prostate tumors. Mutation screening was performed as well as a case-control study to examine if polymorphisms in CAV-1 are associated with prostate cancer aggressiveness in a German population. METHODS. We sequenced the CAV-1 promoter region and its open reading frame in prostate cancer families with linkage to chromosome 7q31-33. Additionally, 105 unrelated familial prostate cancer probands, 190 sporadic cases, and 191 controls were genotyped at four intronic single nucleotide polymorphisms. Resulting haplotypes were tested for association using age at diagnosis, tumor grade, TNMstage, and follow up information to stratify for aggressive disease. RESULTS. No mutation was found in the CAV-1 coding region or in the promoter. One of the 11 observed haplotypes showed an increased frequency in cases with high tumor stage (P=0.03). CONCLUSIONS. This is the first report providing evidence for CAV-1 being involved in predisposition to aggressive prostate cancer. The association of a potential risk haplotype agrees well

with a role of CAV-1 in tumor progression but needs further confirmation.

Introduction

Prostate cancer (MIM 176807) has become the most frequently diagnosed malignancy among men and one of the most common causes of cancer death in men in recent years. The etiology of prostate cancer is largely unknown with both genetic factors and environmental factors likely to be involved [Brawley OW, Giovannucci E, Kramer BS. Epidemiology of prostate cancer. In: Vogelzang SP, Shipley WU, Coffey DS, editors. Comprehensive textbook of genitourinary oncology. Philadelphia: Lippincott Williams and Wilkins; 2000. pp 533–544]. Nevertheless, confirmed risk factors for prostate cancer include age, ethnicity, country of origin, and family history.

In tumors, several genetic alterations have been associated with allelotyping, chromosome deletion, and loss of heterozygosity (LOH) studies of specific chromosomal regions were performed to identify genomic sites harboring tumor suppressor genes (TSGs) [Latil A, Cussenot O, Fournier G, Baron JC, Lidereau R. Loss of heterozygosity at 7q31 is a frequent and early event in prostate cancer. Clin Cancer Res 1995;1:1385–1389; Alers JC, Krijtenburg PJ, Rosenberg C, Hop WC, Verkerk AM, Schroder FH, van der Kwast TH, Bosman FT, van Dekken H. Interphase cytogenetics of prostatic tumor progression: Specific chromosomal abnormalities are involved in metastasis to the

bone. Lab Invest 1997;77:437–448; Nupponen NN, Kakkola L, Koivisto P, Visakorpi T. Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998;153:141–148; Cui J, Deubler DA, Rohr LR, Zhu XL, Maxwell TM, Changus JE, Brothman AR. Chromosome 7 abnormalities in prostate cancer detected by dual-color fluorescence in situ hybridization. Cancer Genet Cytogenet 1998;107:51–60; Alers JC, Rochat J, Krijtenburg PJ,HopWC,Kranse R, Rosenberg C, Tanke HJ, Schroder FH, van Dekken H. Identification of genetic markers for prostatic cancer progression. Lab Invest 2000;80:931–942; Tobias ES, Hurlstone AF, MacKenzie E, McFarlane R, Black DM. The TES gene at 7q31.1 is methylated in tumors and encodes a novel growth suppressing LIM domain protein. Oncogene 2001; 20:2844–2853; Zenklusen JC, Conti CJ, Green ED. Mutational and functional analyses reveal that ST7 is a highly conserved tumor suppressor gene on human chromosome 7q31. Nat Genet 2001;27:392–398]. In the region of 7q31, several potential TSGs have been suggested [Chene L, Giroud C, Desgrandchamps F, Boccon-Gibod L, Cussenot O, Berthon P, Latil A. Extensive analysis of the 7q31 region in human prostate tumors supports TES as the best candidate tumor suppressor gene. Int J Cancer 2004;111:798–804]. One of them may play a critical role for the clinical aggressiveness and progression of prostate tumors [Takahashi S, Shan AL, Ritland SR, Delacey KA, Bostwick DG, Lieber MM, Thibodeau SN,

Jenkins RB. Frequent loss of heterozygosity at 7q31.1 in primary prostate cancer is associated with tumor aggressiveness and progression. Cancer Res 1995;55(18): 4114–4119].

Within the critical region, CAV-1 is located and has been implicated in prostate cancer progression. CAV-1 consists of three exons and is located at 7q31.1 telomeric of the microsatellite marker D7S522. Down-regulation or loss of CAV-1 expression has been reported in many human cancers and cancer cell lines [Hatanaka M, Maeda T, Ikemoto T, Mori H, Seya T, Shimizu A. Expression of caveolin-1 in human T cell leukemia cell lines. Biochem Biophys Res Commun 1998;253(2):382–387; Lee SW, Reimer CL,OhP, Campbell DB, Schnitzer JE. Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. Oncogene 1998;16(11):1391–1397; Bender FC, Reymond MA, Bron C, Quest AF. Caveolin-1 levels are down-regulated in human colon tumors, and ectopic expression of caveolin-1 in colon carcinoma cell lines reduces cell tumorigenity. Cancer Res 2000;60(20):5870–5878; Bagnoli M, Tomassetti A, Figini M, Flati S, Dolo V, Canevari S, Miotti S. Downmodulation of caveolin-1 expression in human ovarian carcinoma is directly related to alpha-folate receptor overexpression. Oncogene 2000;19(41):4754–4763; Davidson B, Nesland JM, Goldberg I, Kopolovic J, Gotlieb WH, Bryne M, Ben-Baruch G, Berner A, Reich

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Caveolin-1 is the major structural and functional protein component of caveolae and the marker protein for this organelle [Glenney JR Jr, Soppet D. Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in Rous sarcoma virus-transformed fibroblasts. Proc Natl Acad Sci USA 1992;89(21):10517–10521]. It plays an important role in many signaling pathways, molecular transport, and cellular proliferation and differentiation. The specific functions of the caveolin-1 protein/caveolae are highly cell- and context-dependent [Li WP, Liu P, Pilcher BK, Anderson RG. Cell-specific targeting of caveolin-1 to caveolae, secretory vesicles, cytoplasm or mitochondria. J Cell Sci 2001;114:1397–1408]. Biochemical and molecular analyses of prostate cancer tissues and cell lines identified CAV-1 as being overexpressed in metastatic prostate

cancer [Yang G, Truong LD, Timme TL, Ren C, Wheeler TM, Park SH, Nasu Y, Bangma CH, Kattan MW, Scardino PT, Thompson TC. Elevated expression of caveolin is associated with prostate and breast cancer. Clin Cancer Res 1998;4:1873–1880; Nasu Y, Timme TL, Yang G, Bangma CH, Li L, Ren C, Park SH, DeLeon M, Wang J, Thompson TC. Suppression of caveolin expression induces androgen sensitivity in metastatic androgeninsensitive mouse prostate cancer cells. Nat Med 1998;4:1062–1064]. It has previously been shown that CAV-1 expression is increased in metastatic human prostate cancer and that CAV-1 cellular protein expression is predictive of recurrence of the disease after radical prostatectomy [Yang G, Truong LD, Wheeler TM, Thompson TC. Caveolin-1 expression in clinically confined human prostate cancer:Anovel prognostic marker. Cancer Res 1999;59:5719–5723].

The emerging evidence pointing to the role of cav-1 in carcinogenesis led us to study whether different alleles of this gene are associated with prostate cancer. Thus, 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 prostate cancer susceptibility. To the best of our

knowledge, this is the most valuable study carried out to evaluate the contribution of Cav-1 polymorphisms in prostate oncology in Taiwan.

Materials and Methods

Study population and sample collection. The study population consisted of 375 prostate cancer patients and 375 all cancer-free control volunteers. The patients, diagnosed with prostate cancer, were recruited at the outpatient clinics of general surgery between 2004 and 2009 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. Wu and 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 (我們的論文可放在這邊). To be Brief, the following primers were used: for *Cav-1* C239A (rs1997623), 5'-GTGTCCGCTTCTGC TATCTG-3' and 5'-GCCAAGATGCAGAAGGAG TT-3';

for *Cav-1* G14713A (rs3807987), 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCATAGT-3';

for *Cav-1* G21985A (12672038), 5'-GGTGTCAGCAAGGCTATGCT-3' and 5'-CCAGACACTCAGAATGTGAC-3';

for *Cav-1* T28608A (rs3757733), 5'-GCTCAACCTCATCTGAGGCA-3' and 5'-GGCCTATTGTTGAGTGGATG-3';

for *Cav-1* T29107A (rs7804372), 5'-GCCTGAATTGCAATCCTGTG-3' and 5'-ACGGTGTGAACACGGACATT-3';

and for *Cav-1* G32124A (rs3807992), 5'-GGTGTCTTGCAGTTGAATG-3' and

5’-ACGGAGCTACTCAGTGCCAA-3’. The following cycling conditions were performed: one cycle at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 30 s, 55^{o}C for 30 s, and 72^{o}C for 30 s; and a final extension at 72^{o}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 SNPs 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* single nucleotide polymorphisms (SNP) 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 was 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

Table I. Frequency distributions of characteristics among prostate cancer patients and controls.

Characteristics	Controls (n = 540)			Patients (n = 135)			*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%		

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

Table II. Distribution of *Cav-1* genotypes among 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.

Table III. Distribution of *Cav-1* alleles among 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	23.6%	
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 IV Distribution of *Cav-1* G14713A/ T29107A haplotypes among 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.