Abstract. Aim: To evaluate the relationship between the Caveolin-1 (CAV1) T29107A (rs7804372) polymorphism and the risk of prostate cancer among Japanese populations, and the associations between CAV1 polymorphisms and clinicopathological characteristics, including Gleason grade and prostate-specific antigen (PSA) grade. Materials and Methods: We recruited 134 patients with prostate cancer and 86 healthy controls matched for age and smoking status. The CAV1 T29107A polymorphism status was determined by polymerase chain reaction and restriction fragment-length polymorphism analysis. Results: Genotype distributions (p=0.0045) and allelic frequencies (p=0.0018) differed between prostate cancer and control groups in terms of the CAV1 T29107A polymorphism (Pearson’s χ² test). Logistic regression analysis of case and control outcomes showed an odds ratio of 0.35 (95% Confidence interval=0.13-0.91, p=0.033) between the TT and AA polymorphisms, indicating a reduced risk of prostate cancer to be associated with the AA polymorphism. Subset analysis revealed no significant associations between this polymorphism and clinicopathological characteristics of prostate cancer. Conclusion: The results of this study demonstrated a relationship between the CAV1 T29107A variant and risk of prostate cancer. This polymorphism thus, merits further investigation as a potential genomic marker for the early detection of prostate cancer. Our results support the hypothesis that the CAV1 T29107A (rs7804372) polymorphism may influence susceptibility to prostate cancer; however, prostate cancer progression was not associated with this polymorphism in a Japanese population.

Prostate cancer is one of the most common types of cancer and the sixth leading cause of cancer-related death among Japanese men (1). In most cases, death from prostate cancer results from metastatic disease. Understanding the mechanisms underlying the progression of prostate cancer will facilitate the development of biomarkers and novel therapeutic strategies to control this devastating malignancy. Caveolin-1, encoded by CAV1, is a major structural component of caveolae, which are specialized plasma membrane invaginations involved in multiple cellular processes such as molecular transport, cell adhesion and signal transduction (2). Although CAV1 may suppress tumorigenesis under some conditions (3), it is associated with and contributes to malignant progression through various mechanisms (4, 5).

The role of CAV1 in cancer cells remains controversial. It is down-regulated in tumors such as human ovarian carcinoma (6) and head and neck squamous cell carcinoma (SCC) (7), suggesting a possible tumor suppressor role. Consistent with this, the human CAV1 gene maps to the suspected tumor locus at 7q31.1, which is deleted in many types of human cancers (8). Conversely, however, CAV1 overexpression is associated with more aggressive behavior, increased recurrence rate and poorer prognosis in prostatic cancer (9) and hepatocellular carcinoma (10). These apparent discrepancies mean that it is still unclear whether up- or down-regulation of CAV1 contributes to a biological advantage in tumorigenesis.

Emerging evidence of a role for CAV1 in carcinogenesis prompted us to investigate the relationship between different alleles of this gene and prostate cancer. We, therefore, aimed to determine the genotypic frequency of the CAV1 T29107A polymorphism and its association with prostate cancer susceptibility. We also analyzed the relationship between CAV1 polymorphisms and clinicopathological characteristics such as Gleason grade and Prostate specific antigen (PSA) grade. To the best of our knowledge, this is the first study to evaluate the contribution of CAV1 polymorphisms to prostate oncology in a Japanese population.

Key Words: Caveolin-1, prostate cancer, polymorphism, oncogene.
Materials and Methods

Study participants. The study population consisted of a total of 220 Japanese men, including 134 histologically-confirmed cases of prostate cancer and 86 healthy age-, ethnicity- and smoking status-matched controls. The patients with prostate cancer were treated at the Department of Urology, Miyazaki Medical University Hospital and its related hospitals between August 2011 and October 2012. Tumor grade was evaluated in these samples using the Gleason scoring system. Controls were selected randomly from healthy individuals with no history of cancer. All participants were informed of the details, procedures and objectives of this study. During the study period, critical information such as age and smoking status were collected from the participants using a standardized questionnaire. This study was approved by the Ethics Committee of Miyazaki Medical University and related hospitals.

Assessment of smoking status. Participants were asked about their smoking status and were classified as “smokers” or “non-smokers”. Information on demographics, smoking history, family history of cancer and medical history were collected during the interview. Any individual who had never smoked or had smoked only a few packs of cigarettes during his lifetime was defined as a non-smoker. Any individual who had smoked cigarettes for more than 20 years was defined as a smoker. Both cases and controls were subjected to similar protocols/questionnaires by the same interviewer.

CAV1 genotyping. Genomic DNA was extracted from peripheral blood leukocytes using a DNA Extractor WB kit (Wako Pure Chemical Industries, Ltd. Osaka, Japan.), according to the manufacturer’s instructions, and eluted with 100 μl (TE) buffer (Nacalaitesque, Tokyo, Japan.). CAV1 T29107A genotypes were determined using polymerase chain reaction (PCR)-based restriction fragment-length polymorphism assay, as described previously (11, 12). The following primers were used: CAV1 T29107A, forward, 5’-GCGCTGAAATTGGCAATCTCTGTG-3’; reverse, 5’-ACGGTGTGATTCAACGGGTAAGACGACAAGT-3’. Reactions were performed using KAPA Taq PCR Kits (Nippon Genetics, Tokyo, Japan) in a thermal cycler (TaKaRa PCR Thermal Cycler Dice; Takara, Tokyo, Japan). PCR conditions consisted of 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 overnight with Sau3AI restriction enzyme (TaKaRa) for CAV1 T29107A. The fragments were separated in a 2% agarose gel. The TT genotype (wild-type) yielded two distinct digestion products (172 and 164 bp), the TA genotype yielded three distinct digestion products (336, 172 and 164 bp), and the AA genotype yielded one digestion product (336 bp) (Figure 1).

Statistical analysis. Statistical analysis was performed using the R i386 2.15.1 software package (Wirtschaftsuniversität Wien, Vienna University of Economics and Business, Vienna, Austria). The significance of differences in CAV1 T29107A genotypes among cases and controls were determined by Pearson’s χ² tests. Probability values <0.05 were regarded as statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer were calculated by multivariate logistic regression analysis after adjusting for several confounding variables such as age and smoking status.

Results

The backgrounds of the cases and controls are summarized in Table I. The mean ages of the cases and controls were 68.3±7.4 (range=61-75) years and 66.9±8.3 (range=59-75) years, respectively. There were no significant differences between prostate cancer cases and controls in terms of mean age distribution (p=0.21, not significant (NS)) and relative frequencies of smokers and non-smokers (p=0.24, NS).

CAV1 T29107A genotypic and allelic frequencies are indicated in Table II. The genotype and allele frequencies were in Hardy-Weinberg equilibrium. The genotypic distributions (p=0.0045) and allelic frequencies (p=0.0018) differed significantly between prostate cancer and control groups in terms of the CAV1 T29107A polymorphisms. The frequency of T-allele carriers was higher in case than in control samples. Logistic regression analysis of outcomes (adjusted for age at diagnosis and smoking status) showed that the AA genotype was associated with decreased susceptibility to prostate cancer (OR=0.35, 95% CI=0.13-0.91, p=0.033). Subset analysis to investigate possible associations between CAV1 polymorphisms and clinicopathological characteristics such as the Gleason grade and PSA grade revealed no significant associations.

CAV1 genotype and risk associated with Gleason grade. CAV1 genotypes were further analyzed for risk associated with less-aggressive or highly-aggressive disease, based on Gleason grade. Patients were then categorized into three groups based on this combined score (Gleason score≤6=low-grade; Gleason score 7=intermediate-grade; Gleason score≥8=high-grade). The results demonstrated no significant associations between genotype and Gleason grade (Table III).

CAV1 genotype and risk associated with PSA grade. Patients were categorized into three groups based on PSA values (PSA <10.0 ng/ml=low-grade; 10.0 PSA ≤10–<20.0 ng/ml=intermediate-grade; PSA ≥20.0 ng/ml=high-grade). No significant associations were found between CAV1 polymorphisms and PSA grade in patients with prostate cancer (Table III).

Discussion

Several investigations have demonstrated a critical role for CAV1 in many types of tumors (13, 14), but few studies have reported on the relationship between CAV1 and the genetic predisposition to cancer. In 2004, inactivation of CAV1 by mutation 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 CAV1 were investigated in 74 oral squamous cell carcinomas and 15 oral cancer cell lines, and the CAV1 expression was also examined. Only five
CAV1 mutations (one missense and four silent mutations) were identified in those cases, all of which occurred in exon 3 (16). In contrast, sequencing of exon and promoter regions have failed to reveal any CAV1 variants that might be directly involved in cancer risk. We selected intronic single-nucleotide polymorphisms (SNPs) from the NCBI database and evaluated the role of CAV1 polymorphisms in prostate cancer risk in a Japanese population. CAV1 is thought to act as a suppressor of tumor growth and metastasis in human breast and colon cancer (17, 18). However, CAV1 function may differ among organs, and CAV1 could, thus, exert opposite functions resulting in promotion or suppression of tumor progression, respectively.

For example, CAV1 expression increased in tumor samples from the kidney, prostate and stomach, and re-expression has been found in some advanced adenocarcinomas (16). Elevated CAV1 expression is associated with progression in some adenocarcinomas, such as prostate carcinoma (19), and in adult T-cell leukemia (20). Interestingly, activated CAV1 expression was associated with higher grades of prostate cancer, although few significant relationships have been identified between CAV1 expression and tumor multiplicity, recurrence and progression, or overall survival (21).

Li et al. showed that CAV1 was secreted by mouse and human prostate cancer cell lines, and that secreted CAV1 promoted cancer cell survival and clonal growth in vitro (22, 23). They further showed that tumor cell-secreted CAV1 promoted pro-angiogenic activities in prostate cancer through the Phosphoinositol-3-kinase (PI3K) -Protein Kinase B (AKT)-endothelial nitric oxide synthase (eNOS) signaling module (24). With regard to the underlying mechanisms responsible for CAV1-mediated oncogenic activities, they showed that CAV1 maintained activated AKT in prostate cancer cells through binding to and inhibiting the serine/threonine protein phosphatases PP1 and PP2A (25). Thus, engagement of CAV1 as a tumor metastasis promoter depends on the specific cellular context and, at the molecular level, by the signaling molecules interacting with and the signaling pathways affected and regulated by CAV1. We hypothesize that altered CAV1 expression may result in failure of homeostatic maintenance, leading to an increased frequency of prostate cancer.

A recent study of eleven CAV1 SNPs only identified one (rs9920, chr7: 115987328) as being associated with prostate cancer risk among Caucasians, while rs7804372 (chr7: 116194228) was associated with prostate cancer risk in Taiwanese. The present study evaluated the association between the latter SNP and prostate cancer risk in Japanese men. We investigated the potential associations between CAV1 polymorphisms and the risk and progression of prostate cancer.

Table I. Participants’ backgrounds.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years mean±SD</td>
<td>68.3±7.4</td>
<td>66.9±8.3</td>
<td>0.21 (NS)</td>
</tr>
<tr>
<td>Smoking status n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>0.24 (NS)</td>
</tr>
<tr>
<td>Non smoker</td>
<td>64 (47.8)</td>
<td>48 (55.8)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>70 (52.2)</td>
<td>38 (44.2)</td>
<td></td>
</tr>
<tr>
<td>PSA (ng/ml) mean±SD</td>
<td>22.8±18.2</td>
<td>2.5±0.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PSA grade</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Low&lt;10</td>
<td>46 (34%)</td>
<td>28 (21%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate=10&lt;20.0</td>
<td>60 (45%)</td>
<td>60 (45%)</td>
<td></td>
</tr>
<tr>
<td>High≥20</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Gleason grade n (%)</td>
<td>42 (31%)</td>
<td>63 (47%)</td>
<td></td>
</tr>
<tr>
<td>Low≤6</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate=7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

NS: Not significant, PSA: prostate specific antigen. *Based on Students t-test.

Table II. Distribution of Caveolin-1 (CAV1) polymorphism among patients with prostate cancer and controls.

<table>
<thead>
<tr>
<th>Genotype (T29107A; rs7804372)</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>60 (44.8)</td>
<td>25 (29.1)</td>
<td>85</td>
</tr>
<tr>
<td>TA</td>
<td>63 (47.0)</td>
<td>42 (48.9)</td>
<td>105</td>
</tr>
<tr>
<td>AA</td>
<td>11 (8.2)</td>
<td>19 (22.1)</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>86</td>
<td>220</td>
</tr>
</tbody>
</table>

*Based on Pearson’s χ² test

p=0.0045*
cancer. Our results indicate that prostate cancer susceptibility and risk are influenced by genetic polymorphisms of the CAV1 gene; in particular, the AA genotype or presence of the A allele of the CAV1 T29107A gene reduced the risk of prostate cancer. However, further analysis revealed no significant associations between this polymorphism and clinicopathological characteristics associated with prostate cancer progression. This apparent discrepancy could be attributable to the small number of cases (n=134) in the study, and further studies with larger sample sizes are, thus, needed to clarify the relationship between this polymorphism and clinicopathological characteristics in prostate cancer.

No molecular basis for the initiation of CAV1 expression in prostate cancer has been established. Previous studies determined that the CAV1 gene promoter has multiple CpG sites, and alterations in gene methylation status have been shown in prostate cancer (26, 27). However, patterns of CAV1 gene methylation have, thus far, failed to provide a convincing argument for the up-regulation of CAV1 in prostate cancer. In general, CAV1 has been associated with the stimulatory effects of steroid receptors, including the androgen receptor, suggesting a possible starting point for further mechanistic studies (28). The results of the current study, however, support the hypothesis that the CAV1 T29107A polymorphism is associated with transcriptional control. A search of CAV1 motifs revealed that the polymorphic site included the binding site for pre-B-cell leukemia homeobox-1 (PBX1), which belongs to the homeobox family of transcription factors. PBX1 binds to the promoter and is known to regulate transcription. The relationship between PBX1 and splicing has not yet been established.

This is the first study to demonstrate an association between a CAV1 gene polymorphism and risk of prostate cancer in Japanese men. However, this was a pilot study, and further studies in larger cohorts are required to confirm the results.

In summary, this study provided evidence for a relationship between the CAV1 T29107A variant and risk of prostate cancer. This polymorphism therefore merits further study as a potential genomic marker for the early detection of prostate cancer. Moreover, these results suggest that the CAV1 T29107A polymorphism plays an important role in prostate cancer susceptibility in the Japanese population. To our knowledge, this is the first such study to be conducted in a Japanese population, and demonstrated that individuals carrying the A allele of T29107A appear to be at a lower risk of developing prostate cancer. Further studies considering the effects of environmental exposure to specific carcinogens must also be investigated in larger studies to further elucidate the role of CAV1 polymorphisms in prostate tumorigenesis.

Conflicts of Interest
The Authors indicate that no potential conflicts of interest exist.

References


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