Gene variation impact on prostate cancer progression: Lymphocyte modulator, activation, and cell adhesion gene variant contribution

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Abstract

Background: The view of prostate cancer (PCa) progression as a result of the interaction of epithelial cancer cells with the host’s immune system is supported by the presence of tumor infiltrating lymphocytes (TILs). TILs fate and interaction with the tumor microenvironment is mediated by accessory molecules such as CD5 and CD6, two signal-transducing coreceptors involved in fine-tuning of T cell responses. While the nature of the CD5 ligand is still controversial, CD6 binds CD166/ALCAM, a cell adhesion molecule involved in progression and dissemination of epithelial cancers, including PCa. The purpose of the present study was to determine the role of CD5, CD6, and CD166/ALCAM gene variants in PCa.

Methods: Functionally relevant CD5 (rs2241002 and rs2229177), CD6 (rs17824933, rs11230563, and rs12360861) and CD166/ALCAM (rs6437585, rs579565, rs1044243, and rs35271455) single nucleotide polymorphisms (SNPs) were genotyped in germline DNA samples from 376 PCa patients. Their association with PCa prognostic factors, namely biochemical recurrence (BCR) and International Society of Urological Pathology (ISUP) grade was analyzed by generalized linear models and survival analyses.

Result: Proportional hazards regression showed that the minor CD6 rs12360861AA and CD166/ALCAM rs579565AA genotypes were associated with earlier BCR, with hazard ratios of 2.65 (95% CI: 1.39–5.05, p = 0.003) and 1.86, (95% CI: 1.02–3.39, p = 0.043), respectively. Individually, none of the analyzed SNPs was significantly associated with ISUP grade, but haplotype analyses revealed association of the CD5 rs2241002C–rs2229177T haplotype with ISUP grade ≥2, with odds ratio of 1.52 (95% CI: 1.05–2.21, p = 0.026).
1 | INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide. Newly diagnosed prostate tumors are often localized PCa, confined to the prostate. A combination of environmental and lifestyle risk factors in addition to genetic variants influences the development and prognosis of PCa in terms of recurrence and metastasis. Treatment options include active surveillance, radical prostatectomy, radiotherapy, focal therapy, androgen deprivation and, more recently, immunotherapy in case of metastatic disease. Positive surgical margins, high International Society of Urological Pathology (ISUP) grade, and short interval to biochemical recurrence (BCR) are the main adverse clinical prognostic factors.

PCa is generally an indolent disease over long time spans that enable antitumor immune responses. However, PCa is often described as a "cold" tumor with minimal inflammatory or immunosuppressive microenvironment, where tumor infiltrating lymphocytes (TILs) may even contribute to PCa progression. Such TILs include CD4+ T cells skewed toward T regulatory (Treg) and T helper 17 (Th17) phenotypes, and CD8+ T cells with tolerogenic phenotype. Recent immunophenotypical analyses of PCa microenvironment have revealed a subset of "immune-activated" patients with favorable outcomes who may benefit from novel immune checkpoint inhibitors.

TIL function modulates T-cell activation and fate through stable adhesive contacts with cancer cells via accessory immunomodulatory molecules such as CD5 and CD6. These are highly homologous signal-transducing lymphocyte surface receptors expressed by all T cell types from early stages of their development and by the B1a cell subset, with lower expression in other immune cell types (e.g., macrophages, dendritic cells, or natural killer cells). Both CD5 and CD6 fine-tune the intracellular activation and differentiation signals delivered by the T cell receptor complex (TCR), to which they physically associate. This is supported by CD5 and CD6 signalosome's integration of both inhibitory and activator effectors of TCR-mediated signals. The relevance of CD5 and CD6 immunomodulatory properties in cancer immune surveillance is sustained by in vitro and in vivo experimental evidence as well as by clinical transcriptomic studies.

Adhesive contacts between cancer cells and microenvironment components (extracellular matrix, immune, and nonimmune cells) are important for local tumor growth and metastasis, making adhesion molecule regulation relevant in cancer biology. One such molecule is CD166/ALCAM (for activated leukocyte cell adhesion molecule), a bona fide ligand of CD6. CD166/ALCAM is a member of the immunoglobulin superfamily with a broad tissue distribution including normal epithelia, endothelia, neurons, hematopoietic and mesenchymal progenitors, bone marrow stromal cells, and an extensive list of malignancies including PCa. Their homotypic (ALCAM-ALCAM) and heterotypic (ALCAM-CD6) interactions are relevant in several physiological processes (e.g., leukocyte transmigration, T-cell activation, hematopoiesis, osteogenesis, neurite outgrowth, angiogenesis, and embryonic implantation) as well as in cancer growth and metastasis.

Genome-wide (GWAS) and candidate gene-driven association studies support involvement of several CD5 (rs2241002 and rs2229177), CD6 (rs17824933, rs11230563, and rs12360861), and CD166/ALCAM (rs6437585, rs579565, and rs1044243) gene variants as susceptibility and/or disease modifier markers in different autoimmune disorders (i.e., rheumatoid arthritis, lupus nephritis, multiple sclerosis, psoriasis, Behcet's disease, and inflammatory bowel disease). Cancer and autoimmunity are considered two opposite sides of the same coin, but there is still scarce information on the influence of CD5, CD6, and/or CD166/ALCAM gene variation in cancer, except for CD5 in melanoma and chronic lymphocytic leukemia and CD166/ALCAM in breast cancer.

Given the involvement of CD5, CD6, and CD166/ALCAM in TIL function and T-epithelial cell interplay, and the association of some of their gene variants with inflammatory diseases and cancer, we have explored their impact on PCa. Our results show association of certain CD5, CD6, and CD166/ALCAM gene variants with PCa prognosis (BCR and ISUP grade), and support a role for the host's immune response in PCa pathophysiology.

2 | MATERIALS AND METHODS

2.1 | Subjects and samples

We conducted a retrospective study with 376 PCa patients attending the Urology Department of the Hospital Clínic de Barcelona, Spain, between 2008 and 2016. All patients had localized prostate...
adenocarcinoma and were treated by means of radical prostatectomy, radiotherapy, or cryotherapy. Exclusion criteria included the presence of other active neoplasm at the moment of diagnosis. Tumor dissemination was controlled postoperatively by prostate-specific antigen (PSA) measurement. The study was approved by the local Hospital Ethics Committee, in accordance with the Declaration of Helsinki and the Regulation 536/2014 of the European Parliament and of the Council, and written informed consent was obtained from all participants before inclusion (ref. HCB2013/8753).

Clinical data, including age, ISUP grade, stage, treatment (radical prostatectomy, cryotherapy, radiotherapy), BCR, metastases, and PCa-related death, was retrospectively collected from medical records. BCR was defined as increase of PSA >0.2 ng/ml after radical prostatectomy or increase of PSA >2 ng/ml over nadir after radiotherapy or cryotherapy.

2.2 | SNP genotyping

One 10 ml EDTA tube of peripheral blood was collected before treatment. Genomic DNA was extracted from peripheral blood by salting out.38 DNA samples (20 ng) were subjected to real-time polymerase chain reaction (RT-PCR) in a LightCycler® 480 Instrument (Roche) using the TaqMan Genotyping Master Mix and the TaqMan probes for rs2241002, rs2229177, rs17824933, rs11230563, rs12360861, and rs6437585, following manufacturer's instructions (Thermo Fisher Scientific). CD166/ALCAM SNPs rs579565, rs1044243, and rs35271455, which lie in a SNP hotspot spanning 7 bp, were PCR amplified for subsequent sequence-based typing (PCR-SBT) using the Hs00666884_CE assay (Thermo Fisher Scientific).

2.3 | Statistical analyses

Statistical analysis was performed with R 3.6.0 (R Foundation for Statistical Computing), with the packages "SNPassoc", “survival”, “survminer”, and “haplo.stats” available at the Comprehensive R Archive Network (CRAN) repository. Association of individual SNPs (predictor variable) with ISUP grade (binary response variable, codified as ISUP = 1 vs. ISUP ≥ 2) was assessed by logistic regression, using the “association” function included in the “SNPassoc” package. For each analysis, four models were generated (codominant, dominant, recessive, and log-additive), and the model with the lowest Akaike information criterion (AIC) was chosen. For haplotype analyses, putative haplotypes were inferred with the expectation-maximization (EM) algorithm and association with clinical parameters was assessed with generalized linear models, both using functions implemented in the “haplo.stats” package. BCR-free survival curves were estimated by the Kaplan-Meier method with the “survival” package. Hazard ratios for each genotype and their respective confidence intervals were calculated and hazard ratio differences between genotypes were assessed with Cox proportional hazard regression, all using the “survminer” package.

3 | RESULTS

The clinicopathological characteristics of the study cohort are listed in Table 1. Median follow-up time was 62.00 months (interquartile range 31.00–77.00 months), during which 119 patients (31.6%) developed BCR. Median time to BCR was 25.00 months (interquartile range: 12.00–48.00). Five patients (1.3%) developed metastases, and six (1.6%) died due to cancer-related causes.

The Table 2 lists allele frequencies of the CD5 (rs2241002 and rs2229177), CD6 (rs17824933, rs11230563, and rs12360861), and
CD166/ALCAM (rs6437585, rs579565, rs1044243, and rs35271455) SNPs analyzed, which were all in Hardy-Weinberg equilibrium.

Cox regression analysis was used to test association between the selected CD5, CD6, and CD166/ALCAM SNPs and BCR-free survival. As shown in Figure 1, association with shorter BCR-free survival was observed for the minor CD6 rs12360861AA genotype (HR = 2.65, 95% CI: 1.39–5.05, \(p = 0.003\)) and the minor CD166/ALCAM rs579565AA genotype (HR = 1.86, 95% CI: 1.02–3.39, \(p = 0.043\)).

No association was identified between any of the individual SNPs and the ISUP grade (Supporting Information: Table S1). However, some of the studied genetic variants are reported to show stronger association of haplotype–clinical feature association than individual SNPs.\(^{27,34,35}\) We therefore performed haplotypic analyses, which revealed association between CD5 and ISUP grade (Table 3).

TABLE 3 Logistic regression analysis of CD5 haplotype association with ISUP grade

| Haplotype | rs2241002 | rs2229177 | % in cohort | ISUP = 1 (%) | ISUP \(\geq\) 2 (%) | \(p\)-Value | OR (95% CI) |
|-----------|-----------|-----------|-------------|--------------|----------------|----------|------------|
| C C       | 43.2      | 47.3      | 41.1        |              |                |          |            |
| C T       | 34.7      | 28.3      | 38.0        | 0.026        | 1.52 (1.05, 2.21) |          |            |
| T T       | 16.5      | 19.3      | 15.1        | 0.508        | 0.86 (0.55, 1.35) |          |            |
| T C       | 5.6       | 5.1       | 5.9         | 0.508        | 1.34 (0.56, 3.17) |          |            |

CD166/ALCAM (rs6437585, rs579565, rs1044243, and rs35271455) SNPs analyzed, which were all in Hardy-Weinberg equilibrium.

4 DISCUSSION

PCa risk stratification based on tumor size, PSA level, and ISUP grade is conventionally used to predict the clinical outcome of patients. Clinical and biological markers remain insufficient to identify PCa patients at higher risk of progression at the time of treatment initiation (radical prostatectomy, cryotherapy, or radiotherapy). On this basis, individualized genetic and molecular prognostic factors will help to discriminate between high-risk patients who may benefit from adjuvant therapy or closer surveillance and low-risk patients for whom active surveillance may suffice.

The present candidate gene-driven approach has identified genetic variants involved in PCa aggressiveness and recurrence after radical prostatectomy. Our analysis includes functionally relevant SNPs from the functionally related CD5, CD6, and CD166/ALCAM gene products. CD5 and CD6 encode highly homologous lymphocyte co-receptors that modulate the activation and differentiation signals of T lymphocytes.\(^{46,47}\) These co-receptors are critical in the regulation of the immune response and may play a role in the pathogenesis of PCa.

Figure 1: Biochemical recurrence (BCR)-free survival curves according to genotypes. (A) BCR-free survival curve of prostate cancer (PCa) patients according to CD6 single nucleotide polymorphism (SNP) rs12360861 genotype. HR comparing homozygous rs12360861AA with homozygous rs12360861GG was 2.65, (95% CI: 1.39–5.05), \(p = 0.003\). (B) BCR-free survival curve of PCa patients according to CD166/ALCAM SNP rs579565 genotype. HR comparing homozygous rs579565AA with homozygous rs579565GG was 1.86, (95% CI: 1.02–3.39), \(p = 0.043\). [Color figure can be viewed at wileyonlinelibrary.com]
transduced by the TCR complex. Here are reports on the impact of CD5 and CD6 variants on susceptibility and/or severity of autoimmune and malignant disorders, which are considered the two sides of the same coin. Here, we found association of the minor CD6 rs12360861G allele with BCR. Clinical relevance of this SNP is illustrated by their role in immune-mediated diseases such as multiple sclerosis and psoriasis. In the case of CD5, we found association of the rs2241002C–2229177T haplotype with a higher ISUP grade, reminiscent of the higher aggressiveness of melanoma in rs2241002C–2229177T carrier patients. Such findings support the utility of CD5 and CD6 genotyping in risk stratification and discrimination of patients with clinically significant tumors from indolent ones.

The functional impact of CD5 variation on T cell activation has been previously reported. The CD5 rs2229177G allele (Val471) is associated with stronger signaling via CD5, which in turn results in stronger inhibition of TCR-mediated signals compared with the rs2229177T allele (Ala471). Thus, we hypothesize that attenuation of TCR signaling lowers the antitumor activity of T cells, concomitant with higher ISUP grades in rs2241002C–2229177T haplotype carriers. There is no available information on the functional consequences of the CD6 rs12360861G>T SNP. We speculate that it behaves similarly to the CD6 rs11230563C>T and rs17824933G>C SNPs, which have been associated with lower T cell expression of the CD6 receptor and deficient binding to CD166/ALCAM, leading to poor endothelial/epithelial-T cell interplay.

The CD166/ALCAM gene encodes the best characterized CD6 ligand. CD166/ALCAM provides heterotypic interactions (CD6-ALCAM) between T cells and epithelial/endothelial cells, but also homotypic interactions (ALCAM-ALCAM) involved in cell adhesion, migration, and progression of several malignancies such as melanoma, breast, colorectal and bladder cancers. CD166/ALCAM is also necessary for engrafting hematopoietic stem cells into the hematopoietic niche. Expression of CD166/ALCAM is known to impact tumor progression in PCa patients and development of bone metastases in mouse models of PCa. Moreover, PCa metastases occur mainly in the bone, compromising patient survival. Here we observed an association of the minor CD166/ALCAM rs579565A allele with lower BCR-free survival. This is a clinically relevant SNP, as shown by its association to multiple sclerosis risk. To the best of our knowledge, no information is available on the functional consequences of this synonymous CD166/ALCAM rs579565 SNP (e.g., introduction of cryptic splicing sites, changes in transcription efficiency, or linkage disequilibrium with other gene variants) that may account for its clinical significance. It is worth mentioning that other CD166/ALCAM SNPs such as rs6437585 and rs1044243 have been associated to breast and bladder cancer, supporting the role of genetic variation of CD166/ALCAM in cancer.

The PCa immune microenvironment is heterogeneous, leading to different immunotherapy responses (e.g., immune checkpoint inhibitors). Recent immunogenomic work proposes a classification of PCa patients in three immunophenotypes based on gene enrichment signatures: nonimmune, immune-activated, and immune-suppressed. The last two subtypes were dichotomized based on a stromal signature involving Wnt/TGF-β (for tumor growth factor), and C-ECM (for cancer-associated extracellular matrix) genes. The work states that PCa patients with an immune-activated gene signature could benefit from TGF-β inhibitors. It would be interesting to know how CD5, CD6, and CD166/ALCAM expression and/or variation fit in those PCa immunophenotypes. Interestingly, CD166/ALCAM is a TGF-β-responsive marker and functional regulator of PCa metastasis and gene expression studies in patients with resectable non-small cell lung cancer show that higher CD5 and CD6 intra-tumor expression associates to better overall and relapse-free survival.

The strengths of this study include the large cohort of PCa patients, expert pathological review of cases and long-term follow-up of patients diagnosed with clinically localized diseases. Participating researchers were blinded to all clinical information, and genomic information was matched to clinical data only after all patient cases had been processed. We also acknowledge some study limitations. First, the use of BCR as a significant endpoint is questionable as only a proportion of patients with BCR show clinical progression. However, the detection of BCR after radical prostatectomy is usually the indicator for the application of adjuvant therapies. So, an independent validation will be necessary to ascertain the role of CD5, CD6, and CD166/ALCAM gene variants as PCa prognostic and treatment response indicators.

5 | CONCLUSION

We report new genetic variants involved in the modulation of lymphocyte activation and immune-epithelial cell adhesion, which influence PCa aggressiveness and recurrence. The described associations position CD5, CD6, and CD166/ALCAM as putative prognosis markers and/or therapeutic targets. Our findings support that CD5 variants attenuating T-cell activation associate with increased ISUP grade, and that CD6 and CD166/ALCAM variants lowering T-epithelial cell adhesive contacts associate with shorter BCR-free survival. Further studies are encouraged to validate our findings and the mechanism underlying them.

AUTHOR CONTRIBUTIONS

Conceptualization: Francisco Lozano, and Lourdes Mengual. Genetic studies: Sergi Casadó-Llombart, Marta Consuegra-Fernández, Esther Carreras, Fernando Aranda, and Noelia Armiger. Sample and clinical information collection: Tarek Ajami, Antonio Alcaraz, and Lourdes Mengual. Statistical analyses: Sergi Casadó-Llombart, Marta Consuegra-Fernández, Esther Carreras, and Fernando Aranda. Writing original draft: Sergi Casadó-Llombart, Tarek Ajami, Lourdes Mengual, and Francisco Lozano. All authors read, critically revised and approved the final version of the manuscript.
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CONFLICT OF INTEREST
Francisco Lozano is a founding partner at Sepsia Therapeutics SL. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The datasets generated in the present study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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