CCR5 Proinflammatory Allele in Prostate Cancer Risk

A Pilot Study in Patients and Centenarians from Sicily

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Prostate cancer (PCa) is the most common malignant neoplasm in older men in Western countries. The number of affected older men is increasing. Therefore, strategies for prevention of prostate cancer are crucial. To this purpose it is essential to know the mechanisms involved in development and progression of this malignancy. Recently, an increasing body of genetic and epidemiological studies proposed new hypotheses for prostate carcinogenesis. It has been suggested that genetic factors as well as exposure to environmental factors such as infectious agents, dietary carcinogens, and hormonal imbalances participate in PCa development. Besides, chronic inflammation plays a key role in PCa. Taking into consideration this complex scenario, in the present study we evaluated whether CCR5Δ32 deletion of *CCR5* gene might be associated with PCa susceptibility. For the control group we used centenarians, since they represent a disease-free human model. These preliminary results suggest that the CCR5Δ32 anti-inflammatory variant might be a resistance factor for the development of PCa.

Key words: **prostate cancer (PCa); inflammation; CCR5Δ32 deletion**

Introduction

Prostate cancer (PCa) is the most common noncutaneous malignant neoplasm in men from Western populations.[1] Its incidence is increasing rapidly in men over 50 years of age.[1,2] The correlation of PCa incidence with aging suggests that the disease burden associated with prostate carcinoma will increase dramatically over the next several decades.[2] On the other hand, a low incidence of PCa has been observed in men of Southeast Asian and East Asian countries.[3] So, it appears that the disease is not an intrinsic feature of aging, and its pathophysiology reflects a complex scenario with the involvement of both genetic and environmental factors.[4] Taking this into consideration, researchers have recently emphasized the crucial role of genetic factors and host exposure to environmental factors such as infectious agents, dietary carcinogens, and hormonal imbalances in the development of PCa.[4]

Chronic inflammation also seems to play a key role in PCa.[5] Inflammation may be directly carcinogenous by damaging DNA. Additionally, it creates a tissue micro-environment rich

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in cytokines, chemokines, and growth factors that can enhance cell replication, angiogenesis, and tissue repair.[5,6] These molecular mechanisms are complex and involve both the innate and instructive immune systems.[5–7] Furthermore, it has also been suggested that the pathway of chemokines and their receptors is involved in PCa growth and dissemination.[8]

Chemokines are small soluble molecules best known for their potent abilities to induce cellular migration during inflammation.[9] Many types of cancer cells, including PCa cells, express chemokines, and their receptors.[10] Recent studies have shown the local production of the CC-chemokine CCL5 (RANTES), a potent chemotactic factor for inflammatory cells, and production of its receptor (CCR5) in human PCa cell lines.[10,11] Possible functions of CCL5/CCR5 axis in PCa progression have been explored, revealing that CCL5 and CCR5 induce PCa cell proliferation and invasion through basement membrane gels.[11] Vaday *et al.*[10] recently demonstrated that the inflammatory chemokine CCL5 might function as an autocrine factor that, when binding to its CCR5 receptor expressed on PCa's cell surface, activates cellular responses involved in cancer progression.

In light of the key role of the CCL5/CCR5 axis in the pathophysiology of PCa, and with the aim of identifying candidate genes to promote the research of new therapies for both the prevention and treatment of PCa, we examined the frequencies of wild CCR5 and the CCR5Δ32 variant, a nonfunctional allele resulting from a 32-bp deletion in exon 4 (rs333) of the CCR5 gene (NM-U83326) in PCa patients and controls. As controls, we analyzed centenarians, since this group has escaped major age-related diseases, including cancer.[12,13]

Materials and Methods

The study material consisted of 50 malignant human prostate tissues placed into a suitable volume of RNA-*later* (RNA Stabilization Reagent, Applied Biosystems, California, U.S.A.), used to purify genomic DNA and total RNA simultaneously from each single biological sample with AllPrep DNA/RNA Mini Kit (Qiagen, Dusseldorf, Germany). The tissue samples were obtained from 50 patients affected by PCa when they enrolled in the study, that is, at the time of their admission to the Department of Oncology of Palermo ARNAS-Civico Hospital of Palermo (age range 60–80 years). The 50 DNA samples were genotyped for CCR5Δ32 deletion as already published.[14] The control group consisted of 53 male centenarians (>99 years), whose age was confirmed from records at the city hall and/or church registries. No cancer or other age-related diseases were observed in the centenarians, although some had reduced auditory and visual acuity. The control group had been previously genotyped for CCR5Δ32 deletion.[15] Both Civico Hospital and University Hospital Ethics committees approved the study, and all participants gave their written informed consent.

The data were tested for goodness-of-fit between the observed and expected genotype frequencies according to Hardy–Weinberg equilibrium (HWE) by χ^2 test. Differences in allele and genotypic frequencies of the CCR5Δ32 variant between the two groups were evaluated by gene count and χ^2 test. Odds ratio (OR) with confidence interval (CI) was also calculated.

Results and Discussion

The CCR5 genotypes of both patients and centenarians were in HWE. We observed no significant differences in genotype frequency between the two groups (data not shown). Analyzing the allelic frequency of CCR5Δ32 deletion of patients and centenarians, we found a significant difference ($P = 0.03$ by χ^2 test; OR, 0.26; Cl, 0.07–0.98; $P = 0.05$) (Table 1).

TABLE 1. Allelic Frequencies of CCR5Δ32 Deletion[a]

Allele (%)	wt	Δ32
Patients ($n = 50$)	97	3
Centenarians ($n = 53$)	95	11

[a]Data are from 50 PCa patients and 53 centenarians from Sicily. 2 × 2 comparisons between the different groups with OR and 95% CI). Significant differences in allelic frequencies were obtained between PCa patients and centenarians by χ^2 test (2 × 2 table; $P = 0.03$; OR 0.26; Cl, 0.07–0.98, $P = 0.05$).

Thus, our results, which show that the anti-inflammatory CCR5Δ32 allele is overrepresented in centenarians and underrepresented in patients, suggest that the CCR5Δ32 variant is a resistance factor for the development of prostate cancer.

The development of PCa is a complex process based on the interaction between tumor and host cells. The host response includes chronic inflammation with the involvement of both innate and instructive immune cells and molecules. Among these, the chemokine and their receptors seem to play a crucial role in the tumor growth and metastasis process of PCa. In particular, it has been suggested that the CCR5 receptor and its ligand, the CCL5, play a key role in tumor progression.[8,10] So, polymorphisms in genes encoding these molecules may modify the susceptibility of this disease. On the other hand, PCa is a heterogeneous disease with multiple loci contributing to its susceptibility. It has been also demonstrated that more important in terms of inherited susceptibility for PCa are common polymorphisms in a number of low penetrance alleles of several genes—the so-called genetic modifier alleles. The list of these variants is long, but the major pathways currently under examination include those involved in DNA repair, carcinogen metabolism, and inflammation pathways.[16]

In the present study we evaluated the CCR5Δ32 deletion, a nonfunctional allele resulting from a 32-bp deletion in exon 4 (CCR5Δ32).[9] This variant plays a role in a variety of human diseases, ranging from inflammatory diseases to cancer.[9] We have previously suggested a protective effect of this anti-inflammatory deletion in longevity since it determines a positive control of inflammation, allowing the avoidance or delaying the occurrence of age-related diseases such as cardiovascular ones.[15] Here we demonstrated that the CCR5Δ32 deletion is overrepresented in centenarians and underrepresented in PCa patients. This confirms our previous data, which suggest that proinflammatory alleles have an opposite role in longevity and age-related diseases, including cancer.[17]

Hence, these data suggest a key role of CCR5Δ32 deletion in susceptibility to PCa, but, as all association studies, they are influenced by a number of possible confounding factors, such as, among others, the total number of patients and controls. To validate our results, therefore, it will be necessary to perform further studies with larger samples, since this study was performed on a small number of patients and controls. False associations might also occur if the controls are not ethnically matched with the patients. We compared people belonging to the same homogeneous population from Sicily. Therefore, we believe that, although they are based on a reduced number of patients and controls, our results might more reliable than those of studies performed on larger cohorts of patients from Northern Europe and the United States, which are ethnically matched but only refer to Caucasians in general. Moreover, we used as a control a population of men unquestionably PCa-free.

Ongoing studies are aimed at examining the effectiveness of CCR5 receptor antagonists in experimental prostate tumor growth.[18] As chemokine receptor antagonists continue to show promise as novel strategies in cancer treatment, their application as combined therapeutic agents in PCa may be an important area of future study.

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Conflicts of Interest

The authors declare no conflicts of interest.

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