Short Communication

Macrophage Inhibitory Cytokine-1 H6D Polymorphism, Prostate Cancer Risk, and Survival

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Abstract

Macrophage inhibitory cytokine-1 (MIC-1), a member of the transforming growth factor-β superfamily, is important in regulating inflammation. Inflammation of the prostate has been suggested to favor tumor development. A recent study (JNCI 2004, 96:1248-1254) found marginal evidence of an association between the presence of the mature MIC-1 protein nonsynonymous polymorphism H6D C-to-G (rs1058587) with reduced prostate cancer risk [odds ratio, 0.83; 95% confidence interval (95% CI), 0.69-0.99]. We tested this in a population-based study of 819 cases and 731 controls from Australia and found a similar, yet not significant, odds ratio of 0.85 (95% CI, 0.7-1.04; *P* = 0.11). We also tested the potential association between the H6D variant and disease-specific survival in 640 cases followed-up for an average of 8.2 years. We found that cases carrying the H6D G allele had an increased risk of death from prostate cancer than cases carrying two copies of the C allele (hazard ratio, 1.72; 95% CI, 1.06-2.78; *P* = 0.03). Our data suggest that the H6D variant in MIC-1 might play a role in prostate cancer, but it is difficult to explain how a variant can be associated with lower risk of developing prostate cancer but more aggressive growth if cancer develops. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1223–5)

Introduction

Macrophage inhibitory cytokine-1 (MIC-1), also known as placental bone morphogenetic protein, prostate-derived factor, and growth/differentiation factor 15, MIM#605312, is a member of the transforming growth factor-β superfamily that regulates a wide variety of physiologic processes involved in tissue differentiation and maintenance (1). A potential function is inhibition of macrophage activation (required for cell immune and inflammatory responses) in response to proinflammatory monokines (2). A role for the host immune and inflammatory responses in the development of prostate cancer by implicating proliferative inflammatory atrophy of cells in the prostate gland in tumorigenesis has been proposed (3). Gene expression studies show MIC-1 overexpression in prostate cancer (4-6), whereas genome-wide scans of multiple-case families provide evidence for linkage of the chromosomal region surrounding MIC-1 to prostate cancer (7, 8). More recently, serum MIC-1 combined with prostate-specific antigen has been shown to improve the specificity of prostate cancer diagnosis (9).

The *MIC-1* gene, located at band p13.11 on chromosome 19, has two exons that encode the 308-amino acid MIC-1 polypeptide, consisting of a 29-amino acid signal peptide, a 167-amino acid propeptide, and a 112-amino acid mature protein. Cleavage of the propeptide allows the mature protein to be secreted as a disulfide-linked homodimer (2). A single-nucleotide polymorphism at position 6 of the mature protein (codon 202, CAC to GAC) results in a histidine to aspartic acid substitution (H6D, rs1058587; ref. 9). Different amino acid properties and the close proximity of this variant to the critical stabilizing cystine residue at position 7 suggest a role in MIC-1 stability and/or function (10). There has been a recent report of a statistically weak negative association between H6D and prostate cancer risk [odds ratio (OR), 0.83; 95% confidence interval (95% CI), 0.69-0.99; ref. 11]. We tested this putative association and a possible association between the H6D single-nucleotide polymorphism and disease-specific survival using the risk factors for prostate cancer study (12).

Materials and Methods

Study Population. Subjects were recruited between 1994 and 1997 in Perth and Melbourne, Australia. In brief, 1,040 cases diagnosed before age 70 years, presenting with a histopathologically confirmed adenocarcinoma of the prostate with a Gleason score >4, were identified from state cancer registries. Tumor stage (I-IV) and grade (moderate grade, Gleason score 5-7; high grade, Gleason score 8-10) were recorded. A total of 1,052 controls were randomly selected from the State Electoral Rolls and frequency matched to the expected age distribution of the cases. Blood samples were obtained from 862 cases (83%) and 745 controls (71%). Almost all subjects (98.5%) were born in Australia, the British Isles, or Western Europe and are classified as being of European descent. During an average follow-up of 8.2 years to December 31, 2004, 69 (11%) of the 640 Melbourne cases died from

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Table 1. Allele and genotype frequencies of the *MIC-1* H6D (C>G) polymorphism in an Australian population-based case-control study of prostate cancer

	Controls, n = 731 (%)	Cases, n = 819 (%)	OR* (95% CI)	$P^{\dagger}$
Codominant model				0.28
CC	393 (53.8)	473 (57.8)	Reference	
CG	289 (39.5)	294 (35.9)	0.85 (0.68-1.04)	
GG	49 (6.7)	52 (6.3)	0.88 (0.58-1.33)	
P_{HWE}	0.7	0.5		
Dominant model				0.11
CC	393 (53.8)	473 (57.8)	Reference	
CG, GG	338 (46.2)	346 (42.3)	0.85 (0.7-1.04)	
Recessive model				0.78
CC, CG	682 (93.3)	767 (93.7)	Reference	
GG	49 (6.7)	52 (6.4)	0.94 (0.63-1.41)	

*OR and 95% CI from unconditional logistic regression analysis.
†Likelihood ratio test for association between genotype and prostate cancer risk.

prostate cancer. Informed consent was obtained from all study participants, and the Ethics Review Committee of The Cancer Council of Victoria approved the study protocol (Human Research Ethics Committee 9500).

Genotyping. Genotyping the H6D polymorphism (C-to-G) was successfully done on genomic DNA from 819 cases (95%) and 731 controls (98%), blind to case-control status, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. PCR and extension primers, conditions of PCR amplification, and the mass extension reaction are available on request. A random selection of 428 samples was regenotyped with a concordance of 99%. Discordant calls were verified via direct sequencing.

Statistical Analysis. Estimations of allele frequencies and tests of deviation from Hardy-Weinberg equilibrium were conducted using likelihood theory (13). Case-control analyses used unconditional logistic regression (14) and OR estimates and 95% CI derived under likelihood theory. Cox regression models adjusted for age, tumor grade, and stage were used to derive hazard ratios (HR) for associations of genotypes and prostate cancer survival (15). All tests were two sided. Following convention, nominal statistical significance was based on $P = 0.05$ and no adjustments were made for multiple testing.

Results

The frequency of the minor G allele was 24.3% (95% CI, 22.2-26.5) for cases and 26.5% (95% CI, 24.2-28.8) for controls ($P = 0.18$), which was comparable with the Swedish study (26.9% and 28.3%, respectively). No deviation from Hardy-Weinberg equilibrium was observed ($P \geq 0.5$). The genotype distribution was not associated with age, country of birth, or family history of prostate cancer (all $P > 0.1$). Table 1 shows that although the prevalence of G allele carriers was lower for cases (42%) than controls (46%), the OR (0.85) for presence of the G allele and prostate cancer risk (95% CI, 0.7-1.04) was not statistically significant ($P = 0.11$). There was little difference in prevalence of G allele carriers by stage (45% for stage III-IV cancers versus 41% for stage I-II disease; $P = 0.33$) or grade (39% for high-grade tumors versus 43% for moderate-grade tumors; $P = 0.28$). Table 2 shows that, from the analysis of time to death from prostate cancer adjusted for age and tumor stage and grade, men carrying the G allele compared with men carrying the CC genotype had a HR of 1.72 (95% CI, 1.06-2.78; $P = 0.03$).

Discussion and Conclusion

Although the estimated OR for G allele carriers compared with carriers of the CC genotype of the *MIC-1* H6D single-nucleotide polymorphism was close to that previously reported, 0.85 versus 0.83 (11), it was not statistically significant. The numbers of controls were similar in the two studies, although the Swedish study had twice as many cases. Both studies were European based, with controls recruited from the general population. The Swedish cases were almost equally divided between the age groups of 45 to 65 (51%) and 66 to 80 (49%) years at diagnosis, whereas ours were younger, with diagnosis before 55 (14%) years, between 55 and 64 (52%) years, and between 65 and 69 (34%) years. Our cases were selected for a Gleason score of ≥5, but the distributions of tumor stage and grade were comparable. Although the

Table 2. HRs by genotype for death from prostate cancer for cases from the Melbourne arm of the risk factors for prostate cancer study

	Cases, n = 636*	Deaths from prostate cancer, n = 68* (%)	Unadjusted HR† (95% CI)	$P^{\ddagger}$	Adjusted HR§ (95% CI)	$P^{\ddagger}$
Codominant model				0.05		0.09
CC	369	30 (8.1)	Reference		Reference	
CG	227	30 (13.2)	1.55 (0.94-2.57)		1.71 (1.03-2.84)	
GG	40	8 (20)	2.44 (1.12-5.3)		1.75 (0.79-3.86)	
Dominant model				0.03		0.03
CC	369	30 (8.1)	Reference		Reference	
CG, GG	267	38 (14.2)	1.68 (1.05-2.7)		1.72 (1.06-2.78)	
Recessive model				0.09		0.42
CC, CG	596	60 (10.1)	Reference		Reference	
GG	40	8 (20)	2.01 (0.96-4.2)		1.38 (0.65-2.93)	

*Of the 640 cases in the Melbourne arm of the study, 4 (1 death) were excluded from the survival analysis because either tumor stage or grade was missing.
†HRs from Cox models, where the event of interest was death from prostate cancer. Cases that did not die from prostate cancer were censored at death from other causes or at the end of follow-up (December 31, 2004).
‡From likelihood ratio test for association between genotype and disease-specific survival.
§Cox models were adjusted for age at diagnosis, tumor stage (I-II, III, and IV) and grade (Gleason score 5-7 or moderately differentiated and Gleason score 8-10 or poorly differentiated).

power of the survival analysis is limited by the number of reported deaths (n = 68), we found the HR for disease-specific death for cases was ~70% higher in cases carrying the G allele. The results of the association analysis in our study and in the Swedish one together provide some evidence that *MIC-1* H6D G allele carriers have a decreased risk of prostate cancer. This would be in contrast with the effect observed on survival. We do not know the exact effect of this polymorphism on gene expression and function of MIC-1, but one may speculate that the histidine to aspartic amino acid change, adjacent to the critical stabilizing cysteine residue, may alter the function of the mature protein. Ultimately, altered function may accelerate tumor progression but the mechanisms remain to be defined. However, it is difficult to explain how the same variant would be responsible for opposite effects in two different stages of carcinogenesis. A possible hypothesis is that the functional H6D variant may affect patient survival through modulation of the patient's immune system. For this reason, further studies with larger sample size are warranted to understand the role of MIC-1, particularly the H6D single-nucleotide polymorphism, in the pathophysiology of prostate cancer.

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