Prognostic Significance of Immunohistochemical *MSH2* Expression in Prostate Cancer

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Purpose: There are numerous prostate cancer-related genes that involve in carcinogenesis and tumor progression. Among the genes, DNA mismatch repair genes recognize and repair misincorporated nucleotides during DNA replication. In this analysis, we evaluated the association of *hMSH2* which is one of the mismatch repair genes, with risk of aggressive prostate cancer and prostate cancer recurrence.

Materials and Methods: Immunohistochemistry was performed in 46 patients who diagnosed prostate cancer and underwent radical prostatectomy between January 2006 and December 2012 at Kyung Hee University Hospital at Gangdong. We evaluated an association between the degree of *hMSH2* immunohistochemical staining and various clinical variables including prostate-specific antigen (PSA), Gleason score, pathological stage, and biochemical recurrence. The intensity of immunostaining for *hMSH2* was divided into 2 groups: low expression group (immunostaining score <2) and high expression group (immunostaining score ≥2).

Results: Although seminal vesicle invasion was marginally associated with the degree of *hMSH2* immunohistochemical staining, PSA, Gleason score, lymph node metastasis, presence of lymphatic, perineural, vascular invasion, and extracapsular extension were not associated with the degree of *hMSH2* immunohistochemical staining. Furthermore, the association of biochemical recurrence free survival with *hMSH2* expression was not statistically significant.

Conclusions: The *hMSH2* expression was marginally associated with risk of aggressive prostate cancer such as seminal vesicle invasion. Further evaluation with a larger number of cases is needed to verify these results. (Korean J Urol Oncol 2018;16:58-62)

Key Words: Prostate • Gene expression • Prostatic neoplasms • DNA repair • Base pair mismatch

INTRODUCTION

Prostate cancer is one of the 5 most common primary cancers among Korean males. The incidence has rapidly increased to 12.8% in the last decade, resulting 7,848 new cases in 2010.¹

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Although many studies have identified numerous prostate cancer risk factors including genetic components, biological mechanisms and main contributors that affect prostate cancer are still unknown. Identification of risk genes involved in initiation and/or progression of prostate carcinoma will assist in development of clinically useful prognostic markers. Human mismatch repair (MMR) genes play a role in the DNA damage response pathway. The MMR genes recognize and correct misincorporated nucleotides and insertion/deletion mispairs formed during DNA replication. In consequence, the spontaneous mutation rate in genes that suppress cancer initiation or progression increases with deficiency in MMR.^{2,3}

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Hereditary nonpolyposis colon carcinoma and sporadic tumors with inactivating mutations in both alleles of a MMR gene exhibit instability in simple polymorphic repetitive genetic elements known as microsatellites.^{4,6} Microsatellite instability (MSI) and differential expression of a prototype MMR gene, *hMSH2*, have been reported in both benign and malignant prostate tissue.^{7,8} Moreover, *hMSH2* expression is altered in a subset of clinically localized prostate carcinoma specimens independent of pathological stage and Gleason pattern with a statistically significant correlation between the degree of *hMSH2* immunohistochemical staining and MSI.⁸

In this study, we analyzed the association of *hMSH2* with risk of aggressive prostate cancer and prostate cancer recurrence to investigate the role of *hMSH2* as a potential prognostic marker in prostate cancer.

MATERIALS AND METHODS

A total of 46 patients underwent radical retropubic radical prostatectomy with simultaneous bilateral pelvic lymphadenectomy between January 2006 and December 2012 at the Kyung Hee University Hospital at Gangdong. No patient received neoadjuvant therapy. All clinical investigations were conducted according to institutional regulations of the Kyung Hee University Hospital to protect privacy.

Radical prostatectomy specimens were staged in compliance with 2009 TNM classification. Gleason score was calculated based on modified Gleason grading of prostatic carcinoma.⁹ Two pathologists, SJL and KYW, were blinded to the pathological findings and independently evaluated the slides.

A section of formalin-fixed paraffin embedded tumor tissue blocks was stained with the hematoxylin and eosin (H&E) and was screened to identify the viable and representative areas for prostate cancers. The areas on the block corresponding to the cores of tissue were marked for punching out. The tissue microarrays (TMAs) were assembled using a commercially available manual tissue microarrayer (Quick-Ray; UNITMA, Seoul, Korea). Briefly, 2 or 3 tumor cores with a diameter of 3.0 mm were punched out from each tumor tissue block, and were arrayed into 2 or 3 paraffin recipient blocks, respectively. We chose to array 2 or three 3.0-mm cores per case to increase the concordance rate between the immunohistochemistry results of the TMAs and the whole sections. The cores from prostate cancer tissues were placed in each block to produce serial sec-

tioned slides. For each slide, H&E staining was performed to verify the tumor cell content. The slides with stromal tissue only or insufficient cancer tissue in all cores were excluded from the analysis.

Immunohistochemistry was performed by an established and validated protocol. For immunohistochemistry, 4- μ m-thick sections of the formalin-fixed paraffin embedded tumor tissue blocks were deparaffinized using Bond Dewax Solution (Vision BioSystems, Mount Waverly, VIC, Australia), and an antigen retrieval procedure was conducted using Bond ER Solution (Vision BioSystems) for 30 minutes at 100°C. The endogenous peroxidase was quenched with hydrogen peroxide. The sections were incubated for 60 minutes at ambient temperature with the primary *hMSH2* antibody. A biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-max automatic slide stainer (Vision BioSystems) was used for staining.

The intensity of immunostaining for *hMSH2* was scored visually and stratified into 4 staining groups: absent or minimal, weak, moderate, and strong (absent or minimal staining, 0 or 0-1; weak, 1 or 1-2; moderate, 2 or 2-3; and strong, 3, 3-4 or 4). All pathological grading was performed under supervision by an experienced pathologist. After scoring all specimens, a consensus on the less than 10% of specimens with disparate scores was achieved. The *hMSH2* expression was categorized into two groups: absent to weak staining (grades 0 and 1) as low *hMSH2* expression group and moderate to high staining (grades 2 to 4) as high *hMSH2* expression group.

Fisher exact test was used to examine the association between the degree of *hMSH2* staining and the clinicopathological characteristics of the patients. Biochemical recurrence free survival curves were plotted using the Kaplan-Meier method, and log-rank test was used to evaluate the relationship between *hMSH2* expression and biochemical recurrence free survival. SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Values of $p < 0.05$ were declared statistically significant.

RESULTS

Among 46 patients who underwent radical prostatectomy, 10 patients were included in the low *hMSH2* expression group and 36 patients were included in the high *hMSH2* expression group (Fig. 1). The means of age for the low and high *hMSH2* expression groups were 63.0 and 69.8 years, respectively

Fig. 1. Immunohistochemical analysis of *hMSH2* expression in prostate cancer tissue. (A) Moderate to high cytoplasmic staining for *hMSH2* in prostate cancer cells (×100). (B) Absent to weak cytoplasmic staining for *hMSH2* in prostate cancer cells (×100).

Table 1. Clinical and pathological characteristics of patients

Variable	All patients	Low expression	High expression	p-value
Mean age (yr)	68.3	63.0	69.8	0.003
Mean PSA (ng/mL)	16.7	32.0	12.5	0.256
Extracapsular extension	14 (30.4)	5 (50.0)	9 (25.0)	0.129
Seminal vesicle invasion	5 (10.9)	3 (30.0)	2 (5.6)	0.061
Lymph node metastasis	2 (4.3)	1 (10.0)	1 (2.8)	0.437
Positive resection margin	12 (26.1)	3 (30.0)	9 (25.0)	0.750
Perineural invasion	15 (57.7)	1 (10.0)	14 (38.9)	0.085
Biopsy Gleason score				0.163
6	12 (26.1)	3 (30.0)	9 (25.0)	
7	25 (54.3)	3 (30.0)	22 (61.1)	
>7	9 (19.6)	4 (40.0)	5 (13.9)	
pT stage				0.133
T2	31 (67.4)	5 (50.0)	26 (72.2)	
T3a	10 (21.7)	2 (20.0)	8 (22.2)	
T3b	5 (10.9)	3 (30.0)	2 (5.6)	

Values are presented as number (%) unless otherwise indicated.

($p=0.003$). The levels of PSA for the two groups were 32.0 and 12.5 ng/mL, respectively ($p=0.256$). The rates of extracapsular extension (ECE), seminal vesicle invasion (SVI), and lymph node metastasis in patients with the low *hMSH2* expression group were higher than those of the high expression group (50.0% vs. 25.0%, [$p=0.129$], 30.0% vs. 5.6% [$p=0.061$], and 10.0% vs. 2.8% [$p=0.437$], respectively). The positive resection margin rates were not different between the 2 groups (30.0% vs. 25.0% [$p=0.750$]), while the perineural invasion rates were higher in the high *hMSH2* expression group (10.0% vs. 38.9% [$p=0.085$]). The SVI was not significantly associated with the degree of *hMSH2* immunohistochemical staining, but it showed marginal correlation with the degree of *hMSH2* immunohistochemical staining (30.0% vs. 5.6%, $p=0.061$). However, other variables including PSA, Gleason score, lymph node metastasis, ECE, presence of lymphatic, perineural, and vascular invasion

were not associated with the degree of *hMSH2* immunohistochemical staining (Table 1).

The mean follow-up periods of patients was 32.5 months. With the definition of biochemical recurrence as a consecutive level of PSA > 0.2 ng/mL following radical prostatectomy, the number of patients with biochemical recurrence during follow-up periods was 8. Three patients among them were in the low *hMSH2* expression group, whereas 5 patients were in the high *hMSH2* expression group. In analysis of the biochemical recurrence free survival of patients, high *hMSH2* expression group showed better biochemical recurrence free survival than low expression group during follow-up periods. However, it showed no statistical significance between the degree of *MSH2* expression and biochemical recurrence free survival (Fig. 2).

Fig. 2. Biochemical recurrence free survival according to degree of *hMSH2* expression.

DISCUSSION

MMR deficiency increases the risk of developing cancer, due to an elevated rate of spontaneous mutation, and has also been implicated in the differentiation, growth, and invasion of cancer. In our study, we demonstrated that the degree of *hMSH2* expression using immunohistochemical analysis was not significantly associated with variables related to the risk of aggressive prostate cancer and biochemical recurrence-free survival including PSA, Gleason score, lymph node metastasis, ECE, SVI, presence of lymphatic, perineural, and vascular invasion. Those results were contradicted with several prior studies demonstrated that the prostate cancer was correlated with *hMSH2* expression.

Velasco et al.⁸ demonstrated differential *hMSH2* expression in benign and malignant prostate tissue. According to their results, a statistically significant correlation between *hMSH2* immunohistochemical staining intensity and MSI was identified in prostate carcinoma specimens. Furthermore, the time to cancer recurrence was associated with *hMSH2* staining intensity. Taken together, they concluded that *hMSH2* gene expression in prostate carcinoma may be a useful prognostic marker for outcome in men with clinically organ confined prostate carcinoma. Another group also reported that *hMSH2* is differentially expressed in malignant prostate tissue and *hMSH2* immunohistochemical staining intensity correlates with Gleason score, overall and disease-free survival.¹⁰ They analyzed 243 specimens from patients who underwent radical prostatectomy for clinically organ confined prostate cancer. Their results showed higher Gleason score significantly correlated with high-

er *hMSH2* expression ($p < 0.001$) and low *hMSH2* expression correlated with increased overall, disease-free, and biochemical disease-free survival (all $p < 0.01$).

Velasco et al.¹¹ also investigated the association between clinicopathological variables and *hMSH2* expression or MSI. However, no statistically significant differences for any of the clinical or pathologic variables and degree of *hMSH2* staining were identified. They suggested that absent to low *MSH2* staining provided favorable prognostic information for biochemical recurrence independent of pathologic grade, stage, tumor volume, or preoperative serum PSA. Another hypothesis suggests that certain biological properties such as growth rate, response to androgen, ability to metastasize are differentially affected by MMR gene expression. According to the study that evaluated whether single nucleotide polymorphisms (SNP) in key MMR genes are related to prostate cancer outcomes, SNP in *MLH1* was associated with overall prostate cancer risk, more aggressive prostate cancer, and prostate cancer recurrence, but those were not associated with SNP in *MSH2*.¹² Those results and our study possibly made it confused the definite role of *hMSH2* gene in prostate cancer. However, it is clear that *hMSH2* is associated with prostate cancer according to aforementioned studies and those that investigated the gene expression profile of MMR genes. Several mutational analysis of MMR genes suggested that MMR including *hMSH2* mutation are associated with risk of prostate cancer.^{13,14}

In our results, proportion of variables associated with aggressive prostate cancer, and prostate cancer recurrence were higher in low *hMSH2* expression group. Furthermore, p-value of SVI between the 2 groups was 0.061, that is close to statistically significant.

One possible explanation for the differences between our results and other different results is almost all studies of correlation between *hMSH2* and prostate cancer investigated nuclear *hMSH2* expression, while we evaluated cytoplasmic *hMSH2* expression. Another possible explanation for the differences is that method of immunohistochemical analysis might affect to our current results. Indeed, many researchers including our study not only had different process for antibody preparation, staining, or incubation, but also used different peroxidase kit, solution, or reagent. Furthermore, racial differences might affect to those results, because most studies conducted with American or European men. Characteristics of prostate cancer including pathology and oncologic outcomes in Korean men are different

from those of American or European men. Other possible explanation for the differences and also major limitation of our study is small number of patients. Other potential limitations of our study are related to the use of TMA and absence of MSI or other genetic analysis. Thus, to defining the exact mechanisms and role of *hMSH2* gene in prostate cancer, further elucidation of the relation between *hMSH2* gene and prostate cancer with large cohort. In addition, various approaches to MMR gene investigation will be needed. Through these studies, we hope that these can clarify the role of MMR in prostate cancer, and possibly provide prognostic and therapeutic information in the treatment of men with this disease.

CONCLUSIONS

The degree of *hMSH2* expression was not significantly associated with variables related to the risk of aggressive prostate cancer and biochemical recurrence-free survival, however, it was marginally correlated with risk of seminal vesicle invasion. Further evaluation with a larger number of cases is needed to verify these results.

CONFLICT OF INTEREST

The authors claim no conflicts of interest.

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