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Research Article

Investigating *MCPH1* Gene Methylation in Prostate Cancer Tissue

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Abstract

Introduction: Prostate cancer, with a high rate among men, is the second cause of death among men. DNA methylation patterns and chromatin structure change in cancer. Thus, the present study investigates the methylation status of the *MCPH1* gene in samples of malignant prostate cancer tumor tissue in comparison with the surrounding normal tissue and benign prostatic hyperplasia (BPH).

Material and Methods: In this study, 104 samples (47 samples of malignant tumor tissue, 47 samples of normal surrounding tissue, and 10 samples of BPH) were collected. After DNA extraction, all samples were treated with bisulfite. The methylation status of genes was examined through the MS-HRM technique and by designing specific primers for this method on treated DNA samples. Finally, the collected data were analyzed using SPSS software.

Results: The results revealed that regarding the *MCPH1* gene, methylated samples in the malignant tumor group are higher than normal and BPH. There is a significant relationship between the disease status and the methylation level of the samples, so the level of methylation in normal tissue and BPH tissue is lower than that in malignant tumor tissue (p-value<0.001). No significant difference was observed between the methylation status of the samples and their age (P-value: 0.9). Also, in people whose both lobes were involved in the malignant tumor, the samples were in the methylated status regarding the *MCPH1* gene. Also, a significant relationship was observed between the involvement of both lobes and *MCPH1* gene methylation.

Conclusion: Based on the observations of the present study, it can be stated that the methylation changes are proportional to the Gleason score, but this relationship was not significant. *MCPH1* gene methylation changes seem to play a significant role in the pathogenesis of prostate cancer and can be used as a diagnostic marker in prostate cancer.

Keywords: Prostate Cancer; *MCPH1* Gene Methylation; BPH Samples; Gleason Score

Abbreviations

BPH: Benign Prostatic Hyperplasia; *MCPH1*: Microcephalin; TS: Tumor Suppressor; TSGs: Tumor Suppressor Genes; PCR: Polymerase Chain Reaction; MS-HRM: Methylation Sensitive-High Resolution Melting; NTC: No-Template Control; BLAST: Basic Local Alignment Search Tool.

Introduction

Prostate cancer, following lung cancer, is the most common cancer in men. The prostate is a gland found only in men and secretes a liquid into the semen. Prostate cancer can either progress slowly or show clinical symptoms over a long period, or the tumor can grow rapidly and invade surrounding tissues. Most patients with prostate cancer have symptoms such as urinary problems, sexual

weakness, frequent urination, difficulty in holding urine, the feeling of urinating during the night, the presence of blood in the urine, weight loss, and abnormal rectal examination (benign and malignant). These signs help us to diagnose cancer [1].

Microcephalin (*MCPH1*) is a gene expressed during fetal brain development. A specific mutation in this gene in the homozygous state causes primary microcephaly, which is a severe brain defect (a medical condition in which the brain does not develop properly and causes the head to be smaller than normal) [2,3]. Other names of this gene are microcephalin1, MTC, and BRIT1. *MCPH1* gene has a promoter and two CpG islands located at chr8:6406062-6406801. This CpG Island contains 72 CpGs. The other island is located at chr8:6566695-6567367 and includes 53 CPGs [4].

MCPH1 is downregulated in breast, prostate, and ovarian cancers [5]. The overexpression of this gene reduces cell growth and proliferation and the *MCPH1* level is inversely related to the probability of prostate cancer [5]. It has also been reported that this gene can act as a tumor suppressor (TS) gene [6]. *MCPH1* was selected for analysis since its methylation status has not been examined in prostate cancer, while it is expressed in the prostate [5]. This gene may be downregulated in cancerous tissues. This gene methylation has been examined in oral, breast, leukemia, etc. [5,7].

DNA methylation is a crucial epigenetic modification involved in gene regulation, and its dysregulation is common in cancers [8]. Methylation of tumor suppressor genes (TSGs) like *MCPH1* can silence their protective functions, contributing to tumorigenesis [9]. *MCPH1*, which plays a role in DNA repair and cell cycle regulation, is downregulated in several cancers through promoter methylation [10]. In prostate cancer, *MCPH1* methylation is associated with more aggressive disease, and similar patterns have been observed in oral, breast, ovarian, and brain cancers, highlighting its potential as a diagnostic biomarker and therapeutic target [5,11-13].

This study aims to investigate *MCPH1* gene methylation in prostate cancer tissue samples compared to benign prostatic hyperplasia (BPH) samples to better understand its contribution to prostate cancer pathogenesis. The findings from this research could provide valuable insights into the role of *MCPH1* methylation in prostate cancer and its potential as a therapeutic target.

Materials and Methods

Patients and samples

Samples of malignant prostate cancer tumor tissue, normal surrounding tissue, and BPH samples were collected with the personal consent of the patients. About 80 mg of them were collected by different surgeons of Imam Khomeini Hospital and stored in a cryotube inside the freezer at -80 C. The medical records of the patients were categorized and the age and gender of the patients, the Gleason score, PSA level, involvement of the lobes, etc. were collected and categorized in an Excel vial and investigated.

Genomic DNA extraction

DNA was extracted from each fresh frozen tissue using SinaClon DNA extraction kit based on manufacturers' instructions. The quality and quantity of extracted DNA samples were determined through NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and loading on agarose gel (2%).

Bisulfite modification of genomic DNA

The genomic DNA isolated from samples was subjected to bisulfate conversion using the EpiTect Plus DNA Bisulfate Kit (Qiagen, Germany) and each step was performed according to the instructions of manufacturer. Briefly, 500 ng of DNA from each sample was treated with sodium bisulfate mix which converts all unmethylated cytosines into uracil, whilst methylated cytosines remain unchanged. Chemical modification of cytosine leads to a change in primary DNA sequence that permits detection of unmethylated cytosines from 5-methyl-cytosine. Finally, DNA was eluted in the elution bufer and stored at −20 °C till subsequent experimentation.

Primer sequences and design

Forward and reverse primers for MS-HRM analysis were designed according to the guidelines of Wojdacz., *et al*. [14,15] in order to minimize PCR bias. In brief, the design criteria were as follows:

Primers should contain a minimal number of CpG dinucleotides, typically 1 or up to 2 and any CpG present was positioned as close as possible to the 5′ end of each primer.

- Each primer included at least one natural thymidine (T) nucleotide derived from a non-CpG cytosine, placed at or near the 3′end.
- The melting temperature of the primers were matched, ideally within 1°C.

Selected primers were designed to amplify specific region within the promoter of each target gene using various online software (Table 1). The steps involved in primer design were as follows:

- Promoter regions identification of different genes was performed using online available software such as Promoser and TRED, which utilize the GenBank accession numbers as provided by reference sequence database of NCBI.
- *In-silico* specificity checks of sequence specificity were conducted with the Basic Local Alignment Search Tool (BLAST) at NCBI.

Table 1: Primers designed for the MS-HRM method.

Name	seq.(5′ -3′)	CG%	TM (°C)	Ta (°C)	Mer
MCPH1 F	GGGGTTTGAGGTATTAGAGTT	43%	55.92	60	20
MCPH1 R	CCGACACCACCTAAAACTAA	45%	55.25	60	21

To optimize sensitivity for nucleotide difference detection, PCR product size was targeted at 100 bp; larger products would reduce the sensitivity of the method. The primers were examined under standard conditions to ensure they did not form secondary structures such as hairpins or dimers. CpG island sequences were retrieved from the UCSC genome browser. The *MCPH1* gene likewise has a promoter on the chr8:6406062-6406801 CpG island. Primers were designed and validated by employing online platforms, including Meth Primer, Beacon Designer, IDT OligoAnalyzer, Bisearch, and DNA-utah.org. The *MCPH1* gene primer was designed to target the first CpG island, which included 72 to CG.

Methylation Sensitive-High Resolution Melting (MS-HRM) analysis

MS-HRM is an efficient and precise technique used to analyze the methylation status of DNA by comparing the melting profiles of unknown samples to profiles from DNA with known methylation levels, known as standards. In order to accurately determine the percentage of DNA methylation in unknown samples, a standard curve was established for each gene analyzed.

Methylation standards

Human fully methylated (100%) and unmethylated (0%) control DNA (EpiTect Human Control DNA, bisulfite converted, Qiagen, Germany) were used as the standards. To generate a series of DNA standards with varying methylation levels, unmethylated and fully methylated DNA of equal concentrations were mixed in different ratios (0%, 10%, 25%, 50%, 75%, and 100%) to simulate DNA samples with known methylation percentages. Each assay included these standard curves to estimate the methylation percentage in the promoter regions of the genes under investigation.

Polymerase chain reaction (PCR) and MS-HRM optimization

Bisulfite-converted DNA was used for MS-HRM analysis. The optimization aimed to identify the most suitable primer pairs and PCR cycling conditions for analyzing the differentially methylated regions of interest. PCR amplification and HRM analysis were performed on a StepOne Plus Real-Time PCR system (Applied Biosystems, Life Technologies). Each 20 µL reaction mix contained 20 ng of bisulfite-modified control DNA standards, with the following final concentrations: 1x MeltDoctor HRM Master Mix (Applied Biosystems, Life Technologies), 0.25 µM forward primer, 0.25 µM reverse primer, and deionized water. The cycling conditions were as follows: an initial step of 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and an annealing/extension temperature for 60 seconds. Melt curve analysis was performed from 60°C to 95°C. HRM analysis was conducted according to the manufacturer's recommended temperature ramping and fluorescence acquisition settings. Data were analyzed using High-Resolution Melt Software v2.0 (Applied Biosystems, Life Technologies). All DNA samples from participants were analyzed in duplicate per PCR reaction on a

96-well plate, which also included reference methylated DNA standards, a no-template control (NTC), and a negative control (unconverted unmethylated DNA).

Statistical analysis

The statistical analyses were performed using SPSS statistical software. In all statistical analysis, p-value less than 0.05 was evaluated as meaningful. The statistical comparison between groups was performed using a two-tailed independent t-test with Welch's correction to assess the significance of differences between the variables. The correlation between gene-specific DNA methylation percentages and variables examined using Pearson's correlation coefficient. Statistical significance was determined by the p-value.

Results and Discussion

Results

Patient and tumor characteristics

In this study, a total of 47 prostate cancer tissue samples, along with normal adjacent tissue and 10 benign prostatic hyperplasia (BPH) tissue samples, were analyzed. A summary of the patients' characteristics is presented in the table 2. The mean age of the participants was 65.4 ± 8.05 years. Approximately 18% of the patients had a Gleason score greater than 8. Prostate cancer was observed in both lobes of the prostate in more than 54% of the patients (Table 2). The mean con-centration of the samples was 249.1 ± 190.55, with an absorption ratio of 260/280 of 0.085 ± 1.80, and a 230/260 ratio of 2.06 ± 0.11 (Table 3).

Table 2: Summary of statistical analysis of the samples' features by SPSS.

Feature	Number	Mean or Percentage
Specimen		
Adenocarcinoma	47	45.19%
Normal adjacent tissue	47	45.195
BPH	10	9.61%
Gleason Score		
3+3=6	9	15.8%
3+4=7/4+3=7	28	49.12%
3+5=8/4+4=8	5	8.77%
4+5=9	4	7%
5+5=10	1	1.8%
Descriptive Statistics		
Age	57	65.4211 ± 8.04662
PSA level	57	11.4383 ± 10.65953
PSA free	57	1.2277 ± 1.29913
RPS13T	57	2.7692 ± 1.06987
RPS13C	57	2.8095 ± 1.07792
Tumor involvement		
Both lobe	31	54.4%
Left lobe	13	22.8%
Right lobe	3	5.3%
Perineurial invasion		
Negative	5	8.8%
Positive	41	71.9%
Prostatic intraepithelial neoplasia (PIN)		
Negative	5	8.8%
Positive	42	73.7%

Apex and Base		
Free	37	64.9%
Involved	10	17.5%
Circumferential Margins and Capsule		
Free	44	77.2%
Invades to capsule, margins free	3	5.3%
Seminal vesicles		
Free	43	75.4%
Involved	4	7%

Table 3: Mean concentration of samples, absorption 260/280, and absorption 230/260.

	Mean
Concentration	249.1 ± 190.55475
280/260	0.08514 ± 1.80385
230/260	2.05893 ± 0.11177

MCPH1 gene methylation levels

Methylation levels of the *MCPH1* gene were assessed across the three tissue types. As shown in Figure 1, the prostate cancer tissues exhibited significantly higher methylation levels compared to both normal adjacent tissue and BPH tissues. The mean methylation percentage in prostate adeno-carcinoma tissues was 45.19%, while normal adjacent tissues and BPH tissues had mean methylation levels of 45.20% and 9.61%, respectively.

MCPH1 gene methylation status in different groups

The Figure 1 shows the *MCPH1* gene methylation status in different groups of malignant tumors, surrounding normal tissue, and BPH. As seen, the level of methylated samples in the malignant tumor group is higher than in normal and BPH, and a significant correlation (p-value<0.001) was found between the 3 groups regarding the methylation level, so the methylation level in normal tissue and BPH tissue is lower than in the malignant tumor tissue

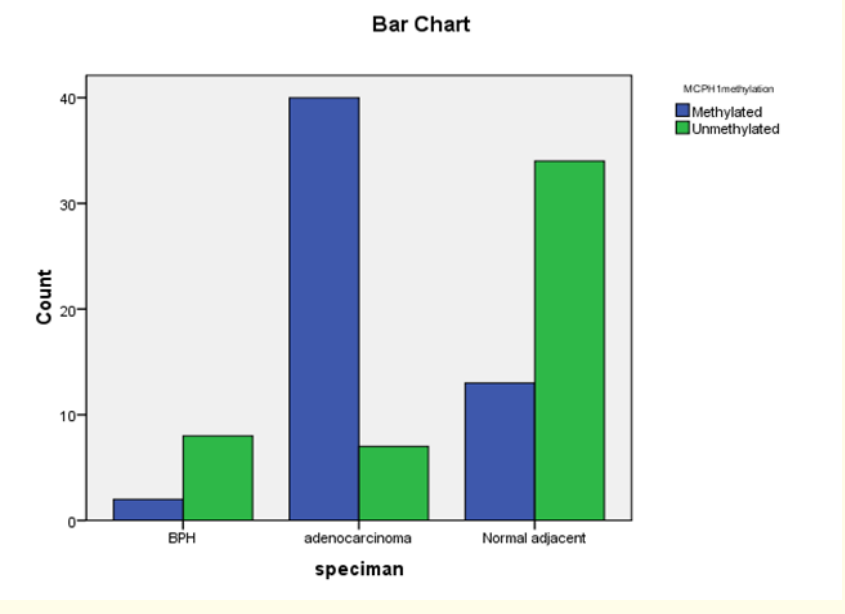

Figure 1: MCPH1 gene methylation column in 104 different samples of malignant tumor, normal surrounding tissue, and BPH.

shown in Figure 1. The results revealed no significant relationship between prostate cancer samples with free PSA regarding *MCPH1* gene methylation status (p-value: 0.3).

Relationship between methylation status of samples and Gleason score

We also examined the relationship between *MCPH1* gene methylation and the Gleason score in prostate cancer tissues. As illustrated in Figure 2, a positive correlation was observed between higher methylation levels and more aggressive Gleason scores. The highest methylation levels were observed in prostate cancer samples with Gleason scores of 8 and above (mean methylation: 52.3%). In contrast, samples from patients with a Gleason score of 6 or 7 showed significantly lower methylation levels (mean methylation: 41.2%).

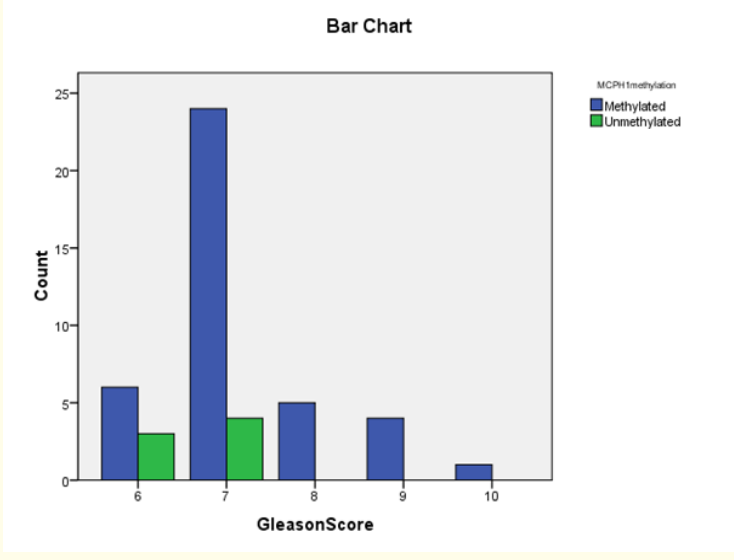

Figure 2: Investigating the relationship between MCPH1 gene methylation status in malignant tumor samples and Gleason score.

Relationship between the age of the samples and different groups

After investigating the age of the samples in three different groups, no significant difference was observed between them regarding their age (P-value: 0.9). The mean age and standard deviation of normal tissue, malignant tumor, and BPH groups are 65.36+6.552, 65.70+13.540, and 65.36+6.552, respectively.

Relationship between methylation status of samples and lobes involved in prostate cancer

The effect of tumor involvement in one or both prostate lobes on *MCPH1* methylation was also analyzed. Figure 3 shows that prostate cancer samples from patients with tumor involvement in both lobes had higher *MCPH1* methylation levels (mean methylation: 50.1%) compared to those with involvement in only one lobe (mean methylation: 41.4%). Figure 3 shows the relationship between *MCPH1* gene methylation status in malignant tumor samples of patients in which only the left lobe was affected by the tumor, only the right lobe was affected by the tumor, and both lobes were affected by the tumor. Interestingly, all the samples were in the methylated status regarding the *MCPH1* gene in people in which both lobes are involved in malignant tumors and have metastasized to the side lobe, and a significant relationship was observed between the involvement of both lobes and the methylation of the *MCPH1* gene (P-value<0.001).

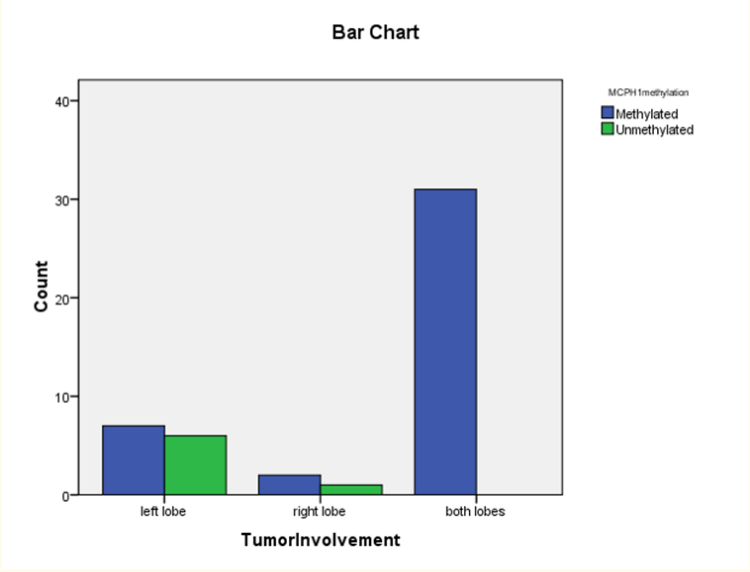

Figure 3: Investigating the relationship between the MCPH1 gene methylation status and lobes involved in malignant tumors in prostate cancer.

Discussion

In this study we indicate that the methylation status of the *MCPH1* gene is significantly higher in prostate cancer tissues compared to normal adjacent tissues and BPH samples. Our findings re-veal a statistically significant difference in methylation levels among the three groups ($p < 0.001$), with the lowest methylation observed in normal tissue and BPH samples, and higher methylation levels in malignant tumor tissues. These results align with previous studies that have shown elevated methylation of the *MCPH1* gene in various cancer types, suggesting that *MCPH1* gene silencing due to DNA methylation may play a critical role in cancer development and progression.

Interestingly, no significant correlation was found between *MCPH1* gene methylation and free PSA levels in prostate cancer samples (p-value = 0.3), contrasting with some previous reports that observed associations between DNA methylation and PSA levels. For example, Moses-Fynn., *et al*. identified a correlation between the methylation of certain genes and PSA levels in prostate cancer patients, though this association was absent in their cancer cohort [16]. Our study, focusing on tis-sue samples rather than serum, did not replicate this correlation, indicating that the relationship be-tween methylation status and free PSA may be more complex or tissue-specific.

The relationship between *MCPH1* gene methylation and the Gleason score was also explored, but no significant association was detected (p-value = 0.38). This is in contrast to other studies, such as those by Delgado-Cruzata., *et al*. who found a significant correlation between increased gene methylation and higher Gleason scores [17]. One possible explanation for the lack of correlation in our study may lie in the relatively small sample size, as previous research with larger cohorts, such as the study by Delgado-Cruzata., *et al*. reported stronger associations between methylation and Gleason scores. Additionally, the methodological differences, including the sensitivity of MS-HRM compared to Pyrosequencing used in other studies, might also contribute to the observed discrepancies.

In this study, methylation levels were significantly associated with tumor involvement in both prostate lobes, with higher methylation levels observed in samples from patients whose tumors

affected both lobes (p-value < 0.001). This finding suggests that *MCPH1* gene methylation might be linked to more advanced stages of prostate cancer, as tumors that affect both lobes typically indicate more aggressive disease. This is consistent with the notion that increased tumor burden and metastatic potential may drive epigenetic changes, including DNA methylation, which could serve as a marker of disease progression.

Our results also showed no significant correlation between *MCPH1* methylation and patient age, consistent with findings by Ghodsi*., et al*. in brain tumors [11]. While age is often associated with cumulative epigenetic changes, the lack of a clear association in this study might reflect the complex nature of age-related methylation changes in cancer, which may become evident only in larger, more diverse populations or in specific cancer types.

The dual role of *MCPH1* in regulating the cell cycle and acting as a tumor suppressor has been well-documented. For instance, studies have shown that low expression of *MCPH1* is associated with increased metastasis in cancers like breast cancer [18,19]. In our study, *MCPH1* methylation in prostate cancer tissues might reflect this gene's role in suppressing tumor progression. It's down-regulation through methylation could contribute to the aggressive nature of prostate cancer, as suggested by previous research on prostate cancer epigenetics [20].

The findings of this study provide valuable insight into the role of *MCPH1* gene methylation in prostate cancer, but further research is needed to better understand the clinical implications of these changes. The lack of a significant correlation with Gleason score or free PSA levels suggests that *MCPH1* methylation may not be a reliable standalone biomarker for prostate cancer diagnosis or prognosis. However, its role in disease progression, particularly in cases involving both lobes of the prostate, warrants further exploration, including studies that combine methylation data with other molecular markers to improve diagnostic accuracy and therapeutic targeting.

Future studies should investigate *MCPH1* gene methylation in larger patient cohorts and explore its potential as a biomarker for treatment response. Moreover, the impact of other methylation testing techniques, such as bisulfite sequencing and methylation-specific PCR, should be considered for comparison to MS-HRM in prostate cancer research.

Conclusion

This study provides compelling evidence that hypermethylation of the *MCPH1* gene is significantly associated with prostate cancer tissue in comparison to normal surrounding tissue and BPH samples. The markedly elevated methylation levels in malignant tumors ($p < 0.001$) highlight the potential role of *MCPH1* as a tumor suppressor gene that may be epigenetically silenced during prostate cancer progression. Although a direct correlation with Gleason score was not statistically significant, a clear trend of increased methylation in higher-grade tumors was observed, suggesting a possible link to disease aggressiveness. Furthermore, the strong association between *MCPH1* methylation and bilateral lobe involvement supports its potential involvement in advanced disease stages.

Importantly, the lack of association with patient age and PSA levels underscores the specific relevance of *MCPH1* methylation as an independent molecular event in prostate tumorigenesis. These findings position *MCPH1* gene methylation as a promising epigenetic marker for prostate cancer detection and disease monitoring. However, validation in larger cohorts and with complementary methodologies is necessary to confirm its diagnostic and prognostic utility. Future investigations integrating *MCPH1* methylation with other molecular markers may pave the way toward more refined and individualized approaches in prostate cancer diagnosis and management.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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