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Original article

Expression and clinical significance of CPS1 in glioblastoma multiforme

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ABSTRACT

Carbamoyl phosphate synthetase-1 (CPS1), the first rate-limiting mitochondrial enzyme in the urea cycle, regulates proliferation and differentiation during tumor progression. However, the detailed function of CPS1 in glioblastoma Multiforme (GBM) is still unclear. Here, we highlight mechanisms for CPS1 upregulation and the effects of upregulated CPS1 on GBM tumorigenesis. The transcriptome data from several public databases, such as Oncomine and GEPIA, revealed that CPS1 transcriptional level was significantly upregulated in GBM tissues and cells. Moreover, CPS1 was hypomethylated in GBM tissues. The Wanderer database, linked to the Cancer Genome Atlas (TCGA), showed the association between CPS1 expression or its methylation values and the clinicopathological parameters in GBM patients. Our work fully demonstrated that CPS1 expression was upregulated in GBM and this gene could be used as a potential diagnostic and prognosis indicator for GBM.

Introduction

Glioblastoma multiforme (GBM), highly malignant brain tumors with frequent genetic and epigenetic alterations, is a serious threat to human health [1]. The incidence of GBM is 3.19 per 100,000 person-years, and the 5-year survival rate is only 4–5% because of poor prognosis [2]. Currently, some factors, like high mean age, tumor location and limited understanding of tumor pathophysiology, always result in unsatisfactory treatment of GBM [3,4]. Thus, search for new targeted molecular markers is imminent for improving the treatment and prognosis of GBM.

Carbamoyl phosphate synthetase 1 (CPS1) is the first rate-limiting mitochondrial enzyme in the urea cycle [5]. Studies have found that up-regulation of CPS1 significantly predicts adverse reactions to neoadjuvant concurrent chemoradiotherapy for locally advanced rectal cancer [6,7]. In addition, CPS1 was down-regulated in hepatocellular carcinoma treated with aflatoxin B1, an effective liver cancer-producing mycotoxins [8]. Furthermore, Pham-Danis C et al. recently found that knockdown of CPS1 with EGFR inhibition could reduce the proliferation of non-small lung cancer cell and prevent cell cycle progression [9]. However, the specific roles and mechanisms of CPS1 in GBM were known limited.

The purpose of our study was to understand the function of CPS1 in GBM and its relationship to clinical prognosis. Here, we used an integrative computational method to identify upregulated CPS1 in GBM tissues and cell lines using data from several bioinformatics databases. The methylation values of CPS1 were also confirmed in clinical colon cancer tissues.

Materials and methods

Data collection and reanalysis of different bioinformatics databases

The bioinformatics network resources, summarized in Table S1, were used to evaluate the detailed roles of CPS1 in GBM tissues and cell lines.

We used two cancer microarray data-mining platforms, Oncomine [10] and Gene Expression Profiling Interactive Analysis (GEPIA) [11], to analyze the expression of CPS1 in GBM tissues. Another database, Encyclopedia of cancer cell lines (CCLE) [12], was used to evaluate the CPS1 expression in GBM cell lines. Through analyzing the data from these public bioinformatics platforms above-mentioned, we clearly understood the expression profiles of CPS1 in human GBM tissues and cell lines.

By reanalyzing data from the Cancer Genome Atlas (TCGA), Wanderer [13] and UALCAN [14] databases could display the

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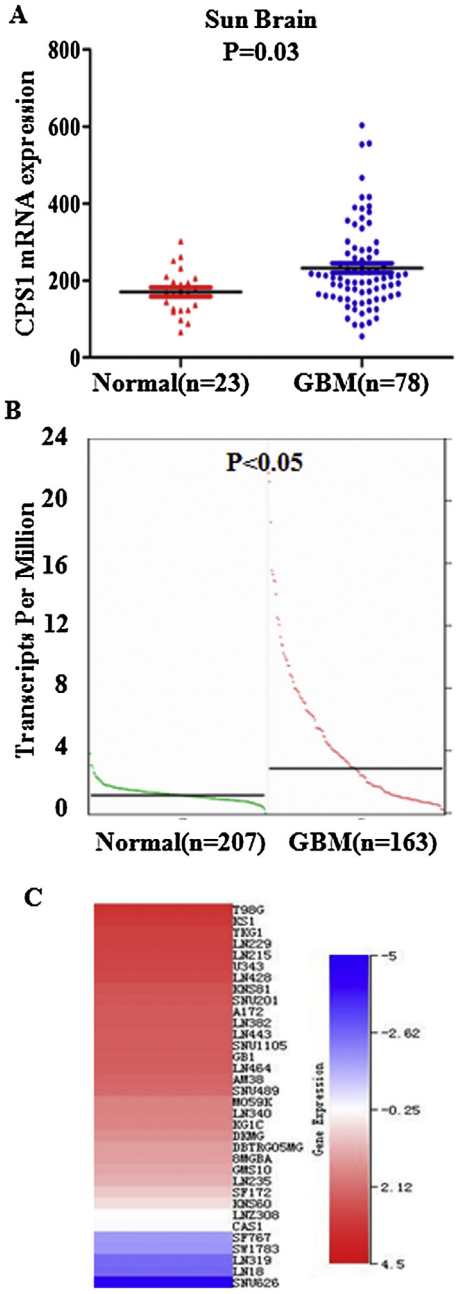

Fig. 1. Analysis of transcriptional level of CPS1 in GBM tissues. (**A**) Expression level of CPS1 in Sun Brain dataset from Oncomine platform. **(B)** The mRNA expression of CPS1 was detected from the GEPIA. (C) Expression level of CPS1 protein in GBM cell lines from CCLE database.

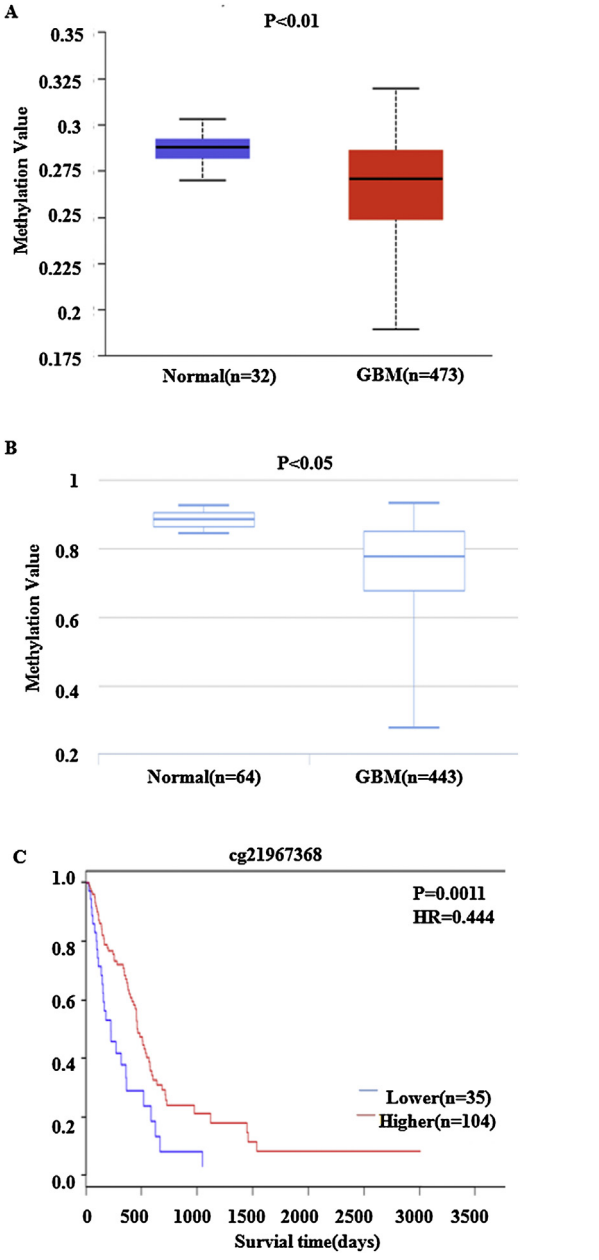

Fig. 2. The relationship between CPS1 methylation and the clinical characteristics of GBM patients. (**A**) Global CPS1 methylation in GBM samples compared with the normal samples through UALCAN database. (**B**) Global CPS1 methylation expression levels were analyzed using the DiseaseMeth database. (**C**) The impact of methylation values of cg21967368 on OS of GBM patients analyzed by the MethSurv web tool.

expression and DNA methylation profiles of candidate biomarkers in human cancers. The DiseaseMeth database is a web based on the aberrant methylomes of human diseases [15]. Then, we used Wanderer to screen for possible methylation values of CPS1 and analyze the association between clinical characteristics of GBM patients and CPS1 expression and methylation values. Using MethSurv, a web tool for survival analysis, we analyzed the relationship between CPS1methylation values and GBM prognosis [16].

The differential co-expression genes of CPS1 in GBM were downloaded from cBioPortal, a web resource for exploring multidimensional cancer genomics data [17]. STRING is a database that collects and integrates known and predicted protein-protein associations from many organisms, including direct (physical) and indirect (functional) interactions. We used STRING database to construct a protein-protein interaction network (PPI) of co-expressed genes [18] and visualized by the cytoscape software, a tool that visually integrates networks with expression profiles, phenotypes, and other molecular states [19].

In order to have a more comprehensive understanding of the biological functions of CPS1 in GBM, we have done a detailed functional analysis of CPS1. Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were conducted by the web-based GEne SeT AnaLysis Toolkit (WebGestalt) [20] and The Database for Annotation, Visualization and Integrated Discovery (DAVID) [21], respectively.

Statistical analyses

Student's *t*-test and statistical package SPSS 12.0 (IBM Analytics) were used to analyze the differential expression of CPS1 between cancer and non-cancer tissues. Meanwhile, the relationship between CPS1 expression and clinicopathological features in GBM patients was analyzed by chi-square test. Correlations between genes were evaluated using Pearson's correlation coefficient. $P < 0.05$ was considered to be statistically significant.

Results

CPS1 was a cancer-promoting biomarker in GBM samples

We used multiple independent databases to evaluate the expression profiles of CPS1 between GBM tissues and non-tumor tissues. First, a microarray dataset, Sun Brain, acquired from Oncomine platform demonstrated that CPS1 expression level was obviously up-regulated in tumor samples (Fig. 1A). Similarly, using GEPIA database, we further confirmed the up-regulated trend of CPS1 in GBM tissues (Fig. 1B). Moreover, the raw data downloaded from CCLE database was performed as a heatmap showing the elevated CPS1 expression level in several GBM cell lines (Fig. 1C). In conclusion, CPS1 plays an oncogenic role in GBM tissues and cell lines.

Relationship between CPS1 methylation and clinical characteristics in GBM patients

It is known to all that there is a negative correlation between DNA methylation and gene expression level [22–24]. UALCAN and the DiseaseMeth database were used to find that CPS1 exhibited global hypomethylation levels (Fig. 2A-B), further indicating the upregulated CPS1 in GBM. Subsequently, we evaluated the methylation site values of CPS1 on the prognosis of GBM patients. In the MethSurv database, we found that the GBM patients with lower methylation level of cg21967368 showed the shorter overall survival (OS) ($P = 0.0011$) (Fig. 2C). Then, the relationship between cg21967368 methylation value and clinical features of GBM patients was examined. As shown in Table 1, the methylation value of cg21967368 was significantly associated with age at initial pathologic diagnosis ($P = 0.047$). Taken together, these data revealed that the methylated CPS1 might be involved in the progression of GBM.

Table 1
The association between cg2196736 methylation value and clinical characters in GBM patients.

Clinical Characters	Population	MEAN ± SD	P-Value
Gender			0.187
Male	64	0.08±0.06	
Female	49	0.10±0.09	
Vital status			0.216
Alive	54	0.11±0.09	
Dead	59	0.08±0.05	
Age at initial pathologic diagnosis			**0.047**
≤60	56	0.08±0.049	
>60	56	0.11±0.09	
Race			0.168
White	93	0.10±0.08	
Blank or African American	1	0.07±0.03	
Primary therapy outcome success			0.755
Complete Response	1	0.06±0	
Partial Response	1	0.05±0	
Progressive Disease	3	0.10±0.06	
Stable Disease	2	0.07±0.00	

Functional enrichment analysis of CPS1-associated coexpression genes

The co-expressed differential genes of CPS1 were downloaded from the cBioPortal database and 256 co-expressed differential genes were screened out by using a criteria of $p \leq 0.05$ and $|LogFC| \geq 0.5$ (Table S2). A PPI network was then constructed using STRING and cytoscape (Fig. 3A). Furthermore, we performed a GO annotation through WebGestalt to identify the major biological processes (biological regulation), cellular components (membrane) and molecular functions (protein binding) associated with CPS1 biology (Fig. 3B). Finally, phosphoinositide 3-kinases (PI3K) - protein kinase B (Akt) signaling pathway was the more preferred KEGG pathways obtained from DAVID database (Table 2).

Discussion and conclusion

Our study used several public databases to discover that CPS1 could be used as a proto-oncogene in GBM occurrence and development. Additionally, the CPS1 methylation level was lower in GBM tissues, and has a negative effect on the patients' prognosis. Moreover, we also analyzed the differential co-expression genes of CPS1 and found the possible signaling pathways using WebGestalt and DAVID algorithms.

The urea cycle signaling pathway can be regarded as supporting processes to sustain increased metabolic processes to meet the high ureagenesis and biogenetic demands of tumor cells. CPS1 has been proved to be the major mitochondrial rate-limiting enzyme in the urea cycle [25,26]. Currently, there is a large body of literatures indicating that CPS1 in human cancer cells specifies aggressive oncological behavior. CPS1 loss reduces the pyrimidine to purine ratio, inducing cell death and reducing tumor growth in lung cancer cells [27]. On the contrary, CPS1 expression appears to be down-regulated in liver cancer tissue and cell lines [28]. DNA sequencing analysis also identified significantly downregulated and hypermethylated CPS1 in hepatocellular carcinoma tissues [29]. However, there are no clear reports on the roles of CPS1 in GBM biology. Considering the possible bias in a single dataset, we used different databases to analyze the methylation of CPS1 from different datasets and its clinical significance. Although the sample size of each sample set is different, the analysis results of individual databases are very similar, indicating that CPS1 in GBM patients is consistently hypomethylated. Through the comprehensive bioinformatics analysis, we first clarified that CPS1 is significantly upregulated and hypomethylated in GBM tissues and cells.

After visualizing the co-expressed gene using cytoscape, we found that CPS1 is closely related to epidermal growth factor receptor (EGFR), matrix metalloproteinase 2 (MMP2) and O6-methylguanine-DNA methyltransferase (MGMT). EGFR has been identified as the first receptor tyrosine kinase (RTK). And studies have shown that EGFR is significantly overexpressed or mutated in many cancers, particularly in breast, ovarian and non-small cell lung cancers [30]. In addition, the genomic analysis using sequencing techniques has identified EGFR gene alterations in more than half of GBM, such as the amplification and mutation of EGFR [31]. MMP2 has been reported as a promising biomarker in a variety of cancers and plays an important role in cancer progression [32]. Some scholars have found that the expression of MMP2 plays a crucial role in the invasion of GBM [33]. MGMT is commonly overexpressed in cancer and is associated with the development of chemoresistance [3]. Many studies have shown that MGMT is closely related to the prognosis of GBM, and patients with MGMT methylation have better OS than patients with unmethylated GBM [34]. Therefore, combined with the results of Fig. 3A, CPS1 may be involved in the occurrence and development

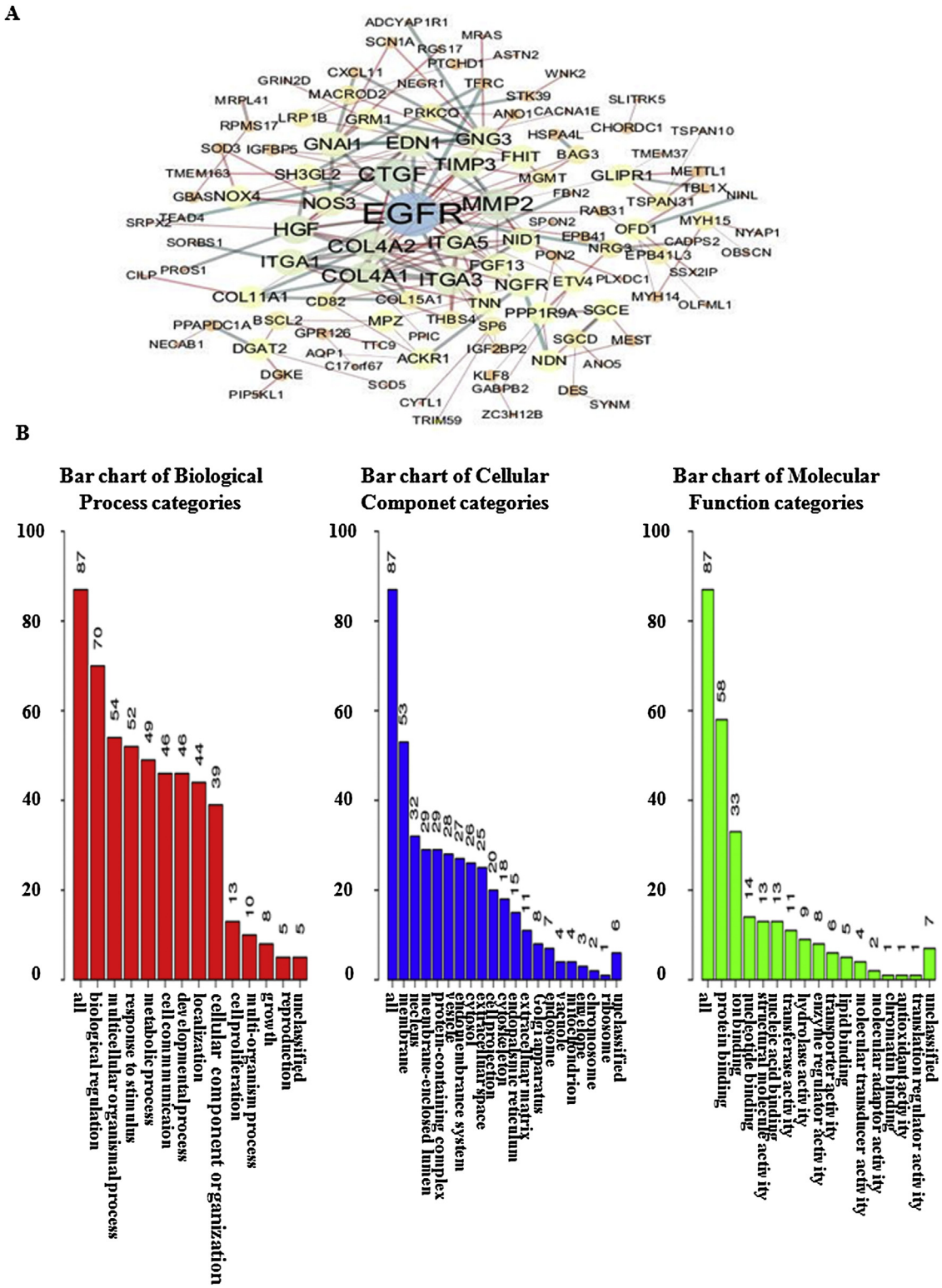

Fig. 3. Co-expression network analysis of CPS1. **(A)** The PPI of CPS1 associated co-expressed genes were constructed by STRING and cytoscape. **(B)**Main molecular functions, biological processes and cell components related to CPS1 were identified by WebGestalt.

of GBM in synergy with these molecules, and may be related to the prognosis of GBM.

In addition, KEGG analysis of CPS1 co-expressed genes showed that the main enriched biological pathway was the PI3K-Akt signaling pathway. Activated Akt induced by mitogen-activated protein kinase 4 (MAPK4) overexpression could induce oncogenic outcomes, including promoting anchorage-independent growth [35]. Overexpressed stearoyl-coenzyme A desaturase 1 confers chemotherapy resistance to GBM cells via enhancing Akt signaling axis [36]. Taken together, these findings suggest that elevated levels of CPS1 might stimulate GBM progression through PI3K-Akt signaling pathway.

Table 2
KEGG pathways associated with CPS1 co-expressed genes in GBM.

Term	Count	P-Value	Genes
hsa04151:PI3K-Akt signaling pathway	15	1.22E-05	EGFR, COL4A2, COL4A1, ITGA1, ITGA3, FGF13, HGF, ITGA5, NOS3, TNN, GNG3, NGFR, THEM4, COL11A1, THBS4
hsa04512:ECM-receptor interaction	8	3.36E-05	COL4A2, COL4A1, ITGA5, ITGA1, TNN, ITGA3, COL11A1, THBS4
hsa04510:Focal adhesion	10	3.00E-04	EGFR, COL4A2, COL4A1, ITGA5, ITGA1, TNN, ITGA3, HGF, COL11A1, THBS4
hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	5	0.005405	DES, ITGA5, ITGA1, SGCD, ITGA3
hsa05410:Hypertrophic cardiomyopathy (HCM)	5	0.009219	DES, ITGA5, ITGA1, SGCD, ITGA3
hsa05414:Dilated cardiomyopathy	5	0.011893	DES, ITGA5, ITGA1, SGCD, ITGA3
hsa04915:Estrogen signaling pathway	5	0.020614	EGFR, GNAI1, NOS3, GRM1, MMP2
hsa04810:Regulation of actin cytoskeleton	7	0.023507	EGFR, ITGA5, MRAS, DIAPH2, ITGA1, FGF13, ITGA3
hsa04924:Renin secretion	4	0.030117	GNAI1, ADCYAP1R1, PDE3A, AQP1

In conclusion, our results indicated that CPS1 was a candidate tumor proto-oncogene for human malignant GBM. The results also showed that CPS1 might be a potential target for GBM therapy. This study method based on a comprehensive analysis of public databases provided new ideas for finding genes related to human malignant diseases.

Author contributions

Acquisition of Data: GT Wu, YL Yan, X Wang.
Analysis and Interpretation of Data: J Wei, X Chen.
Conception and Design: JH Zhou, ZJ Xu.
Data Curation: W Lin, CL Ou, YY Zhou.
Development of Methodology: YL Yan, ZJ Xu.
Writing the Manuscript: GT Wu, YL Yan, ZJ Xu.

Declaration of Competing Interest

No potential conflicts of interest were disclosed.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.retram.2019.08.003.

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