

ORIGINAL RESEARCH ARTICLE

Cyclin-Dependent Kinase 8 (CDK8) Immunoexpression in Uveal Melanoma

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Abstract: Background: Cyclin-dependent kinase 8 (CKD8) is a component of the mediator complex, which regulates the gene transcription of nearly all RNA polymerase II-dependent genes. It is known to be overexpressed in more aggressive cutaneous melanomas, but no data exist for its uveal counterpart. **Methods:** Retrospective study on human UM tissue, performing CDK8 immunostaining ($n=19$). For analysis, a scale of 0-3 staining intensity was used along with an extent score, the percentage of positively staining cells (0-100%), and the confirmed pathological stages. **Results:** No significant change was seen in the CDK8 staining patterns between the different pathologic stages, but there was a trend of higher pathological stage showing lower staining intensity score between the stages ($P=0.06$). **Conclusions:** Lower CDK8 immunoreactivity was associated with a trend for higher grade UM, highlighting the possible tumor suppressor role of CDK8 in UM advancement.

Keywords: Uveal melanoma; Ocular melanoma; Cyclin-dependent kinase 8 immunoexpression; Ocular oncology

1. Introduction

Uveal melanoma (UM) is a malignant entity of the choroid, ciliary body, or iris with the choroid being the most common location. UM is a relatively rare cancer with 5-6 cases per 1 million people per year in the United States, with a predilection for white adults with a mean age of 60 years^[1]. The prognosis of UM is poor even after surgery, with liver metastasis occurring in approximately 50% of patients up to 10-15 years later^[2]. Of patients diagnosed with primary UM approximately 20-30% die of systemic metastases within 5 years of diagnosis and 45% die within 15 years^[3]. Genetic etiologies related to the development of UM include mutations in breast cancer gene 1-associated protein 1 (BAP1), guanine nucleotide-binding protein G(q) subunit alpha gene (GNAQ) and Guanine nucleotide-binding protein subunit alpha-11 gene (GNA11), besides others^[1,4]. Monosomy 3 is frequently correlated with high metastatic risk^[5].

MacroH2A expression has been shown to have an inverse relationship with

cyclin-dependent kinase 8 (CDK8) in cutaneous melanoma^[6]. CDK8 is a coactivator in the mediator complex involved in gene transcription regulation of many RNA polymerase II-dependent genes. It is not directly involved with the cell cycle, however, it indirectly acts to regulate it. Increased CDK8 expression has been associated with various malignancies including breast cancer, colon cancer, acute myeloid leukemia, and myeloproliferative neoplasms^[7]. Cutaneous melanomas have been shown to have decreased levels of macroH2A, and overexpression of CDK8, which is believed to be important in tumor progression. Conversely, in the eye, UM tumors with increased malignancy and progression have overexpression of macroH2A with an unknown expression of CDK8^[8].

This study aims to quantify CDK8 immunoreactivity in human UM tumor tissues of different pathological stages to elucidate the role of CDK8 in the pathogenesis of melanoma, and to possibly offer a new therapeutic target for the disease.

2. Data and methods

2.1. Patient Selection

A retrospective study was performed on 19 UM tumor tissues. All the samples were from enucleations, with patients not eligible for radiotherapy by local therapeutic guidelines^[9]. The Institutional Review Board Committee ruled that approval was not required for this study.

2.2. Study Design

The cases were retrieved from the archives of the Anatomic Pathology Department, Loma Linda University, and the California Tumor Tissue Registry. Several cases were excluded from the study because they either: did not have representative tumor tissue, were incompletely excised, or pre-operatively treated. Paraffin-embedded UM tissue blocks were retrieved from Loma Linda University Medical Center from 2009 to 2020, and from the California Tumor Tissue Registry from 1972 to 1974. No written informed consent was required because of the retrospective nature of the study.

2.3. Histology

Four μ m paraffin-embedded tissue sections were used in our study. We made one hematoxylin and eosin (H&E)

and one CDK8 immunostained slide from each block. Human colorectal adenocarcinoma tissue (4 μ m, paraffin-embedded sections) served as positive controls for our CDK8 staining. The H&E staining was performed by hand (Richard-Allan Scientific, Kalamazoo, MI, USA). For our immunohistochemical study, the slides were stained in the Leica Bond III (Leica Biosystems, Vista, CA, USA) automated stainer using the following: anti-CDK8 antibody (Abcam, Cambridge, USA; rabbit polyclonal, ab124218; 1:500), BOND epitope retrieval solution 1 (Leica Biosystems, Vista, CA, USA), rabbit secondary antibody (Leica Biosystems, Vista, CA, USA), red chromogen (Leica Biosystems, Vista, CA, USA) with a pH of 6.0 setup for the heat-induced epitope retrieval stage.

2.4. Analysis

The tumor location and size were evaluated by ophthalmoscopy and ultrasonography. The tumors were staged using the American Joint Commission on Cancer TNM Classification of tumors of the eye^[10]. CDK8 immunopositivity was called positive if red chromogen staining was visible in the nucleus. Human colorectal adenocarcinoma tissue with pathological T stage 3 served as the positive control.

The H&E samples were used to re-assess morphology. For immunostaining, the intensity of staining (IS) was evaluated by light microscopy (Olympus, Center Valley, PA, USA) using 100 \times objective, and counting 200 cells at least in each tumor tissue. We used two scoring systems. In the first, we simply scored the nuclear staining intensities as follows: 0=no staining; 1=weak, 2=moderate, 3=strong (background melanophage cytoplasmic staining was not included). Our other scoring system with extent score (ES) used five levels of staining intensities, using the proportion of CDK8 positive nuclei/all nuclei as follows: <5% = 0; 5-30% = 1; 31-50% = 2; 51-75% = 3; and >75% = 4. Using these scores, an intensity reactivity score (IRS) was calculated by multiplication of the intensity of the staining (IS) and the percentage of positive cells (IS x ES): when the IRS was ≤ 6 , CDK8 expression was considered to be “low” (L-IRS), while an IRS >6 was considered to be “high” expression (H-IRS).

The evaluation of immunohistochemical expression of CDK8 was performed separately by two pathologists (J.C.K. and J.K.D.), who were blinded to the patient's identity, clinical data, and group identification.

2.5. Statistical analysis

The median age of the patient, tumor thickness, largest diameter, location, extrascleral extension, and cell type were compared between positive staining and negative staining tumors using individual T-tests. The CDK8 IRS scores were compared in between the different stages of the disease (pT2, pT3, pT4), using two-way ANOVA. Factors with a *P*-value <0.05 were considered significant.

3. Results

3.1. Patient Demographics

The patients were 7 females and 12 males with a mean age of 65 years, with a range of 38-99 years. Of the 19 patients, 3 developed metastatic melanoma proven by a

combination of physical examination, computed tomography, and hepatic ultrasound. However, many patients were either lost to follow-up or continued their care and screening outside of the Loma Linda University Health Consortium system (Table 1).

3.2. Tumor cases

Thirteen melanoma tumors were found strictly in the choroid, while 6 were found involving both the choroid and the ciliary body. Extrascleral extension was found in 3 cases. Five cases were classified as epithelioid cell type, 2 cases as spindle cell type, and 12 cases as mixed cell type.

TNM staging in the 12 patients with positively staining UM tumors were as follows: pT2 (1 case, 8.3%), pT2a (1 case, 8.3%), pT2c (1 case, 8.3%), pT2d (1 case, 8.3%), pT3a (4 cases, 33.3%), pT3c (1 case, 8.3%), pT3d (1 case, 8.3%), pT4 (1 case, 8.3%), and pT4e (1 case, 8.3%). TNM staging in the 7 patients with negatively staining UM tumors were as follows: pT2 (1 case, 14.3%), pT2b (1 case, 14.3%), pT3a (1 case, 14.3%), pT4a (1 case, 14.3%), pT4b (2 cases, 28.6%), and pT4e (1 case, 14.3%) (Table 1).

Table 1. Patient demographics, tumor parameters, CDK8 expression parameters in primary uveal melanoma, including intensity of staining (IS), extent score (ES), intensity reactivity score (IRS), and low or high IRSs. Choroid (Ch), Ciliary body (CB), Mixed (Mx), Epithelioid (Ep), Spindle (Sp), Metastasis (M), Extrascleral extension (EE).

Sex	Age (years)	Location	Thickness (mm)	Largest Diameter (mm)	Cell Type	Pathological T Stage	IS	ES	IRS	IRS Low or High
F	38	Ch/CB	7	3	Mx	pT2b	0	0	0	L
F	42	Ch	6	10	Ep	pT2c	3	3	9	H
F	75	Ch	11	13	Mx	pT3a	1	2	2	L
F	73	Ch/CB	6	10	Mx	pT2d (M, EE)	1	3	3	L
F	60	Ch	9	11	Ep	pT3a	0	0	0	L
M	81	Ch	7	12	Ep	pT2a	3	3	9	H
M	99	Ch/CB	9	14	Mx	pT4e (EE)	0	0	0	L
M	65	Ch	17	19	Ep	pT4	1	2	2	L
M	83	Ch	11	12	Mx	pT3a	1	1	1	L
M	76	Ch	8	14	Mx	pT3a (M)	2	2	4	L
M	85	Ch	12	17	Sp	pT3a	1	2	2	L
M	59	Ch	9	12	Mx	pT2	0	0	0	L
M	62	Ch	13	18	Mx	pT4e (EE)	1	2	2	L
F	59	Ch	8	10	Ep	pT2	2	3	6	L
M	82	Ch/CB	9	20	Mx	pT4b	0	0	0	L
M	62	Ch	22	20	Mx	pT4a	0	0	0	L
M	45	Ch	4	12	Mx	pT3c	1	1	1	L
F	64	Ch/CB	9	13	Mx	pT3d (M)	1	2	2	L
M	68	Ch/CB	20	16	Sp	pT4b	0	4	0	L

3.3. Immunohistochemistry

The control tissue stained with CDK8 was colonic mucosal epithelial cells with higher intensity in higher stage colorectal adenocarcinomas. In the control, staining was predominantly seen in the nucleus with occasional, relatively milder staining in the cytoplasm. UM tumors with increased pigmentation showed heavy, nonspecific staining in melanophages. When intensity and extent

scores were evaluated, CDK8-staining melanophages were not included. The UM tumor samples showed variable CDK8 expression between different samples.

When the CDK8 positively staining groups were compared to the negatively staining groups, no significant differences were seen between these two groups in median age, location, largest diameter, tumor thickness, cell type, and extrascleral extension (Table 2).

Table 2. Medians and ranges of patient demographics, tumor parameters, and CDK8 intensity reactivity score. Choroid (Ch), Ciliary body (CB), Mixed (Mx), Epithelioid (Ep), Spindle (Sp)

	Sex m-f	Age (years)	Location	Thickness (mm)	Largest		Extrascleral Extension	Pathological T Stage	CDK8 IRS
					Diameter (mm)	Cell Type			
All (n=19)	12-7	65 (38-99)	Ch 13 Ch/CB 6	9 (6-22)	13 (3-20)	Ep: 5 Sp: 2 Mx: 12	Yes: 3 No: 16	pT2: 6 pT3: 7 pT4: 6	2 (0-9)
CDK8 Positive (n=12)	7-5	69 (42-81)	Ch 10 Ch/CB 2	9 (6-12)	12.5 (10-20)	Ep: 4 Sp: 1 Mx: 7	Yes: 2 No: 10	pT2: 4 pT3: 6 pT4: 2	2 (1-9)
CDK8 Negative (n=7)	5-2	62 (38-99)	Ch 4 Ch/CB 3	13 (7-22)	16 (3-20)	Ep: 1 Sp: 1 Mx: 5	Yes: 1 No: 6	pT2: 2 pT3: 1 pT4: 4	
P (CDK8 positive vs negative)		0.69	0.062	0.387	0.87	0.8	>0.99	0.06	

In UM tumors, CDK8 IS was intense/intermediate in 4 cases (21.1%) and mild in 8 cases (42.1%), while in 7 (36.8%) cases no immunoreactivity was observed (Figure 1).

ES was >50% in 4 cases (21.1%), 31-50% in 6 cases (31.6%), 5-30% in 2 cases (10.5%). The interobserver agreement was very high (Kappa = 0.894). Considering the whole group (n = 19), the median CDK8 value was 2: H-IRS was observed in 2 (10.5%) melanomas and L-IRS in 17 (89.5%).

Between pathological stage groups there was a tendency for lower CDK8 IRS in the higher stages of the disease (P=0.06) (Figure 1). Representative IHC images are shown in Figure 2.

Only three cases of metastasis were identified due to frequent change of providers and loss to follow-ups, therefore the tumor staining pattern in patients with or

without metastasis could not be included in our statistics.

**Mean Intensity Reactivity Score (IRS)
vs Stage Groups**

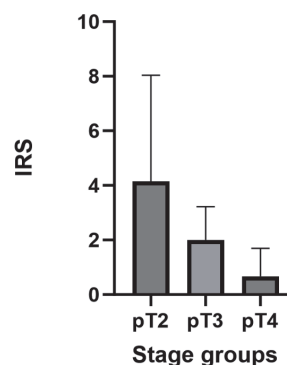


Figure 1. Mean intensity reactivity (MIR) score of each pathologic stage group. Two-way ANOVA P-value=0.06. Standard deviation (SD)

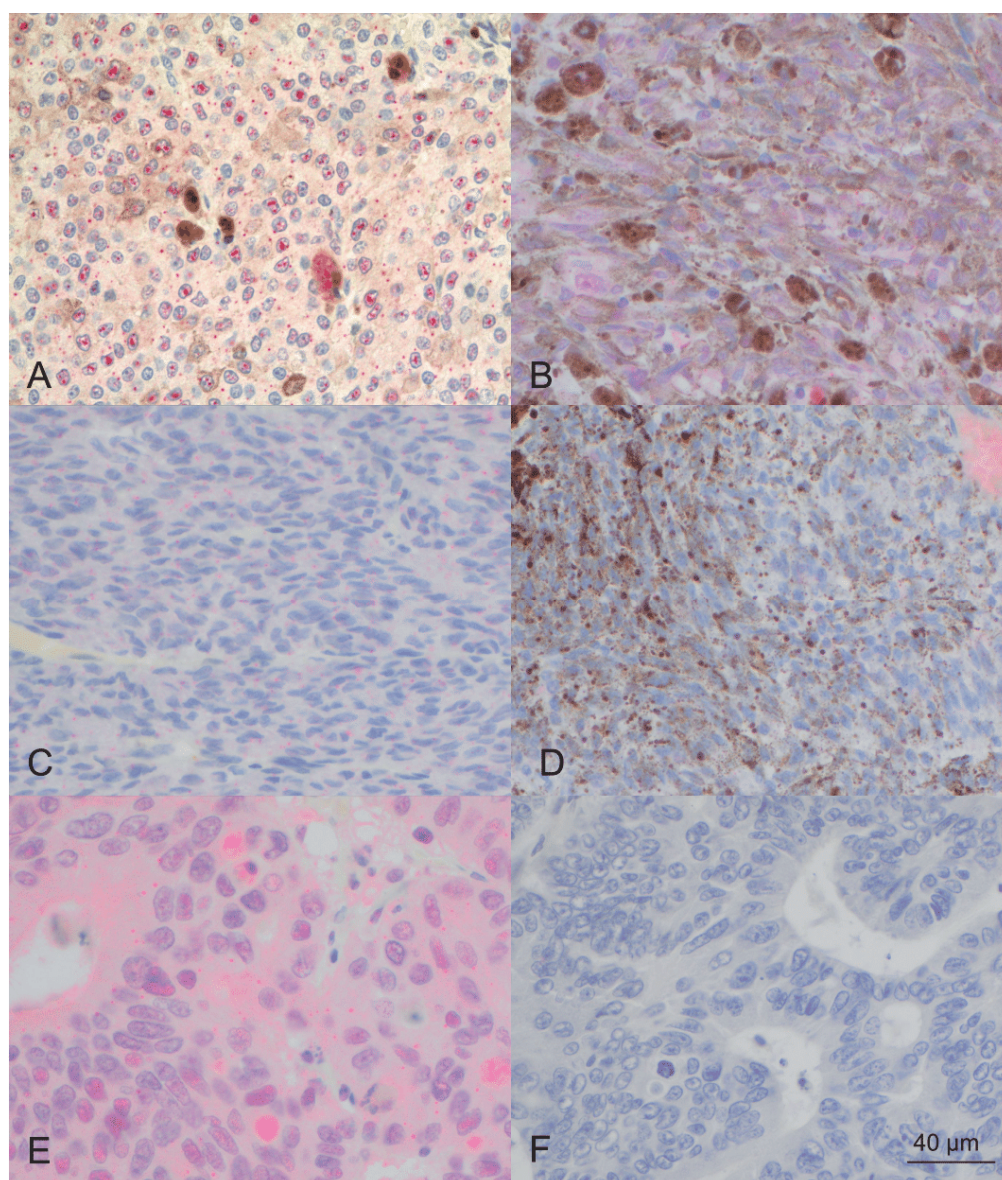


Figure 2. 2A: 6 mm thick tumor staining positively for CDK8 (40 \times). 2B. 4 mm thick tumor staining positively for CDK8 (40 \times). 2C. 20 mm thick tumor staining negatively (40 \times). 2D. 22 mm thick tumor staining negatively (40 \times). 2E. Positive control in colonic adenocarcinoma (40 \times). 2F. Negative control in colonic adenocarcinoma (40 \times).

4. Discussion

In the current study, investigating CDK8 immunoreactivity in UM, variable protein expression was seen in the individual cases studied. We found no significant difference in the immunoreactivity with age, tumor size, cell type or location; but there was a trend ($P=0.06$) of decreased CDK8 immunoreactivity in the higher stages of the disease. It is to note that UM is a rare malignancy, making the histologic studies, such as ours, somewhat limited due to the low number of cases

available. Our results indicate, however, that CDK8 is either not a significant factor in UM oncogenesis or that it might have a tumor suppressor function. Of note, no prognostic analysis could be done in our study due to limited follow-up information available.

As previously mentioned, CDK8 is a component of the mediator complex, which is a general coactivator of RNA polymerase II. The mediator complex is composed of 30 subunits in humans^[11] with four structural modules: the head and middle (together they form the core mediator/ cMed) interact with the RNA polymerase II;

the tail; and the CDK8 kinase modules (CKM) – latter containing CDK8^[12-18], whereas CDK8 phosphorylates the polymerase II C-terminal repeat domain^[19] and other transcription factors having both positive and negative effects on gene transcription^[20-24].

Specifically, CDK8 is known to regulate DNA synthesis and switching the cell cycle from G1 to S phase^[24]. Additionally, CDK8 modulates transcriptional elongation cooperatively with positive transcription elongation factor b^[25]. These functions of CDK8 are found in physiological processes, with its elevated levels being associated with tumorigenesis implicated in cutaneous melanoma, myeloid neoplasms, pancreatic-, colorectal-, and breast cancers^[4]. Also, CDK8 alters cellular metabolism, helping tumorigenesis and tumor progression^[23,26].

To put the changes in CDK8 expression in context of other previously studied markers in UM, we discuss the importance of BAP-1 and histone H2A in relation to this disease. *BAP-1* is known as a tumor suppressor that encodes a deubiquitination enzyme, regulating several key cellular pathways, and besides others, deubiquitinates histone H2A, resulting mostly in transcriptional activation, with evidence of its involvement in transcriptional repression^[27-32]. Consequently, BAP-1 regulates several physiological processes, and it is important in carcinogenesis. Importantly, a 2010 study from Harbour and co-workers reported inactivating somatic mutations in the encoding region of *BAP1* in approximately 84% of metastasizing uveal melanomas, suggesting that the *BAP1* functions as a tumor suppressor in UM^[33,34]. In addition, loss of BAP-1 immunoreactivity has been linked to an increased risk of metastasis in UM. Interestingly, even a focally decreased BAP-1 expression was associated with a more invasive behavior within the UM tissue^[34-38]. Interestingly, in cutaneous melanoma, loss of BAP-1 is associated with a clinically and morphologically distinct type of neoplasm^[30].

H2A is part of the large histone family that includes variants such macroH2A1 and macroH2A2, which can be associated with repression of chromatin transcription^[39,40]. The H2A variants, such as macroH2A1 and macroH2A2 can replace the

conventional H2A in nucleosomes, resulting in transcription repression^[41]. Past studies have correlated tumoral macroH2A2 expression with clinical pathological features^[42-44], suggesting that it can act both as an oncogene or a tumor suppressor, depending on the type of tumor and on the degree of stemness^[42-45]. As for its oncogenic role, macroH2A's downstream epigenetic effects were studied by Kapoor and co-workers in a murine skin melanoma cell line, who found that several genes increased more than two-fold with the loss of macroH2A, including *Integrin alpha 4*, *CDK8*, and *Cited1*^[42]. The same study group proved that an inverse relationship existed between macroH2A2 and *CDK8* mRNA levels^[42]. Supporting these results, melanoma cells with overexpression of macroH2A were shown to have increased levels of G2/M arrest leading to cell death by apoptosis. The tumor-promoting function of macroH2A loss was also shown to be mediated partially through direct upregulation of CDK8, increasing progression into mitosis without undergoing correct DNA synthesis^[42]. Kapoor *et al.* postulated that CDK8 induces progression in cutaneous melanoma by enhancing cell growth and migration^[46].

Conversely to cutaneous melanoma, it has been demonstrated that macroH2A1 protein expression is higher in more advanced UM^[5]. Similarly, the loss of macroH2A1 was shown to inhibit UM cell proliferation and aggressiveness^[47]. In addition, macroH2A1 loss was associated with impaired mitochondrial replication in UM patients^[47]. It is possible therefore that increased macroH2A in UM could alter tumor progression in a different way than in its cutaneous counterpart, such as via metabolic changes, or inhibition of cell cycle arrest in a different way than seen in cutaneous melanoma. Interestingly, the previously reported decreased BAP-1 expression in UM should decrease the deubiquitination of H2A in UM, meaning that the increased H2A protein expression may reflect increased amount, but nonfunctional protein, or more likely, it can result in an increased amount of functional H2A in UM, which participates in tumorigenesis in a different way than seen in cutaneous melanoma. This is still to be elucidated.

Furthermore, in UM, if the same regulatory

mechanism is upheld between macroH2A and CDK8 as in cutaneous melanoma, it is hypothesized that the CDK8 levels would be decreased in more malignant cases of uveal melanoma (Figure 3), which is in line with our current findings.



Figure 3. Previous data suggest that in advanced cutaneous melanomas, decreased macroH2A expression results in increased cyclin-dependent kinase 8 expression. Based on our results and previous data on macroH2A, the opposite is suggested, with increased macroH2A expression resulting in decreased cyclin-dependent kinase 8 expression.

Although CDK8 can potentially participate in UM tumorigenesis, it plays the role of a tumor suppressant in advanced disease. This clearly shows a different way of how UM is evolved and progresses compared to several other tumors, such as cutaneous melanomas. It is likely that in UM, similarly to endometrial carcinoma, CDK8 regulates tumorigenesis in a different way than in other tumors, such as cutaneous melanomas. This can be on the cell cycle regulation, cancer metabolism, or cancer metastasis level, and can be due to different driver mutations or tumor microenvironment and metabolism^[48-50]. In support of this, an *in vivo* mouse model showed that although *CDK8* loss by itself did not result in tumorigenesis, it shortened the animals' survival and increased the tumor size without tumor initiation in the *Apc^{Min}* intestinal tumor model. Interestingly, repressed EZH2 activity was associated with the accelerated tumorigenesis in the latter model of *CDK8* deletion, possibly by at least part via diminished β -catenin signaling^[51].

5. Conclusions

In conclusion, we found no significant difference in CDK8 immunoreactivity when compared the thickness, largest diameter, cell type, or extrascleral extension in our human UM tissue samples. There was, however, a tendency of lower CDK8 immunoreactivity in higher stages of the tumor. This latter is in line with a previous

study, showing increased macroH2A in UM, which usually has an inverse relationship with CDK8. The prognostic value of CDK8 could not be assessed in our study due to loss of long-term follow-ups. Targeted CDK8 inhibition, however, might be a therapeutic choice in the future in CDK8 positive, less advanced UMs.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: DMR, EAWG

Methodology: DMR, EAWG, JCK

Formal analysis: DMR, EAWG

Writing – original draft: DMR, EAWG, AMR

Writing – review & editing: DMR, EAWG, CH, JCK, JKD

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All original data can be requested by the corresponding author.

Further disclosure

None.

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