miR-1207-3p Is a Novel Prognostic Biomarker of Prostate Cancer[1]

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Abstract

MicroRNAs (miRNAs) have been found to be dysregulated in prostate cancer (PCa). In this study, we investigated if miR-1207-3p is capable of distinguishing between indolent and aggressive PCa and if it contributes to explaining the disproportionate aggressiveness of PCa in men of African ancestry (moAA). A total of 404 patients with primary adenocarcinoma of the prostate were recruited between 1988 and 2003 at the Moffitt Cancer Center, Tampa, FL, USA. Patient clinicopathological features and demographic characteristics such as race were identified. RNA samples from 404 postprostatectomy prostate tumor tissue samples were analyzed by real-time quantitative reverse transcription polymerase chain reaction for the mRNA expression of miR-1207-3p. miR-1207-3p expression in PCa that resulted in overall death or PCa-specific death is significantly higher than in PCa cases that did not. The same positive correlation holds true for other clinical characteristics such as biochemical recurrence, Gleason score, clinical stage, and prostate-specific antigen level. Furthermore, miR-1207-3p expression was significantly less in moAA in comparison to Caucasian men. We also evaluated whether miR-1207-3p is associated with clinical outcomes adjusted for age at diagnosis and tumor stage in the modeling. Using competing risk regression, the PCa patients with a high miR-1207-3p expression (≥ 6 vs 3) had a high risk to develop PCa recurrence (hazard rate = 2.5, $P < .001$) adjusting for age at diagnosis and tumor stage. In conclusion, miR-1207-3p is a promising novel prognostic biomarker for PCa. Furthermore, miR-1207-3p may also be important in explaining the disproportionate aggressiveness of PCa in moAA.

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Introduction

Prostate cancer (PCa), with nearly 240,000 men diagnosed in the United States in 2014, is a major cause of cancer morbidity and mortality [1–3]. The annual morbidity continues to steadily grow as the rate of this cancer has increased by 14% over the last two decades [3–5]. One of the challenges faced in combating PCa is its

considerable heterogeneity. PCa usually begins as being androgen dependent, and initially, most men are responsive to pharmacological and surgical androgen deprivation therapies [6]. However, in spite of these efforts, in a subset of patients, the cancer inevitably reoccurs and advances, with approximately 30% of men developing clinical recurrence [3]. Ultimately, it is this highly aggressive form of PCa that leads to death [6]. Therapeutic options for advanced PCa are currently limited and largely ineffective [7].

Furthermore, for reasons still unclear, men of African ancestry (moAA) have the worlds' highest incidence of PCa [8]. moAA have approximately two-thirds increased incidence and two times the increased mortality rate compared with Caucasian men (CM) [9,10]. Furthermore, moAA are more frequently diagnosed with worse clinicopathological features (prostate-specific antigen [PSA] levels, Gleason grades, advanced tumor stage, etc.) than CM [8,11,12]. To decrease the excess mortality, effective early detection and therapeutic strategies are required.

MicroRNAs (miRNAs) have potential as effective early detection and prognostic biomarkers in PCa [4]. miRNAs are small (18-22 nucleotides) nonprotein coding regulatory RNAs [13]. They are well conserved and endogenously synthesized and have been shown to regulate the expression of nearly 60% of human genes [13]. Many miRNAs have been reported to be implicated in cancer pathogenesis, functioning as either oncogenes or tumor suppressors according to the roles of their target genes [13–15]. Their expression is usually found to be aberrant in many cancers including PCa [1,14,16]. Studies of different cancers have demonstrated that miRNAs can be useful tumor markers. For example, specific oncogenic and tumor suppressor miRNAs have been detected in the serum and urine of patients with breast, ovarian, and bladder cancer [17–20]. However, only few reports exist in literature describing the role of miRNAs in PCa aggressiveness and in PCa racial disparities.

miR-1207-3p is encoded by the PVT1 nonprotein coding gene located at the 8q24 PCa susceptibility locus [21]. In this study, we aimed to assess the role of miR-1207-3p in PCa aggressiveness because aggressive PCa is the life-threatening form of PCa [2]. At diagnosis, clinical and pathologic features, such as PSA level, pathologic Gleason grade, and tumor stage, are used to inform clinical decision making [4,5,22]. To determine if miR-1207-3p can be useful in PCa clinical decision making, we examined the expression of miR-1207-3p in prostate tumor tissue of patients by real-time quantitative reverse transcription polymerase chain reaction (qPCR). Then, we evaluated the possible prognostic implication of miR-1207-3p. Consequently, this study investigated the clinical significance of miR-1207-3p in PCa and determined if it contributes to explaining the disproportionate aggressiveness of PCa in moAA.

Materials and Methods

Patients and Samples

To evaluate miR-1207-3p expression in clinical samples, we used tissue specimens from 404 patients (389 CM and 15 moAA) with primary adenocarcinoma of the prostate from 1988 to 2003 at the Moffitt Cancer Center, Tampa, FL, USA. Institutional Review Board approvals were obtained for the study protocol at the University of South Florida and the Moffitt Cancer Center, Tampa, FL. Signed informed consent was obtained from all study participants. Clinicopathological and demographic information of the patients are summarized in Tables 1 and 2.

Table 1. Correlation of miR-1207-3p PCa Tissue Expression with Clinical Characteristics of PCa in 404 Patients

Variables		N	Mean ± SD	P
Recurrence	Yes	155	6.53 ± 3.75	
	No	249	4.49 ± 3.54	<.0001
Death	Yes	129	7.05 ± 4.37	
	No	275	4.44 ± 3.09	<.0001
Death specific	Alive	273	4.48 ± 3.08	
	Dead	79	6.85 ± 4.60	
	PCA specific	50	7.38 ± 4.02	<.0001
Race	Black	15	3.00 ± 2.65	
	White	389	5.36 ± 3.76	.016
Gleason score	≤6	167	4.81 ± 3.40	<.0001
	7	135	3.85 ± 2.52	
	≥8	29	6.33 ± 4.30	
Stage	1-2	302	4.84 ± 3.34	
	3-4	95	6.51 ± 4.44	<.0001
PSA	4	278	3.97 ± 3.79	.002
	4-10	168	4.03 ± 2.80	
	10	51	5.73 ± 3.58	
Marital status	Divorced	22	3.80 ± 3.05	.154
	Married	349	5.37 ± 3.75	
	Single	22	5.47 ± 3.79	

N, number; SD, standard deviation; P, P value. The level of significance was established using a P value less than .05.

RNA Extraction and qPCR Analysis

Prostate tumor tissues were collected at prostatectomy from 404 patients with primary PCa. After histopathologic confirmation, PCa tissues were obtained from unstained slides with macrodissection. Total RNA was extracted from PCa tissues using superscript IV First-strand Synthesis system (Life Technology No. 18091200) and quantified using UV spectrophotometer NanoDrop 2000 (Thermo Scientific, USA). The cDNA templates were synthesized from RNA samples by SuperScript using random hexamers. RNA samples from the 404 patient samples were analyzed by qPCR to examine the expression of miR-1207-3p.

Gene expression was determined using power SYBR Green Real-Time PCR Master Mix (Life Technology No. 4367649) and 2.0 μl of cDNA template. qPCR was performed on an ABI7900 machine using the following amplification conditions: 10 minutes at 95°C followed by 45 cycles of 15 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C. All assays were carried out in triplicate to control for technical variance. Cycle threshold values were determined using the SDS ver2.3 software (Bio-Rad). MicroRNA 1207-3p expression was normalized with U6 expression within each sample. Relative quantification of target gene expression was evaluated using the comparative cycle threshold method.

Statistical Analysis

Differences in two and three clinical outcome groups were compared using the two-tailed Student's t test and analysis of variance test, respectively. For evaluating the association of miR-1207-3p expression with PCa death and PCa recurrence in CM, a competing risk approach was applied. miR-1207-3p expression was categorized into three groups: low (<3), median (3-6), and high (≥6) based on the tertile cut points. Factors associated with PCa death were evaluated using competing-risk regression [23]. The cumulative incidence curves of PCa death were

Table 2. PCa Tissue Expression of miR-1207-3p in moAA and CM

Variables		N	Mean ± SD	P
Race	Black	15	3.00 ± 2.65	
	White	389	5.36 ± 3.76	.016

The level of significance was established using a P value less than .05.

generated by the subgroups of miR-1207-3p expression. For evaluating miR-1207-3p expression associated with PCa survival, PCa death was the primary interest, and non-PCa death was treated as a competing risk. Survival time was defined as the time from date of PCa diagnosis to date of death. For evaluating association of miR-1207-3p expression with PCa recurrence, PCa recurrence was the primary interest, and nonrecurrence death was treated as a competing risk. Time to event was defined as time from the date of PCa diagnosis to PCa recurrence or nonrecurrence death, whichever comes first. Data were censored at the last follow-up date. The same approach was applied to evaluate the association of miR-1207-3p expression with PCa recurrence. The candidate factors taken into consideration in modeling included age at diagnosis, Gleason score, and tumor stage. PSA was excluded from the models because the PSA values were missing in 150 CM. The level of significance was established using a P value less than .05.

Results

Positive Correlation between miR-1207-3p Expression and Clinicopathological Features of PCa

To determine miR-1207-3p expression pattern in PCa and to investigate its ability to predict clinical progression (e.g., biochemical recurrence, PCa-specific death, etc.), miR-1207-3p expression pattern in PCa tissues was analyzed using tissues collected from 404 primary PCa patients at the Moffitt Cancer Center. Correlations between miR-1207-3p expression and known clinical and pathological parameters were determined. We observed that increased miR-1207-3p expression in PCa tissue is significantly positively associated with aggressive clinicopathological features. As shown in Table 1, miR-1207-3p overexpression is positively correlated with overall death and PCa-specific death in patients with PCa. miR-1207-3p expression in PCa tissues of patients that died from any cause (7.05 ± 4.37 vs 4.44.18 ± 3.09, P < .0001) or experienced PCa-specific death (7.38 ± 4.02 vs 4.48 ± 3.08, P < .0001) is significantly higher than expression in PCa tissues of patients that remained alive. Next, we investigated the expression of miR-1207-3p in PCa tissues of patients that had recurrent versus those that had nonrecurrent PCa to explore the involvement of miR-1207-3p in PCa progression. We observed that the expression of miR-1207-3p is significantly higher in recurrent PCa in comparison to nonrecurrent PCa (6.53 ± 3.75 vs 4.49 ± 3.54, P < .0001). This indicates that miR-1207-3p overexpression is associated with progression of PCa patients and may serve as a novel prognostic biomarker for PCa. Indeed, miR-1207-3p is significantly overexpressed in PCa tissues from patients with a Gleason score ≥ 8 (6.33 ± 4.30) in comparison to patients with a Gleason score ≤ 6 (4.81 ± 3.40) or Gleason score 7 (3.85 ± 2.52, P ≤ .0001), further confirming miR-1207-3p as a prognostic biomarker in PCa. In addition, miR-1207-3p expression's positive correlation with advanced disease was also borne out for PCa stage (stage 1-2: 4.84 ± 3.34 vs stage 3-4: 6.51 ± 4.44, P < .0001) and PSA levels (PSA levels < 4: 3.97 ± 3.79, vs PSA levels 4-10: 4.03 ± 2.80 vs PSA levels > 10: 5.73 ± 3.58, P = .002). To our knowledge, this is the first observation of miR-1207-3p as a prognostic biomarker in PCa.

miR-1207-3p Is Differentially Expressed in PCa in moAA compared with CM

It is noteworthy that, in this cohort, moAA had a significantly decreased expression of miR-1207-3p in their PCa tissues in comparison to miR-1207-3p expression in the PCa tissue of CM.

miR-1207-3p expression was significantly less in PCa tissues of moAA in comparison to the PCa tissues of CM (3.00 ± 2.65 vs 5.36 ± 3.76, P = .016) (Table 2). This suggests that miR-1207-3p may play a role in explaining the disproportionately increased aggressiveness of PCa in moAA.

miR-1207-3p Expression Is Associated with PCa Recurrence and PCa-Specific Death in CM

Among CM, there are 118, 131, and 140 patients with a low (<3), median (3-6), and high (≥6) miR-1207-3p expression, respectively. In addition to miR-1207-3p expression, the candidate factors taken into consideration in modeling included age at diagnosis, Gleason score, and tumor stage. Age at diagnosis significantly correlated with PCa-specific death (hazard rate [HR] = 1.9, P < .01). As expected, stage is significantly correlated with recurrence (HR = 2.7, P < .01). Although Gleason score values for some patients were missing (missing n = 71 in CM), Gleason score was nevertheless highly correlated with tumor stage (Fisher's exact P value < .001). We evaluated the association of miR-1207-3p with clinical outcomes adjusting for age at diagnosis and tumor stage in the modeling. Using competing risk regression, the CM with a high miR-1207-3p expression (≥6 vs 3) had a high risk of developing PCa recurrence (HR = 2.5, P < .001) adjusting for age at diagnosis and tumor stage. Also, miR-1207-3p expression had a marginally significant impact on PCa survival (HR = 1.8 P = .06) (Table 3, Figures 1 and 2).

Discussion

Approximately 30% of PCa patients develop clinical recurrence, and the survival period of this phase of PCa is limited and extremely variable [3]. The challenge lies in the identification of those patients most at risk for relapse. Currently, factors such as serum PSA level, Gleason score, and tumor stage are used for diagnosis, prognostication, and treatment decision making [4,24]. However, none of these alone or in combination are adequate indicators for accurate clinical decision making [3]. It is likely that heterogeneity of PCa at the molecular level may account for the fact that even those patients displaying similar PSA levels, tumor stage, and Gleason score can still have different clinical outcomes [3]. Furthermore, current measures for predicting/detecting recurrence such as increased serum PSA levels may actually lead to false-positive diagnosis [25]. Moreover, increased PSA following radical prostatectomy can be caused by the presence of residual benign prostate tissue [3]. These issues with the use of PSA are considered so problematic that the US Preventive Services Task Force has advised against the widespread use of PSA based on their

Table 3. miR-1207-3p Expressions Associated with PCa Death and Recurrence for CM

Variables	PCa Death		PCa Recurrence	
Factor	Unadjusted	Adjusted	Unadjusted	Adjusted
	HR (95% CI)[1]	HR (95% CI)[1]	HR (95% CI)[1]	HR (95% CI)[1]
		$N = 378$		$N = 368$
Age at diagnosis (in 10 years)	1.9 (1.2-2.9)[**]	1.9 (1.2-3.0)[**]	1.1 (0.9-1.4)	0.9 (0.8-1.2)
Stage (3/4 vs 1/2)	1.5 (0.8-2.6)	1.3 (0.7-2.4)	3.1 (2.2-4.3)[**]	2.7 (1.9-3.8)[**]
miR-1207-3p <3	1	1	1	1
3-6	1.0 (0.4-2.6)	1.0 (0.4-2.7)	1.2 (0.8-2.0)	1.3 (0.8-2.1)
≥6	2.1 (0.9-4.6)	1.8 (0.8-4.3)	2.7 (1.8- 4.2)[**]	2.5 (1.6-4.0)[**]

[1] Hazard rate (95% confidence interval) analyzed using competing risk regression.
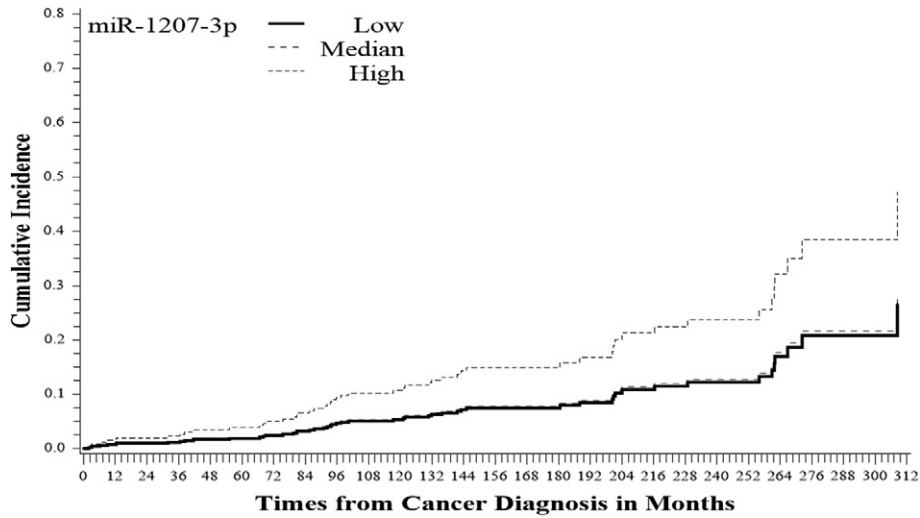[*] $P < 0.05$.
[**] $P < 0.01$.

Figure 1. Cumulative incidence of PCa death by miR-1207-3p expression level ($P = .062$).

opinion that the service has no real clear benefits and in actuality may cause more harm than good [26]. Consequently, further understanding of the molecular abnormalities underlying PCa progression is critical to the discovery of good molecular biomarkers for diagnosis, prognostication, and clinical decision making.

Understanding the molecular mechanisms of PCa progression is essential for the discovery of robust prognostic markers which are capable of helping to identify those patients that are at the greatest risk of relapse. This will, in turn, enable us to optimize management strategies to control PCa progression. In recent studies, several putative biomarkers have been suggested which, thus far, have only achieved limited success. Among them are prostate cancer antigen 3, prostate-specific membrane antigen, and altered expression of apoptotic regulators Bcl-2 and Bax [7,24,27]. These proposed prognostic biomarkers either have not been validated in multiple studies/large scale studies or have failed to reliably and accurately estimate the clinical outcome due to the heterogeneity of PCa [7]. Considering these current limitations, it remains a priority in PCa research to discover novel biomarkers to add to our "tool box" to

improve the clinical decision-making process. Identification of biomarkers which can efficiently predict recurrence after radical prostatectomy would be of great clinical significance. Besides, a biomarker that can predict distinct outcomes regardless of similar clinical characteristics would be invaluable. To contribute to this effort, in the present study, we evaluated the expression of miR-1207-3p in the PCa tissues of patients with both aggressive and nonaggressive PCa.

The importance of miRNAs in cancer biology has become apparent in many different cancers, including PCa [14–16]. Typically, they regulate biological processes such as proliferation, migration, apoptosis, and differentiation by negatively regulating gene expression at the posttranscriptional level via binding to the 3′ untranslated region of their mRNA target [4,14]. There are several advantages to the discovery of microRNA prognostic biomarkers [22]. First, they have been shown to be able to distinguish differentiation states of several malignancies including breast, lung, and colon cancers [13,22]. Hence, their aberrant expression can be exploited as a prognostic tool to distinguish between less aggressive
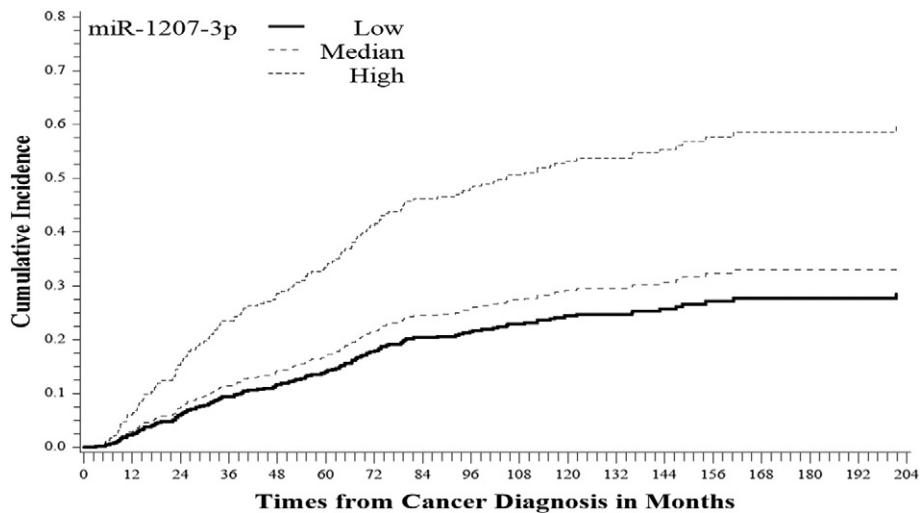

Figure 2. Cumulative incidence of PCa recurrence by miR-1207-3p expression level ($P < .001$).

and more aggressive forms of the same cancer type. Consequently, investigations of miRNA differential expression in cancers are proving to be very beneficial. Second, they are relatively stable in tissues and upon their release into a variety of biofluids such as blood and urine [28,29]. Repeated measurements of miRNA levels in biofluids are very feasible, are minimally invasive, and can provide critical information on a disease's molecular signature status over time through the course of treatment and recurrence [2]. Real-time qPCR assays are able to detect specific RNAs indicative of disease status from a simple noninvasive sampling without the need for a biopsy or surgically resected tissue [2,22]. Consequently, we assessed the role of miR-1207-3p expression in PCa patients with a focus on a potential role in prognostication.

Our results demonstrate that it may be possible to predict clinical behavior of PCa based upon miR-1207-3p expression in primary PCa. To our knowledge, this is the first study to evaluate the expression of miR-1207-3p in any human disease. PCa is a heterogeneous disease, with the exact mechanisms for its development and progression unclear [30]. However, our data show that the expression of miR-1207-3p in PCa tissues displays a significant and distinctly different pattern between indolent and aggressive PCa. Overexpression of miR-1207-3p is clearly positively related to PCa progression and worse prognosis. Therefore, our data support the potential use of miR-1207-3p as a prognostic biomarker in PCa. We anticipate that this beneficial use will advance into the clinical setting soon.

Despite progress being made in the field, significant PCa racial disparity persists [26,31,32]. And the aggressiveness of the disease may be due to specific biological factors [31,32]. Here, we present data that suggest that miR-1207-3p could possibly contribute to the disproportionate aggressiveness of miR-1207-3p in moAA. The discovery of a miRNA biomarker that is capable of distinguishing between indolent and aggressive PCa and displays differential expression in men of different ancestry would be of great significance for the development and optimization of personalized treatment approaches [33]. Moreover, as moAA have a two-fold higher chance of getting PCa and aggressive PCa, it is of great importance to enhance our understanding of the molecular mechanisms which may explain the disproportionately increased aggressiveness of PCa in moAA [8,31,32]. Differential expression of miRNAs such as miR-26a has been reported in PCa [9]. We also previously reported that miR-21 is associated with recurrence, especially in obese cases [16]. However, the importance of miRNAs in PCa in moAA is still unclear. In this regard, we have discovered that miR-1207-3p is differentially expressed in primary tumors of moAA tissues compared with primary tumors of CM. To our knowledge, this is the first report of a microRNA that directly correlates with aggressive PCa but has divergent expression between CM and moAA. These data suggest that it may be possible to predict clinical behavior of PCa based upon miRNA-1207-3p expression in primary PCa. We anticipate that this beneficial use will advance into the clinical setting.

Although the overall sample size of the present study is relatively large, a limitation is the small sample size of the moAA subgroup. Although challenging because of issues related to recruitment, a larger study enriched with a substantial proportion of moAA is warranted. In addition, the present study did not examine miR-1207-3p expression in nontumor versus tumor tissues. Therefore, the current data do not provide information regarding whether miR-1207-3p is differentially expressed in normal prostate versus PCa tissue. Likewise, because miRNAs exert functions via regulating the expression levels of their target genes, it is of great importance to identify the target genes of miR-1207-3p in PCa and to investigate the involvement of miR-1207-3p target genes in PCa. Further studies are needed to address these issues.

Conclusion
Our study identifies miR-1207-3p as a novel prognostic biomarker of aggressive PCa that is differentially expressed between CM and moAA. As such, an individual PCa patient's miR-1207-3p PCa tissue expression level has the potential to provide a novel and clinically relevant basis for making decisions in PCa. It is also possible that miR-1207-3p–based therapeutic strategies may have efficacy in PCa but must be applied in a personalized fashion. Further studies are required to establish this.

Human and Animal Rights
Signed informed consent was obtained from all study participants.

Conflict of Interest
The authors declare no potential conflicts of interests. Olorunseun O. Ogunwobi and Jong Park had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Author contributions
Jong Y. Park led data acquisition. Dibash K. Das, Jong Y. Park, and Olorunseun O. Ogunwobi contributed to data analysis. Dibash K. Das, Joseph R. Osborne, Jong Y. Park, and Olorunseun O. Ogunwobi contributed to data interpretation and manuscript writing. Hui-Yi Lin contributed to biostatistical analysis and manuscript writing.

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