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Original Article

miR-340-5p is reduced in laryngeal carcinoma and hinders tumor survival by targeting ROCK1

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Abstract: Objective: To test the action of miR-340-5p on laryngeal carcinoma cells through the targeted regulation of ROCK1. Methods: We determined the caspase-3, Bax, and Bcl-2 levels, and we studied the proliferation, invasion, and apoptosis mechanisms by determining the levels of miR-340-5p and ROCK1 in the serum, tissues, TU212, and Hep-2 of patients with laryngeal carcinoma, and by enhancing or reducing and transfecting miR-340-5P and Rock1. The correlations of the serum miR-340-5p levels and the clinicopathological features and prognoses in patients with laryngeal carcinoma were analyzed. Results: The miR-340-5p levels were low in the serum and tissues of the laryngeal carcinoma patients, but the ROCK1 levels were evidently high, and there was a negative correlation between them. A multivariate Cox analysis revealed that low miR-340-5p levels, low pathological differentiation, lymph node metastasis, and a tumor diameter ≥2.5 were independent risk factors affecting the prognosis of laryngeal carcinoma patients. Enhancing miR-340-5p and silencing ROCK1 hinders proliferation and invasion but evidently accelerates the apoptosis and improves the apoptosis-related proteins. Compared with the miR-340-5p-mimics + sh-ROCK1 group and the miR-NC group, the proliferation and invasion of cells transfected with the miR-340-5p-mimics were reduced, but the apoptosis was accelerated. Conclusion: miR-340-5p can hinder the malignant progression of laryngeal carcinoma by down-regulating ROCK1, so it may be a new direction in the treatment of laryngeal carcinoma.

Keywords: miR-340-5p, laryngeal carcinoma, down-regulation, targeted regulation of ROCK1, hindering tumor survival

Introduction

Laryngeal malignant tumors are common in the adult upper respiratory tract [1], and laryngeal squamous cell carcinoma (LSCC) is a common pathological type of laryngeal carcinoma with a high incidence rate [2]. Patients with laryngeal carcinoma often have hoarseness, dysphagia, cough, and other symptoms, and there is a high incidence of the cancer among long-term smokers and drinkers [3]. At present, a total laryngectomy or radiotherapy and chemotherapy are the most common clinical treatments. Although the technology for diagnosing and treating laryngeal carcinoma has made great progress, the prognosis of patients with laryngeal carcinoma is still very poor [4]. So clarifying the relevant mechanisms of laryngeal carcinoma and finding targets for improving the prognoses of laryngeal carcinoma patients are crucial.

miRNA, a small non-coding RNA, affects many biological events, especially the occurrence and progression of malignant tumors, and it can be used as a biomarker of the invasion and metastasis of several carcinomas [5, 6]. Moreover, studies have shown that miRNA acts in tumor-related processes, including the proliferation, apoptosis, differentiation, invasion, and angiogenesis of laryngeal carcinoma [7, 8]. For example, serum miR-155 is highly expressed in LSCC and has a high specificity and sensitivity, indicating that miR-155 can be used in determining the prognosis of LSCC [9]. miR-4497 is evidently reduced in LSCC, so enhancing miR-4497 can evidently hinder proliferation and pro-

mote apoptosis, indicating that miR-4497 can act in LSCC by targeting GBX2 [10]. miR-340-5p is a miRNA, and it has a correlation with tumor invasion, metastasis, and drug resistance [11, 12]. Furthermore, miR-340-5p acts in LSCC. For example, miR-340-5p is lowly-expressed in LSCC, but miR-340-5p can evidently hinder cell viability and glycolysis and promote apoptosis [13]. ROCK1 is often over-expressed during the development of carcinoma, and the over-expression of ROCK1 can accelerate the progression of carcinoma by regulating the behaviors of carcinoma cells, such as proliferation, migration and invasion. In addition, ROCK1 can be regulated by some miRNAs to mediate the development of carcinoma [14, 15]. For example, miR-195 can hinder the progression of LSCC by directly targeting ROCK1. Therefore, we suspected that miR-340-5p can participate in laryngeal carcinoma by regulating ROCK1.

We found that miR-340-5p and ROCK1 had targeting sites in the target gene online website, and there are a few studies on their regulation in laryngeal carcinoma patients at present. We speculated that miR-340-5p may regulate ROCK1 and thus regulate the disease progression of laryngeal carcinoma patients, so we carried out the following verification.

Data and methods

General data

Altogether 108 patients with laryngeal carcinoma in The First Affiliated Hospital, Medical College, Xiamen University from May 2017 to January 2019 were placed in the disease group (DG), and 61 healthy people during the same period were placed in the normal group (NG). The two groups of participants were selected in strict accordance with the inclusion and exclusion criteria. Inclusion criteria of the DG: patients 30-70 years old, patients diagnosed with laryngeal carcinoma [16], and patients confirmed as being in the early stage according to the AJCC TNM staging standard. There was no significant difference in the patients' ages in the DG and the NG. This study was approved by the Medical Ethics Committee, and the patients signed the informed consent form. Exclusion criteria of the DG: patients suffering from other severe diseases, patients who underwent radiotherapy or chemotherapy, surgery, or antibiotics within the six months before their admission, patients who suffered from autoimmune defects, transfer patients, patients who dropped out of the experiment halfway, patients lost to follow up. Inclusion criteria of the NG: patients 30-70 years old, patients with no major previous medical history, patients with normal physical examination results, patients with no chronic diseases, patients who agreed to participate in the study.

Cell sources

TU212 and Hep-2 human laryngeal carcinoma cells and NP69 human normal nasopharyngeal epithelial cells were all purchased from ATCC (item numbers: AC340714, BNCC245666, and XY-XB-3198). The cells were cultured in RPMI-1640 medium (Yipu Biotechnology Co., Ltd., Wuhan, China, PM150110) containing 10% FBS (Mito Biotechnology Co., Ltd., Shanghai, China, A3160801/A3160802) with 95% air and 5% CO_2 at 37°C.

Collection of the serum and tissue samples

When the patients in the DG were admitted, and when the patients in the NG underwent their physicals, 5 mL blood was extracted from each patient, centrifuged at 3000×g for 10 min, and the serum was preserved. With the consent of the patients and the hospitals, carcinoma tissues and normal adjacent tissues from the 108 EOC patients were obtained and placed in liquid nitrogen for subsequent study.

Cell culture and transfection

The miR-340-5p-inhibitor, si-ROCK1, the over-expression plasmids (miR-340-5p-mimics, sh-ROCK1), and miR-NC and si-NC were established, and the established drug-resistant cell lines were transferred to the 24-well plate. After 48 hours, a Lipofectamine 2000 kit was used to transfect the cell plasmid, and 100 nM over-expression, inhibition, and a blank control were transfected into the cells. The operation referred to the instructions of miR-340-5p, and the ROCK1 mRNA was tested using qRT-PCR. The total RNA was extracted using TRIzol reagent (Yiji Industrial Co., Ltd., Shanghai, China, YIJ102666) and reverse transcribed into cDNA. Then qPCR was performed using

Table 1. Primer sequences

Gene	Upstream primer sequence	Downstream primer sequence
miR-340-5p	5iR-340-5p primer sequence	5'-AGGCCGCGCGTAGTGATGCAACA-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
ROCK1	5'-AGGAAGGCGGACATATTAGTCCCT-3'	5'-AGAGATAGTTGGGTCCCGGC-3'
β-actin	5'-AGGTAGTCAGTCAGGTCCCGG-3'	5'-AGGTAGTCAGTCAGGTCCCGG-3'

a SYBR Green kit (Jingke Chemical Technology Co., Ltd., Shanghai, China, JKG039). Reaction conditions: 95°C for 2 min; 94°C 20 s; 60°C for 34 s, for a total of 40 cycles. The target gene was tested using $2^{-\Delta\Delta Ct}$, and the primer sequence is shown in **Table 1**.

An MTT assay was applied to test the proliferation

The cells were cultivated in a 96-well plate (1×10^4 cells/well), and three multiple wells were set up. MTT solution (Chreagen, 120752) was put into each well at 24 h, 48 h, 72 h, and 96 h respectively, and the culture was continued for 4 h. Then, the culture solution was absorbed, 100 μL DMSO (Qiaoyu Biotechnology Co., Ltd., Shanghai, China, QY-Xz102) was added, and the OD value of each well was tested at 570 nm using an ultraviolet spectrophotometer (Spectrum Standard Laboratory Equipment Technology Co., Ltd., Dongguan, China, UV752/UV752N), and the cell growth curve was visualized.

Cell invasion test (transwell)

Cells transfected for 24 h were obtained, adjusted to $5*10^4$, inoculated on a 6-well plate, washed with PBS (Biolab, Beijing, China, KFS069-AGP), and the 200 μL DMEM culture medium (Chreagen, 120002) was put in the upper chamber, and 500 mL DMEM was put into the lower chamber (including 20% FBS). After culturing, the matrix and cells that did not pass through the membrane were removed, rinsed with PBS three times, fixed with paraformaldehyde for 10 min, washed with double distilled water three times, and then dried and dyed with 0.5% crystal violet, and then the invasion was tested.

Apoptosis quantification (flow cytometry)

The cells were treated with 0.25% trypsin (Yuanye Biotechnology, R20109), rinsed twice with PBS, mixed with 100 μL binding buffer to prepare $1*10^6$ cells/mL suspension, and then mixed with Annexin V-FITC (Kemin Biotechnology, DXT-130-097-928), incubated in the dark for 5 min, tested using flow cytometry (Shanghai Ranger Apparatus Co., Ltd., Shanghai, China, NovoCyt), and the experiment was repeated 3 times.

Determination of the targeting correlation of miR-340-5p with ROCK1 using double fluorescein reporter enzyme

TargetScan was applied to test the miR-340-5p and ROCK1. Then a fragment of ROCK1-3'-UTR containing ROCK1 Wt or ROCK1 Mut 3'-untranslated region (3'-UTR) was cloned on the vector. After the DNA sequencing verification, the miR-340-5p-mimics and miR-NC were transfected into the TU212 and Hep-2 cells according to the Lipofectamine™2000 instructions. The cells were obtained 48 h after the transfection and analyzed using the double luciferase reporting system.

Statistical analysis

SPSS 21.0 (EASYBIO, China) was used for the statistical analysis. The intra-group count data were represented as cases/percentage [n (%)] and compared using chi-square tests. Independent sample t tests were applied for the pair-wise comparisons, and one-way ANOVA were used for the multiple comparisons and were represented as F. LSD-t tests were applied for the comparisons after the event, repeated measurement ANOVA for the multiple time comparisons, which were expressed as F. Bonferroni corrections were applied for back testing. The diagnostic value of miR-340-5p and ROCk1 was tested using a receiver operating curve (ROC). A Pearson test was applied to test the correlation of miR-340-5p with ROCK1 in serum. A Cox regression equation was used to test the independent prognostic fac-

Table 2. The diagnostic value of miR-340-5p and ROCK1 expressions in laryngeal carcinoma patients

Diagnostic index	AUC	95% CI	Standard error	Cut off value	Sensitivity (%)	Specificity (%)
miR-340-5p	0.922	0.879-0.964	0.022	0.931	91.67	77.05
ROCK1	0.911	0.868-0.955	0.022	0.718	69.44	98.36

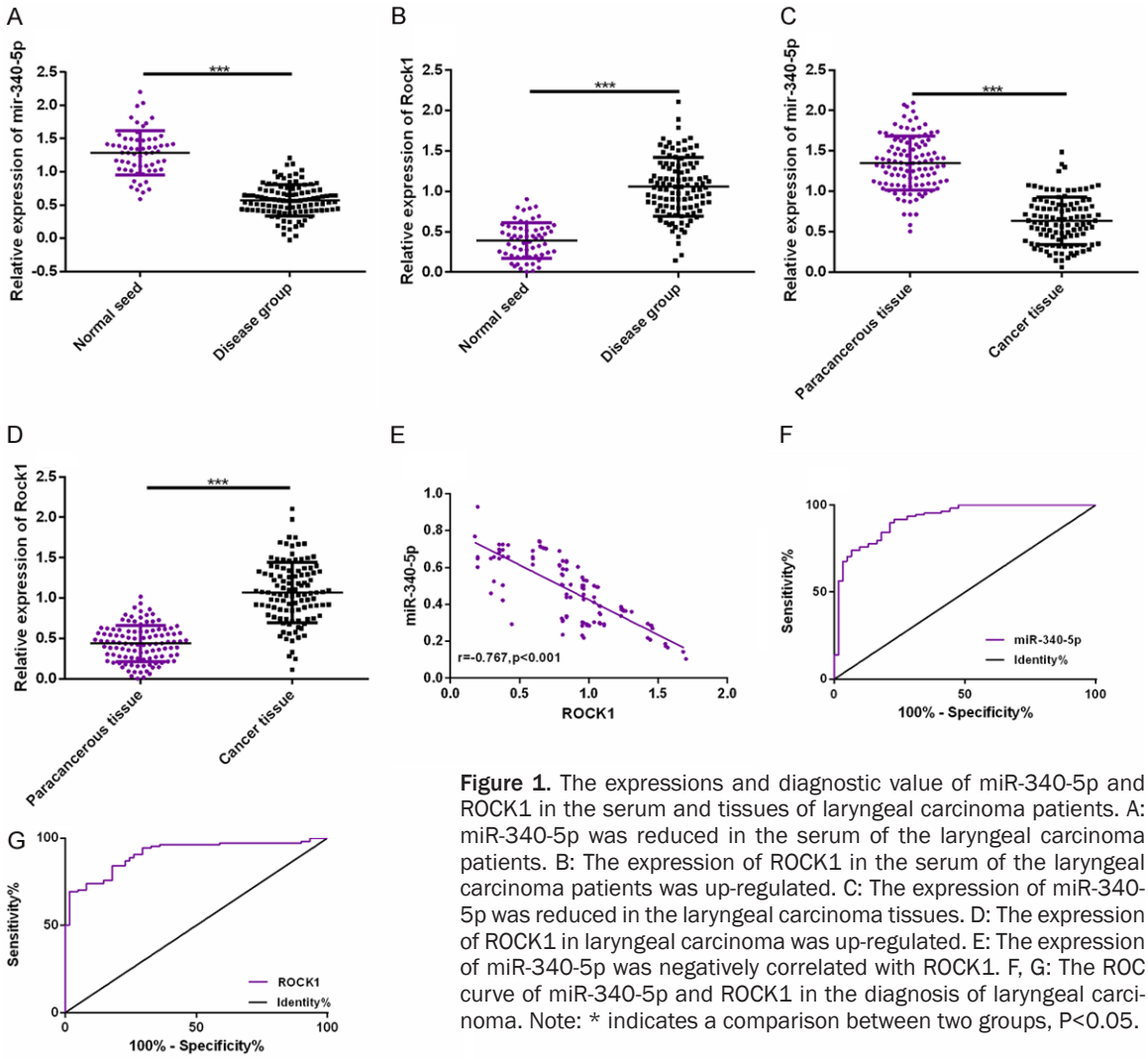

Figure 1. The expressions and diagnostic value of miR-340-5p and ROCK1 in the serum and tissues of laryngeal carcinoma patients. A: miR-340-5p was reduced in the serum of the laryngeal carcinoma patients. B: The expression of ROCK1 in the serum of the laryngeal carcinoma patients was up-regulated. C: The expression of miR-340-5p was reduced in the laryngeal carcinoma tissues. D: The expression of ROCK1 in laryngeal carcinoma was up-regulated. E: The expression of miR-340-5p was negatively correlated with ROCK1. F, G: The ROC curve of miR-340-5p and ROCK1 in the diagnosis of laryngeal carcinoma. Note: * indicates a comparison between two groups, P<0.05.

tors of laryngeal carcinoma. P<0.05 indicates a statistical difference.

Results

The expression and diagnosis of miR-340-5p and ROCK1 in patients with laryngeal carcinoma

The miR-340-5p serum levels in the laryngeal carcinoma patients were reduced, and the ROCK1 was up-regulated (P<0.05). Then we tested miR-340-5p and ROCK1, and found that the miR-340-5p in the carcinoma tissues was lower than it was in the adjacent tissues, but the ROCK1 was evidently higher than it was in the adjacent tissues (P<0.05). A Pearson correlation was applied to test the correlation of miR-26a with IGF-1 (r=-0.767, P<0.001). The ROC curves of the laryngeal carcinoma patients were visualized, and the AUC of the laryngeal carcinoma patients were 0.922 and 0.911, respectively. See **Table 2** and **Figure 1**.

Table 3. The relationship between miR-340-5p and the clinicopathological features of the laryngeal carcinoma patients [n (%)]

Factor	n	miR-340-5p		t value	*P* value
		High expression group (n=54)	Low expression group (n=54)		
Gender				0.686	0.408
Male	59	29 (49.15)	28 (57.14)		
Female	49	30 (50.85)	21 (42.86)		
Age (years)				0.375	0.540
<65	46	21 (45.65)	32 (51.61)		
≥1	62	25 (54.35)	30 (48.39)		
Drinking history				1.363	0.243
Yes	57	31 (54.39)	22 (43.14)		
No	51	26 (45.61)	29 (56.86)		
Pathological differentiation				4.180	0.041
Moderately and highly differentiated	72	39 (54.17)	12 (33.33)		
Poorly differentiated	36	33 (45.83)	24 (66.67)		
T classification				0.289	0.590
T1-T2	58	33 (56.90)	31 (62.00)		
T3-T4	50	25 (43.10)	19 (38.00)		
Lymph node metastasis				9.348	0.002
Yes	52	21 (40.38)	39 (69.64)		
No	56	31 (59.62)	17 (30.36)		
Tumor size (cm)				7.240	0.007
≥.00	61	27 (44.26)	33 (70.21)		
<2.5	47	34 (55.74)	14 (29.79)		

Table 4. Cox regression analyses

Factor	Univariate COX			Multivariate COX		
	P value	HR	95% CI	*P* value	HR	95% CI
Gender	0.382	2.189	1.095-4.378			
Age	0.793	1.179	0.589-2.358			
Drinking history	0.162	1.948	0.974-3.896			
Pathological differentiation	0.009	2.837	1.419-5.674	0.014	4.782	2.391-9.564
T classification	0.152	1.268	0.634-2.536			
Lymph node metastasis	0.006	2.893	1.446-5.786	0.001	5.067	2.533-10.134
Tumor size	0.031	2.791	1.395-5.582	0.012	3.073	1.536-6.146

Correlation of miR-340-5p with the patients' clinicopathological features

We tested the patients' pathological data and miR-340-5p using a multivariate Cox regression analysis. We found that low miR-340-5p, low pathological differentiation, lymph node metastasis, and a tumor diameter ≥2.5 were independent risk factors affecting the prognosis of laryngeal carcinoma patients. See **Tables 3**, **4**.

Enhancing miR-340-5p hindered the proliferation and invasion and promoted apoptosis

The miR-340-5p in each group of cells revealed that the TU212 and Hep-2 levels in the laryngeal carcinoma patients were evidently lower than NP69 (P<0.001). TU212 was transfected with Hep-2, and the miR-340-5p-mimics was tested. The expression of miR-340-5p in the miR-340-5p-mimics group was evidently higher than it was in the miR-NC group (P<0.001). The

proliferation and invasion of the miR-340-5p-mimics was evidently lower than the proliferation and invasion of the miR-NC, but the apoptosis was evidently higher (P<0.001). Next, we also explored the influence of miR-340-5p on the apoptosis-related proteins and found that an over-expression of miR-340-5p can increase caspase-3 and Bax and decrease Bcl-2. See **Figure 2**.

The inhibition of ROCK1 hindered proliferation and invasion and accelerated apoptosis

The ROCK1 in each group revealed that the TU212 and Hep-2 levels in the laryngeal carcinoma patients were evidently higher than NP69 (P<0.001). TU212 and Hep-2 were selected for transfection, and the ROCK1 in transfected si-ROCK1 was evidently lower than it was in miR-NC (P<0.001). The proliferation and invasion of si-ROCK1 were evidently lower than the proliferation and invasion of si-NC, but the apoptosis was evidently higher (P<0.001). Then we explored the influence of ROCK1 on the apoptosis-related proteins and found that the inhibition of ROCK1 could increase caspase-3 and Bax and decrease Bcl-2. See **Figure 3**.

Detection of the target gene of miR-340-5p

We predicted the target gene of miR-340-5p using Targetscan 7.2 and found that there was a targeted binding site between ROCK1 and miR-340-5p. Therefore, we tested the double luciferase activity and found that the luciferase activity of ROCK1 3'UTR-Wt was significantly reduced after up-regulating miR-340-5p (P< 0.001), but it had no correlation with the luciferase activity of ROCK1 3'UTR-Mut (P>0.05). WB revealed that the ROCK1 protein was evidently reduced after the transfection of the miR-340-5p-mimics (P<0.001). See **Figure 4**.

Rescue experiment

TU212 and Hep-2 were co-transfected with the miR-340-5p-mimics + sh-ROCK1 to determine their biological functions. The results revealed that there was no difference in the proliferation, invasion, or apoptosis between the transfected miR-340-5p-mimics + sh-ROCK1 cells and the transfected miR-NC cells, but comparing the miR-340-5p-mimics + sh-ROCK1 group and the miR-NC group, we found the proliferation and invasion of the miR-340-5p-mimics transfected cells were reduced, but the apoptosis was enhanced. See **Figure 5**.

Discussion

Laryngeal carcinoma is a malignant neoplasm of the neck and head, with a high mortality worldwide [17]. At present, the most common clinical treatment methods for laryngeal carcinoma are surgery and chemotherapy, but the treatment is often unsuccessful, so the recurrence rate is high [18]. Therefore, it is urgent to identify specific molecular markers that can better predict clinical outcomes and markers that are suitable therapeutic targets. Many studies have concluded that miR-340-5p and ROCK1 participate in the regulation of laryngeal carcinoma. For example, Yu et al. [19] found that serum miR-340 is reduced in LSCC and Hep-2, and the transfection of miR-340 could evidently hinder the proliferation, invasion, and migration of Hep-2 cells and accelerate the apoptosis, indicating that targeting EZH2 can prevent the progression of LSCC. ROCK1 has been shown to be highly expressed in human carcinomas, and its expression in LSCC is enhanced, indicating that its expression has a correlation with tumor size and lymph node metastasis, and the down-regulation of ROCK1 can hinder the proliferation, migration, and invasion of LSCC [20]. However, the miR-340-5p-targeted regulation of ROCK1 helps to regulate laryngeal carcinoma patients' disease state.

miR-340-5p is reduced in laryngeal carcinoma patients, but ROCK1 is evidently enhanced, suggesting that miR-340-5p and ROCK1 may act in laryngeal carcinoma. Therefore, we tested the correlation of the two, and the results revealed that the expressions of the two are negatively correlated, that is, a decrease of miR-340-5p may lead to the up-regulation of ROCK1. Then we further analyzed the possibility of diagnosing laryngeal carcinoma. The results revealed that the AUCs of miR-340-5p and ROCK1 were 0.922 and 0.911, respectively, indicating that they both have the potential to serve as highly sensitive diagnostic indicators for laryngeal carcinoma patients. After that, we analyzed the relationship between the pathological data of the laryngeal carcinoma patients and miR-340-5p using Cox, and we found that miR-340-5p has a low expression,

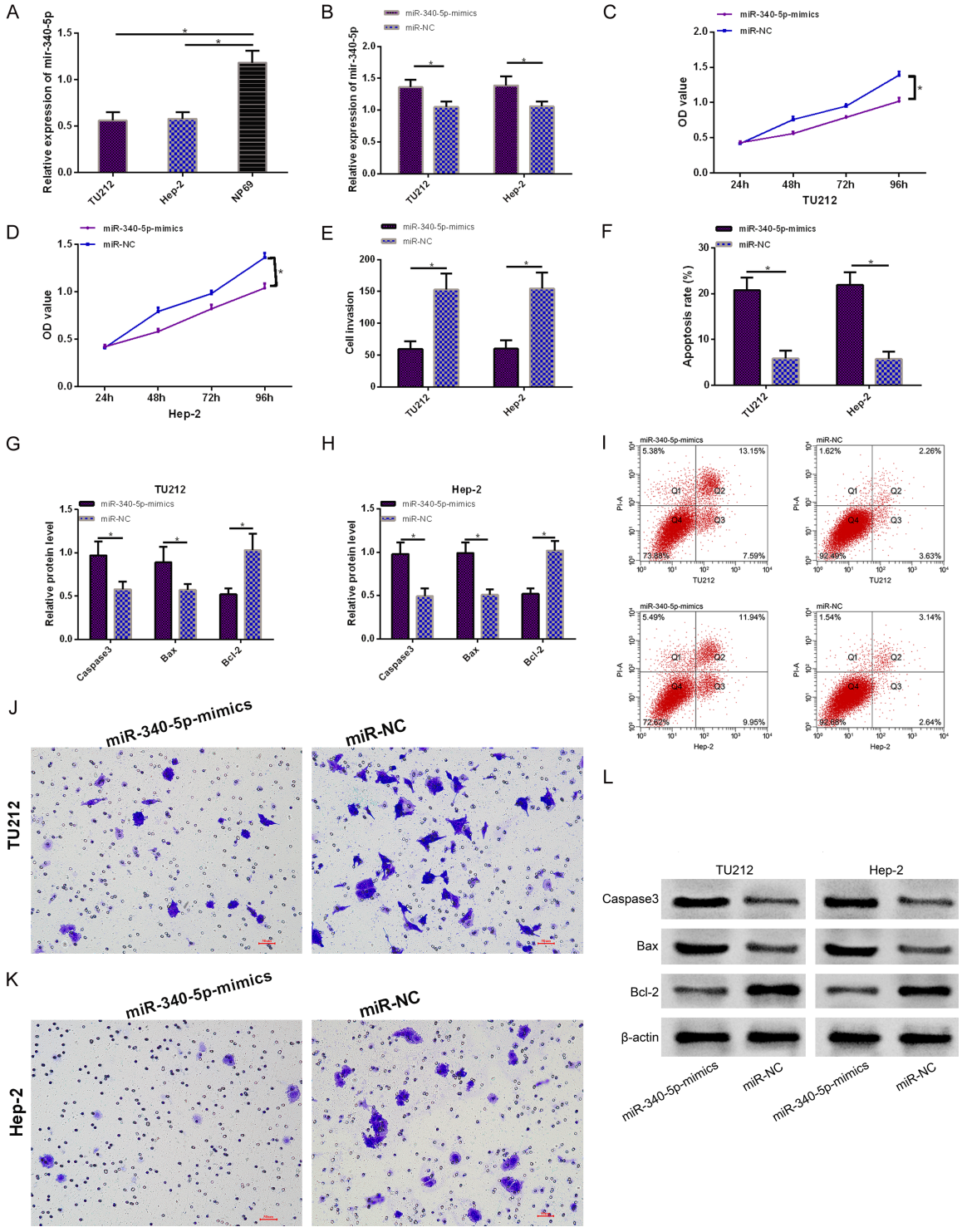

Figure 2. The over-expression of miR-340-5p hinders the proliferation and invasion of laryngeal carcinoma cells and promotes apoptosis. A: The expression of miR-340-5p in laryngeal carcinoma cell lines. B: The expression of miR-340-5p in the TU212 and Hep-2 cells after the transfection. C: The proliferation of the TU212 cells after the transfection. D: The proliferation of the Hep-2 cells after the transfection. E: The invasion of the TU212 and Hep-2 cells after the transfection. F: The apoptosis of the TU212 and Hep-2 cells after the transfection. G, H: The effect of the over-expression of miR-340-5p on the apoptosis-related proteins in the TU212 and Hep-2 cells. I: Flow cytometry of apoptosis. J: Invasion picture of the TU212 cells after the transfection. K: Invasion picture of the Hep-2 cells after the transfection. L: Cell related protein map. Note: * indicates P<0.05.

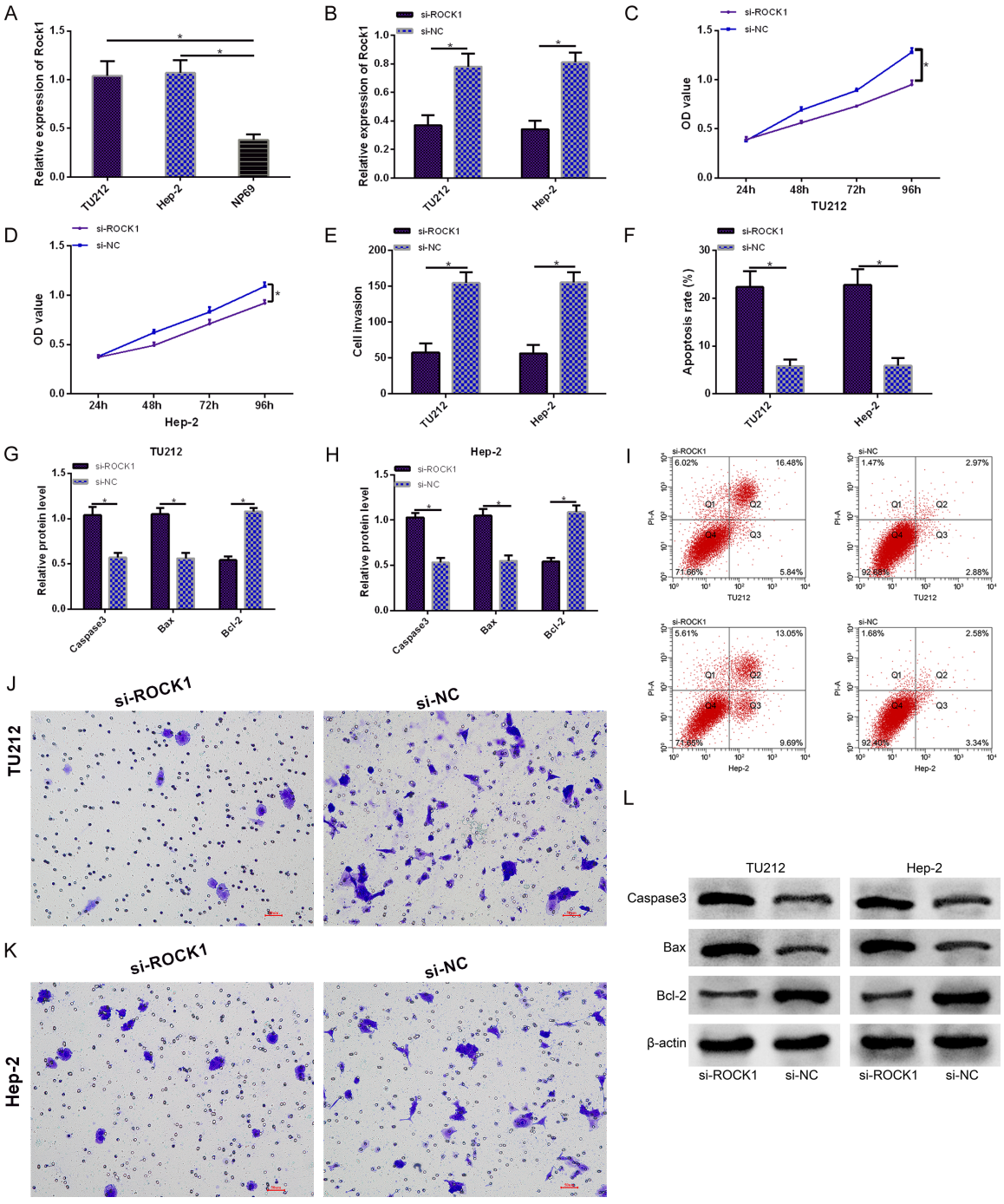

Figure 3. The inhibition of ROCK1 hinders the proliferation and invasion of laryngeal carcinoma cells and promotes apoptosis. A: The expression of ROCK1 in the laryngeal carcinoma cell lines. B: The expression of ROCK1 in the TU212 and Hep-2 cells after the transfection. C: The proliferation of the TU212 cells after the transfection. D: The proliferation of the Hep-2 cells after the transfection. E: The invasion of the TU212 and Hep-2 cells after the transfection. F: The apoptosis of the TU212 and Hep-2 cells after the transfection. G, H: Inhibiting the effect of ROCK1 on the apoptosis-related proteins in the TU212 and Hep-2 cells. I: Flow cytometry of the apoptosis. J: Invasion picture of the TU212 cells after the transfection. K: Invasion picture of the Hep-2 cells after the transfection. L: Cell related protein map. Note: * indicates $P<0.05$.

that low pathological differentiation, lymph node metastasis, and a tumor diameter ≥2.5 are independent risk factors affecting the prognoses of laryngeal carcinoma patients. However, Lee et al. revealed that [21] lymph node metastasis and hyperlipidemia are related to

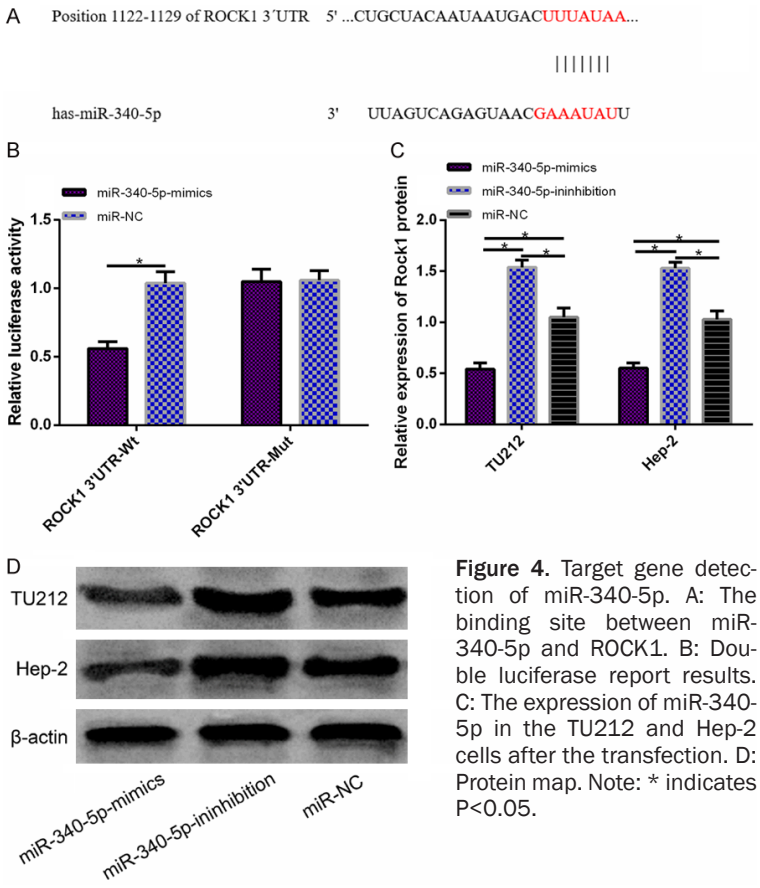

Figure 4. Target gene detection of miR-340-5p. A: The binding site between miR-340-5p and ROCK1. B: Double luciferase report results. C: The expression of miR-340-5p in the TU212 and Hep-2 cells after the transfection. D: Protein map. Note: * indicates P<0.05.

the poor prognoses of laryngeal carcinoma patients. This finding is similar to our research results. Therefore, we speculated that miR-340-5p and ROCK1 participate in the occurrence and development of laryngeal carcinoma, so we conducted cell biology experiments for further verification. It has been pointed out that miR-340-5p can hinder the proliferation and invasion and promote the apoptosis of ESCC by directly binding with and hindering PIK3C3 [22]. However, its specific mechanism in laryngeal carcinoma is still unclear. Therefore, we tested miR-340-5p in laryngeal carcinoma cells and normal nasopharyngeal epithelial cells, and we found that miR-340-5p is also reduced and ROCK1 was enhanced in laryngeal carcinoma cells, a finding consistent with our above results. Then, the cells' biological behavior was tested. MTT and flow cytometry revealed that miR-340-5p was over-expressed, and the proliferation and invasion of the cells were evidently hindered, but the apoptosis was enhanced, and the apoptosis-related factors caspase-3 and Bax (except Bcl-2) had higher levels. Hindering ROCK1 could hinder the proliferation and invasion of laryngeal carcinoma cells and promote apoptosis, indicating that enhancing miR-340-5p and inhibiting ROCK1 act as a tumor suppressor in laryngeal carcinoma.

Studies have shown that miRNA can participate in biological processes by adjusting its downstream target genes [23, 24]. We predicted the potential target genes of miR-340-5p using TargetScan to verify the mechanism of miR-340-5p in regulating the biological behavior of laryngeal carcinoma cells, and we found that there is a targeted binding site between ROCK1 and miR-340-5p. ROCK1, a member of the AGC kinase family, acts in mammalian cell morphology and movement. It acts on the cytoskeleton, and it is related to cell growth and intercellular adhesion in addition to its correlation with cell movement [25]. In this research, after the transfection of the miR-340-5p-mimics, we found that ROCK1 in the TU212 and Hep-2 cells was evidently reduced, indicating that there is a targeted regulatory correlation between miR-340-5p and ROCK1. In addition, we also provided a co-transfection experiment for further verification. The proliferation, invasion, and apoptosis of the transfected miR-340-5p-mimics + sh-ROCK1 cells were not different from those of the transfected miR-NC cells, but compared with the miR-340-5p-mimics + sh-ROCK1 group and the miR-NC group, the proliferation and invasion of the miR-340-5p-mimic transfected cells were reduced, but the apoptosis rate was enhanced. This proved once again that there is a targeted regulatory correlation between miR-340-5p and ROCK1, that is, enhancing miR-340-5p can hinder ROCK1, thus reducing proliferation and invasion and promoting apoptosis.

In this experiment, we proved that miR-340-5p is lowly-expressed in laryngeal carcinoma and can mediate the cells' biological function by regulating ROCK1. However, this study still has

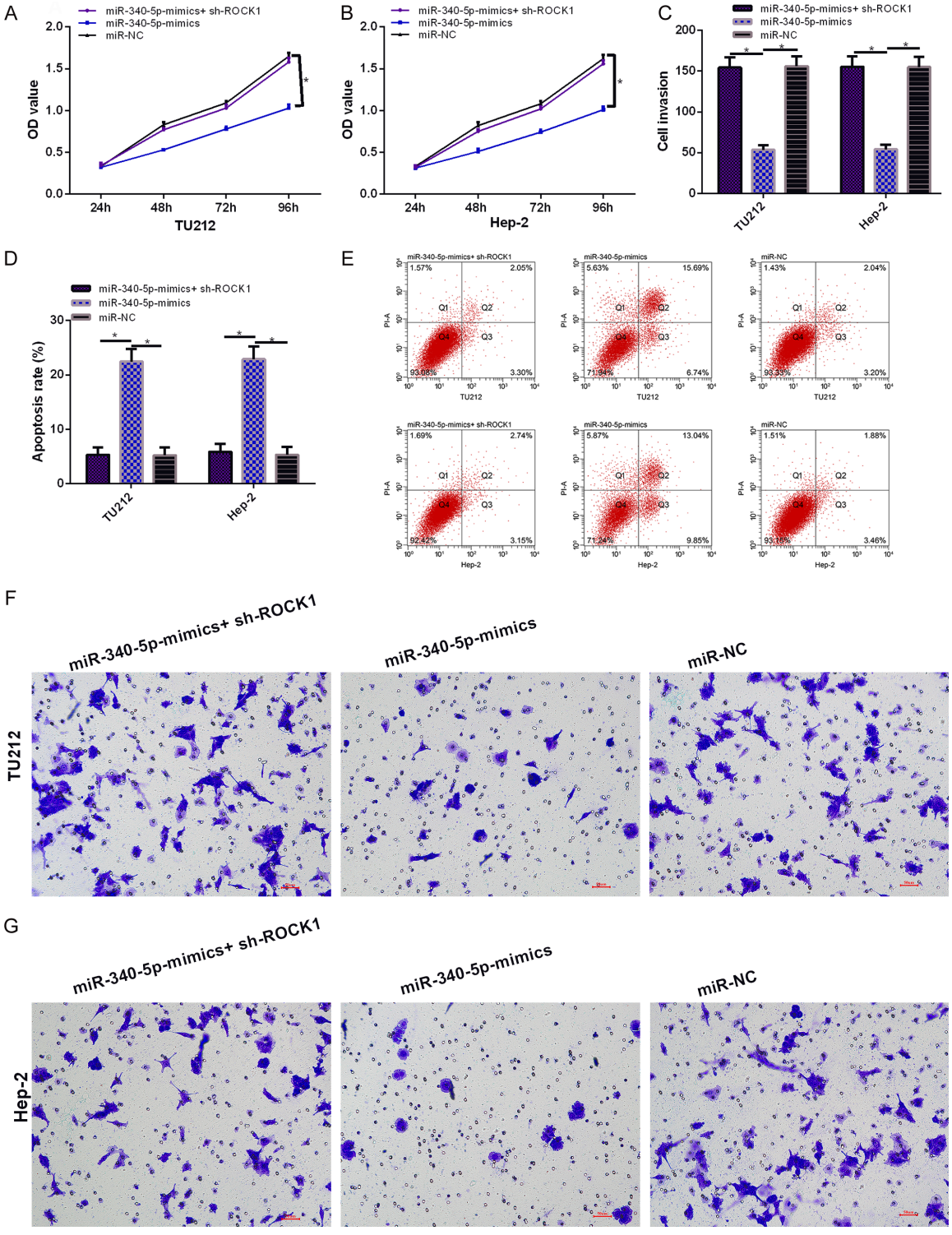

Figure 5. Rescue experiment. A, B: There was no difference in the proliferation between the transfected miR-340-5p-mimics + sh-ROCK1 cells and the transfected miR-NC cells, but the proliferation ability of the transfected miR-340-5p-mimics was lower than it was in the miR-340-5p-mimics + sh-ROCK1 group and the miR-NC group. C: There was no difference in the invasion between the transfected miR-340-5p-mimics + sh-ROCK1 cells and the transfected miR-NC cells, but the invasion ability of the transfected miR-340-5p-mimics + sh-ROCK1 cells was lower than it was in the miR-NC groups. D: There was no difference in apoptosis between the transfected miR-340-5p-mimics + sh-ROCK1 cells and the transfected miR-NC cells, but the apoptosis of the transfected miR-340-5p-mimics + SH-ROCK1 cells was higher than it was in the miR-340-5p-mimics + sh-ROCK1 and miR-NC groups. E: Flow cytometry of the apoptosis. F: Invasion picture of the TU212 cells after transfection. G: Invasion picture of the Hep-2 cells after the transfection. Note: * indicates $P<0.05$.

some limitations. First, because this was basic research, we did not carry out any tumor-forming experiments in nude mice, so whether miR-340-5p is consistent with cell experiments in vivo needs further verification. So we will carry out more basic research in the future, analyze additional potential mechanisms of miR-340-5p using bioinformatics, and collect more kinds (cell lines) and different types (serum) of samples to test our research results.

In conclusion, miR-340-5p can be used as a diagnostic biomarker for laryngeal carcinoma patients, and it can hinder the malignant proliferation and invasion of laryngeal carcinoma cells and promote apoptosis by regulating ROCK1.

Disclosure of conflict of interest

None.

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