MYBPC1 is a key regulator for laryngeal carcinoma formation

Jing Liu[a], Jinlan Song[b] and Chao Li[a]

Laryngeal carcinoma represents one of the most common types of tumor of the respiratory tract. The aim of the present study was to evaluate the functions of myosin-binding protein C1 (MYBPC1) in the progression of laryngeal carcinoma and to unravel the potential underlying molecular mechanism(s). Significantly differentially expressed mRNAs and miRNAs were analyzed, and potential genes were verified using clinically recruited patients with laryngeal carcinoma. The human laryngeal carcinoma cell lines TU686, TU212 and AMC-HN-8, as well as the control nasopharyngeal epithelial cell line NP69, were selected for the functional analysis of MYBPC1. The interaction between MYBPC1 and miR-451a was also explored in depth. The functions of MYBPC1 in the laryngeal carcinoma cell lines were examined using colony formation assay, cell proliferation and invasion assays, and via measuring the extent of apoptosis. The intracellular function of MYBPC1 was subsequently confirmed by constructing an *in vivo* xenograft model through the subcutaneous injection of laryngeal carcinoma cells into 4-week-old male nude mice. Compared with normal tissue, MYBPC1 was found to be the most significantly downregulated gene, whereas activating transcription factor-2 (ATF-2) was the most significantly upregulated one. At the same time, miR-451a was found to be the most significantly downregulated miRNA in laryngeal squamous cell carcinoma tissues. According to the WHO classification system, we found that the level of MYBPC1 was significantly decreased in grade IV tissues compared with grade II and grade III tissues, a finding that was consistent with the observed activity of miR-451a. MiR-451a was found to cause a marked enhancement of the activity of MYBPC1 in TU212 cells, which in turn was attenuated by ATF overexpression, suggesting that miR-451a could indirectly modulate the function of MYBPC1 through the ATF2-dependent signaling axis. MYBPC1 suppressed the invasion of cells induced by ATF2 in laryngeal carcinoma cells. Moreover, subcutaneous injection of MYBPC1 to construct an *in vivo* xenograft mouse model enabled rescue of the mice from laryngeal carcinoma formation. Taken together, the results of the present study have shown that MYBPC1 fulfills a pivotal role in laryngeal carcinoma formation, and these findings may provide both a new avenue for research planning and a potential therapeutic target for laryngeal carcinoma. *Anti-Cancer Drugs* 34: 1–8 Copyright 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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[a]Department of Otolaryngology, The Second Hospital of Tianjin Medical University and [b]Department of Otolaryngology, Tianjin Nankai Hospital, Tianjin, China

Correspondence to Chao Li, Department of Otolaryngology, The Second Hospital of Tianjin Medical University, 23 Pingjiang Road, Hexi District, Tianjin, China
Tel: +86 13302103773; e-mail:earnose1@163.com

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Introduction

Laryngeal carcinoma has been shown to be one of the most common types of tumor of the respiratory tract, which occurs more frequently in men than in women (estimated as 5.8 cases per 100 000 in men compared with 1.2 per 100 000 in women, respectively [1]). The diagnosis rate of laryngeal carcinoma varies widely across the globe, with a significant ethnic disparity [2,3]. The 5-year relative survival rate for patients with laryngeal carcinoma varies widely according to tumor site and stage. However, it should be noted that laryngeal carcinoma has been shown to be one of the oncological diseases for which the 5-year survival rate has declined gradually over the past 40 years, from 66 to 63% [4]. To date, cessation of smoking and alcohol consumption remains the predominant risk factor for laryngeal carcinoma. In addition, other potential factors, including outdoor or indoor air pollution, nutritional status, as well as hereditary susceptibility, contribute to a considerable extent to the onset of the disease [5,6]. Currently, the successful management of laryngeal carcinoma mainly relies on an accurate pretreatment evaluation and diagnosis staging of patients. The recommended treatments for laryngeal carcinoma are surgical resection (including transoral laser microsurgery, open partial laryngectomy and total laryngectomy), potential chemotherapy and radiotherapy (RT) [7]. Furthermore, the development of new treatment options, such as immunotherapy, holds great promise for this patient population. In addition, other types of novel immunotherapies that are focused on the host's

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own immune system against cancer cells, with similar approaches, such as peptide vaccination, have attracted an increasing level of attention. Nevertheless, even after decades' worth of such efforts, improving the overall prognosis for patients has not met with a great deal of success, and effective biomarkers for this disorder are still lacking, which is mainly due to the heterogeneous underlying molecular mechanisms of laryngeal carcinoma and the existence of redundant signaling cassettes.

MicroRNAs (miRNAs) are small non-coding RNAs (ncRNAs) of 21–25 nucleotides in length, which are highly conserved among distinct species [8]. miRNAs have been suggested to manipulate targeted gene expression through translational inhibition and degradation of mRNAs, and are associated with the full spectrum of human disorders, including several types of cancer [9–11]. A growing body of evidence stands in support of there being connections between miRNAs and laryngeal carcinoma. Previously, an oligonucleotide microarray by Liu *et al*. [12] was developed to assess the differential expression profiles of miRNAs and mRNAs in laryngeal carcinoma tissues compared with normal tissues. microRNA-21 (miR-21), as a potential oncogenic miRNA, was shown to be upregulated in laryngeal carcinoma tissues targeting a pan-cell cycle regulator and tumor suppressor (BTG2). Subsequently, miR-21 and miR-221 were shown to be co-overexpressed and to modulate phosphatase and tensin homolog protein (PTEN) [13]. That study confirmed that co-inhibition of miR-21 and miR-221 synergistically triggered laryngeal carcinoma cell apoptosis *in vitro*. Additionally, altered PTEN-Akt signaling and p53-mediated amplification of the transcription of pro-apoptotic miRNAs were found to serve critical roles in the synergistic effects.

Myosin-binding protein-C (MyBP-C) refers to a family of accessory proteins, which are closely associated with cardiac, slow skeletal, as well as fast skeletal muscle functions [14]. There are three isoforms in this family, sharing structural and sequence homology. The cardiac isoform of MyBP-C (cMyBP-C), encoded by MYBPC3, is a well-established isoform that is expressed exclusively in cardiac muscle for the maintenance of normal cardiac functions [15]. The fast skeletal isoform of MyBP-C (fMyBP-C) is encoded by MYBPC2. Subsequently, the slow skeletal isoform (sMyBP-C), encoded by the MYBPC1 gene, was recently identified. Although cMyBP-C is exclusively expressed in cardiac muscle, fMyBP-C and sMyBP-C have been shown to exist in several different tissue types [16]. Dysregulation and mutation of MYBPC1 have been suggested to be associated with inherited myopathies, especially with severe and lethal forms of distal arthrogryposis myopathy [17,18]. However, to date, our understanding of how the MYBPC1 gene may be associated with carcinoma remains incomplete.

Considered altogether, and given the poor understanding of the underlying molecular mechanisms of laryngeal carcinoma as it affects patients, in the present study an in-depth investigation of the differentially expressed miRNAs for this disease was performed. As the most significantly differentially expressed gene, MYBPC1 was shown to exert a vitally important role in regulating the cellular functions of laryngeal carcinoma, and these functions have been shown to be regulated by miR451a through activating transcription factor-2 (ATF-2).

Material and methods

Study subjects

Forty tumor specimens from patients newly diagnosed with laryngeal carcinoma were included in the present study. These patients underwent tumor biopsy or resection in the Second Hospital of Tianjin Medical University between 2017 and 2020. The patients were categorized into grades I-IV using the widely accepted WHO classification system. This study was approved by our institutional review committee.

Cell culture

The human laryngeal carcinoma cell lines TU686, TU212 and AMC-HN-8, as well as the control nasopharyngeal epithelial cell line NP69, were obtained from American type culture collection. The TU686 cell line was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum (FBS). The AMC-HN-8 cell line was cultured in dulbecco's modified eagle medium basic medium containing 10% FBS, whereas the TU212 cell line and the human immortalized NP69 nasopharyngeal epithelial cells were cultured in RPMI-1640 medium containing 10% FBS.

Reverse transcription-quantitative PCR analysis of mRNA and miRNA expression.

TRIzol reagent was used to extract total RNA from cells. The Applied Biosystems high-capacity RNA to cDNA kit (Thermo Fisher Scientific, Inc., Shanghai, China) was used to reverse-transcribe RNA (1000 ng) into cDNA and qPCR amplification was performed with SYBR Green Master Mix (Qiagen, Inc.). The data values were normalized against those of the internal reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of each parallel sample. For miRNA expression analysis, the Applied Biosystems TaqMan Advanced MicroRNA Assay kit (Thermo Fisher Scientific, Inc.) and miRNA-specific reverse-transcribed RNA primers (100 ng) were used in the present study. The Applied Biosystems TaqMan MicroRNA detection primers of miR-451a (Thermo Fisher Scientific, Inc.) were utilized to measure the relative expression level of miR-451a. All experiments were performed in triplicate.

Transfection of miR-451a mimics and inhibitors, MYBPC1 siRNA and plasmids

For functional experiments, a specific Ambion miR-451a mimic/inhibitor and a corresponding Ambion negative control (Thermo Fisher Scientific, Inc.) were transfected into the laryngeal carcinoma cells. For siRNA transfection, a specific ATF2 siRNA, a specific MYBPC1 siRNA

(50 nmol/l) (OriGene Technologies, Inc.) and a negative control were utilized. The pcDNA3/ATF2 and pcDNA3/MYBPC1 plasmids containing either the ATF2 or the MYBPC1 open reading frame were purchased from Addgene, Inc. (Cambridge, Massachusetts, USA).

Colony formation assay

Laryngeal carcinoma cells (100 cells) were seeded on to a 6-well tissue culture plate. After 10–14 days, the visible colonies were fixed, and 0.1% crystallization was used for staining.

Cell proliferation, invasion and apoptosis measurement.

The cells from each group were collected and evaluated for cell proliferation assay using the Cell Counting Kit-8 (CCK-8) method (Thermo Fisher Scientific, China). The absorbance was evaluated at 450 nm using a plate reader purchased from Thermo Fisher Scientific, Inc. For the apoptotic measurements, cell apoptosis was measured using the Annexin V fluorescein isothiocyanate/propidine iodide double-staining flow cytometric method.

Western blot analysis

Western blot analysis was performed as previously reported [19]. The primary antibodies used were as follows: anti-MYBPC1 (1:1,000 dilution), anti-ATF2 (1:1,000 dilution) and anti-GAPDH (1:1,000 dilution), all purchased from Cell Signaling Technology, Inc. (Denvers, Massachusetts, USA). Cell Signaling Technology The western blotting system of MilliporeSigma was used to detect polyclonal goat anti-rabbit antibodies.

Xenograft model construction

A total of 5×10^6 laryngeal carcinoma cells (negative control or transfected with MYBPC1 siRNA) in 100 ml of sterilized saline were injected subcutaneously into the left armpit of a 4-week-old male nude mice ($n = 3$ for each group). After 4 weeks, the injected mice were sacrificed, and the according tumor weights were calculated.

Statistical analysis

All the experiments were repeated three times independently. The statistical software, SAS 9.4 version 19.0, was developed for data analysis. The continuous variables were tested for normal distribution, and Student's *t*-test in SAS 9.4 was developed to analyze the data, where $P < 0.05$ was considered to indicate a statistically significant difference.

Results

MYBPC1 is a negative factor associated with different grades of laryngeal carcinoma patients

To analyze the differentially expressed genes for patients with laryngeal carcinoma, a chip from the GEO database (GSE137308, which contains three laryngeal squamous cell carcinoma tissues and three associated adjacent normal tissues) was investigated. Compared with normal tissue, the laryngeal squamous cell carcinoma tissues exhibited 536 differentially expressed mRNAs, of which 359 were downregulated and 177 were upregulated (Fig. 1a,b). In addition, MYBPC1 was the most significantly downregulated mRNA, whereas ATF2 was the most significantly upregulated one (denoted by the blue and red arrowheads in Figure 1A, respectively). It was also noted that miR-451a was the primary downregulated miRNA for the laryngeal squamous cell carcinoma tissues. To further determine whether the expression of MYBPC1 was associated with the tumor grade of the patients with laryngeal squamous carcinoma, reverse transcription-quantitative PCR (RT-qPCR) was performed, based on TaqMan microRNA detection to evaluate the expression of MYBPC1. Following the WHO classification method, the level of MYBPC1 was found to be significantly decreased in grade IV tissues compared with grade II and III tissues (Fig. 1c), a finding that was consistent with the activity of miR-451a (Fig. 1d). Conversely, the expression pattern of ATF2 was found to follow the opposite trend compared with MYBPC1 (Fig. 1c).

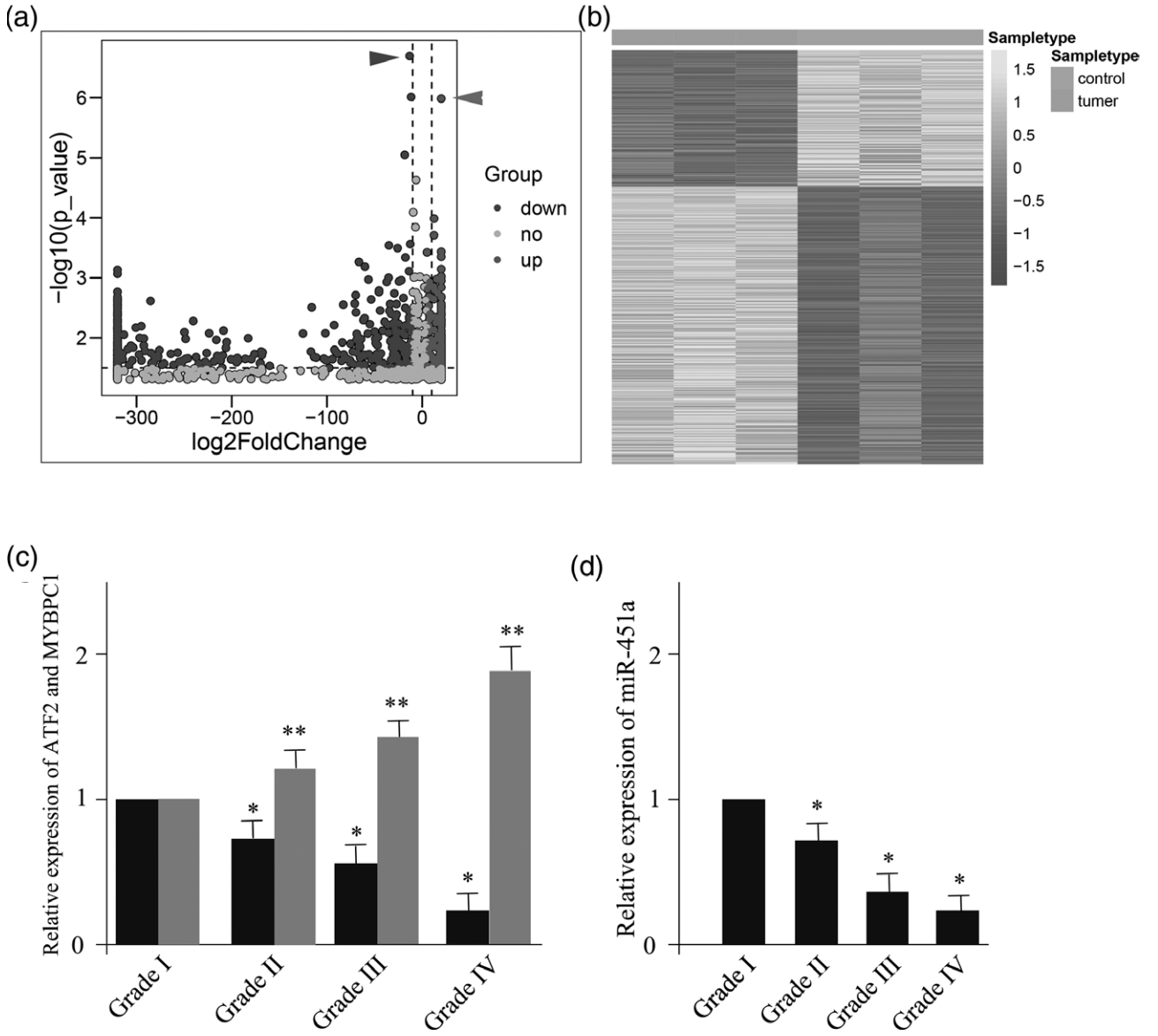
MYBPC1 is modulated by miR-451a via the ATF2-dependent signaling pathway.

RT-qPCR was subsequently used to examine the levels of MYBPC1 in a control nasopharyngeal epithelial cell line (NP69) and in three human laryngeal carcinoma cell lines (TU686, TU212 and AMC-HN-8). As shown in Fig. 2a, all three laryngeal carcinoma cell lines expressed different levels of MYBPC1, which were all significantly lower than that in the NP69 cells. Given that the TU212 cell line was found to have the lowest expression level of MYBPC1, TU212 cells were therefore used for subsequent experiments in this study. Subsequently, bioinformatics analysis using three different microRNA target prediction tools (miRDB, Targetscan, miRTarBase) was performed for miR-451a. However, MYBPC1 was not found to be a potential target for miR-451a. Instead, ATF contained several binding sites for miR-451a, a finding that was consistent with results reported in a previous study [20]. Upregulation of miR-451a in TU212 cells significantly reduced the expression of ATF2, and increased the activity of MYBPC1. At the same time, eliminating miR-451a led to both an increased expression of MYBPC1 and a suppression of ATF2 activity (Fig. 2b). MiR-451a caused a marked increase in the activity of MYBPC1 in the TU212 cells (Fig. 2c), and this increase was shown to be attenuated by ATF overexpression (Fig. 2d). Taken together, these results suggested that miR-451a could indirectly manipulate the function of MYBPC1, and that this influential role was mediated via the activity of ATF2.

MYBPC1 exerts its function via regulating the invasion of laryngeal carcinoma cells

Because the low expression of MYBPC1 in patients with laryngeal carcinoma is associated with poor overall

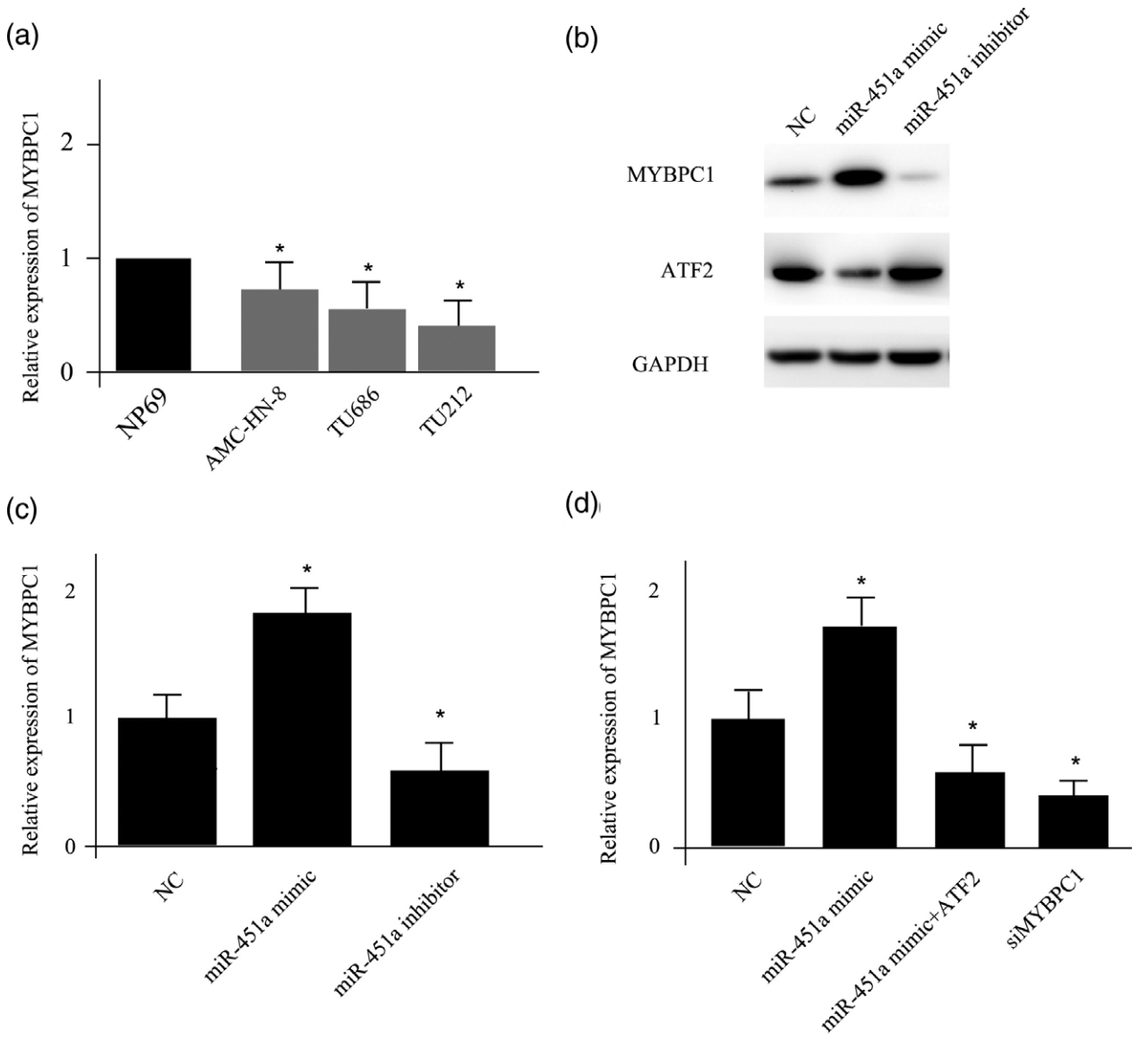
Fig. 1



MYBPC1 was negatively associated with grades of laryngeal carcinoma patients. (a) The volcano map of differentially expressed genes for laryngeal carcinoma patients. MYBPC1 is indicated as a blue arrowhead while ATF2 as a red arrowhead respectively. (b) The heat map of differentially expressed genes for laryngeal carcinoma patients. (c) Expression of MYBPC1 and ATF2 in grade I to grade IV laryngeal carcinoma patients detected by qRT-PCR. N=3, * $P<0.05$ compared expression of MYBPC1 in grade I laryngeal carcinoma patients.** $P<0.05$ compared expression of ATF2 in grade I laryngeal carcinoma patients. (d) The miR-451a expression in laryngeal carcinoma patients of different grades detected by qRT-PCR.. N=3, * $P<0.05$ compared with grade I. ATF, activating transcription factor; MYBPC1, myosin-binding protein C1; qRT-PCR, quantitative reverse transcription-PCR.

survival, we speculated that MYBPC1 might serve a critical role in the progression of laryngeal carcinoma. To explore the functions of MYBPC1 in laryngeal carcinoma cells, TU212 cells were transfected with a negative control, ATF2 analogue or a combination of ATF2 and MYBPC1 analogue. Colony-forming assay analysis demonstrated that ATF2 overexpression produced more clones compared with the numbers of clones formed in the negative control-transfected cells. By contrast, MYBPC1 treatment led to a marked reduction in the number of colonies that resulted from ATF2 transfection (Fig. 3a). Subsequently, the role of MYBPC1 in cell invasion was verified using the Transwell Matrigel assay, which suggested that ATF2 overexpression led to an increased rate of invasion of the laryngeal carcinoma cells, whereas MYBPC1 overexpression reduced the cell invasion rate (Fig. 3d). Interestingly, it was not possible to observe any significant differences with respect to the

Fig. 2



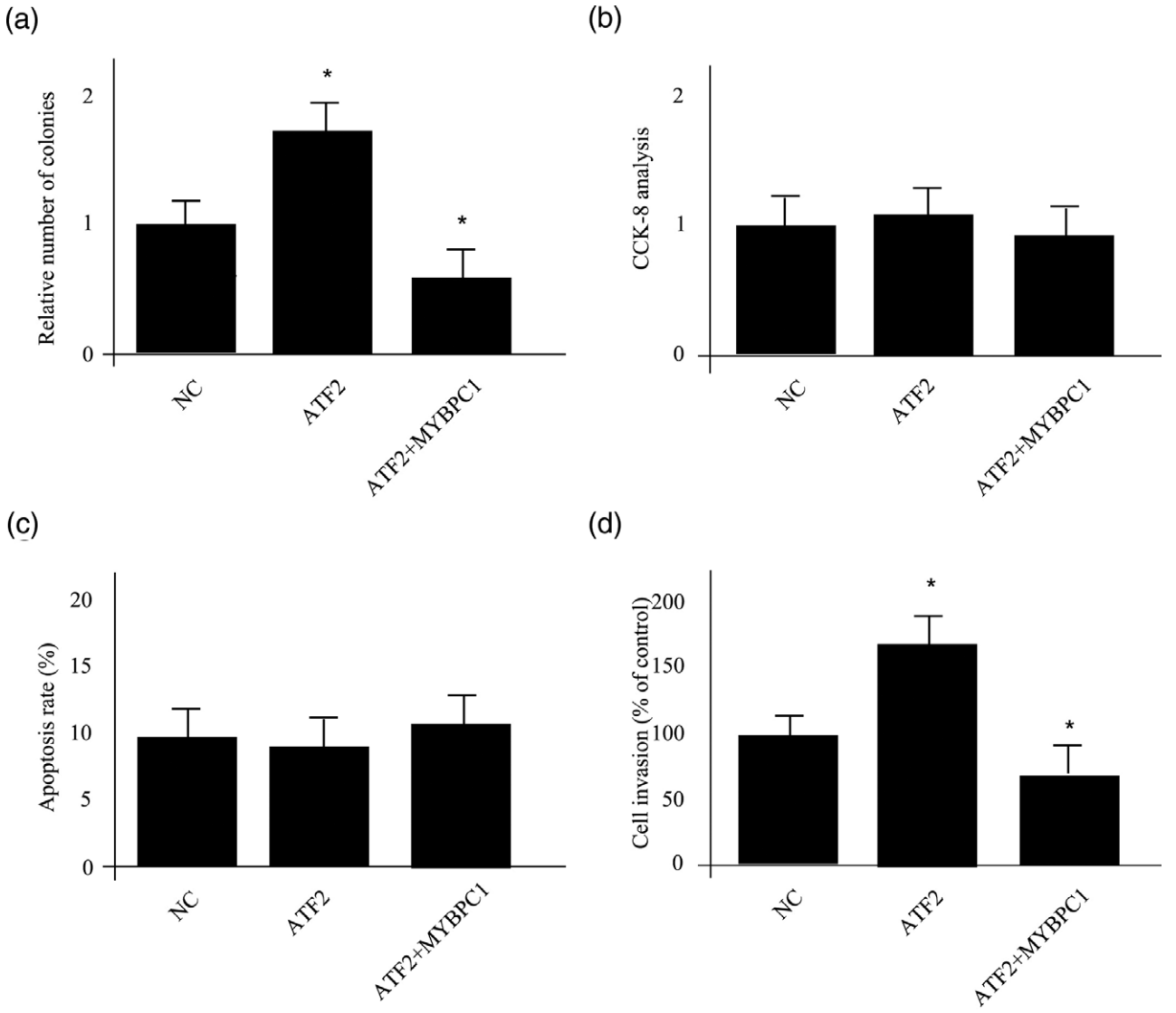
MYBPC1 was a indirectly target of miR-451a. (a) Expression of MYBPC1 in different cell lines detected by qRT-PCR. N = 3, * $P<0.05$ compared NP69 cells. (b) Western blotting analysis of MYBPC1 and ATF2 expression levels in TU212 cells transfected with negative control (NC), miR-451a mimic or miR-451a inhibitor. (c) Expression of MYBPC1 in TU212 cells transfected with NC, miR-451a mimic or miR-451a inhibitor based on qRT-PCR measurement. N=3, * $P<0.05$ compared NC treatment. (d) Expression of MYBPC1 in TU212 cells transfected with NC, miR-451a mimic, miR-451a mimic and ATF2 plasmid, or MYBPC1 siRNA. N=3, * $P<0.05$ compared NC treatment.. ATF, activating transcription factor; MYBPC1, myosin-binding protein C1; qRT-PCR, quantitative reverse transcription-PCR.

results obtained from the cell proliferation and apoptosis assays (Fig. 3b,c). These in-vitro data suggested that ATF2 mainly promotes the invasion of laryngeal carcinoma cells through an MYBPC1-dependent signaling axis.

MYBPC1 influences laryngeal carcinoma formation *in vivo*

With respect to the functions of MYBPC1 for laryngeal carcinoma formation, TU212 cells transfected either with negative control or MYBPC1 analogue were subcutaneously implanted into 4-week-old nude mice to develop tumors. After 4 weeks, the mice were sacrificed, dissected and the tumor tissues were examined. As shown in Fig. 4A,b, the mean tumor weights in the mice that were treated with MYBPC1 were appreciably smaller compared with those that received an injection of the negative control, supporting the notion that MYBPC1 acts as a suppressor of laryngeal carcinoma formation *in vivo*.

Fig. 3

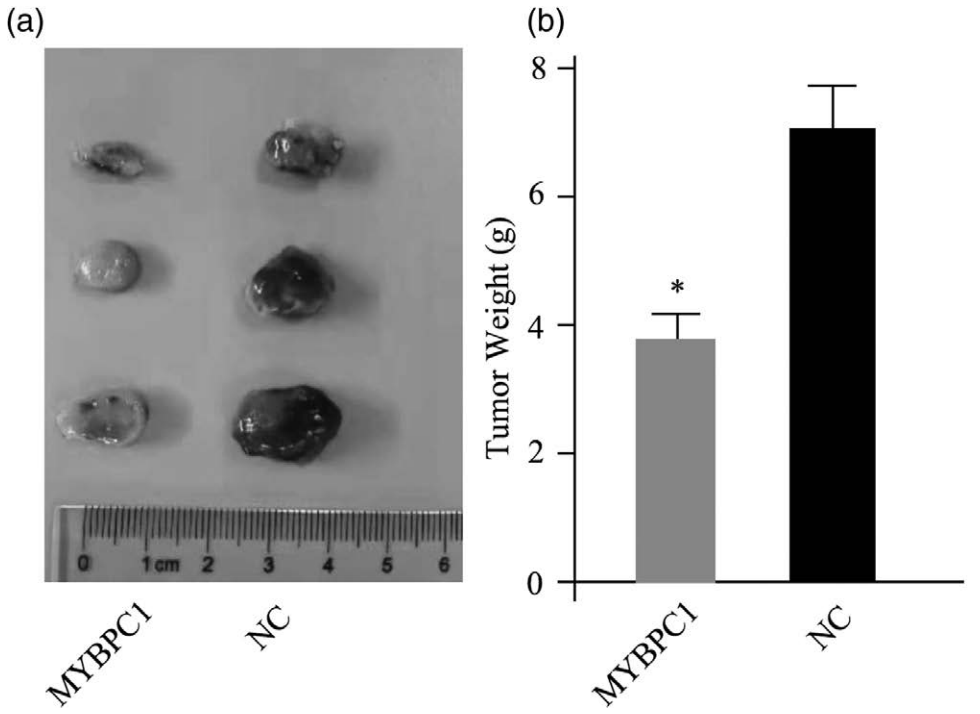


MYBPC1 played a central role in laryngeal carcinoma cell invasion. TU212 cells treated with NC, ATF2 plasmid, or a combination of ATF2 and MYBPC1 plasmids following subsequent colony-forming analysis (a), CCK-8 examination (b), apoptosis measurement (c) as well as cell invasion evaluation (d) respectively. N=3, *$P<0.05$ compared with NC treatment. ATF, activating transcription factor; CCK, Cell Counting Kit; MYBPC1, myosin-binding protein C1.

Discussion

Laryngeal carcinoma comprises ~33% of all cases of head-and-neck cancer, and it constitutes a significant source of morbidity and mortality. Currently, researchers are striving to identify breakthroughs in the prevention, early screening, diagnosis and treatment of laryngeal cancer; however, the overall outcomes continue to fail to meet these expectations. Compared with other types of cancer, the molecular mechanism(s) underpinning the disease remain controversial. Previously, Lyu *et al [21].* conducted a transcriptome-wide RNA sequencing analysis based on three pairs of laryngeal carcinoma tissues and adjacent normal tissues to explore the differential expression profiles of lncRNAs, miRNAs and mRNAs [21]. Using RT-qPCR, they suggested that seven lncRNAs, eight mRNAs and three miRNAs were identified as being dysregulated in the tissues from laryngeal carcinoma patients. Moreover, three miRNAs (namely, miR-1-3p, miR-449a and miR-451a) were verified as being downregulated. Similarly, we also detected a significant downregulation of miRNA-451a in the present study, which displayed a specific expression pattern according to the different tumor grades of patients with laryngeal carcinoma (as shown in Fig. 1c,d). As the primary downregulated gene for patients with laryngeal carcinoma, our attention became focused on MYBPC1. Using three

Fig. 4



The *in vivo* investigation of MYBPC1 functions for laryngeal carcinoma. (a) Tumors that developed upon subcutaneous implantation of TU212 cells (NC or MYBPC1 analogue transfected) were harvested. Each treatment group included three 4-week-old male nude mice. (b) The average weight of harvested tumors was calculated respectively. N=3, **P*<0.05 compared with NC treatment. MYBPC1, myosin-binding protein C1.

different microRNA target prediction tools (miRDB, Targetscan and miRTarBase), MYBPC1 was found not to be a direct target for miRNA-451a. In a previous study featuring non-small cell lung cancer (NSCLC) cells, dual-luciferase reporter assay was performed to confirm the binding conditions between miR-451a and ATF2 [20]. Moreover, consistent with these results and as identified with laryngeal carcinoma in the present study, miR-451a was shown to be downregulated in NSCLC tissues and in the lung cancer cell lines A549 and NCI-H460, whereas ATF2 was upregulated. The mRNA level of miR-451a was negatively correlated with that of ATF2. In addition, miR-451a modulated cell migration and invasion through targeting ATF2. At the same time, ATF2 could reverse the inhibitory patterns of migration and invasion of A549 cells that were induced by miR-451a. As shown in Fig. 3, ATF2 is considered to be an oncogene for the progression of laryngeal carcinoma, whereas MYBPC1 acts more like a tumor repressor.

The proper maintenance of transcriptional regulation is key for the preservation of normal functions of organismal development, disturbances of which result in numerous pathologies, including diverse types of cancer. ATF2, or activating transcription factor-2, also termed cyclic AMP (cAMP) response element (CRE) binding protein 2 (CREB2) and CRE-BP1, is considered to operate as a paradigm for the mechanistic complexity regulating transcription factor function in carcinoma progression [22]. ATF2 has been suggested to manipulate target genes by homo-dimerization or hetero-dimerization with other AP1 family members, including the CREB, Fos, Maf and Jun family transcription factors [23]. Owing to its pivotal role in transcriptional regulation, it is not surprising that ATF2 has attracted increasing levels of attention in carcinoma research. At present, there is accumulating evidence to demonstrate that alterations of ATF2 in several different types of cancer cells could initiate resistance to genotoxic stress-inducing chemical therapeutics [24,25]. For example, ATF2 was shown in liver cancer cells to modulate resistance to the tyrosine kinase inhibitor sorafenib [26]. However, the functions of ATF2 in carcinoma are more complicated than researchers initially considered they would be, as the transcription factor does not have the same role in all types of cancer. For example, ATF2 functions as a tumor suppressor in breast cancer, contributing to the sensitivity and specificity of tamoxifen-based treatments [27].

In the present study, MYBPC1 was assumed to be a central target for ATF2. However, any direct interaction between the two factors has not yet been fully explored. The type of regulation observed may either be direct (i.e. at the transcriptional level) or indirect (at the level of phosphorylation, because the sMyBP-C protein encoded by MYBPC1 undergoes phosphorylation to complete

its final functional operations [28,29]). This will be an interesting question to address in future studies. To date, MYBPC1 is primarily recognized as a myopathic gene, mutations of which have been shown to result in malformed proteins that are made manifest in a dominant-negative manner after incorporation into sarcomeres [30]. Over the last four decades, researchers have emphasized the functions of cMyBP-C based on the facts of direct involvement in congenital heart disease using multiple animal models [31]. Until only recently, Ha *et al*. [32] claimed that knocking down MYBPC1 in a zebrafish model led to severe ventral body curvature, decreased mobility and early lethality, along with impaired sarcomeric developments and a reduced number of myofibrils

Besides the zebrafish model, however, no mammalian MYBPC1 in-vivo models have been generated yet. With respect to carcinoma, the functions of MYBPC1 remain poorly understood. In a study of bioinformatic analysis based on four GEO datasets (GSE5764, GSE7904, GSE20711 and GSE29431) MYBPC1 was shown to be a crucial prognostic candidate gene for breast cancer, and the gene was significantly associated with overall survival for the disorder [33]. Other from this, any evidence in support of the connection between MYBPC1 and laryngeal carcinoma is missing. It is worth noting that MYBPC1 was only able to affect laryngeal carcinoma cell invasion and colony formation, and not cell proliferation or apoptosis (Fig. 3), findings that may be associated with the endogenous characteristics of sMyBP-C encoded by MYBPC1.

In conclusion, the present study has highlighted MYBPC1 as a central factor for laryngeal carcinoma formation, which is potentially regulated by miR-451a via the ATF2-dependent signaling pathway. The innovative findings presented herein have provided novel insights for future studies on laryngeal carcinoma.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

Reference

1 Baselga J. Why the epidermal growth factor receptor? The rationale for cancer therapy. *Oncologist* 2002; (7 Suppl 4):2–8.
2 Shin JY, Truong MT. Racial disparities in laryngeal cancer treatment and outcome: a population-based analysis of 24,069 patients. *Laryngoscope* 2015; **125**:1667–1674.
3 DeSantis C, Naishadham D, Jemal A. Cancer statistics for African Americans, 2013. *CA Cancer J Clin* 2013; **63**:151–166.
4 Steuer CE, El-Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. *CA Cancer J Clin* 2017; **67**:31–50.
5 Kuper H, Boffetta P, Adami HO. Tobacco use and cancer causation: association by tumour type. *J Intern Med* 2002; **252**:206–224.
6 Kawakita D, Matsuo K. Alcohol and head and neck cancer. *Cancer Metastasis Rev* 2017; **36**:425–434.
7 Obid R, Redlich M, Tomeh C. The treatment of laryngeal cancer. *Oral Maxillofac Surg Clin North Am* 2019; **31**:1–11.
8 Correia de Sousa M, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M. Deciphering miRNAs' action through miRNA editing. *Int J Mol Sci* 2019; **20**:6249.
9 Wu M, Wang G, Tian W, Deng Y, Xu Y. MiRNA-based therapeutics for lung cancer. *Curr Pharm Des* 2018; **23**:5989–5996.
10 Michlewski G, Cáceres JF. Post-transcriptional control of miRNA biogenesis. *RNA* 2019; **25**:1–16.
11 Tiwari A, Mukherjee B, Dixit M. MicroRNA key to angiogenesis regulation: MiRNA biology and therapy. *Curr Cancer Drug Targets* 2018; **18**:266–277.
12 Liu M, Wu H, Liu T, Li Y, Wang F, Wan H, *et al*. Regulation of the cell cycle gene, BTG2, by miR-21 in human laryngeal carcinoma. *Cell Res* 2009; **19**:828–837.
13 Salido-Guadarrama I, Romero-Cordoba S, Peralta-Zaragoza O, Hidalgo-Miranda A, Rodríguez-Dorantes M. MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in cancer. *Onco Targets Ther* 2014; **7**:1327–1338.
14 Moss RL. Cardiac myosin-binding protein C: a protein once at loose ends finds its regulatory groove. *Proc Natl Acad Sci U S A* 2016; **113**:3133–3135.
15 Ackermann MA, Kontrogianni-Konstantopoulos A. Myosin binding protein-C: a regulator of actomyosin interaction in striated muscle. *J Biomed Biotechnol* 2011; **2011**:636403.
16 Ackermann MA, Kontrogianni-Konstantopoulos A. Myosin binding protein-C slow: a multifaceted family of proteins with a complex expression profile in fast and slow twitch skeletal muscles. *Front Physiol* 2013; **4**:391.
17 Li X, Zhong B, Han W, Zhao N, Liu W, Sui Y, *et al*. Two novel mutations in myosin binding protein C slow causing distal arthrogryposis type 2 in two large Han Chinese families may suggest important functional role of immunoglobulin domain C2. *PLoS One* 2015; **10**:e0117158.
18 Bhuiyan MS, Gulick J, Osinska H, Gupta M, Robbins J. Determination of the critical residues responsible for cardiac myosin binding protein C's interactions. *J Mol Cell Cardiol* 2012; **53**:838–847.
19 Zhang B, Sun N, Mu X, Zhi L, Zhai L, Jiang Y, *et al*. G protein alpha S subunit promotes cell proliferation of renal cell carcinoma with involvement of protein kinase A signaling. *DNA Cell Biol* 2017; **36**:237–242.
20 Shen YY, Cui JY, Yuan J, Wang X. MiR-451a suppressed cell migration and invasion in non-small cell lung cancer through targeting ATF2. *Eur Rev Med Pharmacol Sci* 2018; **22**:5554–5561.
21 Lyu K, Li Y, Xu Y, Yue H, Wen Y, et al. Using RNA sequencing to identify a putative lncRNA-associated ceRNA network in laryngeal squamous cell carcinoma. *RNA Biol* 2020; **17**:977–989.
22 Watson G, Ronai ZA, Lau E. ATF2, a paradigm of the multifaceted regulation of transcription factors in biology and disease. *Pharmacol Res* 2017; **119**:347–357.
23 Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 2002; **4**:E131–E136.
24 Lau E, Sedy J, Sander C, Shaw MA, Feng Y, Scortegagna M, *et al*. Transcriptional repression of IFNβ1 by ATF2 confers melanoma resistance to therapy. *Oncogene* 2015; **34**:5739–5748.
25 Maekawa T, Sano Y, Shinagawa T, Rahman Z, Sakuma T, Nomura S, *et al.* ATF-2 controls transcription of Maspin and GADD45 alpha genes independently from p53 to suppress mammary tumors. *Oncogene* 2008; **27**:1045–1054.
26 Rudalska R, Dauch D, Longerich T, McJunkin K, Wuestefeld T, Kang TW, *et al*. *In vivo* RNAi screening identifies a mechanism of sorafenib resistance in liver cancer. *Nat Med* 2014; **20**:1138–1146.
27 Rudraraju B, Droog M, Abdel-Fatah TM, Zwart W, Giannoudis A, Malki MI, *et al*. Phosphorylation of activating transcription factor-2 (ATF-2) within the activation domain is a key determinant of sensitivity to tamoxifen in breast cancer. *Breast Cancer Res Treat* 2014; **147**:295–309.
28 Moss RL, Fitzsimons DP, Ralphe JC. Cardiac MyBP-C regulates the rate and force of contraction in mammalian myocardium. *Circ Res* 2015; **116**:183–192.
29 Previs MJ, Mun JY, Michalek AJ, Previs SB, Gulick J, Robbins J, *et al*. Phosphorylation and calcium antagonistically tune myosin-binding protein C's structure and function. *Proc Natl Acad Sci U S A* 2016; **113**:3239–3244.
30 Sadayappan S, Gulick J, Osinska H, Martin LA, Hahn HS, Dorn GW 2nd, *et al*. Cardiac myosin-binding protein-C phosphorylation and cardiac function. *Circ Res* 2005; **97**:1156–1163.
31 Carrier L, Mearini G, Stathopoulou K, Cuello F. Cardiac myosin-binding protein C (MYBPC3) in cardiac pathophysiology. *Gene* 2015; **573**:188–197.
32 Ha K, Buchan JG, Alvarado DM, McCall K, Vydyanath A, Luther PK, *et al*. MYBPC1 mutations impair skeletal muscle function in zebrafish models of arthrogryposis. *Hum Mol Genet* 2013; **22**:4967–4977.
33 Xu YH, Deng JL, Wang LP, Zhang HB, Tang L, Huang Y, *et al*. Identification of candidate genes associated with breast cancer prognosis. *DNA Cell Biol* 2020; **39**:1205–1227.