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MicroRNA-106b regulates the tumor suppressor RUNX3 in laryngeal carcinoma cells

Ying Xu [a,1], Kai Wang [b,1], Wei Gao [a], Chunming Zhang [a], Fuhui Huang [a], Shuxin Wen [a], Binquan Wang [a,*]

[a] Department of Otolaryngology, Head and Neck Surgery, The First Hospital, Shanxi Medical University, Taiyuan, Shanxi, China
[b] Department of Otolaryngology, Head and Neck Surgery, The Third Affiliated Hospital of Soochow University, Changzhou, China

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ABSTRACT

Our study focuses on a set of laryngeal tumors that show reduced RUNX3 expression in the absence of transcriptional silencing of tumor suppressor gene RUNX3 by aberrant methylation of CpG islands. We report that the loss of expression of RUNX3 correlates with up-regulation of miR-106b in human laryngeal carcinoma tissue. The downregulation of RUNX3 is mediated by miR-106b through binding of its 3′UTR. Moreover, miR-106b can promote the proliferation and invasion of laryngeal carcinoma cells by directly targeting RUNX3, and RUXN3 knockdown can abolish this phenotype. These results shed a new insight into the mechanism of miRNA regulation in laryngeal carcinoma.

1. Introduction

Laryngeal carcinoma is a common head and neck malignancy with high incidence because it accounts for approximately 2.4% of new malignancies worldwide every year [1,2]. To further improve its survival and cure rates, the carcinogenic mechanisms of laryngeal carcinoma need to be elucidated.

Various genetic alterations associated with laryngeal carcinoma, such as hTERT, CD24, PTEN, and RUNX1–3, have been described [3–5]. RUNX3, which is located on chromosome 1p36, has been identified as a critical tumour suppressor in many human cancer types [6–11]. The loss of RUNX3 gene expression has been reported to contribute to the tumourigenesis of many human tumour types, including gastric cancers, colorectal cancers, breast cancers, endometrial cancers, and laryngeal carcinomas [12–15]. The downregulation of RUNX3 has been mainly attributed to the hypermethylation of its promoter region [10]. However, the results of a previous study [16] demonstrated that there remains a proportion of laryngeal carcinoma that shows reduced RUNX3 expression in the absence of its methylation regulation mechanism, which suggests that other factors may be involved in the regulation of RUNX3.

MicroRNAs (miRNAs) were recently characterised as endogenous phylogenetically conserved small RNA molecules of approximately 21–25 nucleotides in length that regulate target gene expression by affecting the mRNA translation and stability or by modulating the promoter activity of the target gene [17]. An emerging body of evidence indicates that miRNAs play an important role in a variety of pathogenic conditions, including cancer [18,19]. A differential expression of miRNAs between tumour tissues and normal tissues has been observed in various cancer types [20], which suggests a possible link between miRNA expression and the development of cancer. For example, the members of the let-7 miRNA family have been found to be underexpressed in lung cancer. The let-7 miRNA targets the oncogene RAS, and the loss of let-7 expression results in the overexpression of RAS [21]. In addition, miR-15 and miR-16 are downregulated in chronic lymphocytic leukaemia and cause the overexpression of the tumour suppressor gene BCL2, which protects cells from apoptosis [22]. The aims of this study were to determine whether the dysregulation of miRNAs that target RUNX3 in laryngeal carcinoma is responsible for the observed downregulation of RUNX3 and to evaluate the role of this mechanism in laryngeal carcinoma.

* Corresponding author. Fax: +86 0351-4639114.
E-mail address: wangbinquan121314@163.com (B. Wang).
[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Clinical samples and cell lines

The 17 paired laryngeal carcinoma tissues used in this study were obtained from The First Hospital of Shanxi Medical University in China. The specimens, which included seven laryngeal carcinomas at stage I and II and seven laryngeal carcinomas at stage III and IV, were snap-frozen in liquid nitrogen. The collection and use of the patient samples were reviewed and approved by the Institutional Ethics Committees, and written informed consent was appropriately obtained from all of the patients. The Hep-2 and TU212 cells were purchased from ATCC.

2.2. Selection of candidate miRNAs

The candidate miRNAs that bind to RUNX3 were identified using three web-based bioinformatics algorithms. TargetScan, DIANA LAB, and microRNA predict miRNA-binding sites based on the complementary nucleotide sequence in the 3′-untranslated region of the RUNX3 mRNA.

2.3. Luciferase reporter assay

The luciferase reporter assay was performed as described in the Supplementary materials.

2.4. In vivo tumour xenograft studies

The tumorigenicity in nude mice was determined as described previously [23,24], and the details of this procedure are described in the Supplementary materials.

2.5. Cell proliferation, colony formation, and invasion assays

The details of these procedures are described in the Supplementary materials.

2.6. Statistical analysis

The data are expressed as the means ± standard deviation (S.D.), and differences that were found to have $P < 0.05$ using the Students–Newman–Keuls test are considered statistically significant. All of the data were analysed with the SPSS 17.0 software to confirm their statistical significance.

3. Results

3.1. The downregulation of RUNX3 in laryngeal carcinoma cells is independent of DNA methylation

Twelve laryngeal carcinoma tissues were analysed by methylation-specific PCR (MSP) to detect the methylation status of the RUNX3 CpG islands. As shown in Fig. 1A, tumours 2–4, 6, 9, 10, and 11 were unmethylated at the RUNX3 CpG islands, whereas tumours 1, 7, 8, and 12 were fully methylated at this locus. We then determined the expression level of RUNX3 protein in these unmethylated and methylated laryngeal carcinoma tissues. The Western blot results showed that the level of RUNX3 protein, which was normalised to that of GAPDH, was always lower in laryngeal carcinoma tissues compared to normal laryngeal epithelium, regardless of the methylation status of the RUNX3 CpG islands (Fig. 1B). This result was confirmed with an immunohistochemistry assay. As shown in Fig. 1C, RUNX3 exhibited a loss of expression in the laryngeal carcinoma tissues compared to the normal laryngeal epi-

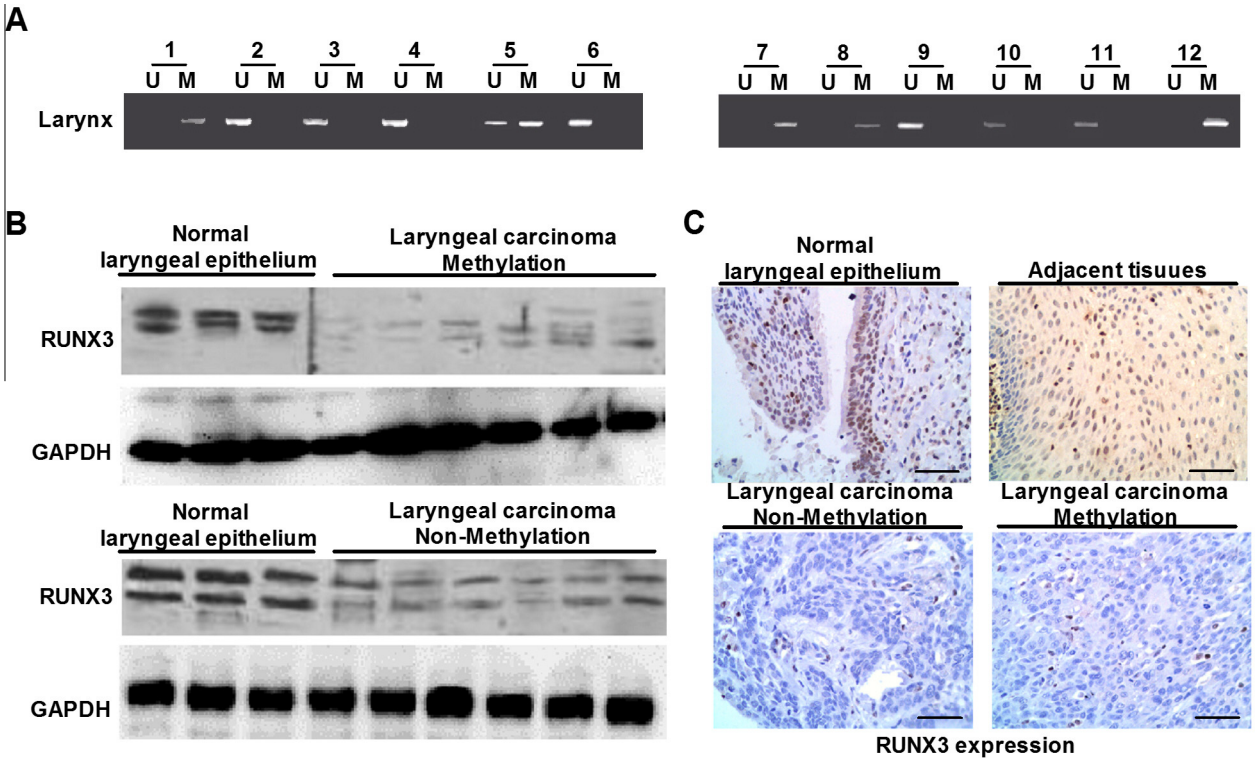

Fig. 1. (A) Representative samples of MSP analysis. After bisulfite modification, the products that were amplified with primers specific to the unmethylated or methylated alleles of RUNX3 were loaded onto 2.5% agarose gels. (B) A Western blot assay was performed to detect the RUNX3 protein levels in these tissues. The RUNX3 protein levels were normalised to those of GAPDH, and representative images are shown. (C) Immunohistochemistry (IHC) was performed using anti-RUNX3 antibody to stain, and representative images from the four groups (normal laryngeal epithelium, adjacent larynx tissues, methylated RUNX3 laryngeal carcinoma, and non-methylated RUNX3 laryngeal carcinoma), are shown.