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Research article

MiR-124 down-regulation is critical for cancer associated fibroblasts-enhanced tumor growth of oral carcinoma

Xia Li[a,*], Qinqiao Fan[b], Jinyun Li[c], Jing Song[a], Yangcong Gu[a]

[a] Department of Stomatology, School of Stomatology and medicine, Foshan Stomatology Hospital, Foshan University, Foshan 528000, PR China
[b] Department of Hepatobiliary Surgery, Cancer Center, Chenzhou No.1 People's Hospital, Chenzhou 423000, PR China
[c] Department of Head and Neck Surgery, The Affiliated Cancer Hospital of Xiangya School of Medicine, Center South University, Changsha 410013, PR China

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ABSTRACT

Cancer associated fibroblasts (CAFs) are known to be involved in initiation, progression and metastasis of various cancers. However, the molecular mechanism of how CAFs affects the biological function of oral cancer (OC) has not been fully-addressed. In this study, we demonstrated that miR-124 was downregulated in oral CAFs and oral cancer cells (OCCs) when compared with matched normal fibroblasts (NFs). Hypermethylation in the promoter region of miR-124 genes was accounted for its downregulation. Interestingly, CAFs but not NFs exerted promotion effect on OCCs cell proliferation, migration and tumor growth in CAFs/NFs-OCCs co-culture. Furthermore, we identified Chemokine (C-C motif) ligand 2 (CCL2) and Interleukin 8 (IL-8) as two direct targets of miR-124. Over-expression of miR-124 in CAFs-OCCs co-culture abrogated CAFs-promoted OCCs cell growth and migration, and this inhibitory effect can be rescued by addition of CCL2 and IL-8. Finally, we showed that restoration of miR-124 expression by lentiviral infection or formulated miR-124 injection inhibited oral tumor growth in vivo suggesting miR-124 rescue could be a potential rationale for therapeutic applications in oral cancer in the future.

1. Introduction

Oral squamous cell carcinoma (OSCC) account for around 40% of the head and neck malignancies and are the sixth most frequently diagnosed cancer in the world. Patients with OSCC have poor clinical outcomes and the 5-year survival rate for OSCC have not been improved in the last three decades [1,2]. understanding molecular determinants of OSCC is critical for development of novel therapeutic strategies for OSCC. Previous studies have shown that cancer cells and adjacent stromal cells are critical for tumor development. The adjacent stromal cells include fibroblasts, endothelial cells and various bone marrow-derived progenitor cells [3,4]. Cancer associated fibroblasts (CAFs), the most common stromal cell type in tumor, play assignable roles in the tumor initiation, progression and metastasis. CAFs cross-interaction with tumor cells to secrete cytokines, chemokines, growth factors and extracellular matrix (ECM) components to create a favorable tumor microenvironment for tumor growth [5].

MicroRNAs (miRNAs) are a class of short non-coding RNAs of 21–23 nucleotides that regulate gene expression by binding to miRNAs recognition elements. miRNAs can function as a proto-oncogene or as a tumor suppressor depending on their target genes [6,7]. Previous reports have shown that aberrant expression levels of miRNAs are not only specific for tumors including oral cancer, but also in the adjacent stromal cells. For example, Down-regulation of miR-148a in endometrial cancer CAFs stimulates the motility of endometrial cancer cells [8]. Down-regulation of miR-26b in breast CAFs promotes breast cancer cell migration and invasion [9]. MiR-21, a well-known oncomiRNA, was found significantly up-regulated in colorectal CAFs and the latter contributed to colorectal cancer growth and invasion [10]. However, how miRNAs are involved in the cross-talk between fibroblasts (CAFs and NFs) and oral cancer cells remains largely obscure.

The aim of this study was to investigate the regulatory mechanism underlying the interaction between OCCs and fibroblasts (CAFs and NFs). In our preliminary study, we found MiR-124 was down regulated in oral cancer cells. Although MiR-124 was found down regulated in animal model of oral cancer and play important role in oral carcinogenesis by other researchers [11,12], the MiR-124 level in CAFs and how the MiR-124 in CAFs affect the oral cancer remain unclear. CCL2 expression was found upregulated in oral squamous cell carcinoma and patient plasma, and inhibition of CCL2 reduced OC tumor burden in

Abbreviations: CAFs, Cancer associated fibroblasts; NFs, normal fibroblasts; OC, Oral cancer or (OCC), oral cancer cells; C-C motif, Chemokine ligand 2 (CCL2); IL-8, Interleukin 8; OSCC, Oral squamous cell carcinoma; CM, Conditional medium; IHC, Immunohistochemistry

* Correspondence to: Foshan Stomatology Hospital, NO. 5 Hebin Road, Foshan city, Guangdong 528000, China.
E-mail address: dentistlx@163.com (X. Li).

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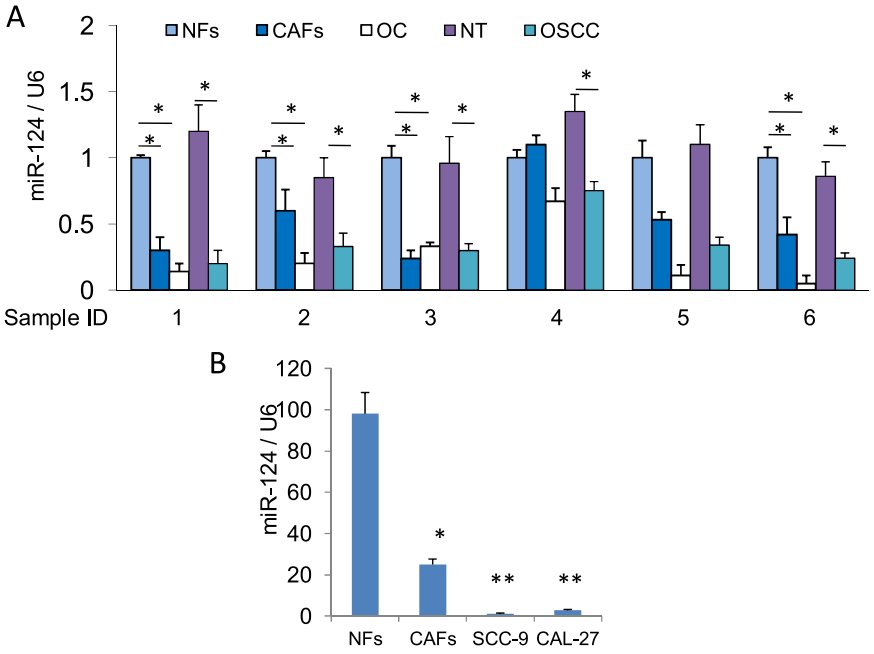

Fig. 1. MiR-124 was down-regulated in oral CAFs and cancer cells. A. The expression levels of miR-124 in 6 sets of cells (CAFs, NFs, and OCCs) as well as their corresponding OSCC and adjacent normal tissues (NTs) were analyzed by Taqman qRT-PCR assay, and normalized to the U6 levels. B. The expression levels of miR-124 in CAFs, NFs, SCC-9 and CAL-27 were analyzed by Taqman qRT-PCR assay, and normalized to the U6 levels. The graphs show the mean ± SD of the relative levels from three replications. $^{*}p < 0.05$.

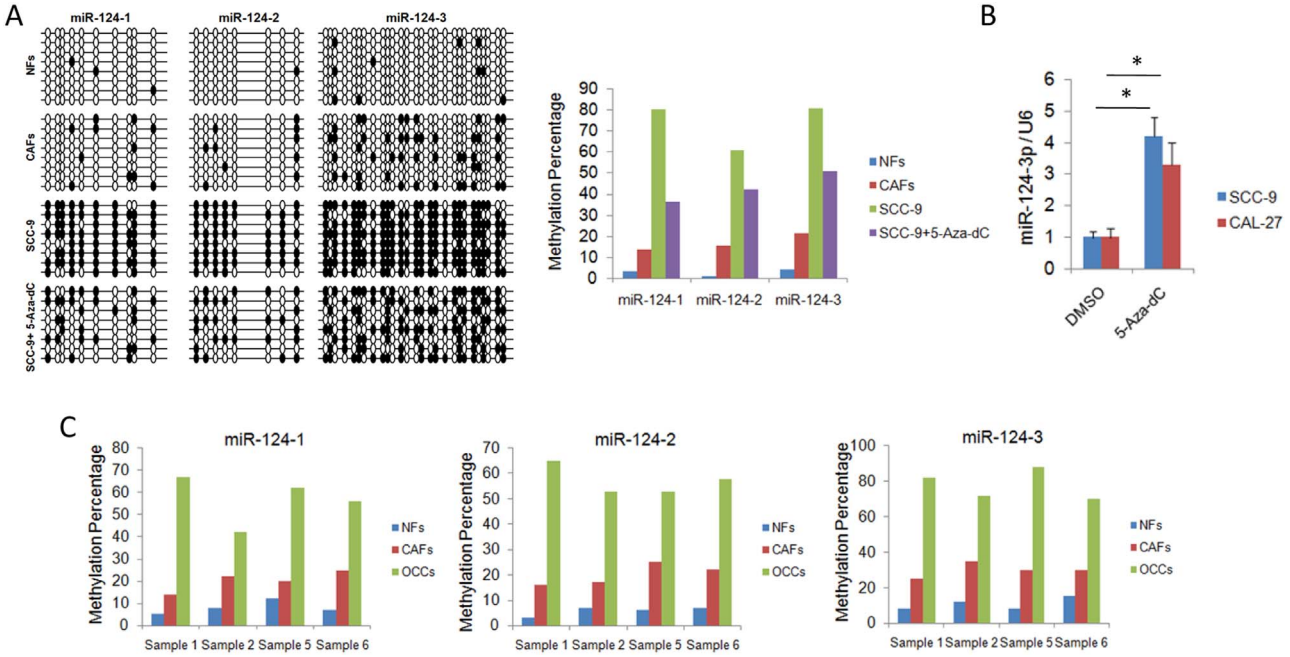

Fig. 2. Methylation status of miR-124 genes in OC cells and tumors. A. Results of bisulfite sequence of miR-124-1, miR-124-2 and miR-124-3 promoter regions in NFs, CAFs, SCC-9 and SCC-9 treated with 5-Aza-dC. Each row represents a single clone for each individual genomic. Open and filled ellipse represent unmethylated and methylated CpG sites, respectively (left panel). The methylation percentages of 8 clones from each of the cell lines (right panel). B. SCC-9 and CAL-27 cells were treated without or with 5-Aza-dC for 5 days. miR-124 expression levels were measured by qRT-PCR. All results represent the mean ± SD from three independent experiments. C. Results of bisulfite sequence of miR-124-1, miR-124-2 and miR-124-3 promoter regions in 4 sets of cells (NFs, CAFs, and OCCs) isolated from indicated human oral tissues. $^{*}p < 0.05$.

mice [13]. CCL2 represents a potential therapeutic target for treatment of OC. Many studies have illuminated that IL-8 is one of prognostic factors of oral carcinoma [14]. According to the bioinformatics search, CCL2 and IL-8 might be the target proteins of MiR-124. In the present research, the relationship between MiR-124 and CCL2 and IL8 were clarified.

We found that down-regulation of miR-124 plays an important role in promoting OCCs cell growth, migration and tumor growth in CAFs/NFs-OCCs co-cultured system through regulation of CCL2 and IL-8 expression. These results indicated that restoration of miR-124 may serves as a potential therapeutic approach for treatment of OSCCs.

2. Materials And Methods

2.1. Cell Culture and Reagents

SCC-9 and CAL-27 cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). SCC-9 and CAL-27 cells