miR145 Targets the SOX9/ADAM17 Axis to Inhibit Tumor-Initiating Cells and IL-6–Mediated Paracrine Effects in Head and Neck Cancer

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Abstract

ALDH1+CD44+ cells are putative tumor-initiating cells (TIC) in head and neck squamous cell carcinomas (HNC). miR-145 regulates tumorigenicity in various cancers but the breadth of its mechanistic contributions and potential therapeutic applications are not completely known. Here, we report that ALDH1+CD44+-HNC cells express reduced levels of miR145. SPONGE-mediated inhibition of miR-145 (Spg-miR145) was sufficient to drive tumor-initiating characteristics in non-TICs/ALDH1+CD44-negative HNC cells. Mechanistic analyses identified SOX9 and ADAM17 as two novel miR145 targets relevant to this process. miR-145 expression repressed TICs in HNC in a manner associated with SOX9 interaction with the ADAM17 promoter, thereby activating ADAM17 expression. Notably, the SOX9/ADAM17 axis dominated the TIC-inducing activity of miR-145. Either miR-145 suppression or ADAM17 overexpression in non-TICs/ALDH1+CD44+-HNC cells increased expression and secretion of interleukin (IL)-6 and soluble IL-6 receptor (sIL-6R). Conversely, conditioned medium from Spg-miR145–transfected non-TICs/ALDH1+CD44+-HNC cells was sufficient to confer tumor-initiating properties in non-TICs/ALDH1+CD44+-HNC and this effect could be abrogated by an IL-6–neutralizing antibody. We found that curcumin administration increased miR-145 promoter activity, thereby decreasing SOX9/ADAM17 expression and eliminating TICs in HNC cell populations. Delivery of lentiviral-miR145 or orally administered curcumin blocked tumor progression in HNC-TICs in murine xenotransplant assays. Finally, immunohistochemical analyses of patient specimens confirmed that an miR-145low/SOX9high/ADAM17high phenotype correlated with poor survival. Collectively, our results show how miR-145 targets the SOX9/ADAM17 axis to regulate TIC properties in HNC, and how altering this pathway may partly explain the anticancer effects of curcumin. By inhibiting IL-6 and sIL-6R as downstream effector cytokines in this pathway, miR-145 seems to suppress a paracrine signaling pathway in the tumor microenvironment that is vital to maintain TICs in HNC. Cancer Res; 73(11): 3425–40. © 2013 AACR.

Introduction

Head and neck squamous cell carcinomas (HNC) represent the sixth most common cancer type worldwide (1). A subpopulation of cells termed tumor-initiating cells (TIC), which possess stem-like properties, were shown to be enriched after therapeutic treatments and presumably contribute to the high rate of relapse and metastasis of HNC tumors (2). Recent reports suggested that CD44 or ALDH1 could be the markers to identify the TICs from HNC (3). ALDH1+CD44+-HNC cells not only present elevated epithelial–mesenchymal transition (EMT) markers but also are highly metastatic, tumorigenic, and resistant to radiotherapy and chemotherapy (4). Thus, an effective therapeutic approach targeting these ALDH1+CD44+-HNC cells may help to improve current treatment regimens for HNC-related malignancies.

miRNAs have recently been linked to regulate the properties of TICs or cancer stem cells (CSC; ref. 5). miR145 modulates embryonic stem cell differentiation by simultaneously regulating multiple genes involved in stem-like properties, including KLF4, OCT4, and SOX2 (6). miR145 is also a tumor-suppressive miRNA that has been shown to be involved in tumor growth and metastasis in several types of cancer (7), as well as modulate TIC- and CSC-like

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-12-3840

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www.aacajournals.org

Published OnlineFirst April 2, 2013; DOI: 10.1158/0008-5472.CAN-12-3840
properties in lung adenocarcinoma and glioblastoma cells (8, 9). However, the roles of miR145 as well as its downstream targets in the regulation of ALDH1+/CD44+/HNC cells remain unclear.

SOX9 (SRY-box containing gene 9) seems to contribute to tumor development through cell-cycle regulatory mechanisms (10), and coexpression of SOX9 and Slug was shown to confer extensive self-renewal capacity upon dormant micrometastasis-forming cells, thereby allowing these cells to spawn macrometastasis (11). ADAM17 (a disintegrin and metalloprotease 17) is a member of the metalloproteinase family and is upregulated in a variety of tumors. Functionally, ADAM17 has been shown to contribute to tumor formation, proliferation, invasion, and antiapoptosis (12–15). In this report, we applied a bioinformatics-based analysis on HNC-TICs and HNC non-TICs, and showed that miR145 as well as its downstream targets, SOX9 and ADAM17, play critical roles in regulating tumor-initiation properties in HNC cells. We showed direct targeting by miR145 to the 3′-untranslated region (UTR) regions of SOX9 and ADAM17 in HNC-TICs, and further showed a direct interaction between Sox9 and ADAM17-promotor by CHIP assay. In addition, it has been reported that ADAM17 mediates the shedding of the IL-6-receptor (IL-6R), which produces a soluble-form of IL-6 (sIL-6R) that could induce a transactivation of the IL-6-pathway in a paracrine manner (16). Notably, elevated IL-6 and sIL-6R levels were induced by miR145-knockdown ALDH1+CD44+/HNC cells, co-knockdown of ADAM17 reversed this effect. Moreover, we showed that the secretion levels of IL-6 and sIL-6R from ALDH1+CD44+/SPONGE-mediated miR145-inhibition-ALDH1+CD44+ cells, as part of the microenviromental niches, are vital to maintain the self-renewal and tumorigenic properties in HNC. Our studies illustrate a novel-regulatory role of the miR145-SOX9/ADAM17 signaling axis in the regulation of TICs-properties in HNC.

Materials and Methods

Reagents

Curcumin was purchased from Sigma Chemical Co. and was dissolved in dimethyl sulfoxide (DMSO) as a stock solution of 100 μmol/L. Just before use, curcumin was further diluted in culture medium to the appropriate final concentrations.

Tumorsphere-forming assay

Tumor cells were dissociated and cultured as tumorspheres in modified Dulbecco’s Modified Eagle Medium (DMEM)/F-12 supplemented with N2 (R&D Systems), 10 ng/mL EGF (Invitrogen), 10 ng/mL basic fibroblast growth factor (bFGF; Invitrogen), and penicillin/streptomycin at 10^5 live cells/low-attachment 6-well plate (Corning Inc.). and the medium was changed every other day until the tumor-sphere formation was observed in about 2 weeks. For serial passage of spheroid cells, single cells were obtained from accurtase-treated spheroids and the cell density of passage was 1,000 cells/mL in the serum-free medium as described earlier.

ALDEFLUOR assay

An ALDEFLUOR assay kit was purchased from StemCell Technologies, Inc. For this assay, 1 × 10^5 cells were suspended in 50 μL of assay buffer, and ALDEFLUOR was added to the cell suspensions for a final concentration of 1 μmol/L. For ALDH1 inhibitor control, diethylaminobenzaldehyde (DEAB) was added to a final concentration of 150 μmol/L. Cells were then incubated at 37°C for 45 minutes and were stained with 7-aminoactinomycin D (7-AAD) on ice for 5 minutes. After washing the cells with PBS, live cells (7AAD-) positive for green fluorescence were analyzed by flow cytometry (FACSCalibur; BD Biosciences) to compare the fluorescence intensity of the DEAB-treated samples. High fluorescence was associated with high ALDH activity (ALDH+ cells).

In vivo GFP imaging of tumor growth

All procedures involving animals were in accordance with the institutional animal welfare guideline of Taipei Veterans General Hospital (Taipei, Taiwan). Cells were subcutaneously injected into 8-week-old immunocompromised C57BL/6j mice (BALB/c strain). In vivo GFP imaging was conducted using an illuminating device [LT-9500 Illumatool TIS equipped with an excitation illuminating source (470 nm) and filter plate (515 nm)]. Tumor sizes were measured using calipers, and their volumes were calculated according to the following formula: (length × width^2)/2; these results were subsequently analyzed using Image Pro-plus software.

Immunohistochemistry

This research follows the tenets of the Declaration of Helsinki, and all samples were obtained after informed consent from the patients. Patients’ tissue samples with different stages of squamous cell carcinoma of the head and neck (HNSCC) were spotted on glass slides for immunohistochemical (IHC) staining (Supplementary Table S4). After deparaffinization and rehydration, tissue sections were processed with antigen retrieval by 1 × Trilogy diluted in H2O (Biogenics) with heating. The slides were immersed in 3% H2O2 for 10 minutes and washed with PBS 3 times. Tissue sections were blocked with serum (Vestastain Elite ABC kit; Vector Laboratories) for 30 minutes, then incubated with the primary antibody in PBS solution at room temperature for 2 hours. Tissue slides were washed with PBS and incubated with biotin-labeled secondary antibody for 30 minutes, then incubated with streptavidin–horseradish peroxidase conjugates for 30 minutes, and washed with PBS 3 times. Tissue sections were then immersed with chromogen 3′-3′-diaminobenzidine plus H2O2 substrate solution (Vector DBA/Ni substrate kit, SK-4100; Vector Laboratories) for 10 minutes. Hematoxylin was applied for counterstaining (Sigma Chemical Co.). The tumor sections were mounted with a cover slide with Gurr (BDH Laboratory Supplies) and examined under a microscope. Pathologists scoring the immunohistochemistry were blinded to the clinical data. The interpretation was done in 5 high-power views for each slide, and 100 cells per view were counted for analysis.
Statistical analysis

Statistical Package for the Social Sciences software version 13.0 (SPSS, Inc.) was used for all statistical analyses. The Student t test was used to determine statistical significance of the differences between experimental groups; P values less than 0.05 were considered statistically significant. The level of statistical significance was set at 0.05 for all tests.

Results

miR145 depletion promotes cell proliferation and enhances tumor growth in non-TICs/ALDH1–/CD44+ HNC cells

The ALDH1+ /CD44+, and sphere-forming HNC cells have been shown to exhibit TICs stem-like properties (4), and these markers have been used to identify HNC-derived TICs (HNC-TIC). To identify the miRNA(s) involved in the regulation of TICs properties in HNC-TICs, we evaluated and compared the miRNA expression profiles in 3 pairs of HNC cell populations (ALDH1+ vs. ALDH1− /CD44+ vs. CD44− , and sphere-forming vs. parental) derived from 2 patient samples and 1 immortalized cell line (HNC-1 and HNC-2) and an immortalized HNC cell line (FaDu). The candidate miRNAs that were either upregulated or downregulated more than 2-fold were considered for further analysis (Fig. 1A and Supplementary Fig. S1A). The data revealed 4 miRNAs, including miR145, that were consistently upregulated or downregulated in ALDH1+ /CD44+ , and sphere-forming HNC cells (Fig. 1A and Supplementary Fig. S1A). Among those candidates, miR145 has been previously linked to regulating the maintenance of stem-like properties in both malignant tumors and embryonic stem cells (6, 17). To further investigate whether miR145 plays a role in the identity of HNC-TICs, the quantitative real-time PCR (qRT-PCR) analysis was used to confirm that miR145 levels were low in ALDH1+ /CD44− and sphere-forming HNC cells but high in ALDH1− /CD44+ and parental cells (Fig. 1B, left and middle). To evaluate the role of miR145 on cancer initiation, we knocked down miR145 in ALDH1+ /CD44− cells isolated from HNC-1, HNC-2, and FaDu cells using an miRNA-Sponge strategy (Fig. 1B, right), and subjected these cells to functional and molecular analysis. As shown in Fig. 1C and D, the sphere formation ability and the percentage of ALDH1+ and CD44+ cells was all elevated upon miR145 knockdown (Spg-miR145) as compared with the control (Spg-ctrl) cells. Moreover, silencing of miR145 increased the cell proliferation rate (Fig. 1E) and the tumor growth of ALDH1+ /CD44− /HNC cells (Fig. 1F, left). The repopulating incident of xenograft-tumor in ALDH1+ /CD44− /Spg-miR145− /transplanted mice was higher than that observed in ALDH1− /CD44− /Spg-ctrl-transplanted animals: as few as 100 injected ALDH1− /CD44− /Spg-miR145− cells were capable of regenerating new tumors in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, whereas 100,000 ALDH1+ /CD44− /Spg-ctrl cells were unable to generate xenograft tumors in NOD/SCID mice (Fig. 1F, right). In addition, knockdown of miR145 in ALDH1− /CD44+ /HNC cells increased the invasive ability in vitro (Fig. 1G) as well as the number of metastatic tumor nodules in vivo in tail vein-transplanted mice (Fig. 1H). These data suggested that suppression of miR145 enables HNC cells to acquire TICs properties.

miR145 directly targets the 3′-UTR of SOX9 and ADAM17 and suppresses the tumor-initiating properties of HNC cells

To further show the significance of the miR145 in regulating the TICs properties in HNC cells, miR145 was overexpressed in ALDH1+ /CD44+ /HNC cells by lentiviral-based delivery system conjugated with GFP (pLV/miR-145; Fig. 2A, left). A scrambled vector-transfected control (pLV/miR-Scr) was also generated simultaneously. The expression level of ectopic miR145 in ALDH1+ /CD44+ /HNC cells was validated by qRT-PCR analysis (Fig. 2A, right). The results of functional analysis showed that overexpression of miR145 significantly decreased the percentages of ALDH1+ /CD44− /HNC, vs. CD44+ (Fig. 2B, left) and invasion capacity (Fig. 2B, right) subsets of cells, and further suppressed sphere formation capacity (Fig. 2C, middle and bottom) in ALDH1+ /CD44+ /HNC cells, as compared with their control cells. Animal study showed that overexpression of miR145 effectively inhibited tumor-initiating property of ALDH1+ /CD44+ cells in NOD/SCID mice (Fig. 2D). As miRNAs suppress target gene expression through miRNA cleavage or translation repression, 3 pairwise comparisons of HNC cell populations (ALDH1+ /CD44− vs. ALDH1− /CD44− , Spg-miR145 vs. Spg-ctrl, and sphere-forming vs. parental) were subjected to a cDNA microarray analysis focusing on stemness-related genes, and the results were further subjected to a prediction algorithm of miR145 targets (Supplementary Fig. S1B). Our results identified SOX9 and ADAM17 as potential targets of miR145 that were highly expressed in ALDH1+ /CD44+, sphere-forming, and Spg-miR145 HNC cells in relation to ALDH1− /CD44− parental, and Spg-ctrl HNC cells, respectively (Supplementary Fig. SIB and S1C). Analysis of the expression levels of miR145, SOX9, and ADAM17 in ALDH1+ /CD44+ and ALDH1− /CD44− cell subpopulations isolated from patient specimens revealed an inverse correlation between miR145 and SOX9/ADAM17 (Fig. 2E). Using the Target Scan program, we predicted potential miR145 targeting sites within the 3′-UTR of SOX9 and ADAM17 (Fig. 2F). We then constructed a series of reporter plasmids containing either wild-type (WT), mutated (Mut), or deleted-forms of the SOX9- and ADAM17-3′-UTR (Supplementary Fig. S1D) and subjected them to luciferase reporter assays in the presence or absence of miR145. The results show that miR145 suppressed the luciferase activities of the reporters containing predicted miR145-targeting sites (SOX9-WT, SOX9-D1, ADAM17-WT, ADAM17-D1, and ADAM17-D2) but not the reporters with deleted targeting sites (Fig. 2G). Mutations within the miR145-targeting sites also hindered the inhibitory effect of miR145 on the reporter constructs (Fig. 2H). These data showed that miR145 directly targets SOX9 and ADAM17 through their 3′-UTR regions. In line with these data, overexpression of miR145 in ALDH1+ /CD44+ /HNC cells by lentivirus-mediated transfection decreased the protein levels of SOX9 and ADAM17, whereas Spg-miR145 treatment of ALDH1− /CD44− /HNC cells increased the invasive ability in vitro (Fig. 1G) as well as the number of metastatic tumor nodules in vivo in tail vein-transplanted mice (Fig. 1H). These data suggested that suppression of miR145 enables HNC cells to acquire TICs properties.
CD44+/HNC cells increased protein expression of SOX9 and ADAM17 (Fig. 2). Remarkably, co-knockdown of SOX9/ADAM17 in ALDH1+/CD44+/HNC cells using SOX9- and ADAM17-specific short hairpin RNAs (shRNAs; Fig. 2J) had similar effects as miR145 overexpression, resulting in a reduction in sphere-forming ability (Supplementary Fig. S2A), reduced percentages of CD44+ (Supplementary Fig. S2B) and ALDH1+ cells (Supplementary Fig. S2C), reduced invasive capacity (Supplementary Fig. S2D), and inhibited tumor-initiating property in ALDH1+/CD44+ cells-transplanted NOD/SCID mice (Supplementary Fig. S2E). Collectively, our results suggest that miR145 directly targets the 3’-UTR of SOX9 and ADAM17 to suppress their expression and repress the TIC properties of ALDH1+/CD44+/HNC cells.

SOX9 directly regulates ADAM17 promoter and the SOX9/ADAM17 axis dominates miR145-mediated EMT and TICs properties

SOX9, a high-mobility group box transcription factor, plays critical roles during embryogenesis, development, and differentiation (11). Recent evidence has provided a link between SOX9 and tumor development and cancer progression (18). Herein, we found that knockdown of SOX9 decreased ADAM17 expression in ALDH1+/CD44+/HNC cells (Fig. 3A), whereas overexpression of SOX9 resulted in increased ADAM17 ALDH1+/CD44+/HNC cells (Fig. 3B). In contrast, protein levels of SOX9 were not significantly changed in ADAM17-knockdown ALDH1+/CD44+/HNC or ADAM17-overexpressed ALDH1+/CD44+/HNC cells (Fig. 3A and B). These findings indicated that SOX9 is an upstream molecule regulating ADAM17 expression. A luciferase reporter assay indicated that SOX9 enhanced ADAM17-promoter (wt and del-1) activity; deletion (del-2 and del-3) or mutation (mut) of the promoter region (−506 to −503) in ADAM17-promoter, however, prevented SOX9 from activating ADAM17 promoter (Fig. 3C). By conducting chromatin immunoprecipitation (ChIP) using an anti-SOX9 antibody and subsequent PCR amplification, the ADAM1 promoter fragment was shown to be present in the immunoprecipitates (Fig. 3D). These data confirm that ADAM1 upregulation is partly resulted from transcriptional activation of SOX9 after directly binding on ADAM17 promoter. We next clarify the functional involvement of miR145-SOX9/ADAM17 axis in HNC aggressiveness. The wound-healing (Fig. 3E) and invasion abilities (Fig. 3F) of ALDH1+/CD44+/HNC cells were increased in Spg-miR145 HNC cells. Furthermore, individual silencing of SOX9 or ADAM17 or co-knockdown of SOX9 and ADAM17 in Spg-miR145-treated ALDH1+/CD44+/HNC cells partially counteracted with miR145-SPONGE and slightly inhibited wound-healing and invasion abilities (Fig. 3E and F). ALDH1+/CD44+/HNC cells were previously shown to be present with elevated EMT markers and are of highly metastatic ability (19). With Western blotting, we showed that Spg-miR145 induced a pattern of upregulated mesenchymal-like proteins (fibronectin, vimentin, and Slug) and downregulated epithelial protein (E-cadherin) in ALDH1+/CD44+/HNC cells, which were reversed by Sox9 and/or ADAM17 downregulation (Fig. 3G). Moreover, we examined the incidence of tumor metastasis in mice following the systemic administration of ALDH1+/CD44+/HNC cell with different combination of Spg-miR145, shSOX9, and shADAM17 (Fig. 3H). These data indicated that the Spg-miR145-increased incident and number of metastatic tumor nodules in the lung and liver were suppressed by additional knockdown of Sox9 and/or ADAM17 (Fig. 3H). Combined with these data, SOX9 directly regulates ADAM17 expression, and the SOX9/ADAM17 axis modulates miR145-mediated EMT and tumor metastasis in HNC.

miR145 and ADAM17 mediate IL-6 inflammatory cytokine and soluble IL-6 receptor secretion, which maintains the TIC properties of HNC cells in a paracrine manner

ADAM17 has been shown to be responsible for the ectodomain shedding of transmembrane proteins, resulting in the release of their extracellular domains from the cell membrane (20). For example, ADAM17-mediated shedding of the IL-6R produces a sIL-6R that induces the transactivation of the IL-6 pathway in a paracrine-manner in cells that do not express IL-6R and thus are insensitive to IL-6-activation (16). To elucidate the possible involvement of miR145 and ADAM17 in regulating IL-6, we conducted a microarray analysis of the NC160 tumor cell database focusing on secreted cytokines. Our results showed that mRNA expression of miR145, ADAM17, and...
IL-6 were closely correlated (Fig. 4A). ELISA analysis further indicated that the secreted IL-6 and sIL-6R were low in ALDH1-CD44-HNC cells but high in ALDH1-CD44-HNC cells, in which the secretion level of sIL-6R presented larger difference than that of IL-6 (Fig. 4B, left). Clinically, patients with recurrent HNC had increased concentration of IL-6 and sIL-6R in serum, in comparison with patients with primary HNC (Fig. 4B, right). Because ADAM17 is a miR145 target, we investigated the effects of miR145 on the expression levels of IL-6 and sIL-6R. Western blot analyses (Fig. 4C, left; Supplemental Fig. S2F) and ELISA (Fig. 4C, right) indicated that the overexpression of miR145 suppressed the protein expression of IL-6 in ALDH1-CD44 cells, whereas Spg-miR145 increased IL-6 expression in ALDH1-CD44 cells. To clarify the causal relationship between miR145, ADAM17, and the regulation of IL-6 expression, we manipulated miR145 and ADAM17 expression levels using Spg-miR145 and shADAM17 in ALDH1-CD44-HNC cells. Western blot analyses showed that cellular IL-6 protein levels were elevated by Spg-miR145 but that concomitant knockdown of ADAM17 inhibited the Spg-miR145-induced increase in IL-6 protein expression, indicating that ADAM17 mediates the miR145-dependent regulation of IL-6 (Fig. 4D). ELISAs further showed that the inhibition of miR145 and the overexpression of ADAM17 enhanced the levels of secreted IL-6 and sIL-6R that were detected in the medium of ALDH1-CD44 cells, and this increase was observed to a greater extent for sIL-6R (Fig. 4E). Regardless of whether the elevated IL-6 and sIL-6R levels were induced by Spg-miR145, knockdown of ADAM17 reversed this effect (Fig. 4E). IL-6 has been implicated in the regulation of some TIC properties in epithelial cancers, including human ductal breast carcinomas (21), and the IL-6 inflammatory loop mediates the resistance of breast cancer cells to HER2-targeting antibodies (22). As such, we hypothesized that secreted IL-6 and sIL-6R may induce TIC-like properties in HNC cells. We incubated ALDH1-CD44-HNC cells for 24 hours in the conditioned medium (CM) derived from ALDH1-CD44-HNC cells (ALDH1-CD44-CM) that had been transfected with or without miR145 and shADAM17, or in ALDH1-CD44-CM pre-incubated with anti-sIL-6R-neutralizing antibodies. The ALDH1-CD44-CM induced the self-renewal capability, the percentage of sphere formation, and the percentage of side population of ALDH1-CD44-HNC cells. These effects were blocked by miR145 overexpression or the anti-IL-6-neutralizing antibody treatment (Supplementary Fig. S3A and S3B). The conditioned medium derived from ALDH1-CD44/Spg-miR145 cells (ALDH1-CD44/Spg-miR145-CM) had similar effects to ALDH1-CD44-CM (Fig. 4F and Supplementary Fig. S3C). Transfection of ALDH1-CD44/Spg-miR145 cells with shADAM17 or preincubation of ALDH1-CD44/Spg-miR145-CM with anti-IL-6-neutralizing antibodies reduced the enhancing effects of ALDH1-CD44/Spg-miR145-CM on self-renewal and side population of ALDH1-CD44-HNC cell (Fig. 4F and Supplementary Fig. S3C). Notably, the phosphorylation status of STAT3, an important downstream effector of IL-6 signaling pathway, in these conditioned medium–treated ALDH1-CD44 cells was closely associated with sphere formation capacity (Fig. 4G), suggesting a role of the IL-6–STAT3 signaling in the paracrine and autocrine regulation of TIC properties. These results collectively suggest that the miR145–ADAM17 pathway mediates the maintenance of TIC properties in HNC in a paracrine/autocrine manner dependent on IL-6 and sIL-6R.

Curcumin increases miR145, decreases SOX9/ADAM17/IL-6/sIL-6R, and eliminates HNC-TICs

Curcumin is known to inhibit TIC-like properties in breast, pancreatic, brain, and colon cancers (23–27). Moreover, curcumin has been shown to suppress the self-renewal of breast TICs (25), reduce the side population of rat glioma cell lines (23), and promote the differentiation of glioma TICs by inducing autophagy (28). Recently, curcumin-regulated miRNAs have been shown to be involved in the epigenetic regulation of TICs properties in several types of malignant cancers (29–32). However, the miRNAs that mediate curcumin-dependent regulatory mechanisms in HNC-TICs remain unclear. We examined the effect of curcumin on 2 normal human oral keratinocyte (NHOK) primary cells and TIC-like ALDH1-CD44-HNC cells isolated from HNC-1 and HNC-2 cells. As shown in Fig. 5A, curcumin inhibited the proliferation rate of ALDH1-CD44-HNC cells in a dose-dependent manner, whereas the inhibition on NHOK cells proliferation was limited (Fig. 5A). These data showed that curcumin was specific and acted almost exclusive on tumor cells, rather than normal, nontransformed cells. We then evaluate the potential role of curcumin in modulating the cancer stem-like properties of HNC-TICs and found that curcumin decreased the self-renewal capacity (Fig. 5B), the

**Figure 2.** miR145 directly targets the 3′-UTR of SOX9 and ADAM17. A, HNC-ALDH1-CD44+ cells were transfected with GFP-tagged miR145 (pLV-miR145) or empty vector (pLV), and the transfection efficiency and the miRNA expression level of miR145 were assessed by fluorescent microscopy (left) and qRT-PCR (right), respectively, B, HNC-ALDH1-CD44+ cells transfected with pLV or pLV-miR145 were assessed by flow cytometry to determine ALDH1 activity (left) and the expression of CD44 (right). C, HNC-ALDH1-CD44+ cells transfected with pLV or pLV-miR145 were subjected to a sphere formation (left) and invasion assay (right). D, various numbers of HNC-ALDH1-CD44+ cells transfected with pLV or pLV-miR145 were injected into NOD/SCID mice (n = 3). Mice were monitored for 4 to 12 weeks for the occurrence of tumor mass and the tumor incidence in each group was calculated and is presented in the chart. E, two groups of HNC patient sample-derived cells (ALDH1-CD44+ and ALDH1-CD44− ) were analyzed for their expression of miR145, SOX9, and ADAM17. Statistical correlation analysis indicated a highly negative correlation between miR145 and Sox9 expression as well as between miR145 and ADAM17 expression. F, miR145 target sites were predicted within the 3′-UTR regions of SOX9 and ADAM17 using TargetScan. The mutation of 4 specific residues in the seed region of the miR145 target site. Both WT and mutated SOX9 3′-UTR were constructed in Luciferase reporter plasmid. G, the WT and deleted forms (D1, D2, and D3) of the SOX9 and ADAM17 reporters were cotransfected with pLV and pLV-miR-145 into HNC cells. The luciferase activity of each combination was assessed and is presented. H, similar reporter assays were conducted in HNC cells with WT and mutated (Mut) reporter plasmids. The results of the luciferase assays indicated that only WT reporter activity was inhibited by miR145. I, the protein expression levels of SOX9 and ADAM17 in miR145-transfected ALDH1-CD44+ and Spg-miR145-transfected ALDH1-CD44+ cells were analyzed by Western blot analysis. J, Western blot analysis of SOX9 and ADAM17 protein levels in ALDH1-CD44+ HNC cells subjected to concomitant knockdown of SOX9 and ADAM17.
Figure 3. SOX9 directly binding to ADAM17 promoter as a dominant axis of miR-145-mediated TICs properties. A, expression level of SOX9 and ADAM17 protein was assessed by Western blot analysis in HNC-ALDH1 CD44- cells transfected with shSox9, shADAM17 individually or concurrently, B, expression level of Sox9 and ADAM17 expression was assessed by Western blot analysis in HNC-ALDH1 CD44- cells transfected with control, Sox9-overexpressing, or ADAM17-overexpressing vector. C, schematic representation of the reporter constructs containing deleted or point mutated human ADAM17 promoter (top). The full-length, deleted, mutated, or deleted ADAM17 promoter reporter constructs were subjected to luciferase reporter assay (bottom). D, ChIP assay of HNC-ALDH1 CD44- cells transfected with control, Sox9-overexpressing, or ADAM17-overexpressing vector. E, PCR amplification of the ADAM17-promoter fragment existed in the Sox9-ADAM17 sample. Input, 2% of total input lysate. F, HNC-ALDH1 CD44- cells were transfected with Spg-ctrl or Spg-miR145, and HNC-ALDH1 CD44- cells transfected with Sh-Luc., Spg-miR145 Sh-ADAM17, or Sh-Sox9, Sh-ADAM17, as indicated, were analyzed by Western blot analysis for the levels of cellular ADAM17 and IL-6, and by ELISA for secreted IL-6 and sIL-6R, respectively (E). F, one thousand of HNC-ALDH1 CD44- cells were subjected to a sphere formation assay in the presence of the conditioned medium derived from ALDH1 CD44- HNC cells that were transfected with indicated plasmids. The number of spheres was counted and presented (right). (, P < 0.01 compared with Spg-Ctrl-CM and #, P < 0.01 compared with Sh-Ctrl-CM as determined by the Student t test.). G, HNC-ALDH1 CD44- cells treated with conditioned medium derived from ALDH1 CD44- HNC cells with differential combination of Spg.Ctrl., Spg-miR145, Spg-miR145 or Sh-Luc, Spg-miR145 or IgG antibody, and Spg-miR145 or IL-6 antibody. These cells were analyzed by Western blot analysis for the protein expression level of indicated p-STAT3 and STAT3.

Figure 4. The miR145-ADAM17 pathway modulates IL-6/sIL-6R trans-signaling. A, bioinformatics analysis of the NCI-60 cancer cell line database showed a negative correlation between the expression of miR145 and that of IL-6 and ADAM17. B, ELISA analysis of the expression of IL-6 and sIL-6R in ALDH1+ and ALDH1- CD44+ HNC cells, as well as in serum of primary (n = 25) and recurrent (n = 25) HNC patient specimens. C, representative images (left) and quantification (middle) of HNC-ALDH1 CD44+ cells transfected with pLV empty vector or pLV-miR145, and HNC-ALDH1 CD44+ cells transfected with Spg-ctrl or Spg-miR145 were then subjected to Western blot analysis for the expression level of cellular IL-6. The levels of secreted IL-6 and sIL-6R from the indicated cells were analyzed by ELISA assay (right). D, HNC-ALDH1 CD44+ cells transfected with Spg-miR145, ADAM17, or ShADAM17, as indicated, were analyzed by Western blot analysis for the levels of cellular ADAM17 and IL-6, and by ELISA for secreted IL-6 and sIL-6R, respectively (E). F, one thousand of HNC-ALDH1 CD44+ cells were subjected to a sphere formation assay in the presence of the conditioned medium derived from ALDH1 CD44+ HNC cells that were transfected with indicated plasmids. The number of spheres was counted and presented (right). (, P < 0.01 compared with Spg-Ctrl and #, P < 0.01 compared with Spg-shLuc as determined by the Student t test.). G, HNC-ALDH1 CD44+ cells treated with conditioned medium derived from ALDH1 CD44+ HNC cells with differential combination of Spg.Ctrl., Spg-miR145, Spg-miR145 or Sh-Luc, Spg-miR145 or IgG antibody, and Spg-miR145 or IL-6 antibody. These cells were analyzed by Western blot analysis for the protein expression level of indicated p-STAT3 and STAT3.

expression patterns indicated that curcumin-treated ALDH1+ CD44+ and ALDH1- CD44-/Spg-miR145 cells were more closely resembled ALDH1+ CD44+ HNC cells and
clustered separately from ESCs, MSCs, and the original ALDH1\(^+\) CD44\(^+\) and ALDH1\(^+/C0\) CD44\(^+/C0\)/Spg-miR145 cells, implying that curcumin treatment modified the genetic signatures of ALDH1\(^+\) CD44\(^+\)-HNC cells, with a shift toward a less malignant profile (Fig. 5D). Consistently, curcumin pretreatment of ALDH1\(^+\) CD44\(^+\)-HNC cells decreased the survival rate of the mice (Fig. 5B), and the expression levels of SOX9 and ADAM17 were decreased by curcumin treatment (Fig. 5C).

Figure 5. Curcumin suppresses the stem-like properties of ALDH1\(^+\) CD44\(^+\) cells by activating miR145 and suppressing SOX9/ADAM17. A, NHOKs and HNC-ALDH1\(^+\) CD44\(^+\) cells were treated with various concentrations of curcumin up to 20 \(\mu\)mol/L for 24 hours. Cell survival was assessed by MTT assay and is presented as percent survival relative to untreated cells. B, representative images (top) and quantification (bottom) of HNC-ALDH1\(^+\) CD44\(^+\) cells treated with or without curcumin were subjected to a sphere formation assay. C, the ALDH1 activity of HNC-ALDH1\(^+\) CD44\(^+\) cells treated with or without curcumin was assessed by flow cytometry. D, miRNA and bioinformatic analyses were conducted to compare the gene expression pattern between the indicated cells. Spg-miR145 shifted the gene expression pattern toward ESC- and MSC-like profiles, whereas curcumin treatment shifted the gene expression pattern toward a profile similar to HNC-ALDH1\(^+\) CD44\(^+\) cells. E, the indicated miRNA expression levels in the curcumin-treated HNC-ALDH1\(^+\) CD44\(^+\) cells were analyzed by qRT-PCR. F, Northern blotting (top) and a miR145 promoter reporter assay (bottom) were conducted in HNC-ALDH1\(^+\) CD44\(^+\) cells with or without curcumin (20 \(\mu\)mol/L) treatment. G, HNC-ALDH1\(^+\) CD44\(^+\) cells were treated with or without curcumin (10 or 20 \(\mu\)mol/L) and were subjected to Western blot analysis to determine the protein expression levels of SOX9 and ADAM17. H, mice were subcutaneously injected with HNC-ALDH1\(^+\) CD44\(^+\) cells (100 to 100,000 cells) that had been treated with various combinations of Spg-miR145, SOX9\(+\)/ADAM17, and curcumin as indicated for 12 weeks (\(n = 3\)). Tumor occurrence is presented in the table.

Figure 6. Treatment with oral-feeding curcumin or miR145 suppresses tumor growth and increases animal survival. GFP-labeled HNC-ALDH1\(^+\) CD44\(^+\) cells were orthotopically implanted in the neck region of nude mice followed by treatment with saline (through oral gavage as control), intratumoral injection of miR145-overexpressing lentivirus (pLV/miR-145) or combined shRNAs against SOX9 and ADAM17 (shSOX9\(+\)/shADAM17), or curcumin (40 mg/kg/d by oral gavage). A, the GFP signal emitted by the implanted tumor cells was monitored for 5 weeks and was photographed, and the growth curve of the GFP-labeled tumors is presented (B). C, the survival rate of the mice was monitored for up to 12 weeks and is presented in the graph (each group; \(n = 12\)). D, mice were sacrificed and tumor sections were stained using specific antibodies against SOX9, ADAM17, SLUG, and IL-6. E, the percentages of positively stained cells for SOX9, ADAM17, SLUG, and IL-6 were compared by Student’s test, and the survival rate up to 12 weeks is presented (each group; \(n = 12\)).

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number of metastatic tumor nodules in the lung of ALDH1+CD44+-HNC–transplanted mice (Supplementary Fig. S3F). Next, we compared the curcumin-treated and nontreated ALDH1+CD44+-HNC cells by miRNA microarray to identify other potential curcumin-regulated miRNAs. Curcumin treatment if ALDH1+CD44+-HNC cells resulted in a dose-dependent increase in the levels of various miRNA, including miR145, miR200b, miR200c, let-7a, and let-7d. Notably, miR145 was the most responsive miRNA in curcumin-treated ALDH1+CD44+-HNC cells (Fig. 5E). A miR145 promoter-driven reporter assay showed that curcumin increased miR145 promoter activity in ALDH1+CD44+-HNC cells (Fig. 5F, bottom). This finding was confirmed by Northern blot analysis, as an increased miR145 mRNA level was detected in curcumin-treated cells (Fig. 5F, top). Importantly, curcumin-treatment of ALDH1+CD44+-HNC cells also suppressed the protein levels of SOX9 and ADAM17, which our data implicated as targets of miR145 (Fig. 5G). Furthermore, an in vivo tumor initiation assay showed that suppression of miR145 or overexpression of SOX9/ADAM17 rendered as few as 100 ALDH1+CD44+-HNC cells capable of forming tumors by subcutaneous injection into nude-mice (Fig. 5H). Moreover, the Spg-miR145- and SOX9/ADAM17-mediated increased in tumor-initiation capacity in ALDH1+CD44+-HNC cells was suppressed by curcumin-treatment (Fig. 5H). To clarify the functional involvement of curcumin and the miR145-SOX9/ADAM17 axis in HNC aggressiveness, Spg-miR145-, SOX9-, ADAM17-, and SOX9/ADAM17-transfected ALDH1+CD44+-HNC cells treated with or without curcumin were subjected to wound-healing and Transwell invasion assays. The wound-healing and invasion abilities of ALDH1+CD44+-HNC cells were increased in Spg-miR145-, SOX9-, ADAM17-, and SOX9/ADAM17-transfected cells compared with controls, whereas the Spg-miR145- and SOX9/ADAM17-dependent increases in migration and invasion were suppressed by curcumin treatment (Supplementary Fig. S4A and S4B). In line with these findings, Western blot analyses showed that the curcumin treatment downregulated pattern of upregulated mesenchymal-like proteins (fibronectin, vimentin, and Slug) and upregulated epithelial protein (E-cadherin) in miR-145–knockdown ALDH1+CD44+ or ALDH1+CD44+-HNC cells in a time-dependent manner (0, 24, 48, and 72 hours; Supplementary Fig. S4C). Furthermore, curcumin time-dependently eliminates IL-6/sIL-6R secretion in ALDH1+CD44+-HNC cells (Supplementary Fig. S4D). Moreover, we examined the incidence of tumor metastasis in mice lungs and livers following the systemic administration of Spg-miR145- or SOX9/ADAM17-transfected ALDH1+CD44+-HNC cells pretreated with or without curcumin. These results indicated that the incidence and number of metastatic tumor nodules were increased in animals that received cells transfected with Spg-miR145 or SOX9/ADAM17, whereas pretreatment with curcumin suppressed the metastatic incidence and number of tumor nodules in both cases (Supplementary Fig. S4E and S4F). Taken together, these results suggest that curcumin has the potential to suppressed EMT and decrease tumor metastasis capacities of HNC-TICs through the activation of miR145 and the suppression of SOX9/ADAM17/IL-6/sIL-6R.

Therapeutic delivery of curcumin or miR145 to ALDH1+CD44+-HNC cells-transplanted mice attenuates tumor progression in vivo

Next, we explored the therapeutic potential of curcumin and miR145 in immunocompromised mice bearing HNC-TIC xenograft tumors. Nude mice that were orthotopically injected with GFP-labeled ALDH1+CD44+-HNC cells in the neck region, followed by intratumoral lesion injections with miR145-overexpressing lentivirus (pLV-miR145) and shSOX9/shADAM17 or by oral gavage with curcumin (40 mg/kg/d) and control (saline). Five weeks after the treatments, mice treated with pLV-miR145 and shSOX9/shADAM17 showed dramatically reduced tumor sizes in comparison with mice that received the empty vector (pLV) or shRNA control (shLuc), and curcumin treatment resulted in an even greater inhibition of tumor growth (Figs. 6A and B). By monitoring the treated mice for up to 12 weeks, we observed that administration of pLV- miR145, shSOX9+shADAM17, and curcumin prolonged animal survival, with a greater extent in LNA-miR145- and curcumin-treated mice (Fig. 6C). Tumor sections obtained from each treatment group were then subjected to IHC analysis to evaluate the expression of miR145 downstream targets. Delivery of LNA-miR145, shSOX9+shADAM17, or curcumin to ALDH1+CD44+-HNC–transplanted mice reduced the expression of SOX9, ADAM17, Slug, and IL-6 (Fig. 6D and E), whereas pLV-miR145 and curcumin treatment significantly increased miR145 mRNA expression in the tumor samples (Fig. 6F). To investigate the therapeutic effects of curcumin on tumor metastasis caused by the suppression of miR145 or the overexpression of SOX9/ADAM17, NOD/SCID (BALB/c-strain) mice were implanted with 2 × 10^5 Spg-miR145- or SOX9+ADAM17–transfected ALDH1+CD44+ cells by tail vein injection, and then the mice were treated with or without curcumin (40 mg/kg/d) by oral gavage. As expected, the numbers of tumor nodules in lung were increased in ALDH1+CD44+/Spg-miR145- and

Figure 7. The miR145/SOX9/ADAM17 signature predicts poor survival in patients with HNC. A, paired tissue samples from tumor (T; n = 75) and adjacent nontumor parts (N; n = 75), as well as lymph node metastatic (LN; n = 50) and local (T; n = 50) lesions in HNC patient tissues were subjected to histologic analysis for the expression levels of miR145, SOX9, and ADAM17. Statistical correlation analysis was applied to the data. B, a panel of HNC patient samples from nontumor (N) and tumor specimens (well, moderate, and poor differentiated) was collected and immunohistochemically stained with anti-SOX9 and anti-ADAM17 antibodies. C, Kaplan–Meier analysis of overall survival period of HNC cases with miR145 versus miR-145 *, SOX9* versus SOX9, and ADAM17 versus ADAM17 in primary tumors. Subgroup analysis of HNC cases according to the expression profile of miR145, SOX9, and ADAM17 in primary tumors. The miR145/SOX9/ADAM17 signature indicates the worst survival rate period when compared with the other groups. P values of the comparison between each group are shown in the inset. D, Kaplan–Meier analysis of lymph node metastasis–free period of patients with HNC according to the expression profile of miR145, SOX9, and ADAM17 in primary tumors. E, a schematic representation of the curcumin-activated miR145-SOX9/ADAM17-IL-6/sIL-6R signaling pathway in the regulation of the TIC properties of HNC cells is shown.

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ALDH1–CD44+/SOX9+/ADAM17–transplanted mice, and the administration of curcumin effectively suppressed them (Figs. 6G and H). In addition, the survival of ALDH1–CD44+/Spg-miR145–/SOX9+–ADAM17–transplanted mice was significantly improved with curcumin treatment (Fig. 6I). Collectively, our data show that miR145 and curcumin treatment impaired tumor growth, reduced miR145 downstream target expression, suppressed metastasis, and improved the survival of HNC tumor-bearing mice.

The clinical significance of the miR145lowSOX9highADAM17high signature in HNC patients

To validate the significance of the miR145-SOX9/ADAM17 axis in clinical specimens, we collected paired samples of tumor (T) and non-tumor (N) tissue from patients with HNC and subjected these samples to histologic analysis. The expression of miR145 in HNC tissues was significantly decreased in the tumor specimens, whereas SOX9 and ADAM17 expression was increased relative to the non-tumor tissue (Fig. 7A, top). In line with these data, the level of miR145 expression was higher in local (T) tumor samples but lower in lymph node metastatic (LN) lesions, whereas SOX9/ADAM17 expression levels were lower in local tumor samples and higher in metastatic lesions (Fig. 7A, bottom). We further compared the expression of SOX9 and ADAM17 in a panel of HNC patient samples from non-tumorous to poorly differentiated tumor specimens (Supplementary Table S4). IHC staining showed that high-grade with poor differentiated HNC tumors had high levels of SOX9 and ADAM17 expression, whereas low-grade tumor samples presented low levels of SOX9 and ADAM17 expression (Fig. 7B). To determine the prognostic significance of miR145, SOX9, and ADAM17 expression levels, a Kaplan–Meier survival analysis of patients with HNC was conducted according to the expression profiles of these genes. Patients with HNC with tumors that expressed high levels of SOX9 and ADAM17 had a reduced survival rate, whereas patients with high tumoral expression of miR145 showed a better survival rate (Fig. 7C). Moreover, patients with tumors that displayed an expression profile of miR145lowSOX9highADAM17high had a lower survival rate compared with patients harboring tumors with other profiles, such as miR145highSOX9lowADAM17low (Fig. 7C). In addition, patients with HNC with miR145lowSOX9highADAM17high expression were also associated with greater metastatic status (Fig. 7D). Overall, these results suggest that the reduced expression of miR145 and elevated expression of SOX9/ADAM17 are strongly associated with advanced-grade HNC and a poor prognosis. Thus, the miR145lowSOX9highADAM17high signature could be used as a predictor of disease progression and clinical outcome in patients with HNC.

Discussion

miR145 is a tumor-suppressive miRNA that inhibits tumor growth and metastasis (33). Here, we showed that miR145 is an important negative regulator of HNC and that suppressing miR145 expression is crucial for maintaining the stem-like and TICs capacities of HNC. SOX9 is a positive regulator of cancer-associated cellular signaling pathways (10, 34–36), and its overexpression is associated with poor prognosis and patient survival in breast and lung carcinoma (11, 37). We showed that the overexpression of miR145 or the knockdown of SOX9 and ADAM17 suppressed the TICs properties of ALDH1–CD44+/HNC cells (Fig. 2). Furthermore, ectopic overexpression of SOX9 and ADAM17 reversed the miR145-mediated inhibition of ALDH1–CD44+/HNC tumorigenicity. In animal models, we confirmed that miR145 suppresses tumor initiation, growth, and metastasis through inhibiting SOX9 and ADAM17 (Fig. 6). Finally, a miR145lowSOX9highADAM17high signature in HNC patient-derived tumor samples correlated with a poor patient survival rate (Fig. 7C). In addition, the conventional anticancer drug curcumin is known to suppress cancer cells by modulating miRNA levels (29), and our findings show that miR145 is one of the major miRNAs upregulated in HNC in response to curcumin (Fig. 5). Functional assays indicated that the miR145-SOX9/ADAM17-axis mediates a curcumin-dependent suppression of tumor-initiating properties in ALDH1–CD44+/HNC both in vitro and in vivo. To our knowledge, this is the first report showing a miR145-targeting SOX9/ADAM17 axis in regulating TICs-properties in HNC, and the miR145-mediated suppression of TIC properties could be partially augmented by the anticarcinogenic effect of curcumin (Fig. 7E).

The inflammatory paracrine effect of IL-6/IL-6R constitutes a critical costimulatory cytokine network and promotes cancer stem-like properties in breast cancer, colorectal cancer, and malignant gliomas (21, 22, 38). Recently, clinical studies showed the importance of IL-6 and sIL-6R in tumor progression, as the levels of IL-6 and sIL-6R are elevated in many patients with cancer, and the IL-6/sIL-6R trans-signaling has also been shown to play an essential role in cancer metastasis and tumor recurrence (16, 39). ADAM17 is known to mediate the ectodomain shedding of IL-6R to generate the sIL-6R (40, 41), thereby activating the IL-6 pathway through a process known as trans-signaling (40–42). Our data indicated that apart from IL-6R shedding, ADAM17 also increased the expression and secretion of IL-6 (Fig. 4). In ALDH1–CD44+/HNC cells, the IL-6 and sIL-6R expression and secretion, as well as the self-renewal capacity, were increased upon treatment with conditioned medium derived from ALDH1–CD44+/HNC or Spg-miR145–transfected ALDH1–CD44+/HNC cells and was decreased upon treatment with shADAM17 and anti-sIL-6R-neutralizing antibodies (Supplementary Fig. S3A and S3B). Clinically, upregulation of serum level of IL-6 and sIL-6R were both observed in recurrent and lymph node metastatic tumor tissues compared with primary and local tumor tissue, respectively (Fig. 4B). Moreover, in line with previous report showing that STAT3 mediates IL-6–dependent promotion of cell proliferation and survival in breast- and glioma stem cells (43), we found that in ALDH1–CD44+/HNC cells, the phosphorylation level of STAT3 was increased upon treatment with conditioned medium derived from Spg-miR145–transfected ALDH1–CD44+/HNC cells and was decreased upon treatment with shADAM17 or IL-6–neutralizing antibody (Fig. 4G). Our data showed that the miR145-ADAM17 regulates IL-6/sIL-6R trans-signaling, which involves STAT3 activation, and paracrinally regulates the self-renewal capacity of HNC. The miR145-low HNC-TICs paracrinally enhance the stem-like and self-renewal properties of miR145-low HNC-TICs.
high HNC-non-TICs through IL-6 trans-signaling, and thus is potentially turn HNC-non-TICs to HNC-TICs.

Sun and colleagues has shown that curcumin alters the miRNA expression profiles in human pancreatic cancer cells (29). The curcumin analog-CDF (difluorinated-curcumin) has also been reported to inhibit pancreatic tumor growth by enhancing the expression of several suppressor miRNAs such as let7, miR26a, miR21, and miR143 (27, 44). We found that curcumin presents a greater inhibitory effect in ALDH1$^+$ CD44$^+$ HNC cells than in NHOK cells (Fig. 3). Our results showed that curcumin-dependent activation of a miR145-SOX9/ADAM17 axis might act as part of the mechanism to suppress TICs properties in HNC. Curcumin has been reported to activate p53 in many human carcinoma cells including breast cancer, prostate cancer, and glioma cells (45–47). Interestingly, Sachdeva and colleagues showed that p53 directly binds the promoter of miR145 at the p53 response element region (48). p53 was shown to suppress EMT and TICs properties by upregulating miR145 (49). These data suggest a possible mechanism that curcumin, by inducing the expression of p53, could increase the expression of miR145 in HNC-TICs. Future research delineates the details of how curcumin regulates its p53–miR145 axis, and how these interactions influence the stemness properties of TICs remains to be determined. Further research effort is needed in this area.

In conclusion, the present study showed that curcumin inhibited HNC tumorigenicity through the miR145 or miR145/5-SOX9/ADAM17 regulatory pathway, which resulted in the inhibition of the self-renewal, tumor initiation, and metastatic properties of HNC-TIC cells. miR145-mediated inhibition of ADAM17 consequently disrupted the maintenance of the cancer stem-like state of HNC-TICs through repressing IL-6/sIL-6R trans-signaling. Therefore, elevating miR145 expression by methods such as curcumin treatment seems to be a promising therapeutic modality to target HNC-TIC cells.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: L.-L. Tsai, C.-H. Yu, M.-Y. Chou, S.-H. Chiou
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Grant Support
This study was funded by the National Science Council (NSC100-2314-B-038-001, NSC100-2314-B-038-001-MY3), NSC100/101-2120-M-001-011, 101-2321-B-039-004, Taipei Veterans General Hospital (STEM Cell Project E99-101), Yen-Tjing-Ling Medical Foundation (CI 99/100), The Department of Health Cancer Research Center of Excellence (DOH101-TD-C-111-007), National Health Research Institutes (NHRI-EX102-10258SI), and The Genomic Center Project and Cancer Center Project of National Yang-Ming University (Ministry of Education, Aim for the Top University Plan), Taiwan.

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Received October 9, 2012; revised March 5, 2013; accepted March 12, 2013; published OnlineFirst April 2, 2013.

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Correction: miR145 Targets the SOX9/ADAM17 Axis to Inhibit Tumor-Initiating Cells and IL6-Mediated Paracrine Effects in Head and Neck Cancer

In this article (Cancer Res 2013;73:3425-40), which appeared in the June 1, 2013, issue of Cancer Research (1), there are errors in Fig. 3. In panels A and B, the Sox9 and GAPDH loading control blots in ALDH1+CD44+ HNC cells and ALDH1−CD44− HNC cells were inadvertently duplicated. The authors provided corrected results; the corrected panels appear below.

These errors do not change either the results or the conclusions of the article. The authors regret these errors.

Reference

Published OnlineFirst June 16, 2015.
doi: 10.1158/0008-5472.CAN-15-1351
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miR145 Targets the SOX9/ADAM17 Axis to Inhibit Tumor-Initiating Cells and IL-6–Mediated Paracrine Effects in Head and Neck Cancer

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