Biochemical and Biophysical Research Communications 454 (2014) 576–580

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Curcumin inhibits oral squamous cell carcinoma SCC-9 cells proliferation by regulating miR-9 expression

Can Xiao [a,b], Lili Wang [b], Lifang Zhu [b], Chenping Zhang [c,*], Jianhua Zhou [a]

[a] Department of Occupational Medicine and Environmental Health, School of Public Health, Soochow University, Suzhou 215123, China
[b] Department of Stomatology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China
[c] Department of Head and Neck Tumors, Shanghai Ninth People's Hospital Affiliated Shanghai JiaoTong University School of Medicine, Shanghai 200011, China

ARTICLE INFO

*Article history:*
Received 18 October 2014
Available online 30 October 2014

*Keywords:*
Oral squamous cell carcinoma
Curcumin
miR-9
Wnt/β-catenin signaling

ABSTRACT

Curcumin, a phytochemical derived from the rhizome of *Curcuma longa*, has shown anticancer effects against a variety of tumors. In the present study, we investigated the effects of curcumin on the miR-9 expression in oral squamous cell carcinoma (OSCC) and explored the potential relationships between miR-9 and Wnt/β-catenin pathway in curcumin-mediated OSCC inhibition *in vitro*. As the results shown, the expression levels of miR-9 were significantly lower in clinical OSCC specimens than those in the adjacent non-tumor tissues. Furthermore, our results indicated that curcumin inhibited OSCC cells (SCC-9 cells) proliferation through up-regulating miR-9 expression, and suppressing Wnt/β-catenin signaling by increasing the expression levels of the GSK-3β, phosphorylated GSK-3β and β-catenin, and decreasing the cyclin D1 level. Additionally, the up-regulation of miR-9 by curcumin in SCC-9 cells was significantly inhibited by delivering anti-miR-9 but not control oligonucleotides. Downregulation of miR-9 by anti-miR-9 not only attenuated the growth-suppressive effects of curcumin on SCC-9 cells, but also re-activated Wnt/β-catenin signaling that was inhibited by curcumin. Therefore, our findings would provide a new insight into the use of curcumin against OSCC in future.



## 1. Introduction

Oral squamous cell carcinoma (OSCC) is the most commonly diagnosed cancer of head and neck neoplasm, and more than 26,000 patients are diagnosed with OSCC globally each year. Although postoperative life quality of the OSCC patients has been improved in recent years, the overall 5-year survival rate has not been raised significantly, reflecting limited advances in our understanding of the pathogenesis of this disease [1]. Similar to other cancers, OSCC involves gradual accumulation of multiple genetic and epigenetic alterations, leading to gain-of-function in oncogenes and loss-of-function in tumor suppressor genes [2,3]. Therefore, a better understanding of the molecular events driving oral carcinogenesis may lead to new diagnostic and therapeutic approaches to the OSCC.

MicroRNAs (miRNAs) are small, highly conserved, non-coding RNA molecules of approximately 22 nucleotides in length. Usually, miRNAs negatively regulate mRNA stability and/or protein expression by imperfect base pairing to the 3′-untranslated regions of target mRNAs. In the previous studies, more and more evidences have suggested that the aberrant expression of miRNAs such as miR-21, -100, -122, -125b, -133a, -133b, -137, -145 and -193a may lead to the development and progression of malignancy [4–6]. MicroRNA-9 (miR-9), initially recognized as a crucial regulators for the development, physiology and pathology of nervous system, has been subsequently confirmed to be significantly down-regulated in many types of cancers, including nasopharyngeal carcinoma, colon cancer and breast cancer [7–9], while up-regulated in hepatocellular carcinoma, brain cancer and Hodgkin's lymphoma [10,11].

In our recent study, the Wnt/β-catenin signaling pathway has been observed as an indispensible role in OSCC development [12]. Aberrant activation of Wnt/β-catenin signaling allows β-catenin to resist degradation and enter the nucleus where it acts as a co-factor to regulate the expression of several genes relevant to cell proliferation and apoptosis. Curcumin, a dietary phytochemical derived from the rhizome of *Curcuma longa*, has been reported as a growth-suppressive compound in different cancer cells by regulating Wnt/β-catenin pathway [13,14]. In addition, a recent study has indicated that curcumin caused a significant and dose-dependent increase of miR-9 expression in SKOV3 ovarian cancer cells, while impeding cell proliferation and stimulating cell apoptosis [15]. These studies suggest that miR-9 may be involved in the anticancer

* Corresponding author at: 639 Zhizaoju Road, Shanghai 200011, China.
*E-mail address:* zhang_cping@163.com (C. Zhang).

http://dx.doi.org/10.1016/j.bbrc.2014.10.122
0006-291X/

effects of curcumin against OSCC development. To test this hypothesis, we investigate the effects of curcumin on the miR-9 expression in OSCC cells and explore the potential effects of miR-9 and Wnt/β-catenin pathway in curcumin-mediated OSCC inhibition.

## 2. Materials and methods

### 2.1. Patient and tissue samples

Paired OSCC and adjacent non-tumor tissues were obtained from 32 patients who underwent primary surgical resection of OSCC with informed consent between September 2012 and August 2013 at The First Affiliated Hospital of Soochow University (China). Tissue samples were immediately frozen in liquid nitrogen after resection and stored at −80 °C until use. Both tumor and non-tumor samples were confirmed by pathological examinations. This study was approved by the Human Research Ethics Committee of The First Affiliated Hospital of Soochow University.

### 2.2. Cell lines and reagents

Human tongue squamous cell carcinoma cell line SCC-9 was obtained from ATCC. The primary antibodies to human SFRP2, β-catenin, GSK-3β, cyclin D1 and β-actin, and the secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Phospho-GSK-3β (Ser9) antibody (Cat: 9336) was purchased from Cell signaling technology (Danvers, MA, USA).

Curcumin was purchased from Sigma–Aldrich (St Louis, MO, USA). For the study on the treatment of curcumin on the SCC-9 cells, curcumin was added into the cell culture supernatant. After another 72 h treatment, cells were harvested for further analyses.

### 2.3. Oligonucleotides and cell transfection

Human pre-miR-9 mimic oligonucleotides (Cat. 4427012) and negative control oligonucleotides (Cat. 4464060) were purchased from Ambion (Life technologies, Shanghai, China). The oligonucleotides sequence of miR-9 was 5′-AUAAAGCUAGAUAACCGAAAGU-3′. For knockdown of miR-9, locked nucleic acid (LNA)-based anti-miR-9 and universal LNA-based negative control were also purchased from Ambion (Life technologies, Shanghai, China). The final concentrations of the oligonucleotides used in this study were 30 nM. Cells were plated overnight at $2 \times 10^5$ cells in 6-well plates. All transfections were performed using Lipofectamine 2000 (Invitrogen) according to the instructions of the manufacturer.

### 2.4. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from cells using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA). The level of mature miR-9 in cells was determined by using Taqman miRNA Assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Relative expression of mRNA was based on the 2-delta delta Ct method, using RNU48 as an internal control. All experiments were performed in triplicate and included negative control that without cDNA.

### 2.5. MTT assay

After treatment with curcumin (20, 40 and 60 μM) for 72 h, cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cell samples were incubated with 5 mg/ml MTT (Sigma) for 4 h. After removal of the MTT solution, formazan crystals were dissolved in dimethyl sulfoxide. The absorbance was measured at a wavelength of 570 nm according to the manufacturer's protocol.

### 2.6. Colony formation assay

Briefly, cultured cells were seeded at a density of 300 $mL^{-1}$ on 35-mm dishes and positive colony formation (more than 50 cells/colony) was counted. The survival fraction of cells was expressed as the ratio of plating efficiency of treated cells to that of untreated control cells. The rate of the colony formation (RCF) was calculated with the following formula: RCF = (number of colony/number of treated cells) × 100%. Data represent the mean ± SD of three independent experiments.

### 2.7. Western blotting

Cells were lysed in ice-cold lysis buffer containing 50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and complete proteinase inhibitor mixture. Protein extracted from the cells were resolved by SDS–PAGE and transferred to polyvinylidene fluoride (Hybond-P, GE Healthcare) membranes, followed by incubation with the indicated primary and secondary antibodies conjugated to horseradish peroxidase (GE Healthcare). Signals were detected using the enhanced chemiluminescence detection system (ECL Plus Western Blotting Detection System, Amersham).

### 2.8. Statistical analysis

Statistical analysis was performed by SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). Differences among multiple groups were performed with one-way analysis of variance followed by Tukey's multiple comparison test. $P < 0.05$ was considered to indicate statistically significant differences.

## 3. Results

### 3.1. Suppression of miR-9 expression in OSCC tissues

In an effort to detect the expression levels of miR-9 in OSCC, 32 clinical OSCC specimens and their adjacent non-cancerous specimens were analyzed by quantitative RT-PCR. The results indicated that the miR-9 expression levels were significantly decreased in the 32 clinical OSCC specimens, compared with that in adjacent non-tumor tissues ($P < 0.001$) (Fig. 1).

### 3.2. Curcumin inhibits SCC-9 cells proliferation in vitro

In order to examine the effect of curcumin on the OSCC cell proliferation, SCC-9 cells were treated with 20, 40 and 60 μM curcumin for 72 h, respectively. As the results shown, curcumin significantly inhibited SCC-9 cells proliferation (Fig. 2). In the present study, the inhibitory effect of curcumin on OSCC cell growth was further confirmed by colony formation and anchorage independent growth assays. Compared to untreated cells, the number of colonies was significantly decreased in cells treated with 20, 40 and 60 μM curcumin (Fig. 2, Table 1).

### 3.3. miR-9 mediates the inhibition of SCC-9 proliferation by curcumin

We further analyzed the role of miR-9 in curcumin-mediated inhibition in SCC-9 proliferation. As shown in Fig. 3, qRT-PCR analysis indicated that curcumin treatment caused a significant elevation of miR-9 expression in SCC-9 cells in a dose-dependent manner, as compared to untreated cells ($P < 0.05$). Furthermore,

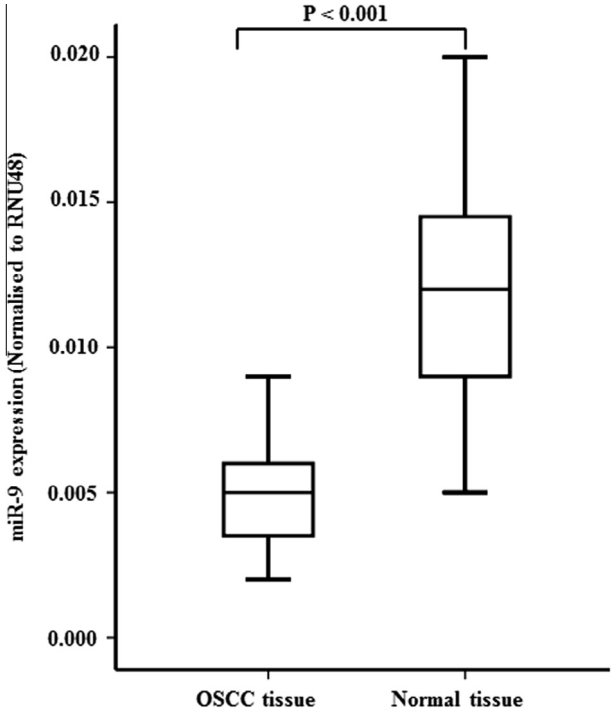


**Fig. 1.** miR-9 expression in oral squamous cell carcinoma specimens. Real-time PCR showed that expression level of miR-9 was significantly decreased in OSCC clinical samples compared with normal adjacent tissues ($n = 32$).

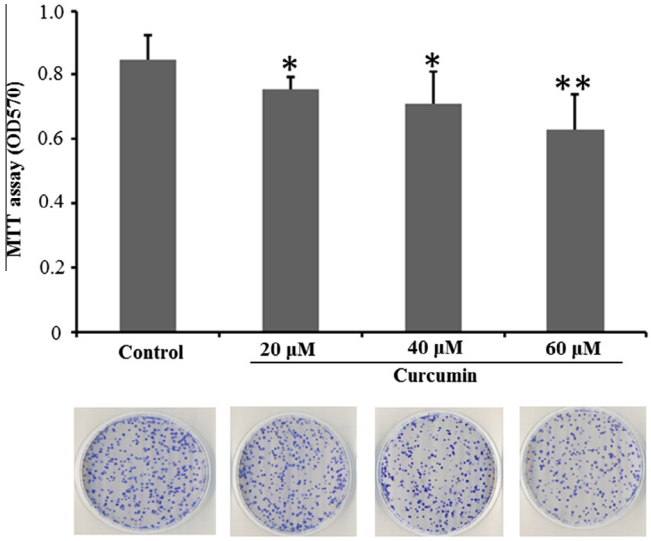


**Fig. 2.** Effects of curcumin on the proliferation of SCC-9 cells and further confirmed by colony formation assay. Cell proliferation was assessed using MTT assay 72 h after treatment. Data represent the mean ± SD of three independent experiments (*$P < 0.05$, **$P < 0.01$).

**Table 1**
Effects of curcumin (0, 20, 40 and 60 μM) on the colony formation rate of SCC-9 cells.

| Curcumin (μM) | Rate of colony formation | $P^*$ |
|---|---|---|
| 0 | 0.8808 ± 0.0172 | – |
| 20 | 0.7810 ± 0.0175 | 0.000 |
| 40 | 0.7113 ± 0.0148 | 0.000 |
| 60 | 0.6283 ± 0.0184 | 0.000 |

* Compared to the control group (0 μM).

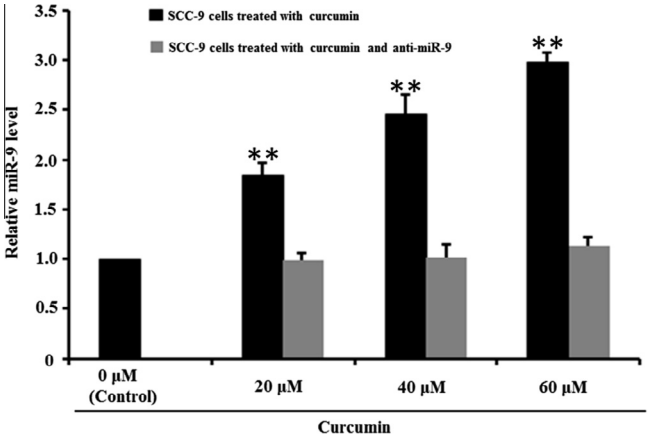


**Fig. 3.** Effects of anti-miR-9 on the upregulation of miR-9 expression in curcumin treated SCC-9 cells. qRT-PCR analysis of miR-9 expression levels in different condition. Results are expressed as fold change relative to untreated control cells (**$P < 0.01$).

**Table 2**
Effects of curcumin on the SCC-9 cells proliferation treated w/o anti-miR-9 (OD570).

| Curcumin (μM) | SCC-9 | SCC-9 with control oligo | SCC-9 with anti-miR-9 |
|---|---|---|---|
| 0 | 0.8492 ± 0.0715 | 0.8492 ± 0.0715 | 1.0338 ± 0.1265 |
| 20 | 0.7555 ± 0.0153 | 0.7342 ± 0.0667 | 1.1217 ± 0.2294* |
| 40 | 0.7113 ± 0.0715 | 0.6961 ± 0.0752 | 1.0612 ± 0.1034* |
| 60 | 0.6283 ± 0.1430 | 0.6351 ± 0.0451 | 0.9415 ± 0.0536** |

* $P < 0.05$ *vs* SCC-9 and SCC-9 with control oligonucleotides.
** $P < 0.01$ *vs* SCC-9 and SCC-9 with control oligonucleotides.

the upregulation of miR-9 by curcumin in SCC-9 cells was significantly blocked by delivering anti-miR-9 but not control oligonucleotides ($P < 0.05$). More important, the results of MTT analysis indicated that downregulation of miR-9 significantly attenuated the growth-suppressive effects of curcumin on SCC-9 cells (Table 2).

### 3.4. Curcumin inhibits Wnt/β-catenin signaling pathway in SCC-9 cells

Aberrant activation of Wnt/β-catenin signaling has been confirmed as a critical role in carcinogenesis by affecting cell growth, cell cycling, cell survival and invasion. Therefore, we further investigated the effects of curcumin (60 μM) on Wnt/β-catenin signaling in SCC-9 cells. As Fig. 4A shown, curcumin treatment resulted in a significant ($P < 0.05$) increased expression levels of the GSK-3β, phosphorylated GSK-3β and β-catenin. However, the growth promoting gene cyclin D1, which was a direct read-out gene of active Wnt signaling pathway, was significantly decreased in curcumin treated SCC-9 cells, compared to untreated control cells.

### 3.5. miR-9 mediates the inhibition of curcumin on Wnt/β-catenin signaling pathway

We further investigated the potential role of miR-9 in curcumin-induced Wnt/β-catenin signaling suppression in SCC-9 cells. Similar to the results observed before, the levels of GSK-3β, phosphorylated GSK-3β and β-catenin were significantly increased in miR-9-overexpressing SCC-9 cells than in those transfected with control oligonucleotides. However, the cyclin D1 expression levels were significantly decreased in the miR-9-overexpressing SCC-9 cells (Fig. 4B). More importantly, our results also indicated that

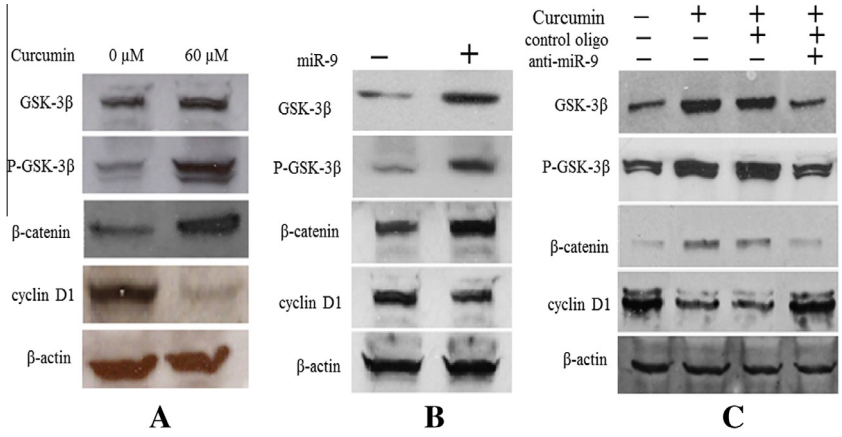


**Fig. 4.** (A) Effects of curcumin on the GSK-3β, phosphorylated GSK-3β (P-GSK-3β), β-catenin and cyclin D1 levels in SCC-9 cells. Cell lysates were collected and subjected to Western blotting assays by using antibodies against GSK-3β, P-GSK-3β, β-catenin and cyclin D1, respectively. β-Actin was used for loading control of Western blotting. (B) Cells were transfected with miR-9 and then collected and subjected to Western blotting assays as described before. (C) Cells were transfected with control oligo and anti-miR-9, respectively. Western blotting assays were used to analyze the effects of anti-miR-9 on the effects of curcumin on the expressions of GSK-3β, P-GSK-3β, β-catenin and cyclin D1. β-Actin was used for loading control.

the effects of curcumin on Wnt/β-catenin signaling in SCC-9 cells could be reversed by the delivery of anti-miR-9, as decreased levels of GSK-3β, phosphorylated GSK-3β and β-catenin, and increased level of cyclin D1 could be detected in anti-miR-9 and curcumin co-treated SCC-9 cells (Fig. 4C).

## 4. Discussion

Curcumin, derived from eastern traditional medicines, *C. longa*, has a variety of beneficial properties such as anti-inflammatory, anti-cancer, antioxidant, and chemotherapeutic activities. Its multiple-target characteristics influence several signaling pathway, including interferon signaling pathway, MAPK signaling pathway, as well as Wnt/β-catenin signaling pathway [16,17]. In the present study, we reported that curcumin inhibited OSCC cells proliferation by up-regulating miR-9 expression and inhibiting Wnt/β-catenin signaling pathway.

In many types of cancer, aberrant miR-9 levels have been reported involved in tumor formation or progression. However, the function of miR-9 in different tumor progression is different according to the previous study [18]. In this study, we demonstrated that miR-9 was significantly downregulated in 32 OSCC tissues, compared to the adjacent non-cancerous specimens. Curcumin-mediated miR-9 overexpression *in vitro* significantly inhibited the proliferation and colony formation of OSCC cells. These findings demonstrated that miR-9 can be classified as a tumor-suppressive microRNA in OSCC and might be a promising target for cancer therapeutics. Additionally, it is well known that miR-9 can regulate the expression of many target mRNAs, such as cyclin D1, Ets1, and CXCR4. Repression of cyclin D1 and Ets1 is involved in miR-9-mediated suppression of the proliferation, invasion and metastasis of gastric cancer cells. Targeting CXCR4 was found to be related with miR-9-mediate suppression of nasopharyngeal carcinoma [19]. Therefore, additional studies are needed to identify the specific target genes of miR-9 that involved in curcumin-mediated inhibitory effects on OSCC development.

The Wnt/β-catenin signaling pathway has been implicated in tumorigenesis at several sites, including the colon, rectum, breast and liver [20,21]. Its central component, namely, β-catenin, plays a critical role in this process. A number of downstream target genes of Wnt/β-catenin signaling have been reported that play critical roles in carcinogenesis by affecting cell growth, cell cycling, cell survival and invasion [22,23]. Aberrant activation of Wnt/β-catenin signaling is a significant feature of human pancreatic adenocarcinoma, as revealed by aberrant β-catenin expression in a significant fraction (30–65%) of tumors [24,25]. Wnt/β-catenin signaling pathway antagonist such as SFRP2, is able to negatively regulate the Wnt/β-catenin signal transduction pathway. Overexpression of SFRP2 expression generally causes Wnt/β-catenin signal pathway suppression and cell proliferation inhibition [26,27]. In our recent study, the relative mechanism underlying the upstream and downstream modulation function of Wnt/β-catenin signal pathway on OSCC progression have already been described [12].

To further identify the molecular mechanism of curcumin-mediated miR-9 overexpression on OSCC progression *in vitro*, the GSK-3β, β-catenin and cyclin D1 expression levels were examined in the present study. Our results showed that the GSK-3β and β-catenin protein expression significantly increased, while cyclin D1 significantly decreased in the SCC-9 cells with curcumin treatment or with miR-9-overexpressed compared with those control groups. However, it is well known that increased the GSK-3β levels usually result in degradation of β-catenin in cells [28]. Therefore, we further investigated the ratio of inactivated GSK-3β (phosphorylated GSK-3β, P-GSK-3β) to the total GSK-3β in the present study. As expected, the level of phosphorylated GSK-3β was increased with total GSK-3β, indicating that the activity of GSK-3β was inhibited in curcumin or miR-9 treated SCC-9 cells. Considering Wnt/β-catenin signaling pathway has been widely reported to be activated in OSCC [29], our presented data suggested that the inhibitory activity of curcumin against SCC-9 cells is largely/partially dependent on the induction of miR-9, which in turn inhibiting the Wnt/β-catenin signaling pathway.

Taken together, our present results suggest that curcumin is capable of inhibiting SCC-9 cells proliferation by inducing miR-9 expression and disrupting Wnt/β-catenin signaling pathway. These findings should provide a new insight into the use of curcumin in the treatment of OSCC. However, miR-9 direct targets mediating the anticancer effects of curcumin on OSCC need to be further identified.

## Acknowledgment

We thank Dr. Chen Dong at the Department of Epidemiology and Statistics, School of Public Health, Soochow University, China for his kind help in manuscript preparation.

## References


[1] W. Meng, Y. Wu, X. He, et al., A systems biology approach identifies effective tumor-stroma common targets for oral squamous cell carcinoma, Cancer Res. 74 (2014) 2306–2315.

[2] F. Xavier, M. Destro, C. Duarte, et al., Epigenetic repression of HOXB cluster in oral cancer cell lines, Arch. Oral Biol. 59 (2014) 783–789.

[3] H. Zhang, X. Nan, X. Li, et al., CMTM5 exhibits tumor suppressor activity through promoter methylation in oral squamous cell carcinoma, Biochem. Biophys. Res. Commun. 447 (2014) 304–310.

[4] J. Bezerra, A. Latronico, MicroRNA Era: the importance for diagnosis and prognosis of adrenocortical tumors, Biomed Res. Int. 2014 (2014) 381917.

[5] J. Wu, Y. Yao, T. Gu, et al., MiR-421 regulates apoptosis of BGC-823 gastric cancer cells by targeting caspase-3, Asian Pac. J. Cancer Prev. 15 (2014) 5463–5468.

[6] Y. Lang, S. Xu, J. Ma, et al., MicroRNA-429 induces tumorigenesis of human non-small cell lung cancer cells and targets multiple tumor suppressor genes, Biochem. Biophys. Res. Commun. 450 (2014) 154–159.

[7] F. Gao, Z. Zhao, W. Zhao, et al., MiR-9 modulates the expression of interferon-regulated genes and MHC class I molecules in human nasopharyngeal carcinoma cells, Biochem. Biophys. Res. Commun. 431 (2013) 610–616.

[8] L. Cekaite, J. Rantala, J. Bruun, et al., MiR-9, -31, and -182 deregulation promote proliferation and tumor cell survival in colon cancer, Neoplasia 14 (2012) 868–879.

[9] S. Selcuklu, M. Donoghue, K. Rehmet, et al., MicroRNA-9 inhibition of cell proliferation and identification of novel miR-9 targets by transcriptome profiling in breast cancer cells, J. Biol. Chem. 287 (2012) 29516–29528.

[10] Z. Sun, Q. Han, N. Zhou, et al., MicroRNA-9 enhances migration and invasion through KLF17 in hepatocellular carcinoma, Mol. Oncol. 7 (2013) 884–894.

[11] L. Zhu, H. Chen, D. Zhou, et al., MicroRNA-9 up-regulation is involved in colorectal cancer metastasis via promoting cell motility, Med. Oncol. 29 (2012) 1037–1043.

[12] C. Xiao, L. Wang, L. Zhu, et al., Secreted frizzled-related protein 2 is epigenetically silenced and functions as a tumor suppressor in oral squamous cell carcinoma, Mol. Med. Rep. (2014) (Epub ahead of print).

[13] Y. Li, T. Zhang, Targeting cancer stem cells by curcumin and clinical applications, Cancer Lett. 346 (2014) 197–205.

[14] C. Prasad, G. Rath, S. Mathur, et al., Potent growth suppressive activity of curcumin in human breast cancer cells: modulation of Wnt/beta-catenin signaling, Chem. Biol. Interact. 181 (2009) 263–271.

[15] S. Zhao, X. Zhang, X. Zhang, et al., Induction of microRNA-9 mediates cytotoxicity of curcumin against SKOV3 ovarian cancer cells, Asian Pac. J. Cancer Prev. 15 (2014) 3363–3368.

[16] Y. Guo, Q. Shan, Y. Gong, et al., Curcumin induces apoptosis via simultaneously targeting AKT/mTOR and RAF/MEK/ERK survival signaling pathways in human leukemia THP-1 cells, Pharmazie 69 (2014) 229–233.

[17] R. VanderBroek, G. Snow, Z. Chen, et al., Chemoprevention of head and neck squamous cell carcinoma through inhibition of NF-κB signaling, Oral. Oncol. 50 (2013) 930–941.

[18] O. Yang, J. Huang, S. Lin, Regulatory effects of miRNA on gastric cancer cells, Oncol. Lett. 8 (2014) 651–656.

[19] J. Lu, H. Luo, X. Liu, et al., MiR-9 targets CXCR4 and functions as a potential tumor suppressor in nasopharyngeal carcinoma, Carcinogenesis 35 (2014) 554–563.

[20] Y. Ma, G. Zhang, X. Fu, et al., Wnt signaling may be activated in a subset of Peutz–Jeghers syndrome polyps closely correlating to LKB1 expression, Oncol. Rep. 23 (2010) 1569–1576.

[21] P. Jabbarzadeh Kaboli, A. Rahmat, P. Ismail, et al., Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer, Eur. J. Pharmacol. 740 (2014) 584–595.

[22] R. Morgan, J. Ridsdale, A. Tonks, et al., Factors affecting the nuclear localization of β-catenin in normal and malignant tissue, J. Cell. Biochem. 115 (2014) 1351–1361.

[23] M. Jalving, D. Heijink, J. Koornstra, et al., Regulation of TRAIL receptor expression by β-catenin in colorectal tumours, Carcinogenesis 35 (2014) 1092–1099.

[24] B. Moon, W. Jeong, J. Park, et al., Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/β-catenin signaling, J. Natl. Cancer Inst. 106 (2014) (djt373).

[25] R. Tarapore, I. Siddiqui, V. Adhami, et al., The dietary terpene lupeol targets colorectal cancer cells with constitutively active Wnt/β-catenin signaling, Mol. Nutr. Food Res. 57 (2013) 1950–1958.

[26] T. Saito, H. Mitomi, A. Imamhasan, et al., Ownregulation of sFRP-2 by epigenetic silencing activates the β-catenin/Wnt signaling pathway in esophageal basaloid squamous cell carcinoma, Virchows Arch. 464 (2014) 135–143.

[27] M. Takeda, T. Nagasaka, S. Dong-Sheng, et al., Expansion of CpG methylation in the SFRP2 promoter region during colorectal tumorigenesis, Acta Med. Okayama 65 (2011) 169–177.

[28] A. Gupta, A. Verma, A.K. Mishra, et al., The Wnt pathway: emerging anticancer strategies, Recent Pat. Endocr. Metab. Immune Drug Discov. 7 (2013) 138–147.

[29] L. Wang, S. Chen, S. Zhuang, et al., Galectin-3 accelerates the progression of oral tongue squamous cell carcinoma via a Wnt/β-catenin-dependent pathway, Pathol. Oncol. Res. 19 (2013) 461–474.