科技部補助專題研究計畫成果報告
期末報告

miR-1246作為口腔黏膜下纖維化症及口腔癌的治療標靶及臨床應用性

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 106-2314-B-040-005-
執 行 期 間 ： 106年08月01日至107年07月31日
執 行 單 位 ： 中山醫學大學附設醫院

計 畫 主 持 人 ： 周明勇

計畫參與人員： 此計畫無其他參與人員

中 華 民 國 107 年 10 月 13 日

中 文 摘 要 ： 流行病學研究指出，臺灣地區口腔黏膜下纖維化症及口腔癌發生與嚼食檳榔有密切關係。口腔黏膜纖維化症為一種口腔癌發生之癌前病變，而口腔癌為台灣男性癌症發生率及死亡率的第四位，患者五年內預後不佳。微型RNA在纖維化及癌化進程扮演重要角色，其致病機制主要在於與標靶基因轉錄子3’ UTR 之結合而抑制其功能。本計畫初步研究結果發現miR-1246於口腔黏膜下纖維化症及口腔癌組織會高度表現，阻斷miR-1246表現降低口腔黏膜下纖維中造纖維母細胞之肌纖維母細胞活性及口腔癌細胞癌幹原性。口腔癌病患若高表現miR-1246其預後差，而降低miR-1246表現可抑制化療抗性。CCNG2為miR-1246直接標的基因而參與miR-1246調控之口腔癌幹性。總結本計畫實驗結果，將能提供將來在臨床上標靶治療的參考方向。

中 文 關 鍵 詞 ： 口腔黏膜下纖維化症，口腔癌，miR-1246

英 文 摘 要 ： It has been revealed in the epidemiological studies that areca nut chewing was the major risk factor associated with oral submucous fibrosis (OSF) and oral cancer (OC) in Taiwan. OSF is a chronic scarring disease which has been considered as pre-cancerous condition of oral mucosa. OC is currently the 4th most common cancer type and leading cause of cancer death in male Taiwanese. And the prognosis of OC patients remains unsatisfactory. MicroRNAs (miRNAs) have been known to play crucial roles in the pathogenesis of OSF and OC through by binding to the 3’ UTR of target genes and inhibiting their expression. In our preliminary data, we showed that miR-1246 expression was increased in OSF and OC specimens. Knockdown of miR-1246 repressed the myofibroblasts activation in fibrotic buccal mucosal fibroblasts. MiR-1246 inhibition also reduced the cancer stemness in oral cancer cells. Kaplan-Meier survival analysis of OSCC patients with high levels of miR-1246 had the worst survival rate compared to their low-expression counterparts. Moreover, we showed that downregulation of miR-1246 decreased chemoresistance. In addition, we verified that miR-1246-inhibited CCNG2 contributed to the cancer stemness of OSCC. As such, this proposal aims to investigate the molecular mechanisms underlying the miR-1246 upregulation in OC and OSF, and the potential of using miR-1246 as a therapeutic target. Our study will provide crucial information regarding how miR-1246 participates in OSF and oral tumorigenesis and determine whether miR-1246 could represent as a therapeutic target.

英 文 關 鍵 詞 ： oral submucous fibrosis; oral cancer; miR-1246

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（□期中進度報告/■期末報告）

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計畫主持人：周明勇
計畫參與人員：

本計畫除繳交成果報告外，另含下列出國報告，共 ___ 份：
□執行國際合作與移地研究心得報告
□出席國際學術會議心得報告

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中　華　民　國 107 年 10 月 13 日

中文摘要

流行病學研究指出，臺灣地區口腔黏膜下纖維化症及口腔癌發生與嚼食檳榔有密切關係。口腔黏膜纖維化症為一種口腔癌發生之癌前病變，而口腔癌為台灣男性癌症發生率及死亡率的第四位，患者五年內預後不佳。微型RNA在纖維化及癌化進程扮演重要角色，其致病機制主要在於與標靶基因轉錄子3'UTR 之結合而抑制其功能。本計畫初步研究結果發現miR-1246於口腔黏膜下纖維化症及口腔癌組織會高度表現，阻斷miR-1246表現降低口腔黏膜下纖維中造纖維母細胞之肌纖維母細胞活性及口腔癌細胞癌幹原性。口腔癌病患若高表現miR-1246其預後差，而降低miR-1246表現可抑制化療抗性。CCNG2為miR-1246直接標的基因而參與miR-1246調控之口腔癌幹性。總結本計畫實驗結果，將能提供將來在臨床上標靶治療的參考方向。

關鍵字：口腔黏膜下纖維化症，口腔癌，miR-1246

Abstract

It has been revealed in the epidemiological studies that areca nut chewing was the major risk factor associated with oral submucous fibrosis (OSF) and oral cancer (OC) in Taiwan. OSF is a chronic scarring disease which has been considered as pre-cancerous condition of oral mucosa. OC is currently the 4th most common cancer type and leading cause of cancer death in male Taiwanese. And the prognosis of OC patients remains unsatisfactory. MicroRNAs (miRNAs) have been known to play crucial roles in the pathogenesis of OSF and OC through by binding to the 3'UTR of target genes and inhibiting their expression. In our preliminary data, we showed that miR-1246 expression was increased in OSF and OC specimens. Knockdown of miR-1246 repressed the myofibroblasts activation in fibrotic buccal mucosal fibroblasts. MiR-1246 inhibition also reduced the cancer stemness in oral cancer cells. Kaplan-Meier survival analysis of OSCC patients with high levels of miR-1246 had the worst survival rate compared to their low-expression counterparts. Moreover, we showed that downregulation of miR-1246 decreased chemoresistance. In addition, we verified that miR-1246-inhibited CCNG2 contributed to the cancer stemness of OSCC. As such, this proposal aims to investigate the molecular mechanisms underlying the miR-1246 upregulation in OC and OSF, and the potential of using miR-1246 as a therapeutic target. Our study will provide crucial information regarding how miR-1246 participates in OSF and oral tumorigenesis and determine whether miR-1246 could represent as a therapeutic target.

Key words: oral submucous fibrosis; oral cancer; miR-1246

Introduction

1. Oral submucous fibrosis and oral cancer

Epidemiological evidence has suggested that areca quid chewing habit as the major etiological factor that confers an increased risk for oral submucous fibrosis (OSF) [1, 2] and oral cancer (OC) [3]. It has been shown that areca nut extract-associated upregulation of vimentin through PI3K/AKT activation could underlie the areca-associated oral pathogenesis[4]. OSF is a progressive scarring disease characterized by chronic inflammation and epithelial atrophy along with submucosal deposition of dense fibrous connective tissue leading to this pre-cancerous condition. OSF could progress to neoplasia--notably oral squamous cell carcinoma (OSCC) with a transformation rate about 7–13% [5, 6] and it greatly increases the mortality rate of these patients. The accumulation of collagen in lamina propria and oral submucosa following dysregulation of

extracellular matrix synthesis and degradation eventually results in OSF. As for oral cancer, it includes a group of malignancies that occur in the following locations: lip, gingiva, anterior 2/3 of the tongue, buccal mucosa, hard palate, retromolar trigone and the floor of the mouth according to the National Cancer Institute [7]. After arising from the primary lesion originating in oral cavity, cancer cells start to invade the neighboring tissues and spread to distant parts of the body via metastasis, causing considerable economic and quality-of-life burdens to the patients. The malignant neoplasms affecting the oral cavity include squamous cell carcinoma, verrucous carcinoma, adenoid cystic carcinoma and mucoepidermoid carcinoma. And more than 90% of all oral neoplasms are OSCC [8]. The estimated incidence of oral cancer was around 170, 000 with the mortality rate about 83,000 in 2008 [9]. Oral cancer is the fourth most frequently occurring cancer and the fifth leading cause of cancer death for men in Taiwan according to the statistics from Ministry of Health and Welfare [10]. Despite the progress in cancer treatment, survival rates have improved only frugally over the past few decades [11]. It has been shown that 3-year overall survival rate of advanced OSCC patients with recurrence was less than 30% [12]. Due to the poor prognosis and high tendency of recurrence and metastasis, investigation of the mechanisms underlying pathogenic fibrotic response in the oral cavity is an emerging issue in order to ameliorate disease progression and develop therapeutic strategies.

2. MiRNAs and fibrosis

Increasing number of studies has unraveled the association between miRNAs and fibrosis. And the advent of microRNAs (miRNAs) research has opened new avenues to understand the regulatory mechanisms of fibrogenesis at the post-transcriptional level. MiRNAs are small non-coding RNA molecules of 19–24 nucleotides (nt) in length and modulate gene expression via binding to the 3' untranslated region (UTR) of target mRNAs, thereby causing degradation or translational inhibition. Although miRNAs account for only around 3% of the human genome, they could regulate 20–30% of the protein coding genes[13]. To date, there are unique subsets of miRNAs implicated in the development of fibrogenic process, including miR-21, miR-29, miR-31, and let-7d, etc. It has been demonstrated that the increased expression of miR-21 were present in the fibrotic lungs of bleomycin-induced mouse and idiopathic pulmonary fibrosis (IPF) patients [14]. And the upregulated miR-21 expression was primarily associated with myofibroblasts, which are extremely critical in fibrotic disease [15]. It is known that miR-21 promotes fibrosis by regulating TGF-β1/Smad7 [14] and ERK /MAP kinase [16] signaling in activated myofibroblasts. In liver fibrosis, inhibition of miR-31 has been proved to reduce hepatic stellate cell activation, while over-expression promoted it. The increased miR-31 expression after TGF-β stimulation was via binding of Smad3 to miR-31's promoter, indicating the pro-fibrotic role of miR-31 was relied on TGF-β1/Smad pathway as well [17]. MiR-29 has also been identified as a potential therapeutic target in systemic sclerosis [18], cardiac fibrosis [19], liver fibrosis [20] and lung fibrosis [21]. It has been indicated that miR-29 levels inversely correlated with the levels of pro-fibrotic genes and severity of fibrosis. MiR-29 is a downstream target gene of Smad3 and negatively regulated by TGF-β/Smad signaling in fibrosis [21]. So far, the function of miR-29 has been suggested to be involved in TGF-β- and NF-κB-dependent pathways with subsequent upregulation of extracellular matrix–associated and remodeling genes following inhibition of miR-29 [20, 22]. Like miR-29, there are other miRNAs constitutively expressed under healthy condition but downregulated in fibrotic tissues, such as let-7d, suggesting that they might possess anti-fibrotic function. It has been demonstrated that TGF-β-induced downregulation of let-7d was mediated through Smad3 binding to the let-7d promoter. And

inhibition of let-7d increased epithelial-to-mesenchymal transition (EMT) markers, such as N-cadherin-2, vimentin and myofibroblast marker α-smooth muscle actin [23].

3. MiRNAs in OC and OSF

Due to the fact that miRNAs participate in various important biological processes, such as cell proliferation, differentiation and apoptosis, many of them have been be unveiled could contribute to not only the development of progressive fibrotic disease but also carcinogenesis. For example, miR-21 has been recognized as an oncogene because it suppresses various tumor suppressor genes. And miR-21 also functions as an antiapoptotic factor in tumor cells [24]. The increased expression of miR-21 has been observed in precancerous lesions leukoplakia that progressed to OSCC [25], indicating the potential role of miR-21 in early OSCC diagnosis. The other important oncogenic miRNAs involved in head and neck squamous cell carcinoma (HNSCC) is miR-31 [26]. One of the studies revealed that miR-31 was significantly up-regulated in tissues of oral cancer and three precancerous condition, including leukoplakia, lichen planus and oral submucous fibrosis [27]. In fact, it has been shown that salivary miR-21 and miR-31 expression were all increased in patients of oral potentially malignant disorder (OPMD). However, patients with recurrent OPMD and/or malignant transformation only expressed higher miR-31, but not miR-21, in the epithelium. Hence, they concluded that upregulation of miR-31 could be used as screening tool and predict the malignant progression for OPMD [28]. They also found high expression of miR-31 was correlated with higher VEGF expression, which was important for tumorigenesis, and lower E-cadherin expression in OPMD tissue [29]. In HNSCC, miR-31 has been demonstrated to enhance the oncogenicity and stemness[30]. Consistent with these findings, it has been shown that salivary miR-31 could be used to detect OSCC [31]. Also, expression of miR-31 was associated with clinico-pathological characteristics, and the expression was higher in early stage as compared to late stage OSCC [32]. Although miR-31 seems to be oncogenic in OSCCs, it holds tumor suppressive potential in other tumor types [33, 34]. On the other hand, miR-29a has been reported to play a pivotal role in cancer metastasis and relapse [35] and its expression was found downregulated in leukoplakia and cancer tissues, which implied a tumor repressive function of miR-29a [36]. Collectively, various microRNAs have emerged as critical determinants in OSF and oral cancer; however, the mechanistic details of other miRNA biogenesis pathways and their function in oral diseases remain to be elucidated.

4. MiR-1246 as a new target in OSF and OC pathogenesis

Among various miRNAs that involve in carcinogenesis, miR-1246 has been identified as cancer stem cell (CSC)-specific miRNA. MiR-1246 could confer not only tumorigenic potential, such as tumor initiation and cancer progression, but also be required for cancer metastasis [37]. When miR-1246s were transferred to poorly metastatic cells using exosomes, the cell motility and invasive ability were all increased [38]. It has been shown that miR-1246 promoted cancer stemness features by activating Wnt/β-catenin pathway in liver CSCs [39]. And inhibition of miR-1246 decreased stemness and EMT markers, and suppressed proliferation, , migration, invasion, sphere-formation and colony formation in non-small cell lung cells [40] and colorectal cancer cells [41]. It also has been discovered that extracellular miR-1246 could act as a signaling molecule between irradiated and non-irradiated cells, contributing to radioresistance by directly suppressing the DR5 genes [42]. Although the researches on miRNA expression signatures in pre-cancerous OSF were limited, the level of miR-1246 was found upregulated following arecoline, the main alkaloids of areca nut, exposure in normal human oral fibroblast cells [43]. In addition, miR-1246 involves in EMT process [40], which has been

implicated in OSF as well [44]. Circulating and tissue miR-1246 have been proved to be correlated with the TNM stage and prognosis, and could be used to distinguish patients from healthy controls in various cancers [37, 45, 46].

Materials and Methods

OSCC tissues and cell culture

Samples of OSCC (T) and normal paired noncancerous matched tissues (N) were collected after obtaining written informed consent and all procedure were approved by The Institutional Review Board in Chung Shan Medical University Hospital. Tumor tissues were snap frozen in liquid nitrogen and stored at −80 °C for quantitative real-time reverse transcription–PCR (qRT-PCR). The OSCC cell lines SAS, GNM, OC3, and Fadu cells were cultivated as previously described [20].

qRT–PCR analysis

For miR-1246 levels, qRT–PCR was performed using TaqMan miRNA assays with a specific primer (Applied Biosystems, Carlsbad, CA, USA) and detection was conducted using a StepOne Plus real-time PCR system [21]. For Sox2 detection, total RNA was prepared from cells using Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). qRT–PCRs of mRNAs were reverse-transcribed by the Superscript III first-strand synthesis system (Invitrogen) and reactions on the resulting cDNAs were carried out on an ABI StepOne™ Real-Time PCR Systems (Applied Biosystems).

Flow cytometry for cancer stem cell isolation and drug resistance analysis

Cells were stained with CD133 (Miltenyi Biotech, Auburn, CA, USA), CD44 antibodies (phycoerythrin conjugated; BioLegend, San Diego, CA, USA) or aldehyde dehydrogenase 1 (ALDH1; StemCell Technologies Inc., Vancouver, BC, Canada) followed by fluorescence-activated cell sorting analysis (FACS) to isolate the cancer stem cells or examine their expression as previously described [22,23]. For drug resistance analysis, cells were stained with ABCG2 antibody (Chemicon, Temecula, CA, USA) according to the manufacturer's instructions. Fluorescence emission from 10,000 cells was measured with FACS Calibur (Becton Dickinson, San Jose, CA, USA) using CellQuest software.

Tumor spheres culture for cancer stem cell selection

Spheroid cells from OSCC were cultured in the serum-free DMEM/F12 medium (Gibco, Grand Island, NY, USA) consisting of N2 supplement (Gibco), human recombinant basic fibroblast growth factor, and epidermal growth factor (R&D Systems, Minneapolis, MN, USA). Cells were plated at 10^4 live cells/10-mm low attachment dishes, and the medium was changed every other day until the tumor sphere formation was observed in about 2 weeks [22].

Downregulation or overexpression of miR-1246

miR-1246 mimic, miR-1246 inhibitor, and scramble (Scr) control were purchased from Applied Biosystems. Lipofectamine™ 3000 transfection reagent (Invitrogen) was used according to the manufacturer’s protocol.

Cancer stemness phenotypic analyses

After transfected with inhibitor or mimics, tumor cells were cultured as spheroid cells to evaluate their

self-renewal ability. Cell density was 1,000 cells/mL in the serum-free medium as described earlier [22].

For migration or invasion capacities, 24-well Transwell system with an 8.0 μm porous transparent polyethylene terephthalate membrane was used. Cells (1×10^5/ well) were added to the upper chambers (filter coated with Matrigel for invasion assay) in the low serum medium. Medium supplemented with higher serum were used as a chemoattractant in the lower chamber followed by 24-hour incubation. Subsequently, cells that had migrated through the membrane to the lower surface were stained with crystal violet and counted from 5 different fields under a fluorescence microscope.

Soft agar colony forming assay was carried out to evaluate the clonogenicity. Each well of a 6-well culture dish was coated with 2 mL bottom agar mixture (Sigma–Aldrich, St. Louis, MO, USA) (DMEM, 10% [v/v] fetal calf serum, 0.6% [w/v] agar). After the bottom layer was solidified, a top agar-medium mixture containing 2×10^4 cells was added and incubated at 37°C for 4 weeks followed by crystal violet staining. The number of total colonies with a diameter ≥100μm was counted from 5 fields per well for a total of 15 fields in triplicate experiments [22].

Subcutaneous xenografts in nude mice

The animal study was approved by the Institutional Animal Care and Use Committee in Chung Shan Medical University. OSCC cells transfected with miR-1245 inhibitors or mimics were injected subcutaneously into BALB/c nude mice (6–8 weeks). Imaging measurement was performed using an IVIS50 animal imaging system (Xenogen Corp., South San Francisco, CA, USA) as the photons emitted from the target site penetrated through the tissue could be externally detected and quantified. Tumor volume was calculated using the following formula: tumor volume (cm^3) = (length × $width^2$)/2.

Cell survival assay

1×10^3 cells were seeded in a 24-well plate, and then MTT solution was added and incubated at 37°C for 3 hours. The supernatant was removed, and 200 μL of dimethyl sulfoxide was added and then the O.D value of the solution was analyzed by a microplate reader set at 570 nm.

Immunoblotting analysis

Cell protein extraction and immunoblotting analysis were conducted to examine the expression of CCNG2 as previously described [23]. Th sample was boiled and separated on 10% SDS-PAGE. The proteins were transferred to PVDF membrane (Amersham, Arlington Heights, IL, USA) by wet-transfer. The immunoreactive bands were developed using an ECL-plus chemiluminescence substrate (Perkin-Elmer, Waltham, MA, USA) and captured by LAS-1000 plus Luminescent Image Analyzer (GE Healthcare, Piscataway, NJ, USA).

Luciferase activity assay

The pmirGLO-CCNG2-Wt reporter was generated by cloning wild-type putative target region of CCNG2 to pmirGLO plasmids (Promega, Madison, WI, USA) following the manufacturer's instructions. pmirGLO-CCNG2-mut reporter was generated by mutagenesis. Cells were co-transfected pmirGLO-CCNG2-Wt reporter, pmirGLO-CCNG2-mut reporter, miR-1246 mimics, or miR-Scr using Lipofectamine 2000 reagent followed by analysis of luciferase activity.

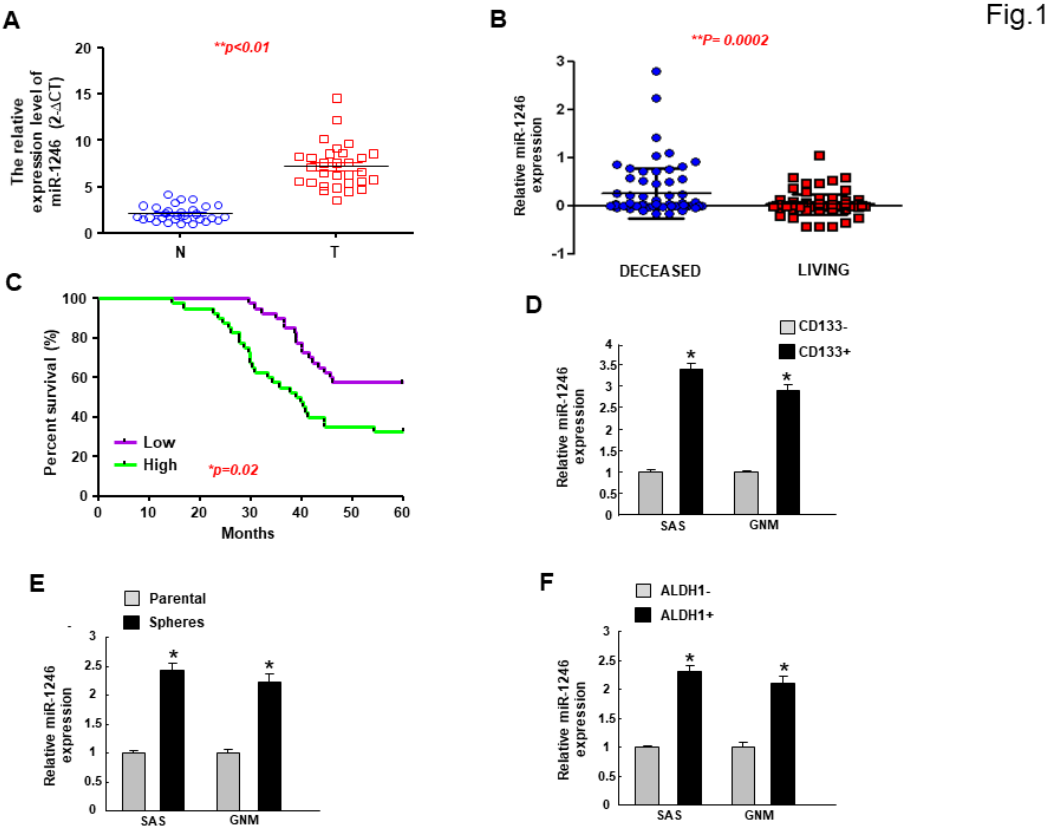
Statistical analysis

SPSS (version 13.0; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Pearson's correlation coeffiecient was used to evaluate the correlation between miR-1246 and stemness markers and CCNG2. Statistical differences were evaluated with the Student's *t*-test and were considered significant at $p<0.05$.

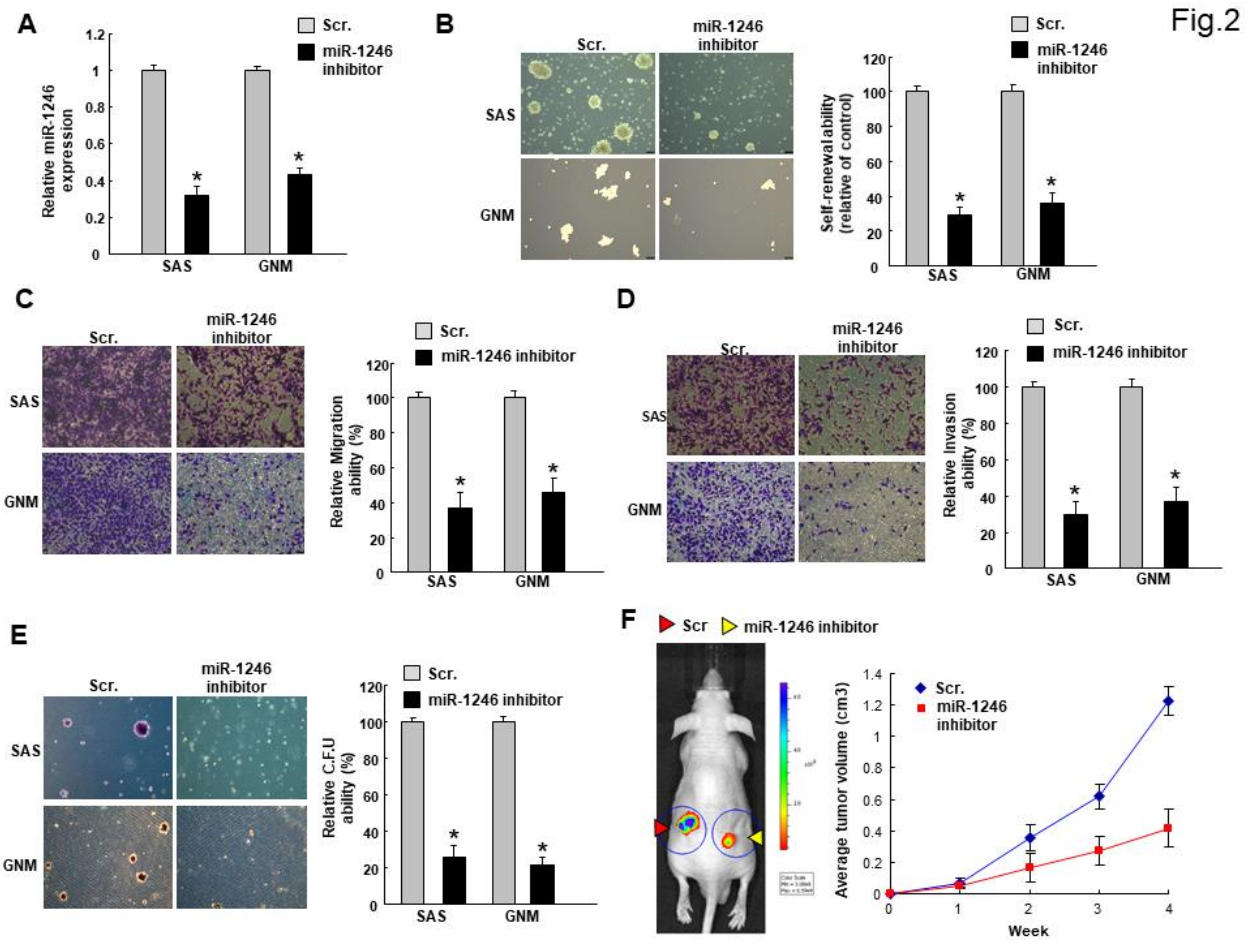
Results

1. The expression of miR-1246 is upregulated in the oral cancer tissues and cancer stem cells

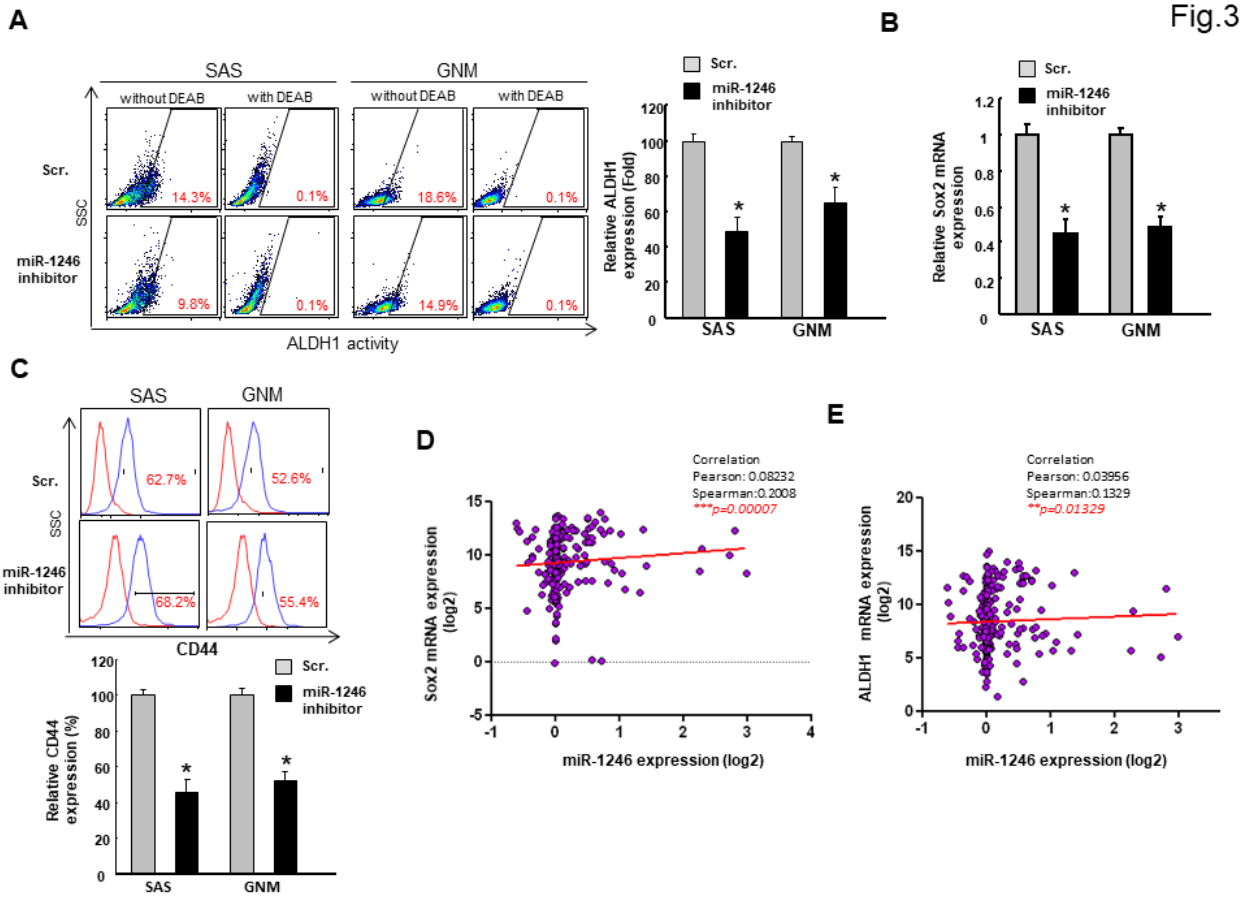
Fig.1



2. Inhibition of miR-1246 downregulates the stemness phenotypes in OSCC

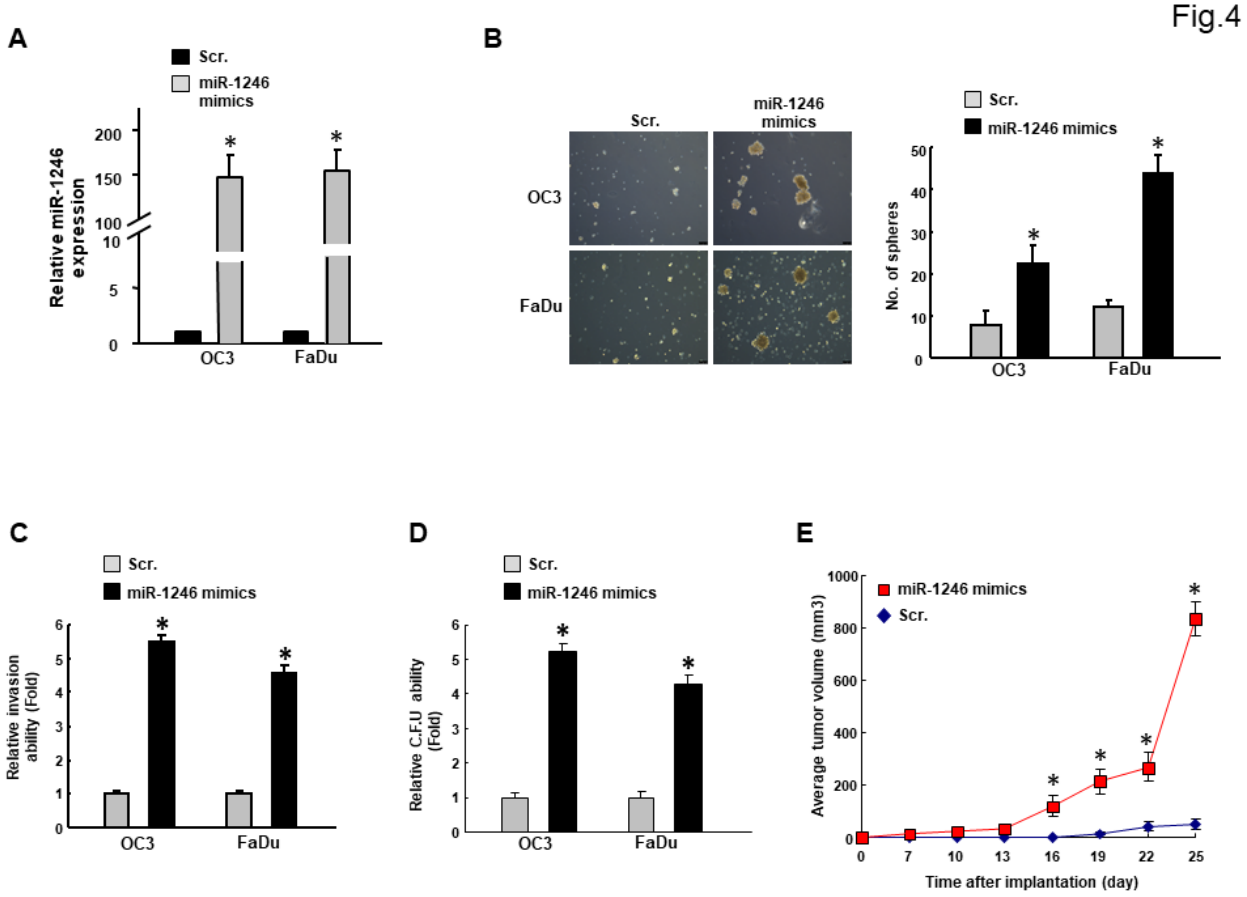


3. Downregulation of miR-1246 reduces the CSC markers in OSCC cells



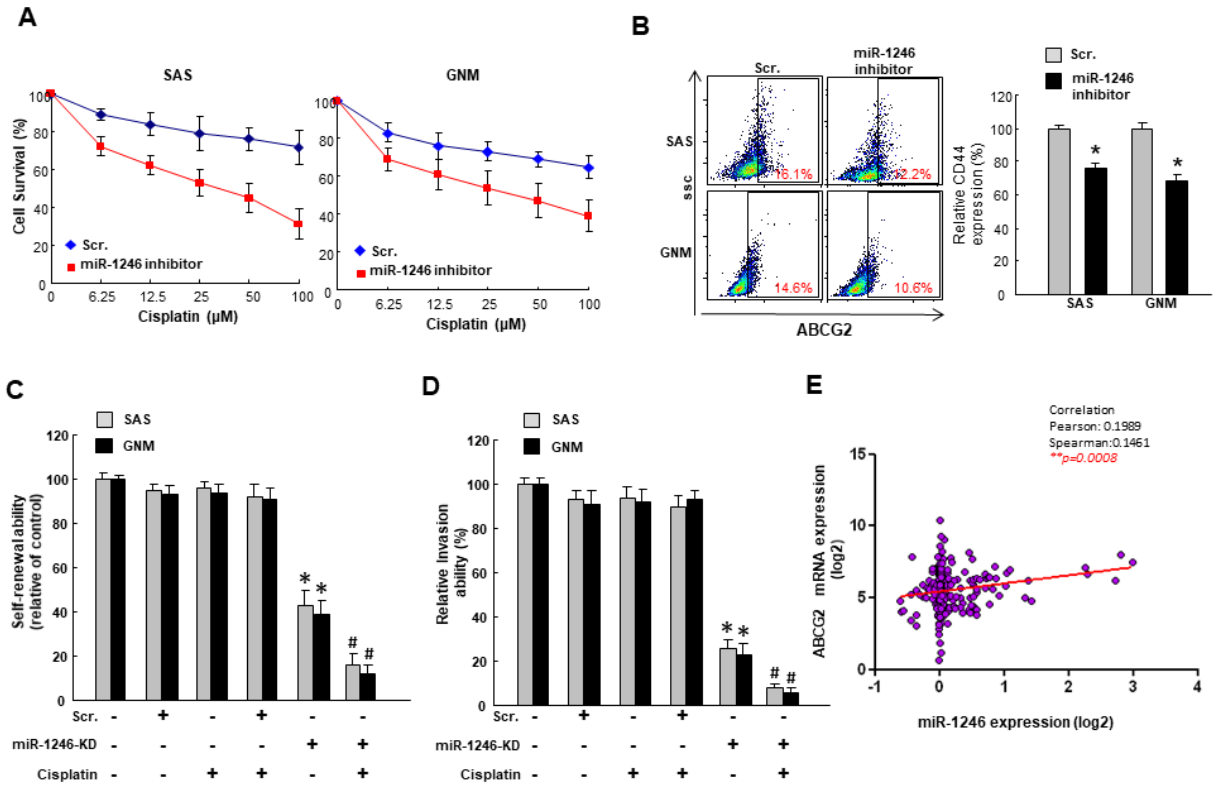
4. Upregulation of miR-1246 enhances the stemness phenotypes in OSCC

Fig.4



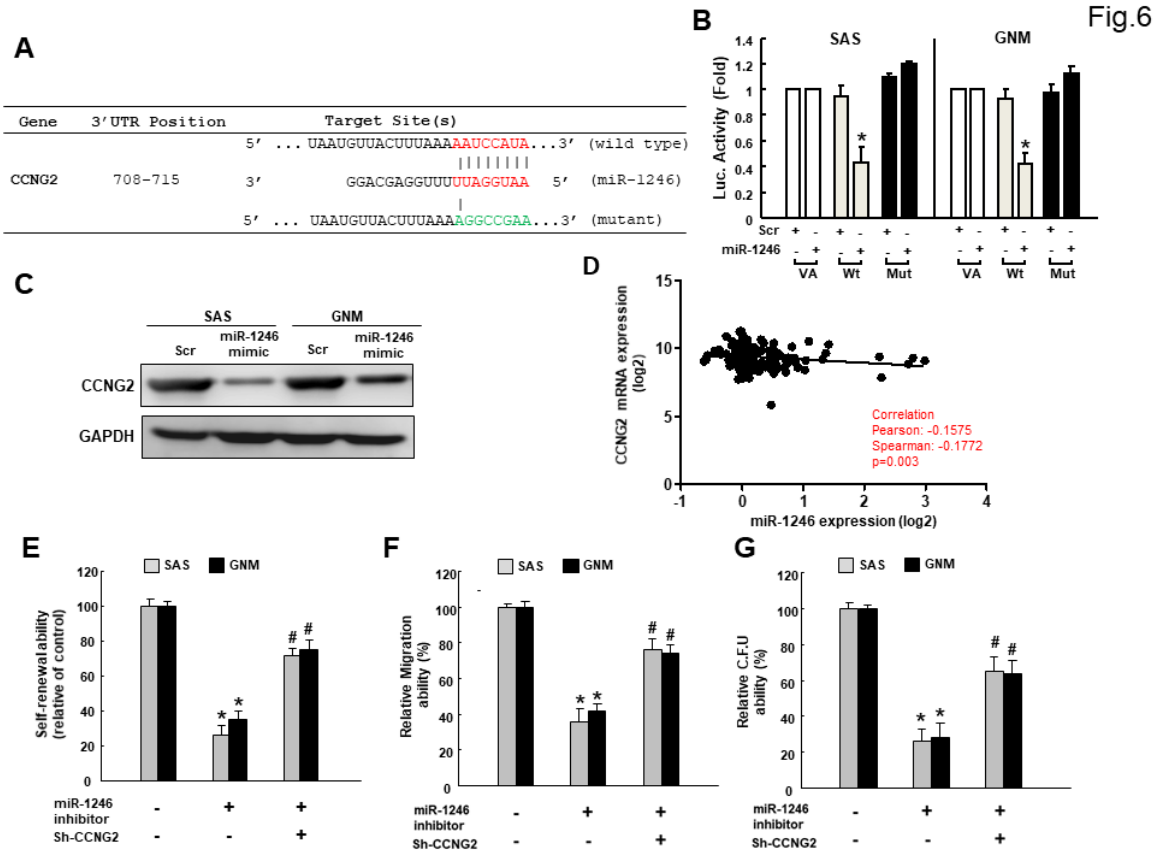
5 Downregulated miR-1246 increases the chemosensitivity of OSCC

Fig.5

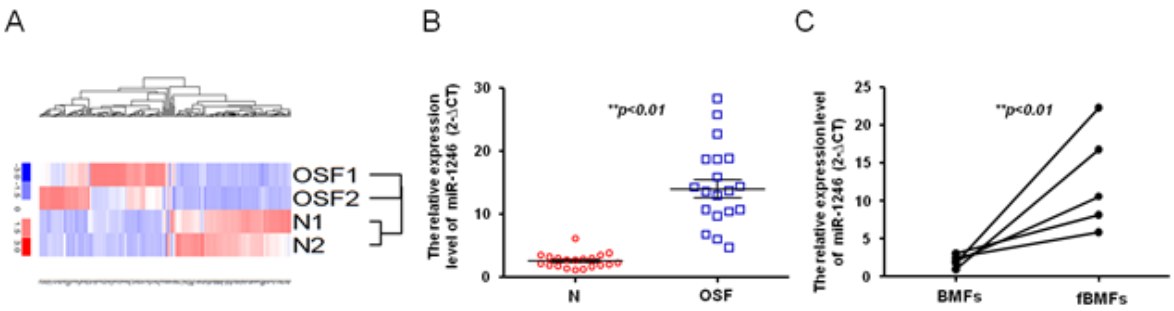


6. Suppression of CCNG2 by miR-1246 contributes to oral cancer stemness

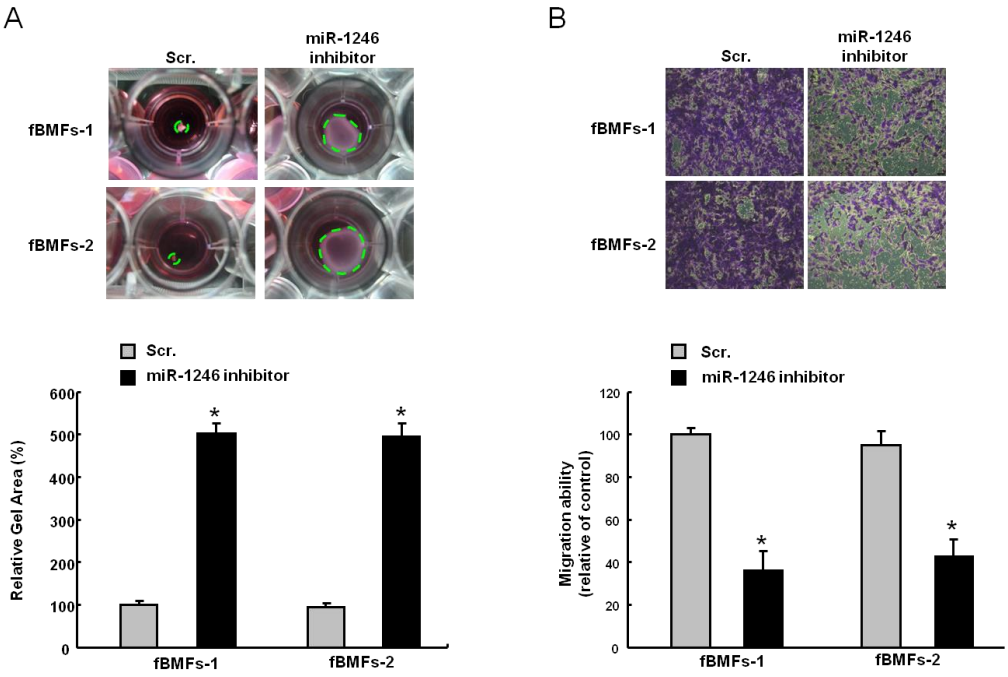
Fig.6



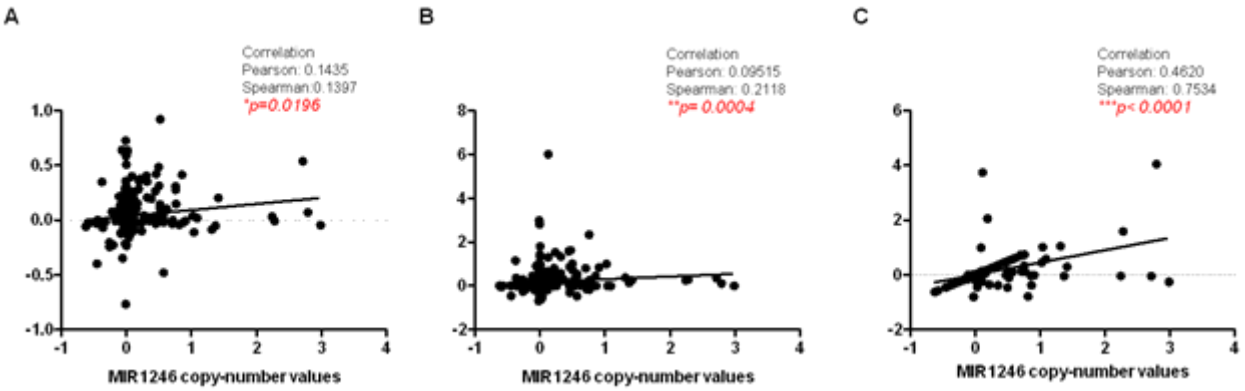
7. Up-regulation of miR-1246 in OSF specimens



8. Targeting miR-1246 represses myofibroblast activity



9. Clinical results revealed that miR1246 was positively correlated with fibrogenic markers



10. The expression of miR-1246 is upregulated in the oral cancer tissues and cancer stem cells

Publications:

1. miR-1246 Targets CCNG2 to Enhance Cancer Stemness and Chemoresistance in Oral Carcinomas. Cancers (Basel). 2018 Aug 16;10(8). pii: E272.

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106年度專題研究計畫成果彙整表

計畫主持人：周明勇	**計畫編號：**106-2314-B-040-005-
計畫名稱：miR-1246作為口腔黏膜下纖維化症及口腔癌的治療標靶及臨床應用性	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	發表於Cancers (Basel). 2018 Aug 16;10(8). pii: E272.
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		

		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）			本研究闡明miR-1246在口腔黏膜下纖維化症及口腔癌進程及的分子機制及臨床病理意義將可提供未來抗口腔黏膜下纖維化症及口腔癌研發的標的及標靶治療發展的可能性。		

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：■已發表　□未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）
   部分研究成果發表於Cancers (Basel). 2018 ;10(8)pii: E272.

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   本研究結果闡明miR-1246在口腔黏膜下纖維化症及口腔癌進程及的分子機制及臨床病理意義, 部分研究成果發表於Cancers (Basel). 2018 ;10(8)pii: E272. ,成果將可提供未來抗口腔黏膜下纖維化症及口腔癌研發的標的及標靶治療發展的可能性。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：□否　□是
   說明：（以150字為限）