科技部補助專題研究計畫成果報告
期末報告

微核糖核酸200家族成員於口腔癌癌症幹細胞之角色探討及微核糖核酸200家族成員於口腔癌臨床病理意義之研究（第3年）

計畫類別：個別型計畫
計畫編號：NSC 100-2314-B-040-010-MY3
執行期間：102年08月01日至103年07月31日
執行單位：中山醫學大學口腔科學研究所

計畫主持人：余承佳
共同主持人：周明勇

處理方式：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢
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中　華　民　國　103年10月29日

中 文 摘 要 ： 口腔癌為台灣男性癌症發生率及死亡率的第四位。口腔癌的診斷及治療方式已改善，但此疾病對化學性及放射性療法仍有高復發性，口腔癌病患預後成果及存活率相當差。因此必須進一步研究口腔癌之致病分子機轉。本實驗室先前已證實癌症幹細胞對口腔癌的起始、增生、轉移、及復發相當重要，因此抑制癌症幹細胞特性期待可發展出口腔癌新穎治療法。微核糖核酸(microRNAs)為重要癌症標記。微核糖核酸可與其標靶基因結合而降解其表現，在癌症中扮演促癌基因或抑癌基因之角色。miR-200 家族成員包括 miR-200a. miR-200b, miR-200c, miR-141,及 miR-429。miR-200 已證實為腫瘤抑制型微核糖核酸，可抑制腫瘤 細 胞 生 長 及 轉 移 。 且 miR-200 為重要 上 皮 - 間 質 細 胞 轉 換 過 程(epithelial-mesenchymal transformation； EMT)調節者。miR-200 家族對正常乳腺及乳癌幹細胞生長及自我更新極重要。近年來，EMT 與幹細胞特性之相互調控作用被相當重視。而 miR-200 對於口腔癌幹細胞、EMT、腫瘤生成特性之角色及其分子機轉仍未知。本研究發現 miR-200a 及 miR-200b 於 ALDH1+CD44+口腔癌幹細胞低度表現。miR-200a 或 miR-200b 表現抑制可使 ALDH1-CD44-細胞獲得癌幹細胞特性。ZEB2 及 TIMP2 為 miR-200b 之標靶基因。過度表現 miR-200b 可降低癌幹細胞特性及活體腫瘤生成力。miR-200a 及 miR-200a 於口腔癌腫瘤組織及淋巴轉移組織低度表現。證實 miR-200 可調節口腔癌癌症幹細胞特性而影響口腔癌致病機轉，以應用於口腔癌的診斷與監測。

中文關鍵詞： 口腔癌；微核糖核酸； 微核糖核酸 200； 癌症幹細胞

英 文 摘 要 ：

英文關鍵詞：

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

微核糖核酸 200 家族成員於口腔癌癌症幹細胞之角色探討及微核糖核酸 200 家族成員於口腔癌臨床病理意義之研究

計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST 100-2314-B-040-010-MY3
執行期間：　100 年 8 月 1　日至 103　年 7　月 31　日

執行機構及系所：中山醫學大學口腔醫學院口腔科學研究所

計畫主持人：余承佳
共同主持人：周明勇
計畫參與人員：張雅雯

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

期末報告處理方式：

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中　華　民　國　103　年　10　月　29　日

一、中文摘要

口腔癌為台灣男性癌症發生率及死亡率的第四位。口腔癌的診斷及治療方式已改善，但此疾病對化學性及放射性療法仍有高復發性，口腔癌病患預後成果及存活率相當差。因此必須進一步研究口腔癌之致病分子機轉。本實驗室先前已證實癌症幹細胞對口腔癌的起始、增生、轉移、及復發相當重要，因此抑制癌症幹細胞特性期待可發展出口腔癌新穎治療法。微核糖核酸(microRNAs)為重要癌症標記。微核糖核酸可與其標靶基因結合而降解其表現，在癌症中扮演促癌基因或抑癌基因之角色。miR-200 家族成員包括 miR-200a. miR-200b, miR-200c, miR-141,及 miR-429。miR-200 已證實為腫瘤抑制型微核糖核酸，可抑制腫瘤細胞生長及轉移。且 miR-200 為重要上皮-間質細胞轉換過程(epithelial-mesenchymal transformation; EMT)調節者。miR-200 家族對正常乳腺及乳癌幹細胞生長及自我更新極重要。近年來，EMT 與幹細胞特性之相互調控作用被相當重視。而 miR-200 對於口腔癌幹細胞、EMT、腫瘤生成特性之角色及其分子機轉仍未知。本研究發現 miR-200a 及 miR-200b 於 ALDH1+CD44+口腔癌幹細胞低度表現。miR-200a 或 miR-200b 表現抑制可使 ALDH1-CD44-細胞獲得癌幹細胞特性。ZEB2 及 TIMP2 為 miR-200b 之標靶基因。過度表現 miR-200b 可降低癌幹細胞特性及活體腫瘤生成力。miR-200a 及 miR-200a 於口腔癌腫瘤組織及淋巴轉移組織低度表現。證實 miR-200 可調節口腔癌癌症幹細胞特性而影響口腔癌致病機轉，以應用於口腔癌的診斷與監測。

二、Abstract

Oral cancer (OC) is a fatal disease, accounting the fourth leading malignancy and cancer death in male population in Taiwan. In spite of improvements in the diagnosis and management of oral cancer, long-term survival rates have improved only marginally over the past decade. To increase patient survival rates, knowledge of the mechanism(s) of tumorigenesis in oral cancer are urgently required. The most recent tumorigenesis theory is based on the idea that cancers arise from a rare population of cells, termed cancer stem cells (CSCs) Our laboratory has prospectively identified oral cancer stem cells and suggested that subsets of oral cancer stem cells are key contributors to chemo-radioresistance, tumor metastasis, and recurrence. Therefore, it is presumed that elimination of oral CSCs might be a novel therapeutic target for oral cancer. MicroRNAs (miRNAs)—highly conserved small RNA molecules that regulate gene expression—can act as cancer signatures, and as oncogenes or tumor suppressors depending on its main target genes. In this report, we found that lower miR-200a or miR-200b expression was detected in OC-derived ALDH+CD44+ cells, and SPONGE-mediated miR-200a or miR-200b inhibition conferred CSCs characteristics upon OC-derived non-TICs ($ALDH1^{-}CD44^{-}$ cells). Reporter assays further revealed that miR-200b directly targets the 3' UTR regions of ZEB2 and TIMP2. In contrast, overexpression of miR-200a or miR-200b dramatically alleviated CSCs properties and tumor progression in CSCs-transplanted mice. Compared with non-tumor samples from the same patient, the expression of miR-200a or miR-200b was decreased in all of the tumor samples. A similar down-regulation of miR-200a or miR-200b was also observed in metastatic lymph nodes when compared with local tumors. miR-200 family may be a useful diagnostic oral cancer tumor marker and novel miRNA-based approach for oral cancer treatment.

三、 前言

The miR-200 family is a tumor suppressive miRNA down regulated in several types of tumors including renal, prostate, breast, lung, bladder, pancreatic, and gastric cancers [1-5]. Accumulating data suggest that members of miR-200 family can reverse EMT process and induce mesenchymal-epithelial transition [6-8]. Down-regulation of miR-200c promotes the EMT of breast cancer cells while overexpression of miR-200c induces

mesenchymal-epithelial-transitions [9]. Notably, miR-200 regulates the EMT and cancer stemness properties by targeting ZEB1/ZEB2, Bmi1, and Sox2 in pancreatic cancer cells [10]. However, the role of miR-200 family in regulating tumourigenicity and metastasis in oral cancer or oral cancer-CSC has not been reported.. It has been considered as a key mechanism responsible for the process of cancer metastasis [11]. The interplay between EMT and stemness signature has gained huge interest in the field of cancer research recently, as they contribute to tumor metastasis and recurrence making cancer difficult to be tackled [12]. It has been suggested that EMT can promote stemness property in normal breast tissues as well as breast cancer cells [12]. Detailed bioinformatics analysis done by Ben-Porath *et al.* discovered an embryonic stem cell-like gene expression signature in poorly differentiated aggressive tumors [13]. Later on, single or combined overexpression of stemness factors, including Oct4 and Sox2, were associated with cancer metastasis [14, 15]. Recently report on the synergetic effect of Oct4 and Nanog regulating Slug promoter activity and EMT process in lung cancer revealing a role of stemness signature controlling EMT mechanism [16]. Recently, miRNAs have emerged as key post-transcriptional regulators of gene expression, involved in diverse physiological and pathological processes [17]. An oncogenic or tumor-suppressor miRNA may have potential as a therapeutic target for cancer treatment [17]. A regulatory network between miRNA, EMT, and stemness signature may update our current knowledge on the development of therapeutic treatments for cancer patients.

四、研究成果

Part1: miR145 targets the SOX9/ADAM17 axis to inhibit tumor initiating cells and IL-6-mediated paracrine effects in head and neck cancer (published in Cancer Res. 2013 ;73(11):3425-40).

Fig. 1. Suppression of miR145 is crucial for ALDH1$^-$/CD44$^-$ HNC cells to retain their tumor-initiating stem-like properties

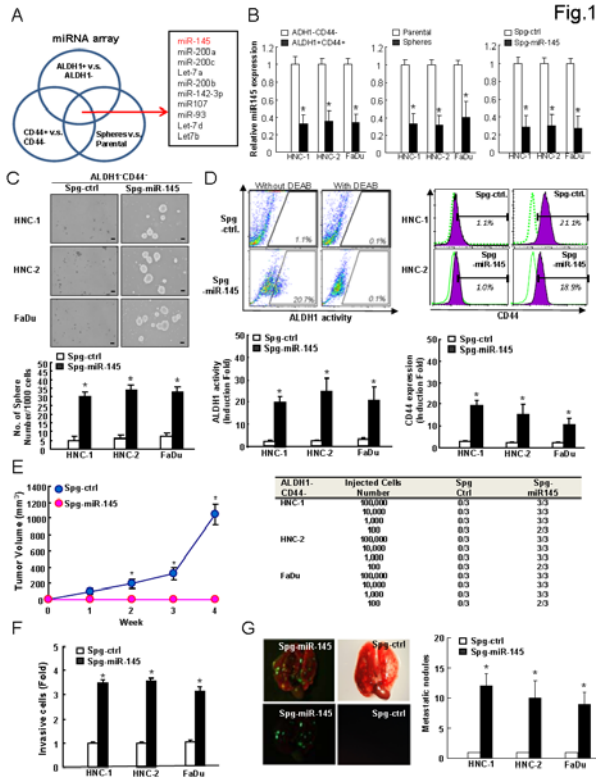


Fig. 2. miR145 directly targets the 3'UTR of SOX9 and ADAM17

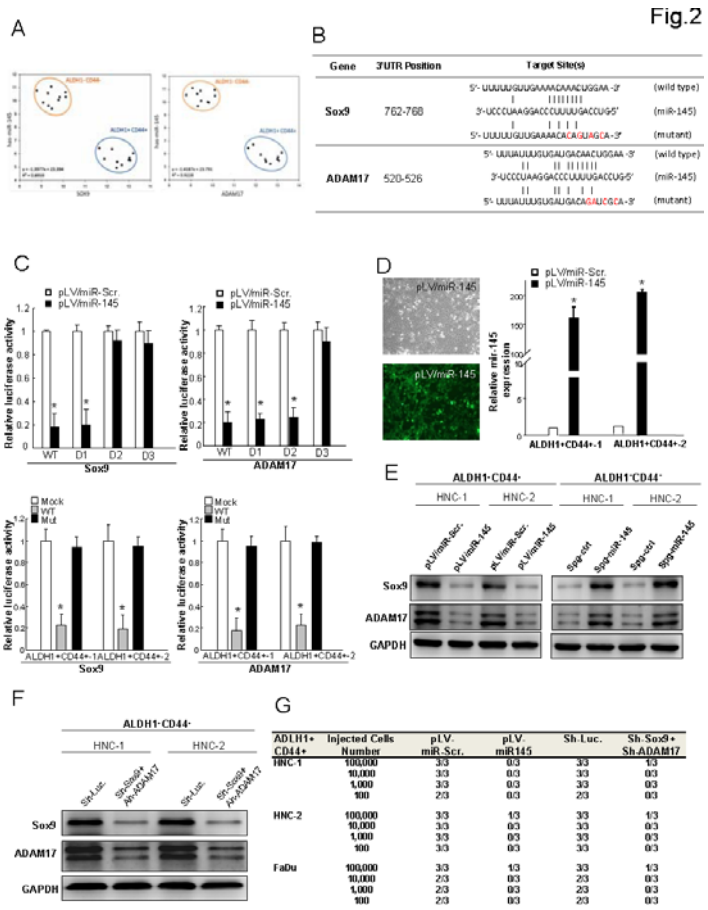


Fig. 3. Curcumin suppresses the stem-like properties of ALDH$^+$CD44$^+$ cells by activating miR145 and suppressing SOX9/ADAM17

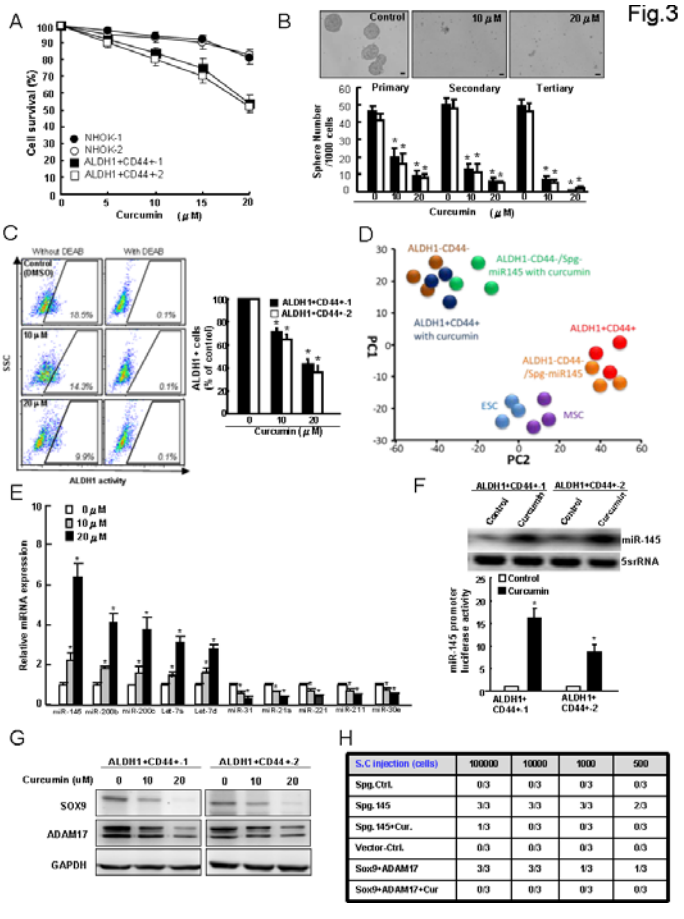


Fig. 4. Curcumin suppresses the migratory capability of ALDH$^+$CD44$^+$ HNC cells through the miR145-SOX9/ADAM17 pathway

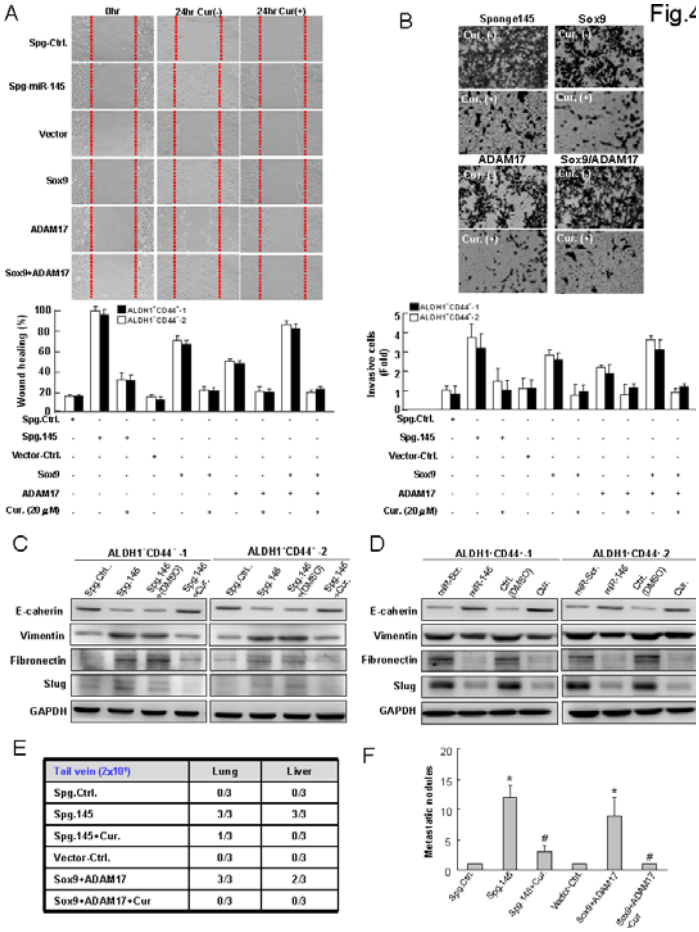


Fig. 5. The miR145-ADAM17 pathway modulates IL6/sIL6R trans-signaling

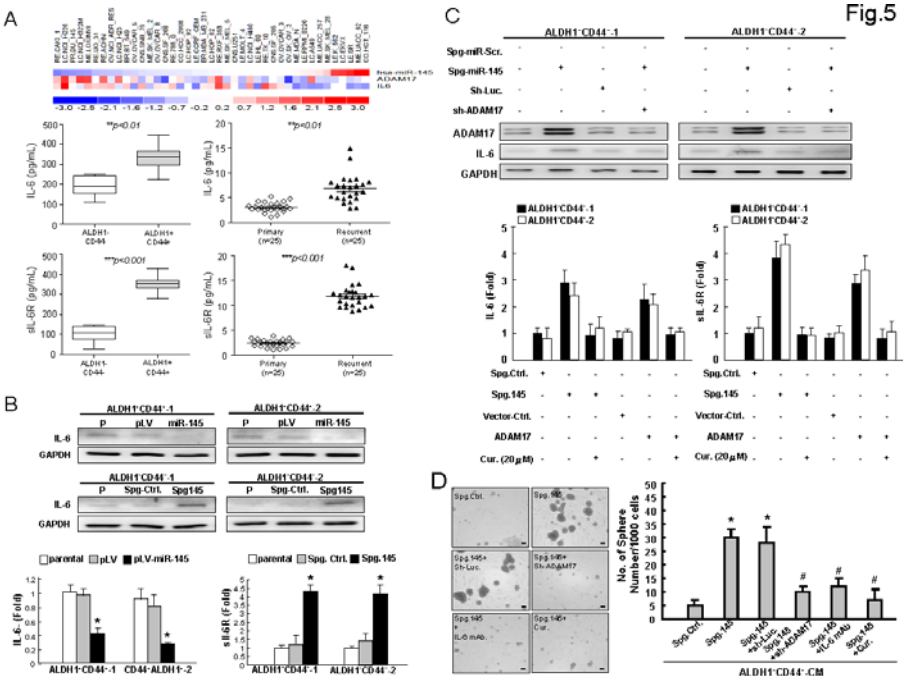


Fig. 6. Treatment with of oral-feeding curcumin or miR145 suppresses tumor growth and increases animal survival.

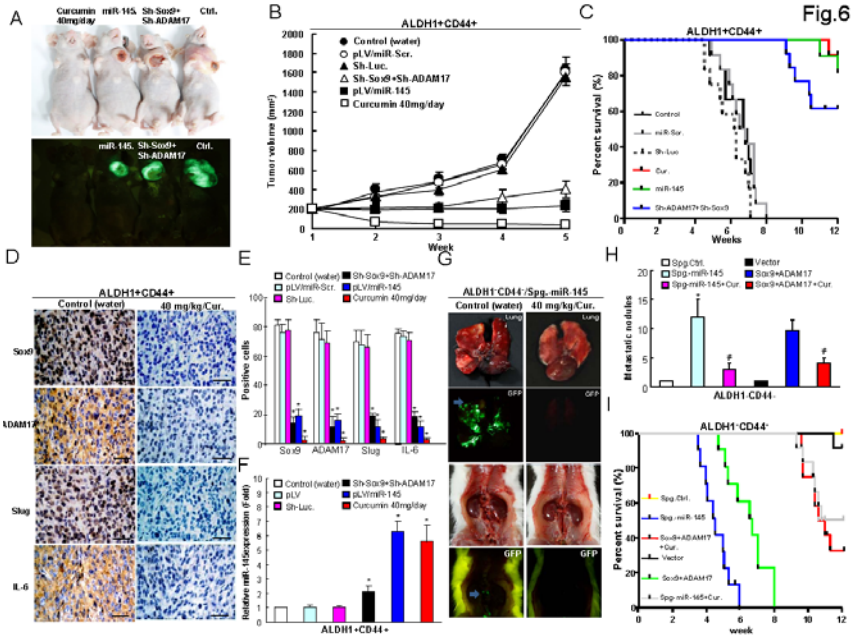
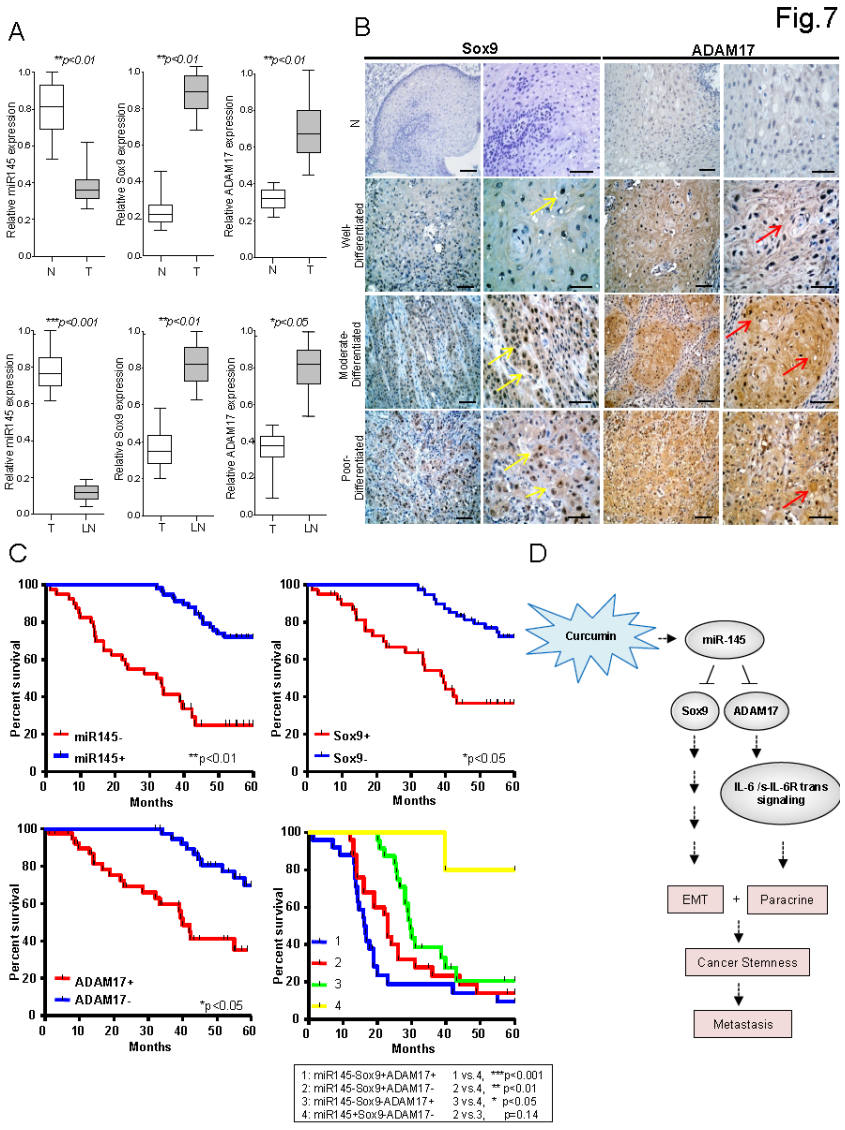


Fig. 7. The miR145^{low}Sox9^{high}ADAM17^{high} signature predicts poor survival in HNC patients



Part2: miR-200a/b impairs tumor initiating stem-like property in oral cancer

Fig.1. The level of miR-200a/b expression was lower in local oral cancer samples lymph node metastatic lesions

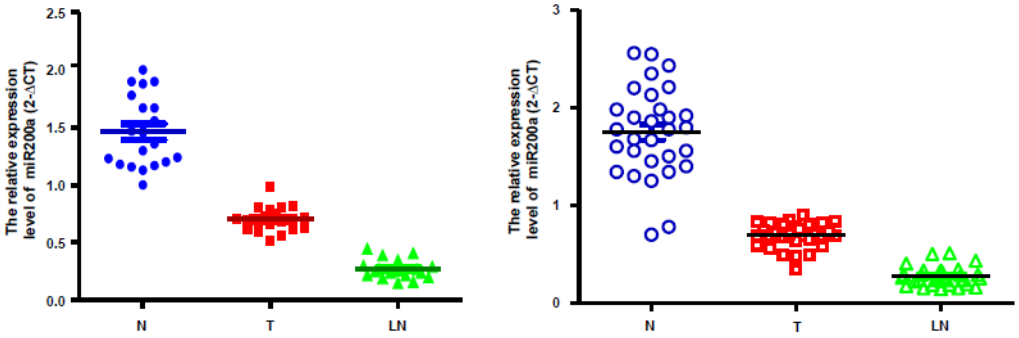
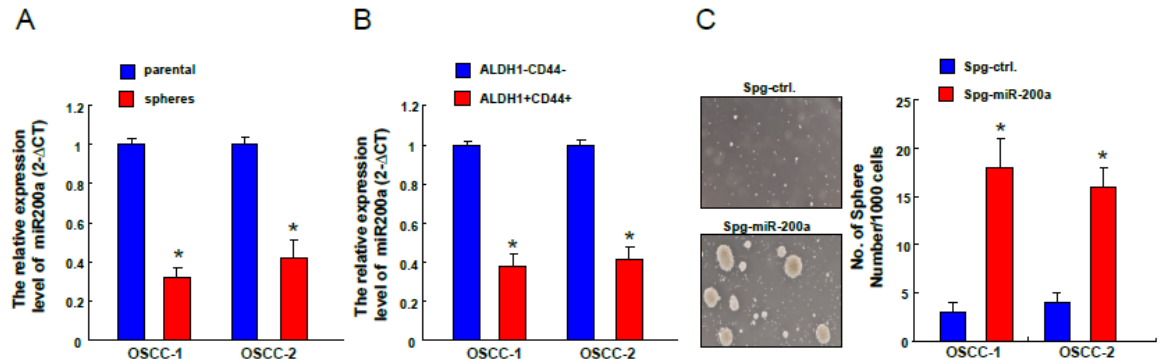


Fig. 2. miR-200a depletion enhances tumor initiating capability in ALDH1$^-$CD44$^-$ non-CSCs oral cancer cells.



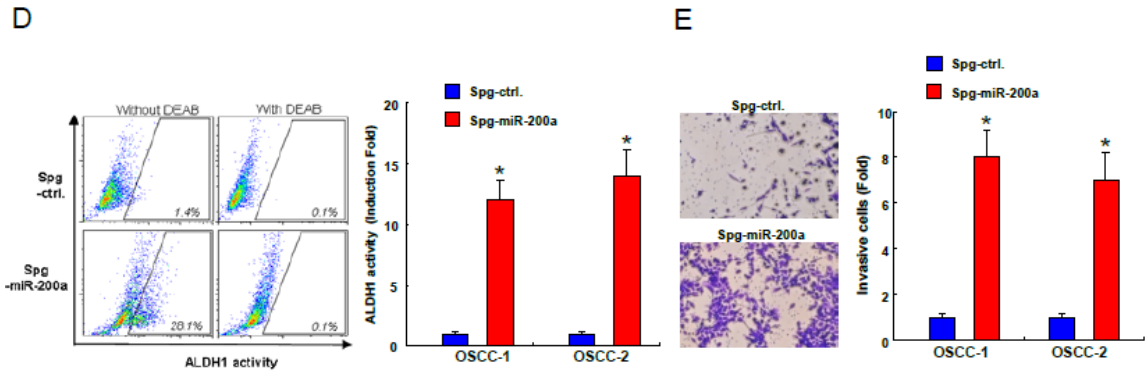


Fig. 3. miR-200b depletion enhances tumor initiating capability in ALDH1$^-$CD44$^-$ non-CSCs oral cancer cells.

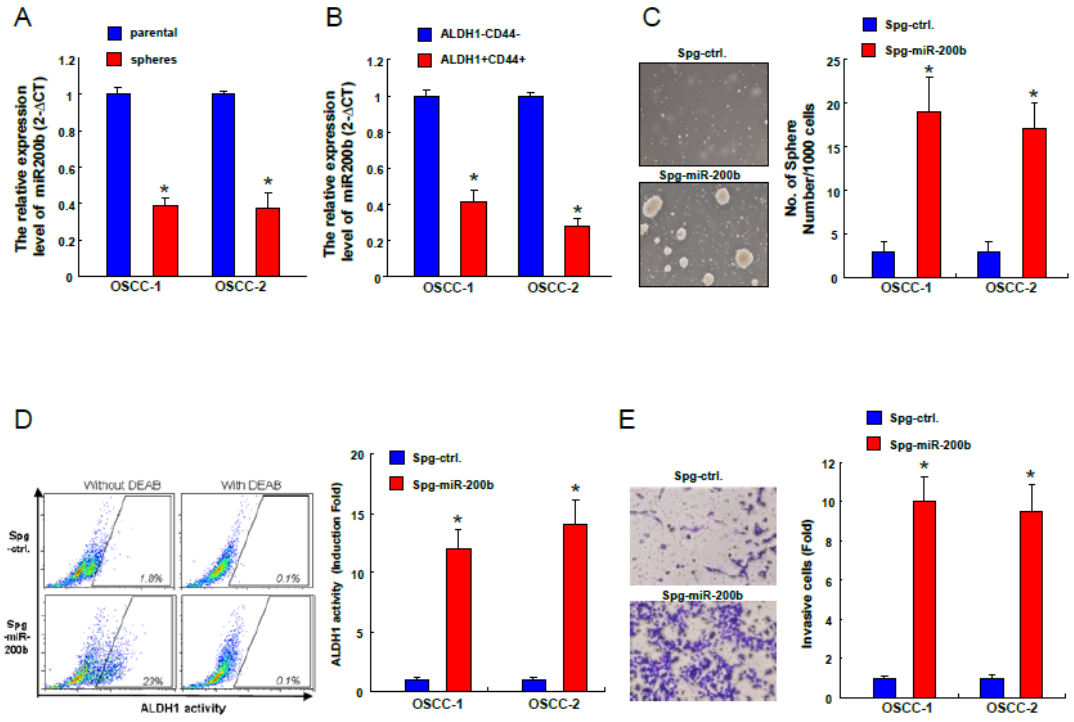


Fig. 4. miR-200b directly targets the 3'UTR of ZEB2 and TIMP2

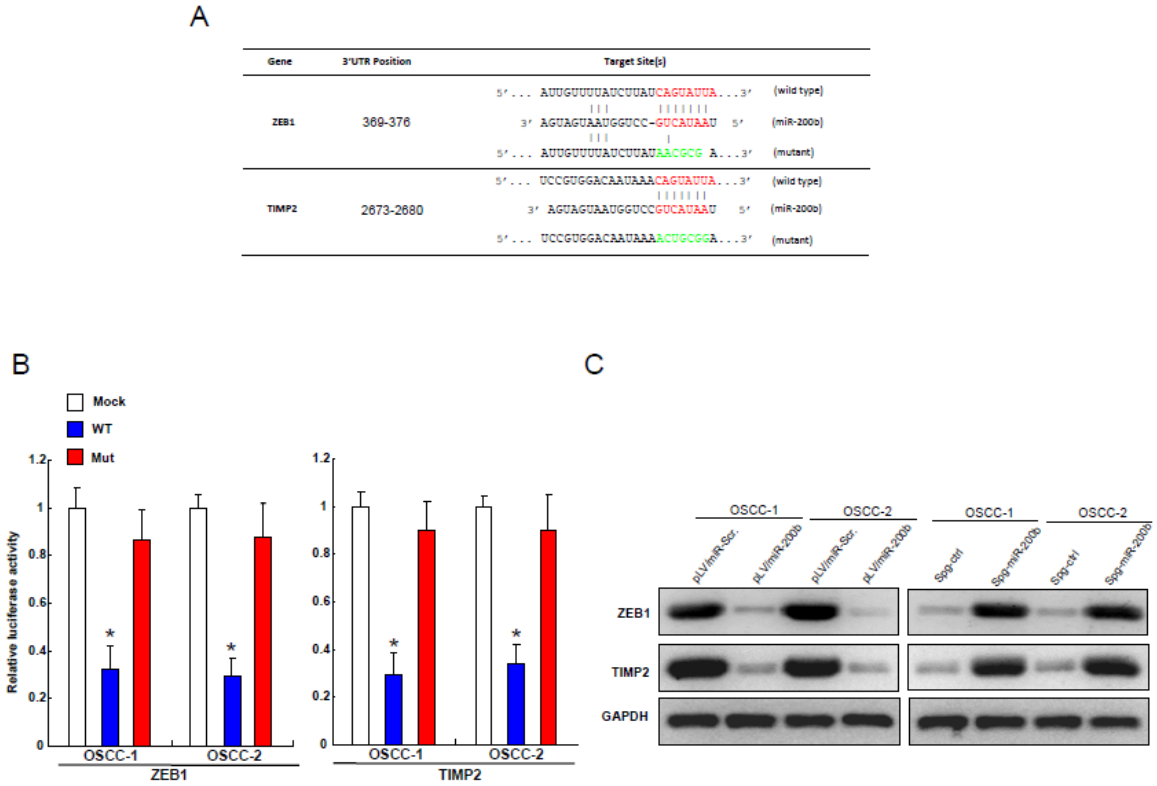
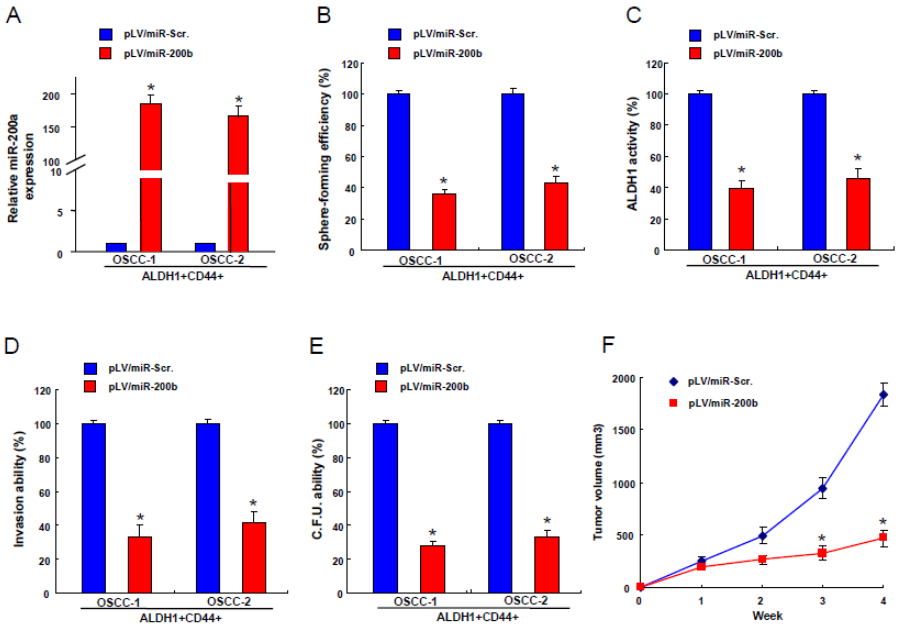


Fig. 5. miR200b impairs CSCs and *in vitro* tumorigenic properties.



五、已發表著作

1. **Yu CC***, Tsai LL, Wang ML, Yu CH, Lo WL, Chang YC, Chiou GY, Chou MY, Chiou SH* (2013). miR145 targets the SOX9/ADAM17 axis to inhibit tumor initiating cells and IL-6-mediated paracrine effects in head and neck cancer. ***Cancer Res*** 73:1-16
2. Chu PY, Hu FW#, **Yu CC#**, Tsai LL, Yu CH, Wu BC, Chen YW, Huang PI, Lo WL* (2013). Epithelial-Mesenchymal Transition Transcription Factor ZEB1/ZEB2 Co-expression Predicts Poor Prognosis and Maintains Tumor-Initiating Properties in Head and Neck Cancer. ***Oral Oncol*** 49:34-41.
3. Chiou GY, Cherng JY, Hsu HH, Wang ML, Tsai CM, Lu KH, Chien Y, Hung SC, Chen YW, Wong CI, Tseng LM, Huang PI, **Yu CC***, Hsu WH, Chiou SH* (2012). Cationic Polyurethanes-Short Branch PEI-Mediated Delivery of Mir145 Inhibited Epithelial-Mesenchymal Transdifferentiation and Cancer Stem-Like Properties and in Lung Adenocarcinoma. ***J Control Release*** 159:240-250
4. Hu FW, Tsai LL, Yu CH, Chen PN, Chou MY, **Yu CC*** (2012). Impairment of Tumor Initiating Stem-Like Property and Reversal of Mesenchymal Transdifferentiation in Head and Neck Cancer by Resveratrol Treatment. ***Mol Nutr Food Res*** 56:1247-58.

六、參考文獻

1. Spaderna S, Brabletz T, Opitz OG. The miR-200 family: central player for gain and loss of the epithelial phenotype. Gastroenterology. 2009;136:1835-7.

2. Roybal JD, Zang Y, Ahn YH, Yang Y, Gibbons DL, Baird BN, et al. miR-200 Inhibits Lung Adenocarcinoma Cell Invasion and Metastasis by Targeting Flt1/VEGFR1. Mol Cancer Res. 2010.

3. Nakada C, Matsuura K, Tsukamoto Y, Tanigawa M, Yoshimoto T, Narimatsu T, et al. Genome-wide microRNA expression profiling in renal cell carcinoma: significant down-regulation of miR-141 and miR-200c. J Pathol. 2008;216:418-27.

4. Kong D, Li Y, Wang Z, Banerjee S, Ahmad A, Kim HR, et al. miR-200 regulates PDGF-D-mediated epithelial-mesenchymal transition, adhesion, and invasion of prostate cancer cells. Stem Cells.

2009;27:1712-21.
5. Wiklund ED, Bramsen JB, Hulf T, Dyrskjot L, Ramanathan R, Hansen TB, et al. Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. Int J Cancer. 2010.
6. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008;22:894-907.
7. Korpal M, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem. 2008;283:14910-4.
8. Xia H, Cheung WK, Sze J, Lu G, Jiang S, Yao H, et al. miR-200a regulates epithelial-mesenchymal to stem-like transition via ZEB2 and beta-catenin signaling. The Journal of biological chemistry. 2010;285:36995-7004.
9. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, et al. Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell. 2009;138:592-603.
10. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol. 2009;11:1487-95.
11. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2:442-54.
12. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133:704-15.
13. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet. 2008;40:499-507.
14. Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, et al. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PloS one. 2008;3:e2637.
15. Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet. 2009;41:1238-42.
16. Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, et al. Coexpression of oct4 and nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. Cancer Res. 2010;70:10433-44.
17. Weidhaas J. Using microRNAs to understand cancer biology. Lancet Oncol. 2010;11:106-7.

科技部補助計畫衍生研發成果推廣資料表

日期:2014/10/21

科技部補助計畫	計畫名稱: 微核糖核酸200家族成員於口腔癌癌症幹細胞之角色探討及微核糖核酸200家族成員於口腔癌臨床病理意義之研究
	計畫主持人: 余承佳
	計畫編號: 100-2314-B-040-010-MY3　　學門領域: 牙醫學

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：余承佳	計畫編號：100-2314-B-040-010-MY3
計畫名稱：微核糖核酸 200 家族成員於口腔癌癌症幹細胞之角色探討及微核糖核酸 200 家族成員於口腔癌臨床病理意義之研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	1	0	100%		
國外	論文著作	期刊論文	4	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

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。)	茲將本計畫主要重要成果分述如下：1.微型核醣核酸 miR-145 可透過抑制 SOX9/ADAM17 訊號軸及 IL-6 旁分泌作用來標靶口腔癌幹細胞。(以通訊作者發表於 Cancer Res, 2013 IF:9.2)；2.利用 PU-PEI 之非病毒載體送入腫瘤抑制微型核醣核酸 miR-145 可降低肺腺癌細胞上皮-間質轉化現象及癌轉移特性微型核醣核酸 miR-145(發表於 J Control Release, 2012 IF:7.2)；3. 發現天然植物萃取物白藜蘆醇能有效抑口腔癌幹細胞特性，提升化療敏感性。(以通訊作者發表於 Mol Nutr Food Res, 2012 IF:4.9)；4.ZEB1/ZEB2 可作為口腔癌診斷標記及維持癌幹細胞特性。(發表於 Oral Oncol, 2013 IF:3)

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

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：

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 100 字為限）

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）

目前已發表四篇 SCI 論文,其中兩篇文章 Impact factor>6, 部分資料持續整理後將投稿於期刊,期待能利用微核糖核酸應用於口腔癌的診斷與監測， 進一步發展出新穎口腔癌治療法。