科技部補助專題研究計畫成果報告
期末報告

檜木醇透過調控微核醣核酸miR-513a-5p標靶癌幹細胞及上皮-間質相互轉換特性作為口腔癌化學輔助性療法之研究(第3年)

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： NSC 102-2628-B-040-001-MY3
執 行 期 間 ： 104年08月01日至105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

中 華 民 國 105 年 10 月 31 日

中 文 摘 要 ： 由於嚼食檳榔的盛行，使得口腔癌為台灣男性癌症發生率及死亡率的第四位。90%的口腔癌為鱗狀細胞癌，其特性為容易復發及轉移。近年來發現，腫瘤組織中細胞的異質性引發癌幹細胞存在之可能性。本實驗室先前研究發現，癌幹細胞與上皮-間質細胞轉換過程(epithelial-mesenchymal transformation; EMT) 之相互調控作用對於傳統化療及放射性療法具備阻抗性及導致癌細胞具有高度轉移力，故認為癌幹細胞應是導致口腔癌病患病灶復發致死的主因。因此，尋找有效標靶癌幹細胞特性之療法將有助於口腔癌臨床上預防、診斷、及口腔癌新穎輔佐療法之開發。本實驗室初步分析研究結果發現檜木醇能有效抑制口腔癌幹細胞的增生能力，對正常口腔細胞無明顯細胞毒性。此外，檜木醇亦可以有效抑制活體內口腔癌幹細胞標記ALDH1活性及CD44的表達、降低幹細胞球體自我更新能力、及細胞轉移侵襲力。於活體裸鼠動物模式中，口服餵食檜木醇可降低活體腫瘤生成能力。綜合上述，檜木醇應為標靶口腔癌幹細胞特性之化學治療物質。微核糖核酸(microRNAs, miRNAs)為重要癌症標記，可與其標的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。初步研究利用微核糖核酸microarray發現檜木醇會誘發miR-513a-5p之表現。過度表現miR-513a-5p可降低口腔癌細胞癌幹性及致癌性。基於上述理由，檜木醇可藉由調控miR-513a-5p及其標的基因表現成為有潛力抑制癌幹細胞特性及上皮-間質細胞轉換過程之標靶化學治療物質。

中 文 關 鍵 詞 ： 檜木醇;口腔癌;癌幹細胞; 微核醣核酸; miR-513a-5p;上皮間質轉換過程

英 文 摘 要 ： Due to the popularity of areca use, oral cancer (OC) has become a prevalent disease in Taiwan. OC is currently the 4th most common cancer type and leading cancer mortality in male Taiwanese. Almost 90% of OC is classified as oral squamous cell carcinoma (OSCC), which is characterized by high recurrence and early metastasis. Most patients relapse within months after current therapeutic treatments. A subpopulation of cells called cancer stem cells (CSCs) possessing stemness properties was shown to enrich after therapy, resulting in the relapse and metastasis of tumors. Our previous studies have demonstrated that oral cancer derived cancer stem cells (OC-CSCs) presented high tumorigenic, chemo-radioresistant, metastatic properties, and coupled with gain of epithelial-mesenchymal transition (EMT) characteristics. Thus, an effective therapeutic approach targeting these OC-CSCs cells may help to improve current treatment regimens for OC-related malignancies. We observed that the treatment of hinokitiol dose dependently down-regulated the ALDH1 activity, CD44 positivity, stemness signatures expression, and self-renewal property in OC-CSCs. Mice model showed that hinokitiol treatment by oral gavage to xenograft tumors reduced tumor growth. 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. Using miRNA-microarray, hinokitiol increased the expression of miR-513a-5p in OC-CSCs. Therefore, elevating miR-513a-5p by methods such as hinokitiol treatment appears to be a promising therapeutic modality to target OC-CSCs.

英文關鍵詞：hinokitiol; oral cancer; cancer stem cells; microRNAs; miR-513a-5p epithelial-mesenchymal transition

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計畫主持人：余承佳
共同主持人： 石尹華、彭榆茜
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 105 年 10 月 31 日

中文摘要

由於嚼食檳榔的盛行，使得口腔癌為台灣男性癌症發生率及死亡率的第四位。90%的口腔癌為鱗狀細胞癌，其特性為容易復發及轉移。近年來發現，腫瘤組織中細胞的異質性引發癌幹細胞存在之可能性。本實驗室先前研究發現，癌幹細胞與上皮-間質細胞轉換過程(epithelial-mesenchymal transformation; EMT) 之相互調控作用對於傳統化療及放射性療法具備阻抗性及導致癌細胞具有高度轉移力，故認為癌幹細胞應是導致口腔癌病患病灶復發致死的主因。因此，尋找有效標靶癌幹細胞特性之療法將有助於口腔癌臨床上預防、診斷、及口腔癌新穎輔佐療法之開發。本實驗室初步分析研究結果發現檜木醇能有效抑制口腔癌幹細胞的增生能力，對正常口腔細胞無明顯細胞毒性。此外，檜木醇亦可以有效抑制活體內口腔癌幹細胞標記ALDH1活性及CD44的表達、降低幹細胞球體自我更新能力、及細胞轉移侵襲力。於活體裸鼠動物模式中，口服餵食檜木醇可降低活體腫瘤生成能力。綜合上述，檜木醇應為標靶口腔癌幹細胞特性之化學治療物質。微核糖核酸(microRNAs, miRNAs)為重要癌症標記，可與其標的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。初步研究利用微核糖核酸microarray發現檜木醇會誘發miR-513a-5p之表現。過度表現miR-513a-5p可降低口腔癌細胞癌幹性及致癌性。基於上述理由，檜木醇可藉由調控miR-513a-5p及其標的基因表現成為有潛力抑制癌幹細胞特性及上皮-間質細胞轉換過程之標靶化學治療物質。

關鍵字：檜木醇;口腔癌;癌幹細胞; 微核醣核酸; miR-513a-5p;上皮間質轉換過程

Abstract

Due to the popularity of areca use, oral cancer (OC) has become a prevalent disease in Taiwan. OC is currently the 4th most common cancer type and leading cancer mortality in male Taiwanese. Almost 90% of OC is classified as oral squamous cell carcinoma (OSCC), which is characterized by high recurrence and early metastasis. Most patients relapse within months after current therapeutic treatments. A subpopulation of cells called cancer stem cells (CSCs) possessing stemness properties was shown to enrich after therapy, resulting in the relapse and metastasis of tumors. Our previous studies have demonstrated that oral cancer derived cancer stem cells (OC-CSCs) presented high tumorigenic, chemo-radioresistant, metastatic properties, and coupled with gain of epithelial-mesenchymal transition (EMT) characteristics. Thus, an effective therapeutic approach targeting these OC-CSCs cells may help to improve current treatment regimens for OC-related malignancies.
We observed that the treatment of hinokitiol dose dependently down-regulated the ALDH1 activity, CD44 positivity, stemness signatures expression, and self-renewal property in OC-CSCs. Mice model showed that hinokitiol treatment by oral gavage to xenograft tumors reduced tumor growth. 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. Using miRNA-microarray, hinokitiol increased the expression of miR-513a-5p in OC-CSCs. We demonstrated that overexpression of miR-513a-5p in OC-CSCs inhibited cancer stemness and oncogenicity Therefore, elevating miR-513a-5p by methods such as hinokitiol treatment appears to be a promising therapeutic modality to target OC-CSCs.

Key words: hinokitiol; oral cancer; cancer stem cells; microRNAs; miR-513a-5p epithelial-mesenchymal transition

Introduction

Oral cancer (OC) represent the sixth most common cancer type worldwide and account for approximately 8 – 10% of all cancers in Southeast Asia (1). In addition, most OC patients relapse within months after receiving treatment. Mounting evidence have showed that the resistance to chemoradiation therapy and other currently available targeted therapies are, in part, due to the survival of a subpopulation of cells, called cancer stem cells (CSCs) or tumor initiating cells (TICs), within the heterogeneous tumor mass. TICs possesses stemness properties and further enriches after therapy, resulting in the relapse, metastasis, and therapeutic resistance of tumors. The oral cancer derived cancer stem cells (OC-CSCs) have been isolated by sorting the cells expressing specific surface markers, CD44 and ALDH1 (aldehyde dehydrogenase) (2). CD44 is a transmembrane glycoprotein involved in many cellular processes. A minority population of $CD44^+$ cell in HNC has previously been reported to possess the unique properties of cancer initiating cells in functional assays for self-renewal and differentiation, and form unique histological microdomains that may aid in cancer diagnosis (3). ALDH1 is a cytosolic isoenzyme which is responsible for oxidizing intracellular aldehydes and contributing to the oxidation of retinol to retinoic acid in early stem cell differentiation. $ALDH1^+$ cells in inflammatory breast cancer has been shown to predict metastasis and poor patient outcome (4). It was shown to be the CSC marker in several types of cancers including lung, colorectal, and breast cancer (4-8). ALDH1 has been demonstrated to be a marker distinguishing malignant from premalignant cells as well as identifying the putative OC-CSCs (9, 10). More importantly, CD44/ALDH-overexpressing OC-CSCs present elevated epithelial-mesenchymal transition markers and are highly metastatic, tumorigenic, and resistant to radio and chemotherapies (2). An effective therapeutic approach targeting the OC-CSCs would be a potential method to improve the treatments for OC-related malignancies.

Hinokitiol, also known as β-thujaplicin, is a tropolone-related natural compound isolated from heartwood cupressaceous plants. It exhibits diverse biological and pharmacological properties including anti-bacterial (11), anti-inflammatory (12), anti-oxidant as well as anti-tumor activities (11, 13-15). It has been shown that hinokitiol inhibited oral bacteria with low cytotoxicity to normal oral cells(11). And several studies have demonstrated its anti-tumor potential in various carcinomas. It was reported that hinokitiol induced apoptosis in teratocarcinoma F9 cells through the activation of caspase-3 (16). Recent studies also showed that hinokitiol suppressed tumor growth in colon cancer (13) or malignant melanoma (15) through induction of cell-cycle arrest and apoptosis. Additionally, hinokitiol induced autophagy through the AKT/mTOR pathway in murine breast and colorectal cancer cells (17). It also has been showed to decrease the OSCC viability(11). The efficacy of hinokitiol in the specific subset of OC-CSCs is still remained elusive.

miRNAs have recently been linked to regulate the properties of cancer stemness (18). For example, miR200a reduced the stem-like state and epithelial-mesenchymal transition through targeting ZEB2 and β-catenin signalings in nasopharygeal carnicoma cells (19). miR34a inhibits prostate tumor regeneration and metastasis through directly repression of the CD44 prostate CSC marker (18). Overexpression of miR145-SOX9/ADAM17 regulatory pathway resulted in the inhibition of the self-renewal, tumor initiation, and metastatic properties of OC-CSCs (20). miR-200c expression is negatively correlated with advancing OSCC grading and that miR-200c negatively regulates Bmi-1 expression (21). The let-7 miRNA family including let-7a (22) let-7d (23), or let-7i (24) regulates self-renewal renewal, cell mobility, and the EMT properties in OSCC cells. miR-494 is down-regulated in clinical biopsy tissues from OSCC patients with high-grade and lymph node metastasis and associated with CSCs properties (25). According to the above findings, miRNAs are critical regulatory factors in tumorigenesis through CSCs modulation.

Materials and Methods

MTT assay

Cell proliferation/survival was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The 570 nm absorbance of DMSO treated group was set as 100% and data were presented as percentage of DMSO control.

Tumor spheres assay

For enrichment of spheres, cells were cultured in tumor sphere medium consisting of serum-free DMEM/F12 medium (GIBCO), N2 supplement (GIBCO), 10 ng/mL human recombinant basic fibroblast growth factor-basic (FGF) and 10 ng/mL Epidermal Growth Factor (EGF) (R&D Systems, Minneapolis, MN).The two cell lines were then cultured in tumor sphere medium consisting of serum-free DMEM/F12 medium (GIBCO), N2 supplement (GIBCO), 10 ng/mL human recombinant basic fibroblast growth factor-basic (FGF) and 10 ng/mL Epidermal Growth Factor (EGF) (R&D Systems, Minneapolis, MN). Cells were plated at a density of 10^3 live cells/low-attachment six-well plate (Corning Inc., Corning, NY), 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 will be obtained from accurtase treated spheroids and the cell density of passage will be 1000 cells/ml in the serum-free medium as described above.

Aldefluor assay

Aldefluor assay kit is purchased from StemCell Technologies, Inc. (Vancouver, BC, Canada) 1×10^5 cells will be suspended in 50 μl of assay buffer and added Aldefluor to final concentration of 1 μM. For ALDH1 inhibitor control, DEAB will be added to final concentration of 150 μM. Cells will be then incubated at 37°C for 45 min and stained with 7-AAD on ice for further 5min. After washed with PBS, green fluorescence positive cells in live cells (7AAD-) will be analyzed by flow cytometry (FACSCalibur^{TM}, BD Bioscience) by comparing the fluorescence intensity of DEAB treated sample and these cells will be represented as cells with high ALDH activity (ALDH+ cells).

miRNAs qRT–PCR anlysis

For miR-145 levels decetion, qRT–PCR was performed using TaqMan miRNA assays with specific primer sets (Applied Biosystems, Carlsbad, Calif). All reagents and protocols were from Applied Biosystems, and detection was performed using a 7900HT fast real-time PCR system.

Western blot

The extraction of proteins from cells and western blot analysis were performed as described. Samples (15 μL) were boiled at 95°C for 5 min and separated by 10 % SDS-PAGE. The proteins were wet-transferred to Hybond-ECL nitrocellulose paper (Amersham, Arlington Heights, IL, USA). The following primary antibodies were used: rabbit anti–human Oct4, rabbit anti–human Nanog, and rabbit anti–human Nestin, (Santa Cruz Biotechnology, Santa Cruz, CA, USA); rabbit anti-GAPDH (MDBio, Inc., Taipei, Taiwan);. Immunoreactive protein bands were detected by the ECL detection system (Amersham Biosciences Co., Piscataway, NJ, USA).

In vitro cell invasion analysis.

The 24-well plate Transwell® system with a polycarbonate filter membrane of 8-μm pore size (Corning, United Kingdom) was employed to evaluate the invasion ability of cells. The membrane was coated with Matrigel^{TM} (BD Pharmingen, NJ, USA).The cancer cell suspensions were seeded to the upper compartment of the Transwell chamber at the cell density of 1×10^5 in 100 μl within serum-free medium. The lower chamber was filled with serum-free medium. or media with 10% serum After 24 hours of incubation, the medium was removed and the filter membrane was fixed with 4% formalin for 1 hour. Subsequently, the remaining cells of the filter membrane faced the lower chamber was stained with Hoechst 33258 (Sigma-Aldrich). The migrated cancer cells were then visualized and counted from 5 different visual areas of 100-fold magnification under an inverted microscope.

Soft agar colony forming assay

Six-well culture dish was coated with 2 ml bottom agar (Sigma-Aldrich) mixture (DMEM, 10% (v/v) FCS, 0.6% (w/v) agar). After the bottom layer was solidified, 2 ml top agar-medium mixture (DMEM, 10% (v/v) FCS, 0.3% (w/v) agar) containing $2x10^4$ cells was added, and the dishes were incubated at 37^oC for 4 weeks. Plates were stained with 0.005% Crystal Violet then the colonies were counted. The number of total colonies with a diameter ≥100 μm was counted over five fields per well for a total of 15 fields in triplicate experiments.

Imaging measurement of tumor growth in nude mice

All procedures involving animals were in accordance with the institutional animal welfare guidelines of the Chung Shan Medical University. For the nude mice xenograft model, 5-6 weeks old immuno-deficient nude mice (BALB/c nu/nu mice) weighing 18-22 g were used. The mice were housed with a regular 12 h light/12 h dark cycle and ad libitum access to standard rodent chow diet (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO) and were kept in a pathogen-free environment at the Laboratory Animal Unit. OC-CSCs (1×10^4 cells/0.2 mL/mouse) were injected subcutaneously into the right front axilla. Eight days postimplantation, the mice were randomly divided into three groups (N = 6 for each group) and fed by oral gavage with saline (control) and hinokitiol (10 and 20 mg/day/kg) suspended in PBS. The day of cell implantation was designated day 0. Imaging measurement was performed using an IVIS50 animal imaging system (Xenogen Corp.). The photons emitted from the target site penetrated through the mammalian tissue and could be externally detected and quantified using a sensitive light-imaging system. The image acquisition time was 1 min. The displayed images of the tumor sites were drawn around and quantified in photons per second using Living Image software (Xenogen Corp.) The volume was calculated (according to the following formula: [length × width^2]/2), and then analyzed using Image-Pro Plus software. Body weight was assessed daily after cell injection. After 20 days, the animals were euthanized, and the primary tumors were isolated and weighed.

Statistical analysis

Statistical Package of Social Sciences software (version 13.0) (SPSS, Inc., Chicago, IL) was used for statistical analysis. Student's 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

1. Hinokitiol preferentially eliminates proliferation rate and self-renewal capacity in OC-CSCs

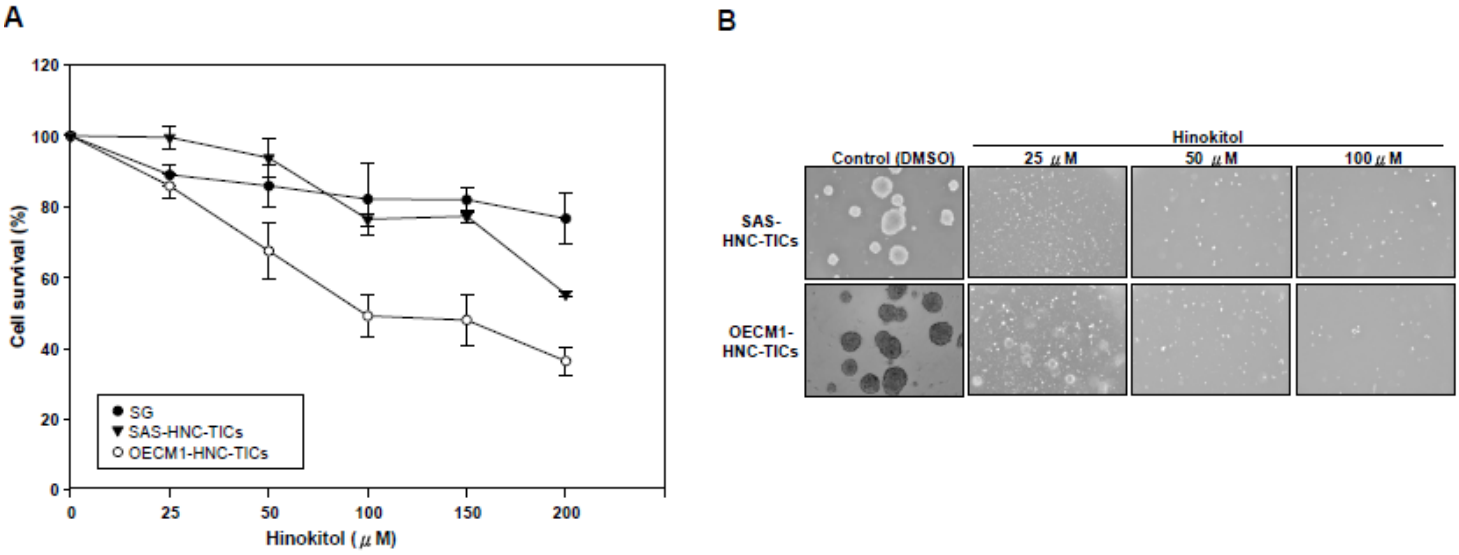


Figure1: (A) SG and $ALDH^{+}CD44^{+}$ OC-CSCs were treated with various concentrations of hinokitiol up to 200 μM for 24 hours. Cell survival was assessed by MTT assay and was presented as percent survival relative to untreated cells. (B) $ALDH1^{+}CD44^{+}$ OC-CSCs treated with or without hinokitiol were subjected to a self-renewal secondary sphere-forming assay.

2. Hinokitiol effectively eliminates ALDH1 and CD44 positivity in OC-CSCs

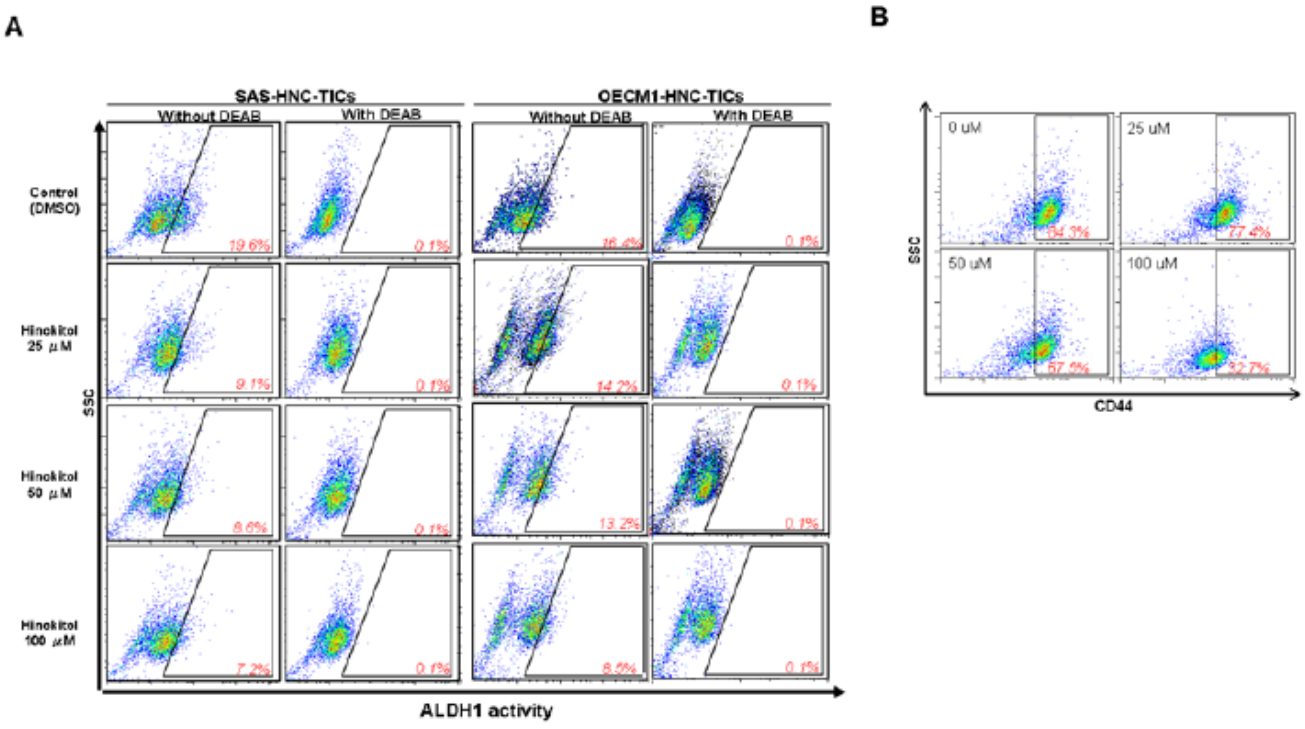


Figure 2: The ALDH1 activity (A) and CD44 positivity (B) of OC-CSCs treated with or without hinokitiol was assessed by flow cytometry

3. Hinokitiol decreased cancer stemness marker expression n OC-CSCs

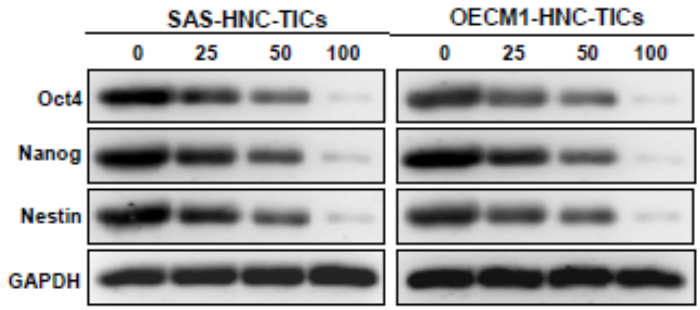


Figure 3: The indicated stemness markers expression levels (Oct4, Nanog, and Nestin) in the hinokitiol -treated ALDH^{+}CD44^{+} OC-CSCs were analyzed by western blotting. Anti-invasive activity of hinokitiol in OC-CSCs

4. Hinokitiol abrogated invasiveness in OC-CSCs

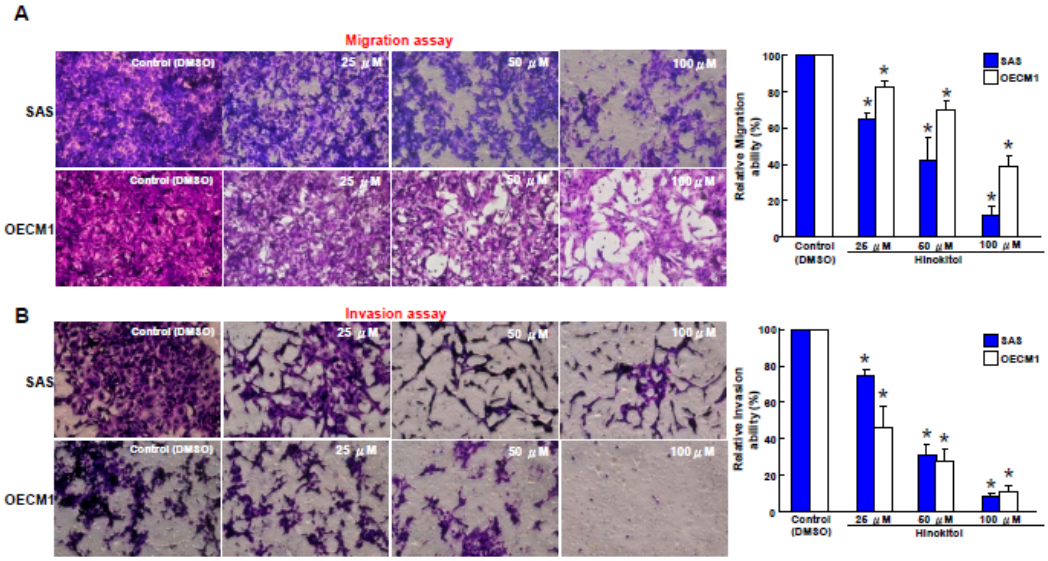


Figure 4: Different concentration hinokitiol-treated OC-CSCs were subjected to (A) migration assay and (B) matrix invasion assay

5. Hinokitiol abrogated clonogenicity in OC-CSCs

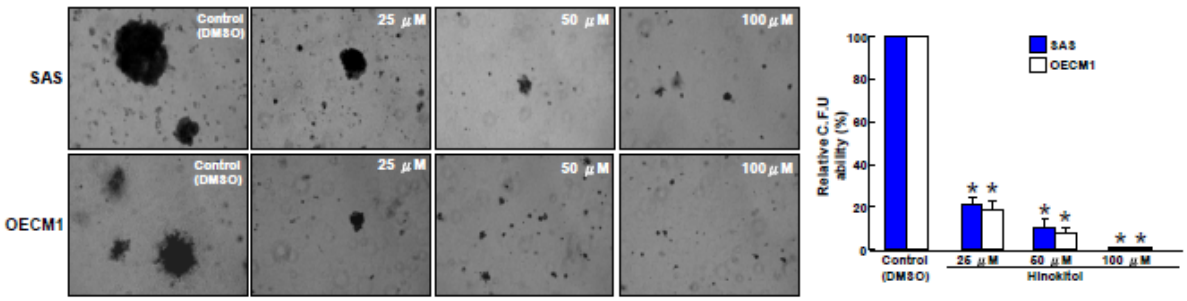


Figure 5: Different concentration hinokitiol-treated OC-CSCs were subjected to soft agar colony formation assay.

6. Hinokitiol induced miR-513a-5p expression

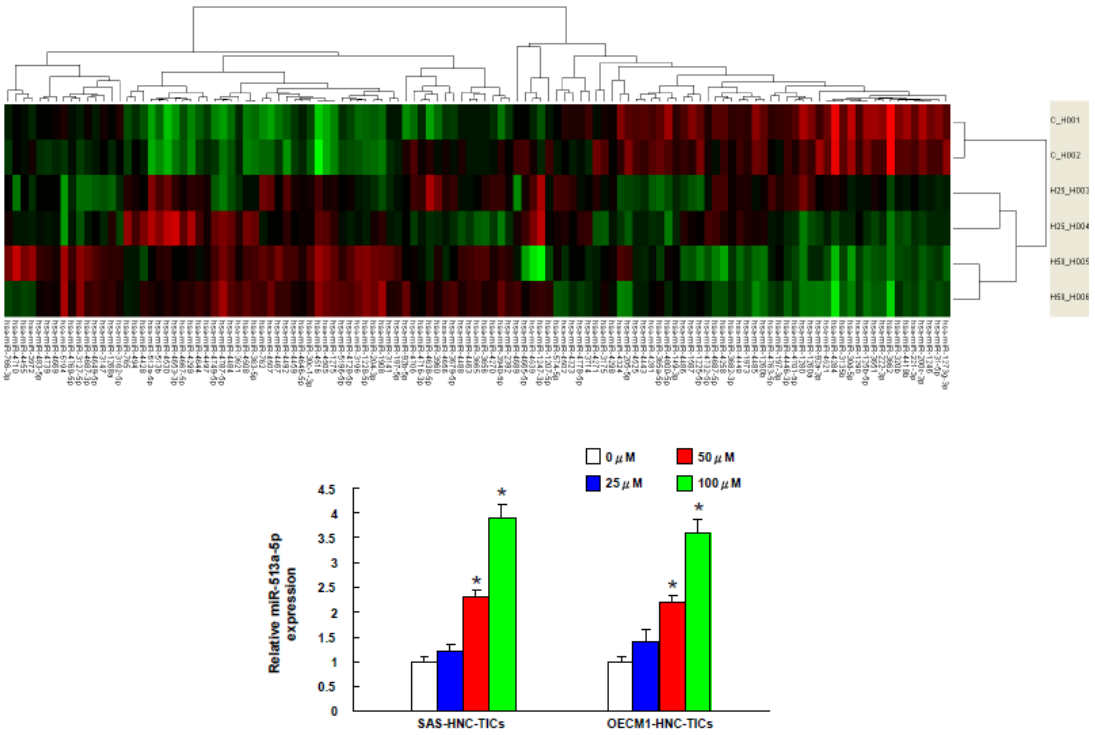


Figure 6: (A) The indicated miRNAs expression levels in the hinokitiol-treated OC-CSCs were analyzed by miRNAs microarray analysis. (B) qPCR analysis was applied to analyzed the relative miR-513a-5p expression level in hinokitiol dose-dependently treated OC-CSCs .

7. Oral-feeding Hinokitiol impaired tumor growth of OC-CSCs tumor-bearing mice

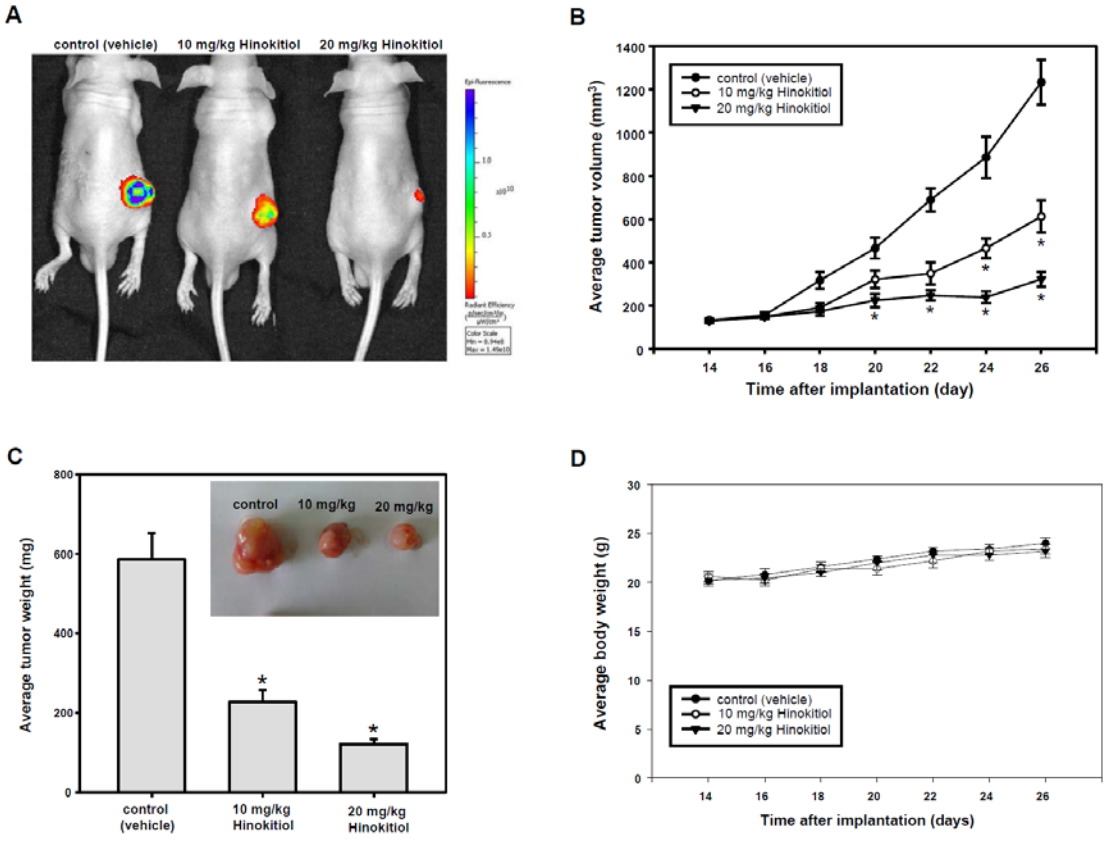
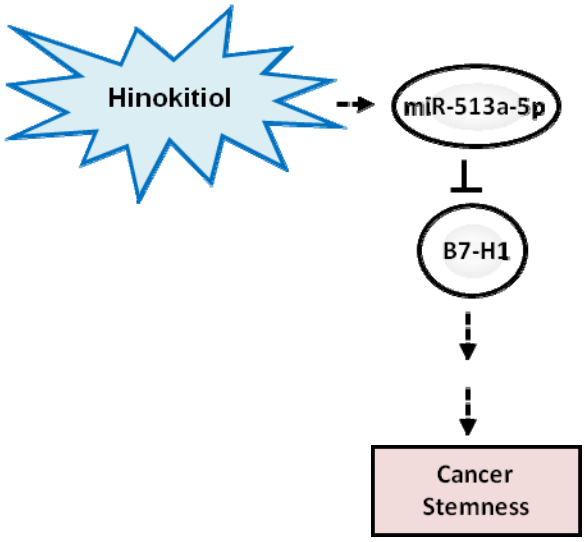


Figure 7: After subcutaneous implantation of OCSCs, BALB/c nude mice (N = 6 for each group) were oral-feeding treated with saline or hinokitiol and then analyzed for the bioluminescence signal (A), growth of

tumor (B), average tumor weight (C) , and average mice body weight (D).

8. A schematic representation of the hinokitiol-activated miR-513a-5p-targeting B7-H1 signaling proposed in the current study.



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2. Shih YH, Chang KW[#], Yu CC[#], Kao MC, Chen MY, Wang TH, Chi TY, Chen YL, Shieh TM* (2015). Hinokitiol suppressed pan-histone expression and cell growth in oral squamous cell carcinoma cells. J Funct Foods 15:452-463. (SCI, [#] Equal Contribution)

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科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/31

科技部補助計畫	計畫名稱: 檜木醇透過調控微核醣核酸miR-513a-5p標靶癌幹細胞及上皮-間質相互轉換特性作為口腔癌化學輔助性療法之研究
	計畫主持人: 余承佳
	計畫編號: 102-2628-B-040-001-MY3　　學門領域: 中醫藥

無研發成果推廣資料

102年度專題研究計畫成果彙整表

計畫主持人：余承佳	**計畫編號：**102-2628-B-040-001-MY3
計畫名稱：檜木醇透過調控微核醣核酸miR-513a-5p標靶癌幹細胞及上皮-間質相互轉換特性作為口腔癌化學輔助性療法之研究	

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	1	件	用於抑制組蛋白基因轉錄及表現之醫藥組合物及其應用，發明專利，台灣，I434681
				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			2	篇	部分結果已發表於Oral Oncol. 2015 51:31-9.及J Funct Foods. 2015 15:452-463.
		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	1		
		專任助理	1		
	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。）			檜木醇調控miR-513a-5p標靶癌幹細胞之研究成果整理及文章撰寫中, 預計可投稿至績優國際學術期刊		

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）

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）
   本研究可應用檜木醇調控微型RNA來開發標靶癌幹細胞藥物，亦可搭配miR-513a-5p作為口腔癌診治生物標記，發展個人化醫療，冀望用於此本土重大疾病之防治。

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）