行政院國家科學委員會專題研究計畫 期末報告

Lipocalin-2 在口腔癌致癌過程的表現及其調控缺氧相關因子的機制探討

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 101-2314-B-040-013-
執 行 期 間 ：101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ：中山醫學大學醫學研究所

計 畫 主 持 人：楊順發
共 同 主 持 人：張育超
計畫參與人員：博士班研究生-兼任助理人員：楊嘉欣

報 告 附 件 ：出席國際會議研究心得報告及發表論文

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

中 華 民 國 102 年 10 月 23 日

中 文 摘 要 ： Lipocalin-2(LCN2)是Lipocalin蛋白家族的一分子。LCN2除了在人類各種發炎反應會上升，近年來也發現其與腫瘤生成有關。但是LCN2與口腔癌的相關性仍不清楚。我們先前發表的文獻發現OSCC病人血漿中LCN2、MMP-9與LCN2/MMP-9複合物的濃度明顯高於健康對照組。並且LCN2的表現量與腫瘤大小、TNM分期有關。因此，我們進一步想釐清LCN2在口腔癌細胞株中的分子機轉。在篩選了穩定表達LCN2的SCC9口腔癌細胞株發現其細胞移動能力降低，也發現到缺氧相關因子，包括缺氧誘導因子1α (Hypoxia-inducible factor 1 alpha；HIF-1α）與碳酸酐酶IX (carbonic anhydrase IX； CAIX)，無論是在mRNA與蛋白表現皆下降，且細胞的移動能力降低是受到CAIX的影響。因此我們推測在OSCC病人血漿中LCN2的表現可以當作一項非侵入性指標來預測OSCC病人的病程，並且LCN2在口腔癌細胞株中是透過調控缺氧相關因子的表現量使得細胞移動能力降低。

中文關鍵詞： 口腔鱗狀細胞癌、缺氧誘導因子、碳酸酐酶、腫瘤轉移

英 文 摘 要 ： Objectives: Lipocalin 2 is a secreted, iron-binding glycoprotein that is abnormally expressed in some malignant human cancers. However, the roles of Lipocalin 2 in oral cancer cells are still unknown.
Methods: Expression of Lipocalin 2 protein and mRNA in human oral cancer cells were determined by western blotting and qRT-PCR. Cell viability and migration ability were determined by MTT and boyden chamber assay.
Results: Functional analyses revealed that the stable overexpression of Lipocalin 2 reduced the migration ability in SCC-9 cells. Moreover, Lipocalin 2 overexpression inhibits focal adhesion kinase (FAK) phosphorylation and downregulation of Src, JNK activity. Furthermore, Lipocalin 2 overexpression potently decreased HIF-1α and CAIX synthesis.
Conclusions: We showed that Lipocalin 2 may function as a putative metastasis suppressor in oral cancer cells.

英文關鍵詞： Lipocalin 2； oral cancer； migration； focal adhesion kinase

Lipocalin 2 reduces cell migration by down-regulating carbonic anhydrase IX in oral cancer cells

Shun-Fa Yang[1,2], Chiao-Wen Lin[3,4],

[1]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[2]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
[3]Institute of Oral Sciences, Chung Shan Medical University, Taichung, Taiwan
[4]Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan

*Corresponding authors:

Shun-Fa Yang, PhD.
Institute of Medicine, Chung Shan Medical University, 110, Section 1, Chien-Kuo N. Road, Taichung, Taiwan, ROC
Tel: +886-4-24739595#34253;
Fax +886-4-24723229;
E-mail: ysf@csmu.edu.tw

Abstract

Objectives: Lipocalin 2 is a secreted, iron-binding glycoprotein that is abnormally expressed in some malignant human cancers. However, the roles of Lipocalin 2 in oral cancer cells are still unknown.

Methods: Expression of Lipocalin 2 protein and mRNA in human oral cancer cells were determined by western blotting and qRT-PCR. Cell viability and migration ability were determined by MTT and boyden chamber assay.

Results: Functional analyses revealed that the stable overexpression of Lipocalin 2 reduced the migration ability in SCC-9 cells. Moreover, Lipocalin 2 overexpression inhibits focal adhesion kinase (FAK) phosphorylation and downregulation of Src, JNK activity. Furthermore, Lipocalin 2 overexpression potently decreased HIF-1α and CAIX synthesis.

Conclusions: We showed that Lipocalin 2 may function as a putative metastasis suppressor in oral cancer cells.

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer, with poor prognosis due to frequent lymph node metastasis and local invasion [1]. Cancer invasion and metastasis, the spread of cancer cells from the primary neoplasm to distant sites and their growth there, are the major causes of death in various cancer patients including OSCC [2]. Metastasis of cancer cells involves multiple processes and various cytophysiological changes, including changing adhesion capabilities between cells and the extracellular matrix (ECM) and damaging intercellular interaction. Thus, degradation of the ECM and components of the basement membrane caused by a concerted action of proteinases, such as matrix metalloproteinases (MMPs), cathepsins, and plasminogen activator (PA), play a critical role in tumor invasion and metastasis [3, 4]. MMPs are overexpressed in almost all human cancers [5, 6] including OSCC [7]. Of the MMPs, MMP-2, MMP-9 and their upstream enzyme, urokinase-PA (u-PA), are the most vital enzymes for the degradation of the main constituent of the basement membrane, type IV collagen [8-10], and deeply involved in cancer invasion and metastasis. The inhibition of migration or invasion mediated by MMP-2, -9 or u-PA could, therefore, putatively provide a preventive measure against cancer metastasis [8].

Lipocalin (LCN)2, also known as neutrophil gelatinase-associated lipocalin, neu-related lipocalin, oncogene 24p3, and uterocalin, is a 25-kDa protein which is stored in the granules of human neutrophils [11]. It belongs to the lipocalin family, which comprises more than 50 members, all of which are characterized by the ability to bind and transport small lipophilic substances [12]

LCN2 participates in iron trafficking [13] and increases cytoplasmic levels of this mineral by capturing and transporting iron particles to cell interiors after interacting with specific membrane receptors (24p3 and megalin) [14]. Its role in iron delivery to cells underlies the multiple effects attributed to LCN2. Released by activated neutrophils, this protein participates in an iron-depletion strategy exploited by the immune defense against bacterial pathogens [15]. In addition, LCN2 seems to be involved in the growth, development, and differentiation of several human tissues, as early as in the embryo, through its regulation of iron-responsive genes which are important in the differentiation of primordial cells [16, 17]. Finally, LCN2 seems to participate in carcinogenesis by favoring iron uptake from extracellular spaces within malignant cells, a fundamental process for maintaining neoplastic cell multiplication [18]. In line with its involvement in carcinogenesis processes, LCN2 synthesis is induced by factors promoting the development of neoplasias [12, 19] and its overexpression was found in several malignancies including breast, gastric,

esophageal squamous cell, colorectal, pancreatic, lung, and ovarian cancers [19-27]. Regarding oral squamous cell carcinoma (OSCC), Hiromoto and coworkers recently investigated the expression levels of LCN2 in oral cancer tissues, found that LCN2 expression is high in well-differentiated cancer, and suggested that LCN2 might be a useful diagnostic marker of tumor-cell differentiation in OSCC [28]. To date, most studies focused on LCN2 and LCN2/MMP-9 tissue expressions, while only a few investigated the clinical utility of their urinary levels. The purposes of our study were to investigate the association of LCN2 and its probable one of downstream genes, carbonic anhydrase IX, and their correlations with metastatic phenotypes such as cells migration and signal transduction pathway in OSCC cells.

2. Materials and Methods

2.1. Materials.

Cell culture materials and fetal bovine serum (FBS) were obtained from Gibco-BRL (Gaithersburg, MD). An enhanced chemiluminescence kit was purchased from Amersham (Arlington Heights, IL). Antibodies specific for CA IX, HIF-1α, lipocalin 2 and β-actin were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Unless otherwise specified, other chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Cell culture

SCC-9, a human tongue squamous cell carcinoma cell line obtained from ATCC (Manassas, VA, USA), was cultured in Dulbecco's modified Eagle's medium supplemented with a nutrient mixture, F-12 Ham's medium (Life Technologies, Grand Island, NY, USA), 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and and 25 mM HEPES (pH 7.4) in a humidified 37 ℃ incubator.

2.3. Cell migration assays

Cell migration was assayed according to the methods described by Yang et al. SCC-9 cell and stable lipocalin 2 overexpression SCC-9 cancer cell lines were harvested and seeded in a Boyden chamber (Neuro Probe, Cabin John, MD, USA) at a density of 10^4 cells/well in serum free medium, and then incubated for 24 h. The invaded cells were fixed and stained with 5 % Giemsa. Cell numbers were counted under a light microscope.

2.4. RNA preparation and TaqMan quantitative real-time PCR

Total RNA was isolated from oral cancer cells using Trizol (Life Technologies,

Grand Island, NY) according to the manufacturer's instructions. Quantitative real-time PCR analysis was carried out using Taqman one-step PCR Master Mix (Applied Biosystems). 100 ng of total cDNA was added per 25 μl reactions with lipocalin 2, carbonic anhydrase IX or GAPDH primers and Taqman probes. The lipocalin 2, carbonic anhydrase IX and GAPDH primers and probes were designed using commercial software (ABI PRISM Sequence Detection System; Applied Biosystems). Quantitative real-time PCR assays were carried out in triplicate on a StepOnePlus sequence detection system. The threshold was set above the non-template control background and within the linear phase of target gene amplification to calculate the cycle number at which the transcript was detected.

2.5. Western blot analysis

The total cell lysates were prepared as previously described. Western blot analysis was performed using primary antibodies against CA IX, HIF-1α, lipocalin 2 and β-actin. The relative photographic densities were quantitated by scanning the photographic negatives using a gel documentation and analysis system (AlphaImager 2000, Alpha Innotech Corporation, San Leandro, CA, USA).

2.6. Transfection and carbonic angydrase IX promoter-driven luciferase assays

SCC-9 cells were seeded at a concentration of 5 x10^4 cells per well in 6-well cell culture plates. After 24 h of incubation, pGL3-basic (vector) and pCA IX-luciferase (Luc) were co-transfected with a β-galactosidase expression vector (pCH110) into cells using Turbofect (Fermentas, Carlsbad, CA). The cell lysates were harvested, and luciferase activity was determined using a luciferase assay kit. The value of the luciferase activity was normalized to transfection efficiency and monitored by β-galactosidase expression.

2.8. Statistical analysis

Statistical significances of differences throughout this study were analyzed by One-way ANOVA test to compare differences between treatments and followed up using Dunnett's multiple comparison post-hoc test. A p value < 0.05 was considered to be statistically significant. Values represent the means ± standard deviation and the experiments were repeated three times.

3. Results

3.1. Relationships among cells migration and invasion and lipocalin 2

We transfect lipocalin 2 gene into SCC-9 cancer cells to establish lipocalin 2 overexpressing cancer cell lines L1 and L7. Stable lipocalin 2 overexpression SCC-9 cancer cell lines (L1 and L7 cancer cells) increased lipocalin 2 protein levels and

mRNA expression as compared to their negative control cell line pcDNA3.1 (P3 cancer cells) (Figure 1A and 1B). Using a cell migration assay with a Boyden chamber, we showed that stable lipocalin 2 overexpression SCC-9 cancer cell lines reduced the migration ability while compared to SCC-9 cancer cells (Figure 2).

3.2. Relationships between carbonic anhydrase IX as well as HIF-1α and lipocalin 2

Figure 3A shows a western blotting analysis of the protein levels of carbonic anhydrase IX and HIF-1α protein levels were adjusted with β-actin. Protein levels of carbonic anhydrase IX and HIF-1α decreased significantly by stable lipocalin 2 overexpression manners. Testing of mRNA, quantitative real-time PCR, and promoter reporter assays evaluated the inhibitory effects of stable lipocalin 2 overexpression on carbonic anhydrase IX expression in SCC-9 cells. The results shown that the mRNA and report activity of carbonic anhydrase IX was decreased ($p < 0.01$ and $p < 0.01$, respectively; Figure 3B and 3C), as compared to their negative control cell line pcDNA3.1 (P3 cancer cells). Furthermore, carbonic anhdrase IX overexpressing cancer cells increased their migration while compared to lipocalin 2 overexpression SCC-9 cancer cells (Figure 4).

3.3. Effect of MAPK and PI3K/Akt pathways by overexpression of lipocalin 2

Because the inhibitory effect of lipocalin 2 overexpression on the cell migration was revealed, the effects of lipocalin 2 overexpression on the expressions of MAPK and PI3K/Akt pathways have been investigated by western blotting to clarify their underlying mechanisms. Western blotting showed that lipocalin 2 overexpression could reduce the phosphorylation of focal adhesion kinase (FAK) and downregulation of Src, JNK activity in SCC-9 cells. (Figure 5)

References:

1. Spiro RH, Alfonso AE, Farr HW and Strong EW (1974). Cervical node metastasis from epidermoid carcinoma of the oral cavity and oropharynx. A critical assessment of current staging. *Am J Surg* **128**: 562-7.
2. Weiss L. (1990) Metastatic inefficiency. *Adv Cancer Res* **54**: 159-211.
3. Westermarck J and Kahari VM (1999). Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* **13**: 781-92.
4. Yoon SO, Park SJ, Yun CH and Chung AS (2003). Roles of matrix metalloproteinases in tumor metastasis and angiogenesis. *J Biochem Mol Biol* **36**: 128-37.
5. Cakarovski K, Leung JY, Restall C, Carin-Carlson A, Yang E, Perlmutter P, Anderson R, Medcalf R and Dear AE (2004). Novel inhibitors of urokinase-type plasminogen activator and matrix metalloproteinase expression in metastatic cancer cell lines. *Int J Cancer* **110** : 610-6.
6. Toda D, Ota T, Tsukuda K, Watanabe K, Fujiyama T, Murakami M, Naito M and Shimizu N (2006). Gefitinib decreases the synthesis of matrix metalloproteinase and the adhesion to extracellular matrix proteins of colon cancer cells. *Anticancer Res* **26** : 129-34.
7. Patel BP, Shah SV, Shukla SN, Shah PM and Patel PS (2007). Clinical

significance of MMP-2 and MMP-9 in patients with oral cancer. *Head Neck* 29, (6), 564-72.

8. Bjorklund M and Koivunen E (2005). Gelatinase-mediated migration and invasion of cancer cells. *Biochim Biophys Acta* **1755**: 37-69.
9. Nelson AR, Fingleton B, Rothenberg ML and Matrisian LM (2000). Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol* **18**: 1135-49.
10. Mackay AR, Corbitt RH, Hartzler JL and Thorgeirsson UP (1990). Basement membrane type IV collagen degradation: evidence for the involvement of a proteolytic cascade independent of metalloproteinases. *Cancer Res* **50**: 5997-6001.
11. Cowland JB and Borregaard N (1997). Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. *Genomics* **45**: 17-23.
12. Bratt T (2000). Lipocalins and cancer. *Biochim Biophys Acta* **1482**: 318-26.
13. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, Erdjument-Bromage H, Tempst P, Strong R and Barasch J (2002). An iron delivery pathway mediated by a lipocalin. *Mol Cell* **10**: 1045-1056.
14. Goetz DH, Willie ST, Armen RS, Bratt T, Borregaard N and Strong RK (2000). Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. *Biochemistry* **39**: 1935-1941.
15. Saiga H, Nishimura J, Kuwata H, Okuyama M, Matsumoto S, Sato S, Matsumoto

M, Akira S, Yoshikai Y, Honda K, Yamamoto M and Takeda K (2008). Lipocalin 2-dependent inhibition of mycobacterial growth in alveolar epithelium. *J Immunol* **181**: 8521-8527.

16. Gwira JA, Wei F, Ishibe S, Ueland JM, Barasch J and Cantley LG (2005). Expression of neutrophil gelatinase-associated lipocalin regulates epithelial morphogenesis in vitro. *J Biol Chem* **280**: 7875-7882.
17. Mori K, Yang J and Barasch J (2003). Ureteric bud controls multiple steps in the conversion of mesenchyme to epithelia. *Semin Cell Dev Biol* **14**: 209-216.
18. Devireddy LR, Gazin C, Zhu X and Green MR (2005). A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. *Cell* **123**: 1293-1305.
19. Stoesz SP, Friedl A, Haag JD, Lindstrom MJ, Clark GM and Gould MN (1998). Heterogeneous expression of the lipocalin NGAL in primary breast cancers. *Int J Cancer* **79**: 565-572.
20. Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N and Friedl A (2008). Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. *Breast Cancer Res Treat* **108**: 389-397.
21. Kubben FJ, Sier CF, Hawinkels LJ, Tschesche H, van Duijn W, Zuidwijk K, van der Reijden JJ, Hanemaaijer R, Griffioen G, Lamers CB and Verspaget HW (2007). Clinical evidence for a protective role of lipocalin-2 against MMP-9 autodegradation and the impact for gastric cancer. *Eur J Cancer* **43**: 1869-1876.
22. Zhang H, Xu L, Xiao D, Xie J, Zeng H, Wang Z, Zhang X, Niu Y, Shen Z, Shen J, Wu X and Li E (2007). Upregulation of neutrophil gelatinase-associated lipocalin in oesophageal squamous cell carcinoma: significant correlation with cell differentiation and tumour invasion. *J Clin Pathol* **60**: 555-561.
23. Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M and Kjeldsen L

(1996). Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. *Gut* **38**: 414-420.

24. Furutani M, Arii S, Mizumoto M, Kato M and Imamura M (1998). Identification of a neutrophil gelatinase-associated lipocalin mRNA in human pancreatic cancers using a modified signal sequence trap method. *Cancer Lett* **122**: 209-214.
25. Laurell H, Bouisson M, Berthelemy P, Rochaix P, Dejean S, Besse P, Susini C, Pradayrol L, Vaysse N and Buscail L (2006). Identification of biomarkers of human pancreatic adenocarcinomas by expression profiling and validation with gene expression analysis in endoscopic ultrasound-guided fine needle aspiration samples. *World J Gastroenterol* **12**: 3344-3351.
26. Friedl A, Stoesz SP, Buckley P and Gould MN (1999). Neutrophil gelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression. *Histochem J* **31**: 433-441.
27. Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, Quinn MA and Rice GE (2007). Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. *Int J Cancer* **120**: 2426-2434.
28. Hiromoto T, Noguchi K, Yamamura M, Zushi Y, Segawa E, Takaoka K, Moridera K, Kishimoto H and Urade M (2011). Up-regulation of neutrophil gelatinase-associated lipocalin in oral squamous cell carcinoma: relation to cell differentiation. *Oncol Rep* **26**: 1415-1421.

Figure 1A

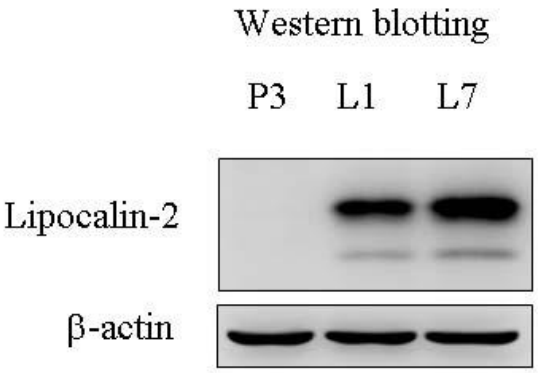


Figure 1B

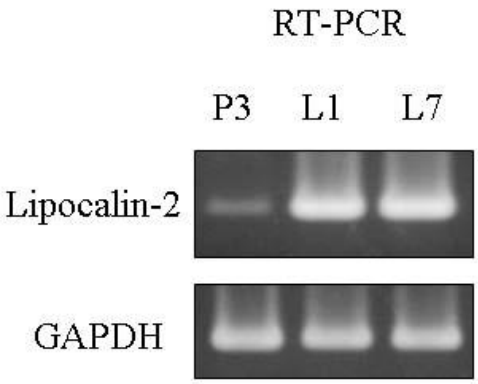


Figure 2

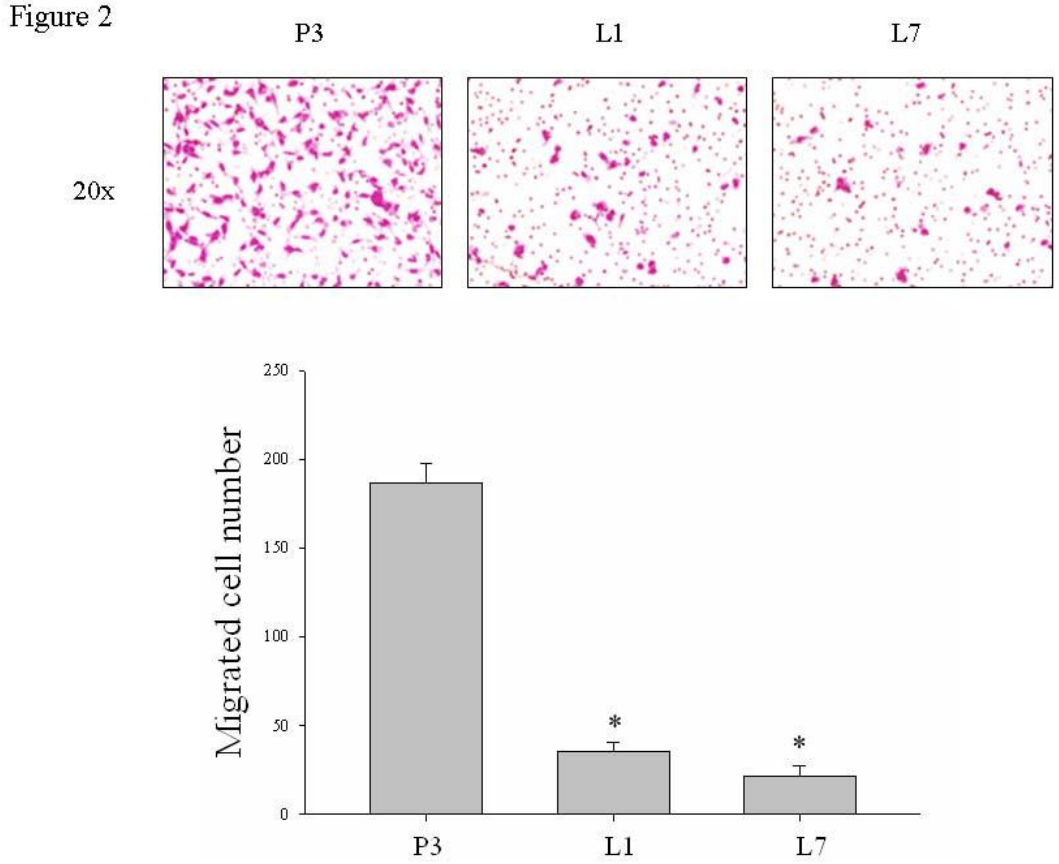


Figure 3A

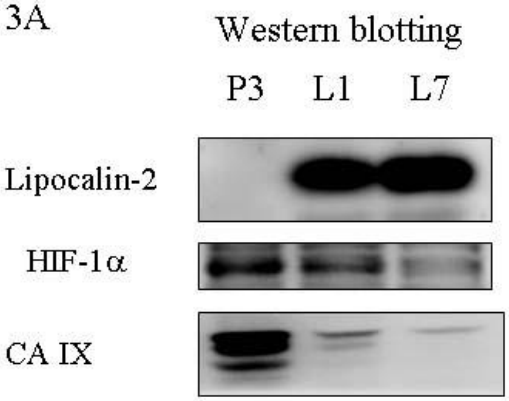


Figure 3B

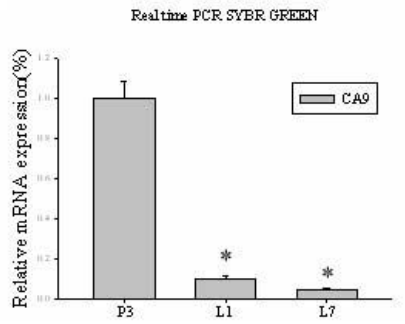


Figure 3C

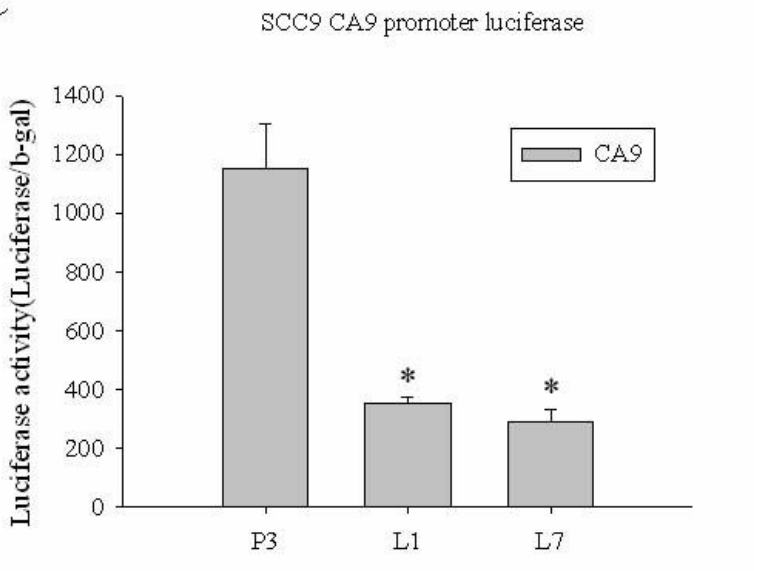


Figure 4

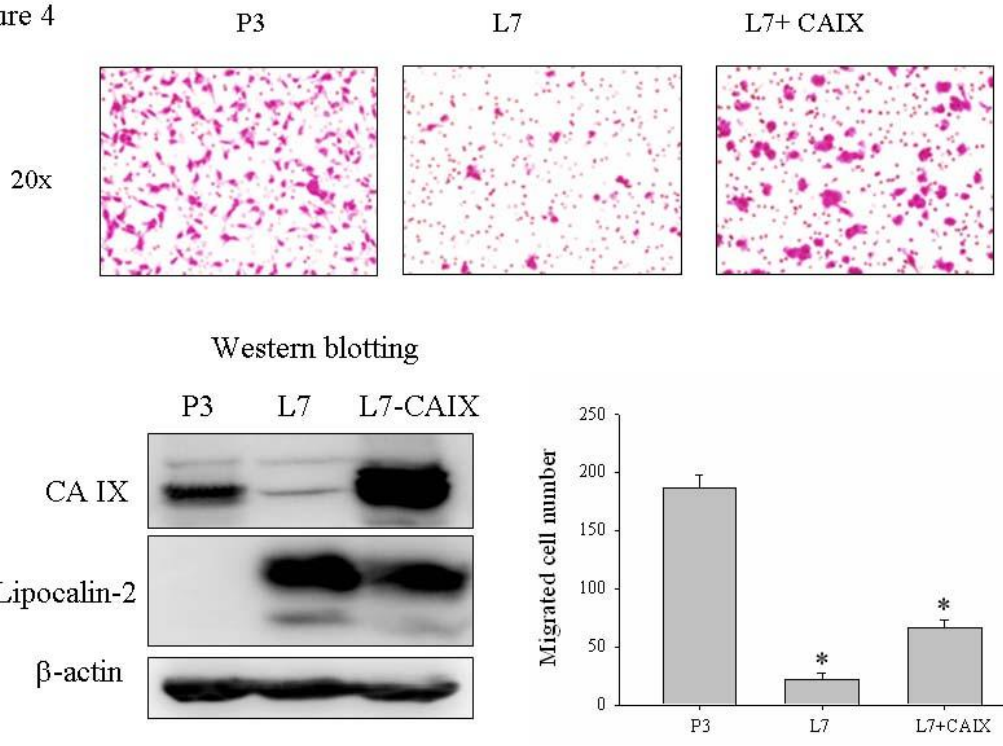
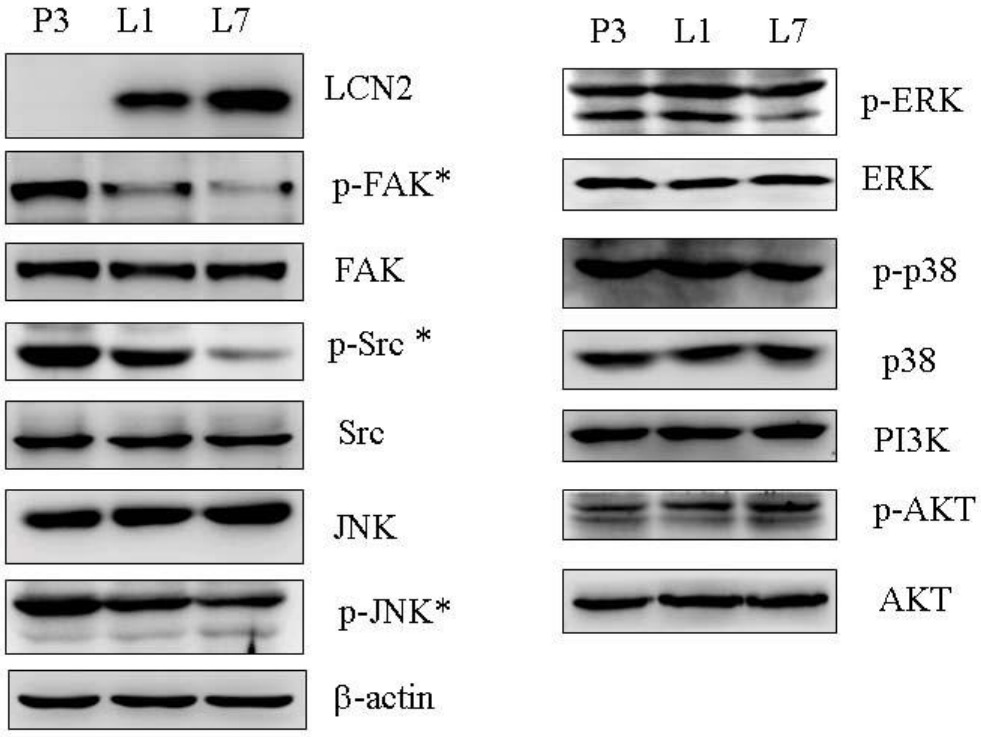

P3
L7
L7+ CAIX
20x
Western blotting
P3
L7
L7-CAIX
CA IX
Lipocalin-2
β-actin
Migrated cell number
250
200
150
100
50
0
*
*
P3
L7
L7+CAIX

Figure 5


P3
L1
L7
LCN2
p-FAK*
FAK
p-Src *
Src
JNK
p-JNK*
β-actin
P3
L1
L7
p-ERK
ERK
p-p38
p38
PI3K
p-AKT
AKT

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

102 年 10 月 23 日

報告人姓名	楊順發	服務機構及職稱	中山醫學大學 醫學研究所 教授
時間 會議 地點	2013/03/20~2013/03/23 美國西雅圖(Seattle, Washington)	本會核定 補助文號	
會議名稱	（中文）第 91 屆國際聯合牙醫研究會議 （英文）91 th General Session & Exhibition of the International Association for Dental Research		
發表論文題目	（中文）CAIX 在嚼食檳榔所引起的口腔癌的探討 （英文）Elevation of CAIX in oral cancer patients with areca-quid chewing		

報告內容應包括下列各項：

一、參加會議經過

3/20 抵達西雅圖開會地點，3/21 早上前往會議中心報到及領取大會議程及摘要手冊，並張貼研究成果海報，之後參觀其他研討成果展覽以及聆聽多場會議報告。下午則在成果海報處講解研究內容。3/22 再度前往會場與其他參加者交流研究報告。

二、與會心得

本人的研究主題主要是 CAIX 在嚼食檳榔所引起的口腔癌的相關性探討，而本次大會的主題除了涵蓋人類口腔癌的基礎研究之外，還加入臨床治療及個案探討，因此藉由此次會議讓我有機會接觸到更實際的臨床領域，獲得不少新觀念及之前未曾有過的一些想法。會中聆聽許多大師級的演講，受益良多。與其他相關研究人員的諸多討論，也獲得很多寶貴的意見及肯定。此類與國外研究人員的溝通及聯繫是很重要的，讓我有機會與國外實驗室有初步之合作構想，並已有初步之計畫，希望能藉此有國際合作的機會。

三、建議

國內應多加舉辦如此大型會議、增加補助出國額度、或盡量補助博士班學生出國開會或短期研究之經費，讓年輕研究學者有機會與大師級學者學習。

四、攜回資料名稱及內容

會議議程手冊
會議摘要手冊

國科會補助計畫衍生研發成果推廣資料表

日期:2013/10/23

國科會補助計畫	計畫名稱: Lipocalin-2在口腔癌致癌過程的表現及其調控缺氧相關因子的機制探討
	計畫主持人: 楊順發
	計畫編號: 101-2314-B-040-013- 學門領域: 牙醫學

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：楊順發	計畫編號：101-2314-B-040-013-
計畫名稱：Lipocalin-2 在口腔癌致癌過程的表現及其調控缺氧相關因子的機制探討	

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	0	0	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	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	1	1	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。)	無

	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	

國科會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)

Lipocalin-2(LCN2)是 Lipocalin 蛋白家族的一分子。LCN2 除了在人類各種發炎反應會上升，近年來也發現其與腫瘤生成有關。但是 LCN2 與口腔癌的相關性仍不清楚。我們先前發表的文獻發現 OSCC 病人血漿中 LCN2、MMP-9 與 LCN2/MMP-9 複合物的濃度明顯高於健康對照組。並且 LCN2 的表現量與腫瘤大小、TNM 分期有關。因此，我們進一步想釐清 LCN2 在口腔癌細胞株中的分子機轉。在篩選了穩定表達 LCN2 的 SCC9 口腔癌細胞株發現其細胞移動能力降低，也發現到缺氧相關因子，包括缺氧誘導因子 1α (Hypoxia-inducible factor 1 alpha；HIF-1α）與碳酸酐酶 IX (carbonic anhydrase IX； CAIX)，無論是在 mRNA 與蛋白表現皆下降，且細胞的移動能力降低是受到 CAIX 的影響。因此我們推測在 OSCC 病人血漿中 LCN2 的表現可以當作一項非侵入性指標來預測 OSCC 病人的病程，並且 LCN2 在口腔癌細胞株中是透過調控缺氧相關因子的表現量使得細胞移動能力降低。