科技部補助專題研究計畫成果報告
期末報告

細胞質中碳酸酐異構酶在口腔癌致癌過程的表現及其調控轉移相關因子的機制探討(第3年)

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中 文 摘 要 ： 上皮細胞間質化 (epithelial-mesenchymal transition, EMT) 是一種上皮細胞轉化成間質細胞的過程，是癌症轉移的主要機制之一。 碳酸酐異構酶 (Carbonic anhydrase; CA) 是一種存在於細胞質中的催化酵素，主要的功能是參與催化可逆性水解反應。近年有多篇文獻證實 Cytosolic CA 中的 CAIII 參與蛋白質的硫化作用同時提供細胞抗氧化的角色，而這些抗氧化的能力可能對腫瘤轉移與侵襲相關。然而對 CAIII 在口腔癌的發展與轉移的過程中許多機制仍不清楚，本研究試探討 CAIII 對口腔癌轉移與 EMT 之間的關係並其詳細機制為何。本研究建立兩株能夠穩定大量表現 CAIII 蛋白的口腔癌細胞株 SCC-9 與 SAS 細胞株，並利用此細胞株做後續的研究探討。首先透過 MTT 及細胞計數實驗發現在大量表現 CAIII 的環境下對細胞生長率存活率沒有顯著的影響。再利用 invasion / migration assay 實驗發現大量表現CAIII對細胞移動、侵襲、貼附和轉移能力的影響都有明顯促進的情形。我們進一步利用mRNA array 方式發現上皮細胞間質化的marker有明顯的變化，同時利用 real time PCR及西方墨點法技術也確認在大量表現CAIII時其EMT相關的 e-cadherin 蛋白表現下降， vimentin 以及 fibronectin 蛋白表現量上升以及轉錄因子 twist 、 snail 及 slug 表現量增加。另外也發現FAK及Src 路徑有明顯地上升。透過以上實驗推論由 CAIII 所造成細胞侵襲與轉移可能是透過影響 FAK/Src 路徑進而造成EMT 引起，此發現未來可當作口腔癌治療轉移的輔助療法。

中 文 關 鍵 詞 ： 上皮細胞間質化、碳酸酐異構酶 、口腔癌、轉移

英 文 摘 要 ： Epithelial-mesenchymal transition (EMT) is highly related to tumor metastasis and it contains several protein makers such as E-cadherin. Carbonic anhydrase III (CA III) is a cytoplasmic enzyme that exhibits a low carbon dioxide hydratase activity and a predominant expression in the cancer. However, the roles of CA III detailed mechanisms in oral cancer are still unknown. This study established a CA III overexpressed stable clone and observe the expression of CA III protein and mRNA in human oral cancer cells SCC-9 and SAS, also migration and invasion abilities were determined by boyden chamber assay. We found that CAIII protein significantly increase the migration and invasion abilities in oral cancer cells and result in activate FAK / Src signaling pathway to down regulate E-cadherin expression but induce vimentin and fibronectin expression. That suggest that CA III may induce migration and invasion in oral cancer cells through activate EMT and are potential related to tumor metastasis in oral cancer.

英 文 關 鍵 詞 ： Epithelial-mesenchymal transition, Carbonic anhydrase, oral cancer, metastasis

Carbonic anhydrase III promotes epithelial-mesenchymal transition associated with tumor invasion and migration by regulate E-cadherin in oral cancer

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Abstract

Epithelial-mesenchymal transition (EMT) is highly related to tumor metastasis and it contains several protein makers such as E-cadherin. Carbonic anhydrase III (CA III) is a cytoplasmic enzyme that exhibits a low carbon dioxide hydratase activity and a predominant expression in the cancer. However, the roles of CA III detailed mechanisms in oral cancer are still unknown. This study established a CA III overexpressed stable clone and observe the expression of CA III protein and mRNA in human oral cancer cells SCC-9 and SAS, also migration and invasion abilities were determined by boyden chamber assay. We found that CAIII protein significantly increase the migration and invasion abilities in oral cancer cells and result in activate FAK / Src signaling pathway to down regulate E-cadherin expression but induce vimentin and fibronectin expression. That suggest that CA III may induce migration and invasion in oral cancer cells through activate EMT and are potential related to tumor metastasis in oral cancer.

Keywords: Carbonic anhydrase III, metastasis, invasion, migration, epithelial-mesenchymal transition, E-cadherin, vimentin.

Introduction

Head and neck squamous cell carcinoma (HNSCC) has developed to a common human cancer in the past few years and more than 90% of cases occurs involve oral squamous cell carcinoma [1, 2]. Tumor metastasis has known as serious difficulties in the clinical treatments in vary cancers [3-5]. Epithelial-mesenchymal transition (EMT) is a process that turned epithelial cells to mesenchymal cells. Several characteristics change were company with EMT as the shape from polygonal to spindle shape, apico-basolateral polarization turns to anterior-posterior polarization, strong cell to cell adhesion comes to focal cell to cell contact and also raise the cell migration potential [6-8]. EMT process contains several proteins expressions include epithelial markers E-cadherin, claudin, occludin and cytokeratins; mesenchymal makers N-cadherin, vimentin, fibronectin and smoth muscle actin [9, 10]. Previous studies showed that E-cadherin expression were connected to invasion and metastasis in head and neck squamous cell carcinoma [11]. And loss of E-cadherin also associated to the EMT process which occurs tumor metastasis [12].

Carbonic anhydrase (CA) is a family of metalloenzymes, the active site contains a zinc ion [13]. The main function of CA is to catalyze carbon dioxide to bicarbonate ($CO_2 + H_2O \leftrightarrows HCO3^- + H^+$), a reversible hydrolysis reaction. CA take participate in carbon dioxide transport, calcification and photosynthesis. In mammals physiology function, CA regulates ion transport, pH value and water homeostasis. And also take parts in synthesize glycogen, urea and lipid in metabolism [14-17].

CA III is located on chromosome 8q22, include two isoforms: CA III A and CA III B and has a strong ability in hydrolyze carbon dioxide [18]. CA III expression obviously in skeleton muscle which help carbonic dioxide could extend to tissue capillaries. Include spleen, kidney, lung and heart have high level of CA III expression

[19].

CA inhibitors as sulphonamide, include: acetazomide、methazolamide、ethoxzolamide、dichlorophenamide、dorzolamide、brinzolamide, the main functions are inhibit active site activity. Clinical commonly used to treat glaucoma, epilepsy, mountain sickness and duodenal ulcers [20]. In recent years, CA inhibitor has connected to laboratory cancer researches. Teicher and Puscas suggested that acetazomide, methazolamide and ethoxzolamide could significant reduce cancer cell growth whether in vivo or in vitro [21-23]. In physiology, CA III has lower carbonic dioxide hydrolysis activity compare to other isoforms, but CAIII has both carboxyl esterase and phosphatase activity. Several studied proved CA III connect to glutathione by disulfide bond and made protein sulfhydrase, and it's as the first react face to oxidative stress [24, 25]. Suggested that protein sulfhydrase through CA III can help cell resist oxidation. However, there less of evidences connect CA III to oral cancer or related to tumor metastasis. Thus, this study established a CA III overexpressed system, to clarified CA III could play roles in oral cancer development and metastasis.

Materials and methods

Cell culture

Human oral squamous cell carcinoma (OSCC) cell lines CA9-22, CAL-27, HSC-3, OC-2, SAS, SCC-9, SCC-25, TW-206 cells were purchased from Japanese Collection of Research Bioresources Cell Bank (JCRB, Shinjuku, Japan) and were cultured in DMEM or DMEM/F-12 medium (Life Technologies, Grand Island, NY, USA) with 10 % fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin, and

100 μg/ml streptomycin. Oral epidermal carcinoma cell line (OECM-1) were acquired from Dr Meng's group [26] and maintained in RPMI 1640 medium (Gibco™, Grand Island, NY, USA) with 10 % FBS. Human oral keratinocytes HOK and human immortalized keratinocytes SG were used as normal cells compared to the cancer cell lines. All cell cultures were maintained at 37 ℃ in a humidified atmosphere of 5 % CO2.

CA III overexpressed system

SAS and SCC-9 cell lines were used to the target cell lines to establish the stable CA III overexpressed cell clones. We choose pEGFPN-1 (Promega Corp., Madison, MI) as the vector and carried CA III promoter sequence. The forward primer 5'-CACGAATTCatggcccaaggagtggggc-3' and reverse primer 5'-GTGGGATCCCTtttgaaggaagctctcacca-3' were used to amplify CA III promoter sequence. The products after treated with EcoRI and BamHI restriction enzymes, using ligase reagent to complete the ligation with pEGFPN-1 vector. Spread 4×10^5 cells in 6 cm culture dish incubated for 16 hours and used Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) transfect the vector plasmid, after incubated 16 hours used G418 antibiotic to select the stable clones and used as the target cells in the following experiments. The empty vector GFP was took as the control group compare to the GFP-CA III group.

Cell growth curve

Cell growth curve were used to compare the cell growth rate affected after CA III expressed. Spread 6×10^4 / well number of cells to 24 well culture plates, incubated for 24, 48, 72 and 96 hours discarded the condition medium and adding MTT reagent

(Sigma chemical Co., Louis, MO, USA) with final concentration 0.5 mg / ml for 4 hours. The living cells were measured proportional by spectrophotometrically at 563 nm with the final production of formazan with purple color [27].

Migration and invasion assay

For wound healing assay, cells with pEGFPN-1 CA III overexpressing vectors were plated in 6-well plates for 16 hours, wounded by scratching with a pipette tip, then incubated with DMEM/F12 medium containing 0.5 % FBS for 12 or 24 hours. Cells were photographed using a phase-contrast microscope. Estimation the cell migration ability briefly by measured the wound recovered area. And used Boyden chamber (Neuro Probe, Cabin John, MD, USA) for migration and invasion assay. For migration assay, cells were harvested and seeded to the chamber at 10^4 cells / ml in serum free medium and then incubated for 24 hours at 37 ℃. For invasion assay, 10 ml Matrigel (25 mg / 50 ml; BD Biosciences, MA, USA) was applied to 8 mm pore size polycarbonate membrane filters and the bottom chamber contained standard medium. Filters were then air-dried for 5 hours in a laminar flow hood. The invaded cells were fixed with 100 % methanol and stained with 5 % Giemsa. Cell numbers were counted under a light microscope. The migration assay was carried out as described in the invasion assay with no coating of Matrigel [28].

Reverse-trancription-PCR

Total RNA was isolated from cultured cells using Geneaid Total RNA Mini Kit (2012-04-05© Geneaid Biotech Ltd.) according to the manufacturer's instructions. For reverse transcription, first-strand cDNA synthesis was performed with random primers (hexamers; Promega, Madison, WI) and 100 U of moloney murine leukemia

virus reverse transcriptase and performed at 42 ℃ for 60 minutes and terminated at 90 ℃ for 10 minutes. To detect CA III mRNA, the cDNA (4 μg) was amplified by PCR with the following primers: CA III forward primer 5'-ATGGCCAAGGAGTGGGGC-3' and reverse primer 5'-TGGTGAGAGCTTCCTTCAAATGA-3'. Samples were subjected to 30 cycles, each involving denaturation at 94 ℃ for 1 minute, annealing at 62 ℃ for 1 minute, and extension at 72 ℃ for 2 minutes, with a final extension phase of 10 minutes performed on a programmable thermal controller. PCR products were subjected to agarose gel electrophoresis, stained by ethidium bromide, and read by a densitometer (AlphaImager 2000; AlphaInnotech Corporation). The quantitative real-time PCR analysis was performed using Taqman one-step PCR Master Mix (Applied Biosystems, Carlsbad, CA) with primer-probe E-cadherin, vimentin, fibronectin, twist, snail1 and snail2.

Western blot

For Western blot analysis, cell lyates were collected as described. Briefly, cells were solubilized with sodium dodecyl sulfate-solubilization buffer for 30 minutes on ice. Then, cell lysates were centrifuged at 12000 g at 4 ℃ and the protein concentrations determined with Bradford reagent using bovine serum albumin as standards. Equivalent amounts of total protein per sample of cell extracts were run on a 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and overnight with polyclonal antibodies CA III, E-cadherin, vimentin, fibronectin, tSrc, pSrc, tFAK,pFAK (397), twist, snail1 and snail2 [29]. Detected protein expression by chemiluminescence using an ECL detection kit (Amersham Biosciences UK Limited, Buckinghamshire, England) Relative photographic density was quantitated by

scanning the photographic negatives on a gel documentation and analysis system (Alpha Innotech Corp., San Leandro, CA, USA).

Gene expression microarray

The total RNA was commission Phalanx Biotech Group work with whole genome array. Each sample should have 6 μg of RNA and the OD260 / OD280 ≥ 1.8; OD260 / OD 230 ≥ 1.5 as standard RNA quality. Using Human OneArray Gene Expression Microarray kits quantify the gene expression and analysis the data with chart.

Luciferase-report assay

SCC-9 and SAS cells were spread 4×10^4 cells per well in 24 well culture plates. After incubated 16 hours, pGL3 - basic vector and E-cadherin promoter plasmid were co-transfected with β-galactosidase expression vector (pCH110) into target cells by Turbofect (Fermentas, Carlsbad, CA) as previous described [30]. Transfection for 24 hours, the cell lysates were harvested, the luciferase activity was determined by luciferase assay kit. The value of the luciferase activity were normalized to transfection efficiency and monitored by β-galactosidase expression

Statistic

Statistically calculated were using student's t-test (Sigmastat, Jandel Scientific, and San Rafael, CA, USA) to compare with each group. Reach statistical significant was set at $p < 0.0.5$, the values are the means ± standard deviation and determent at least three independent experiments.

Result

CA III overexpressed system

Considered the different CA III expression from different oral cancer cell lines, we selected CA9-22, CAL-27, HSC-3, OC-2, OECM-1, SAS, SCC-9, SCC-25, TW-206 several oral cancer cell lines and analyzed the CA III expression. As comparison, we also selected two normal oral cell lines HOK and SG (Fig. 1A). We selected SAS and SCC-9 oral cancer cell lines as the target cell lines which showed lower CA III expression and also connected with tumor metastasis. We construct a CA III overexpression system by pEGFPN-1 vector, and the CA III protein and mRNA expression were significant increased through this overexpressed system (Fig. 1B). Besides, the cell growth curve was used to see if there any affections under the CA III overexpressed system. There is no significant difference between GFP control group and the CA III overexpressed group in both SAS and SCC-9 cell lines (Fig. 1C).

Cell shift ability

Since the CA III overexpressed was not affected cell proliferation, we considered with migration and invasion abilities which similar as tumor metastasis behavior. Simply used wound healing assay to observe the cell shift abilities by recovered the wound. Obviously, the CA III overexpressed group had a great wound area recovered abilities compared to the GFP control group in both cell lines (Fig. 2A, $p<0.05$). Further, used boyden chamber assay to analyzed cell migration and invasion abilities under CA III overexpressed system. And the outcomes showed weather migration or invasion abilities were significant raised in the CA III overexpressed group (Fig. 2B, $p<0.05$).

mRNA arrays analysis

When CA III overexpressed which induced cell shift abilities may related to several mechanisms. In clarification, we selected SCC-9-GFP-CA III overexpression stable clones contrasted the mRNA changes under CA III overexpressed system by mRAN array (Fig. 3A). From the chart, CDH1, VIM, FN1 had obviously expression differences which were related to EMT. Also, we used real-time PCR to detect the mRNA changes in the two group. E-cadherin mRNA expression significant decrease while vimentin and fibronectin mRNA expression obviously raised (Fig. 3B).

Epithelial–mesenchymal transition

Epithelial-mesenchymal transition often company with extracellular matrix degradation. We choose the EMT markers E-cadherin and vimentin, and an extracellular matrix maker fibronection as the target marker. While CA III overexpressed, EMT marker E-cadherin were significant decreased (SCC-9: 0.2; SAS: 0.07) and vimentin showed the opposite way (SCC-9: 4.3; SAS: 6.1). And the extracellular matrix maker fibronection was decreased (Fig. 4A). Further, we also figure out that may though the signaling pathways such as Src and FAK were activated by the phosphate Src and phosphate FAK (397) raised (Fig. 4B).

EMT transcription factors

Transcription factors may through binding on the DNA binding sites to regulate gene translate. Several transcription factors related to EMT such as twist, snail1 and snail2, were considered take parts in the EMT process. After CA III overexpressed, twist, snail1 and snail2 expression were significant higher than the GFP control group

in the protein level and mRNA expression (Fig. 5A, 5B). And we also used luciferase assay to detected protein binding affinity of the E-cadherin promoter transcription binding site. At the CA III overexpressed group, the E-cadherin promoter transcription binding sites with decreased protein binding affinity compared to the GFP control group (Fig. 5C). According to findings above, this might call CA III could through effect transcription factors twist, snail1 and snail2 to block the E-cadherin promoter transcription binding sites results the EMT and stimulate oral cancer cells invasion and migration abilities.

CA III siRNA

Final, we treated with CA III siRNA to inhibit the overexpressed CA III and to clarification the casual relationships caused by CA III. After inhibited the overexpressed CA III, the decreased E-cadherin protein expression were recovered and the raised vimentin, fibronectin protein expression were down regulated (Fig. 6A). And the cells invasion and migration abilities which were turn on by CA III overexpressed also down regulate after treated with CA III siRNA (Fig. 6B). That explained the CA III could through regulate the EMT and ECM related proteins E-cadherin, vimentin and fibronectin to affected cell invasion and migration abilities.

Discussion

According to the findings, we suggested that CA III may influenced EMT process include inhibit epithelial marker e-cadherin gene transcription binding site affinity to decrease e-cadherin expression, also raised mesenchymal markers vimentin, fibronectin and several transcription factors twist, snail1 and snail2 through FAK, Src signaling pathways to stimulate cell invasion and migration abilities which similar to tumor metastasis. In vivo, previous studies suggested that lower CA III expression was suppressed cancerous lesions in hepatoma-bearing rat and that also indicated the suppression of CA III accompanied hepatocarcinogensis [31, 32]. On the other hand, the other study showed CA III could through active FAK signaling pathway, promote downstream hepatoma cell Sk-Hep1 transformation and invasion abilities [33], which similar to our findings. These different may due to the varies CA III functions in the living subjects as regulate ion transport, pH value and water homeostasis to stable the basic living conditions and plays as a tumor suppressor. But in the in vitro environment, CA III may only character a regulator for the culture condition and make the oral cancer cells more active tend to invasion and migration.

EMT has known play a very important role in the metastasis and take part in several cancer metastasis mechanisms [34-37]. EMT may through MEK / ERK and PI3K / Akt signaling pathways promote tumor metastasis in human hepatomacellular carcinoma [38]. In breast cancer, WNT5A could through down regulate EMT to inhibit breast cancer cell invasion and migration [39]. Through GSK-3β / β-catenin pathways, β-arrestin1 inspirit EMT and provide a potential therapeutic target for prostate cancer [40]. With the miRNA-429, also connected to affect EMT to cause the metastasis and poor prognosis in renal cell carcinoma [41]. In the head and neck cancer, previous study suggested that Notch1 was strongly correlated to HNCC

growth, invasion and metastasis and that could traced back to Notch1 enhanced EMT through c-Myc signaling pathway [42]. Following these evidences, EMT has a deep related to metastasis in several cancers and diseases, natural and right, epithelial marker as E-cadherin and mesenchymal markers vimentin and fibronectin were also looks as potential target connect to tumor metastasis.

Previous research suggested that inhibited E-cadherin expression could induced cell invasion and metastasis in osteosarcoma [43]. In clinical, E-cadherin expression in clinical later stage in breast invasive carcinoma of no specific type was significantly lower than that in early stage ($p < 0.01$) and also significantly associated with lymph node metastasis and vascular invasion ($p < 0.01$) [44]. From a molecular pathological epidemiology database of 689 rectal and colon cancer cases study, loss of tumour E-cadherin expression was associated with infiltrative tumour growth pattern and higher pN stage [45]. In hepatocellular carcinoma, loss of E-cadherin correlated with a higher recurrence rate in an epidemiology contain 137 subjects [46]. In vivo, TGF-β could induce the RNA binding protein inhibition down regulate E-cadherin expression to promote EMT and take part in cell invasion and metastasis in lung cancer [47]. Another study also showed that sprouty proteins SPRY4 could induced amphirgulin expression to down regulate E-cadherin to bring about enhanced cell invasion in ovarian cancer [48]. Also, up-regulation miRNA-185 could reversed EMT via the up regulate E-cadherin and down regulate vimentin in epithelial and mesenchymal human hepatocellular carcinoma cells [49]. In vitro, dimethoxy curcumin enhanced E-cadherin expression and suppressed survivin expression and inhibit cell invasion in colon cancer [50]. And in the head and neck carcinoma, a meta-analysis showed definite evidence that high E-cadherin gene expression with a better overall survival and disease-free survival of head and neck squamous cell

carcinoma [51].

On the other hand, mesenchymal markers vimentin and fibronectin also related to tumor metastasis in various cancers [52-54], and were regarded as metastatic suppressor [55]. EMT could be regulated though many of signaling pathways such as PI3K / AKT, MEK / ERK, RhoA / MLC2 and FAK / Src that affected tumor proliferation, differentiation and metastasis [56-59]. Also, FAK-targeting pathways were potentially anticancer strategies [60, 61]. Our findings connected overexpressed CA III through the FAK, Src signaling pathways to up regulate transcription factors twist, snail1 and snail2 which decreased the E-cadherin DNA binding sites binding abilities in the nuclear brought out down regulate E-cadherin expression and up regulate vimentin and fibronectin that enhanced EMT with a highly invasion and metastasis abilities in oral cancer lines.

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Figure 1

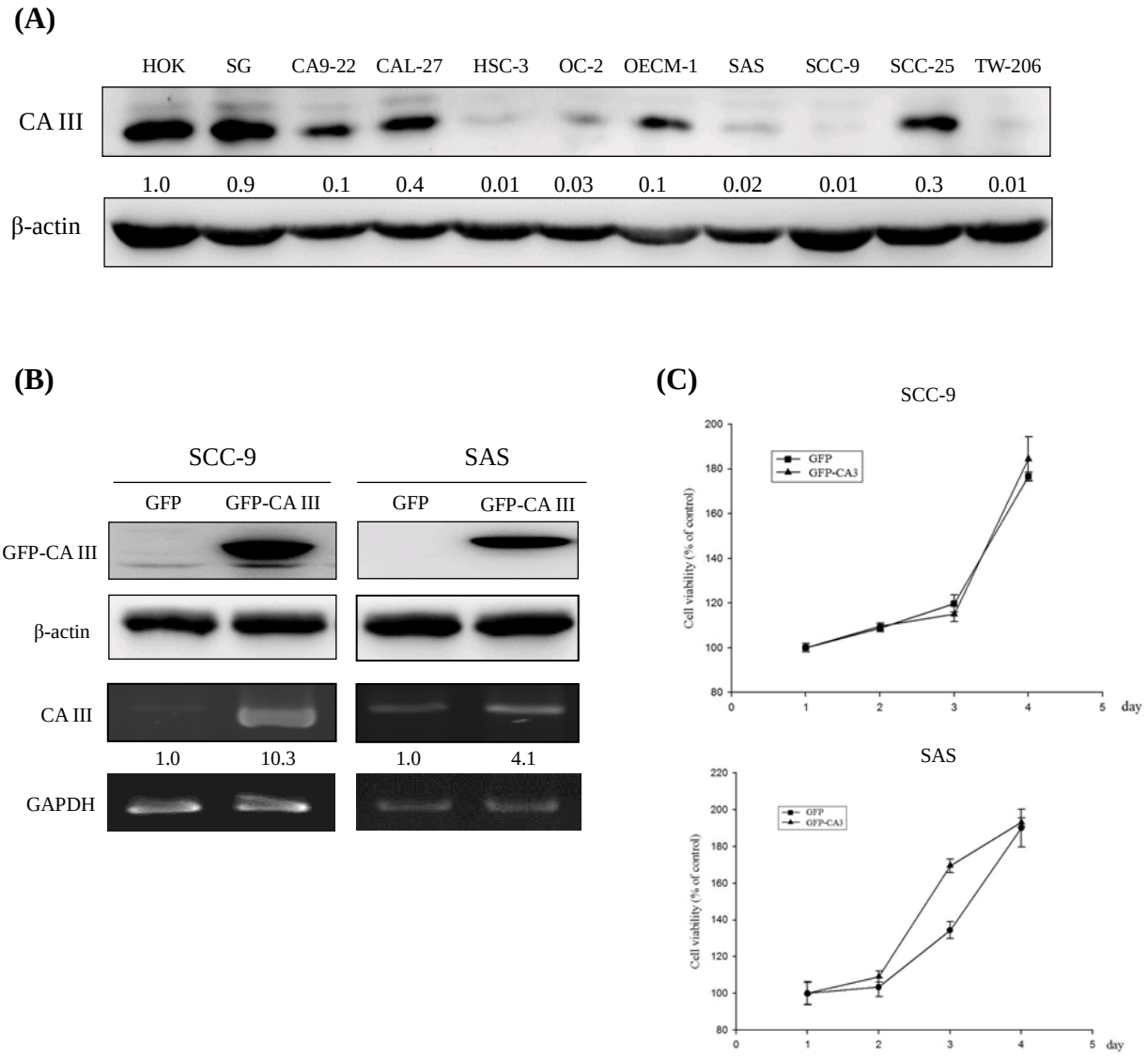


Figure 1. CAIII expressions in oral cancer cell lines. (A)We selected CA9-22, CAL-27, HSC-3, OC-2, OECM-1, SAS, SCC-9, SCC-25, TW-206 oral cancer cell lines and two normal oral cell lines HOK and SG. Compared the CAIII protein expressions in each cell lines, we pick SAS and SCC-9 as targets to build CAIII overexpression system. (B) CA III expression in protein and mRNA levels under CA III overexpressed system**.** We use SCC-9 and SAS oral cancer cell lines as targets, pEGFP-N1 (GFP) fluorescence vector as media to contribute CA III overexpressed system. G418 1000μM used to select stable clones which continuously expressed CA III. Obviously CA III expressions are higher under CA III overexpressed system in both cell lines. (C) Cell viabilities under CA III overexpressed system. Compared to the GFP only group, cell growth curve showed no significant differences under CA III overexpressed system in SCC-9 and SAS oral cancer lines. The first day measurement was used as control.

Figure 2

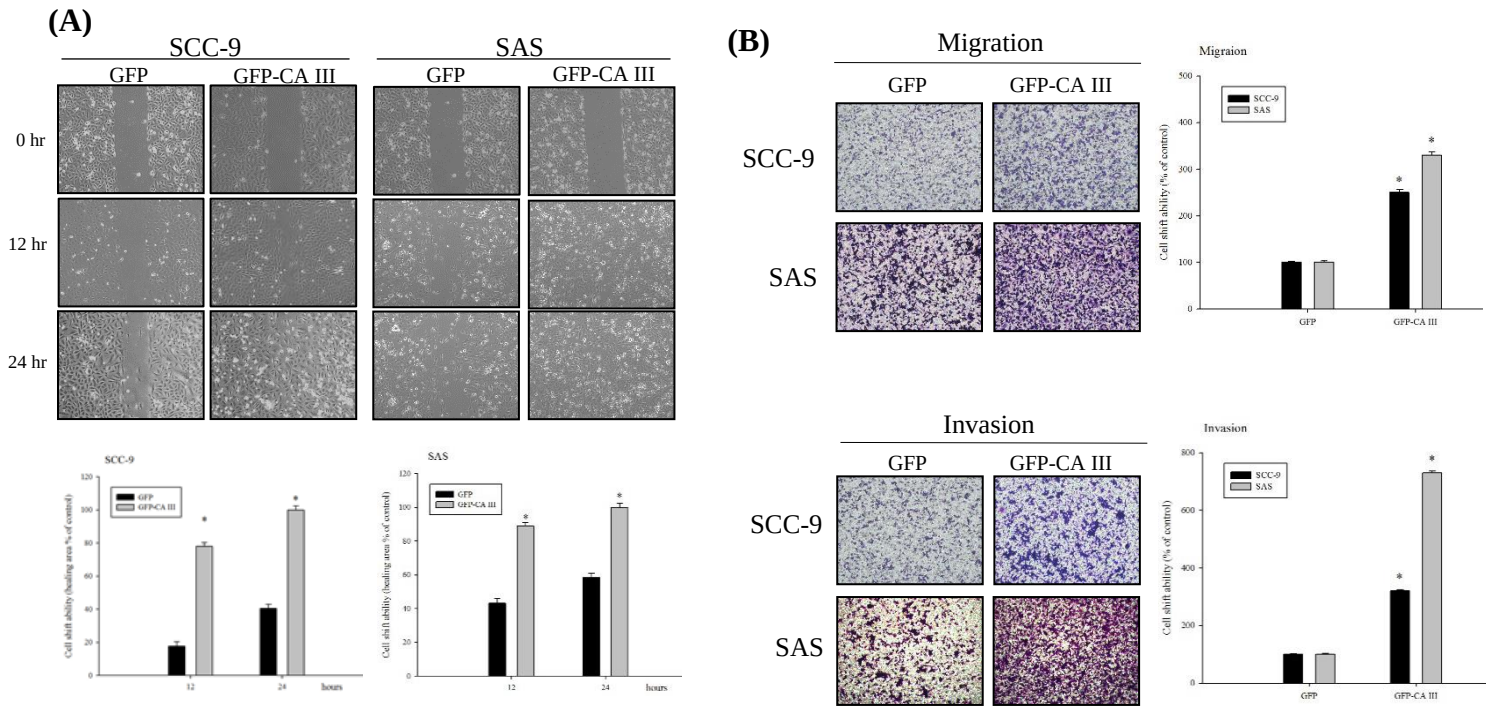
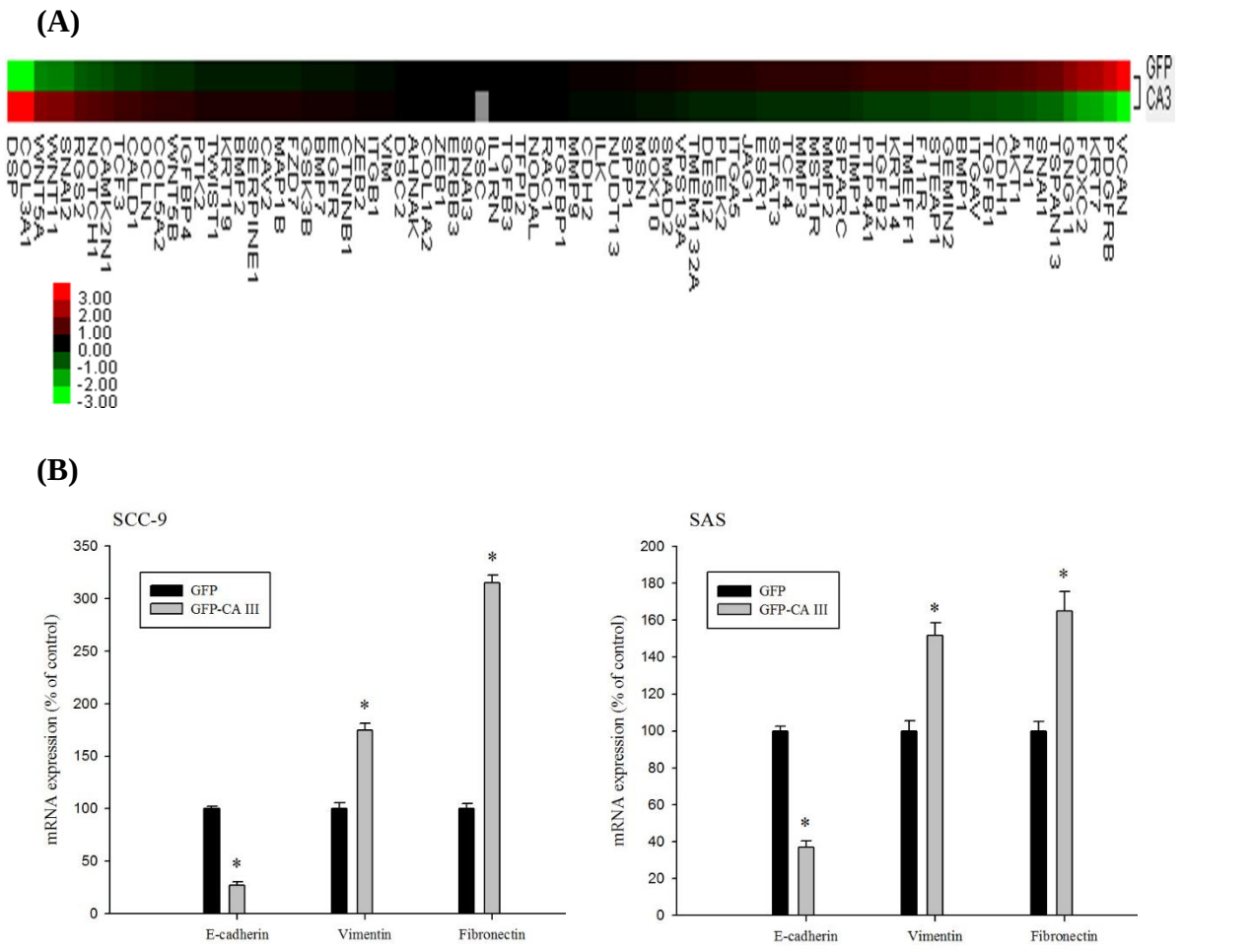


Figure 2. Higher cell shift abilities under CA III overexpressed system. (A) Wound healing assay showed a higher cell shift abilities by disappearing wound areas under CA III overexpressed system in both SCC-9 and SAS oral cancer cell lines (p<0.05). (B) By using boyden chamber assay discovered cell migration abilities obviously improved under CA III overexpressed system in oral cancer cell lines (p<0.05). Invasion abilities also increased under CAIII overexpressed system compared to vector only group (p<0.05).

Figure 3



* p value < 0.05 as statistic significant

Figure 3. We selected SCC-9-GFP-CA III overexpression stable clones using mRNA array analysis the mRNA expression differences under CA III expression system. (A) The comparing chart showed several EMT associated mRNA such as CDH1, VIM, FN1 had obviously expression differences under CA III overexpression system. (B) Extract mRNA from SCC-9 and SAS cells and analysis by real-time PCR we demonstrated E-cadherin mRNA expression significant decrease and vimentin, fibronectin mRNA expression obviously increase under CA III overexpressed system (p<0.05).

Figure 4

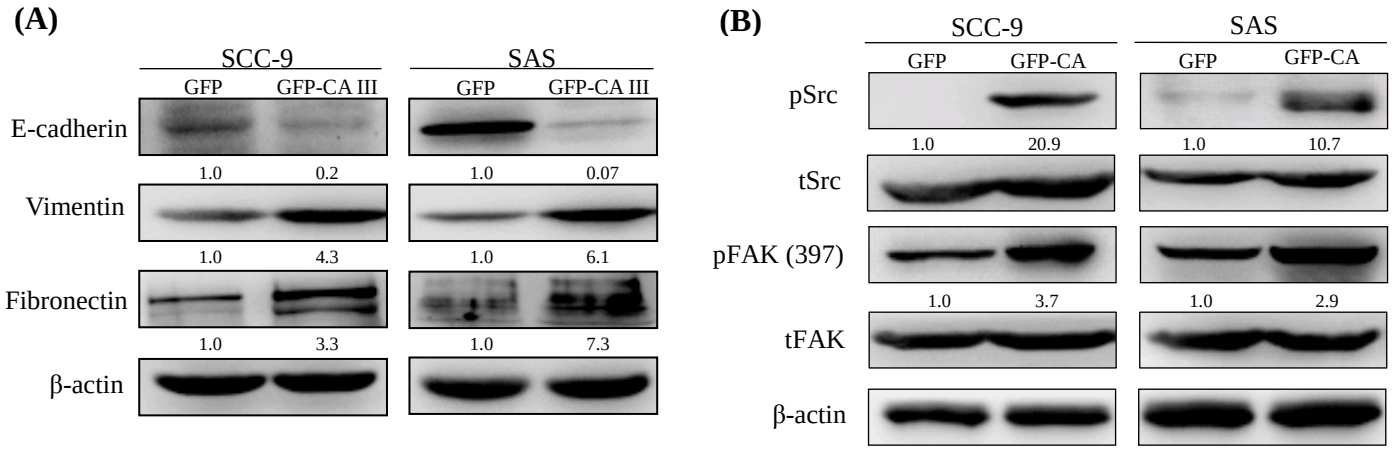


Figure 4. ECM related protein and signaling pathways under CA III overexpression system. Under the CA III expression system (A) ECM related protein E-cadherin was significant decrease (0.2 and 0.07 fold) while vimentin and fibronectin expressions were raised (vimentin 4.3 and 6.1 fold; fibronectin 3.3 and 7.3 fold) in both SCC-9 and SAS cell lines. And the signaling pathways (B) Src and FAK pathways were significant stimulated by CA III overexpression system (pSrc 20.9 and 10.7 fold; pFAK 3.7 and 2.9 fold).

Figure 5

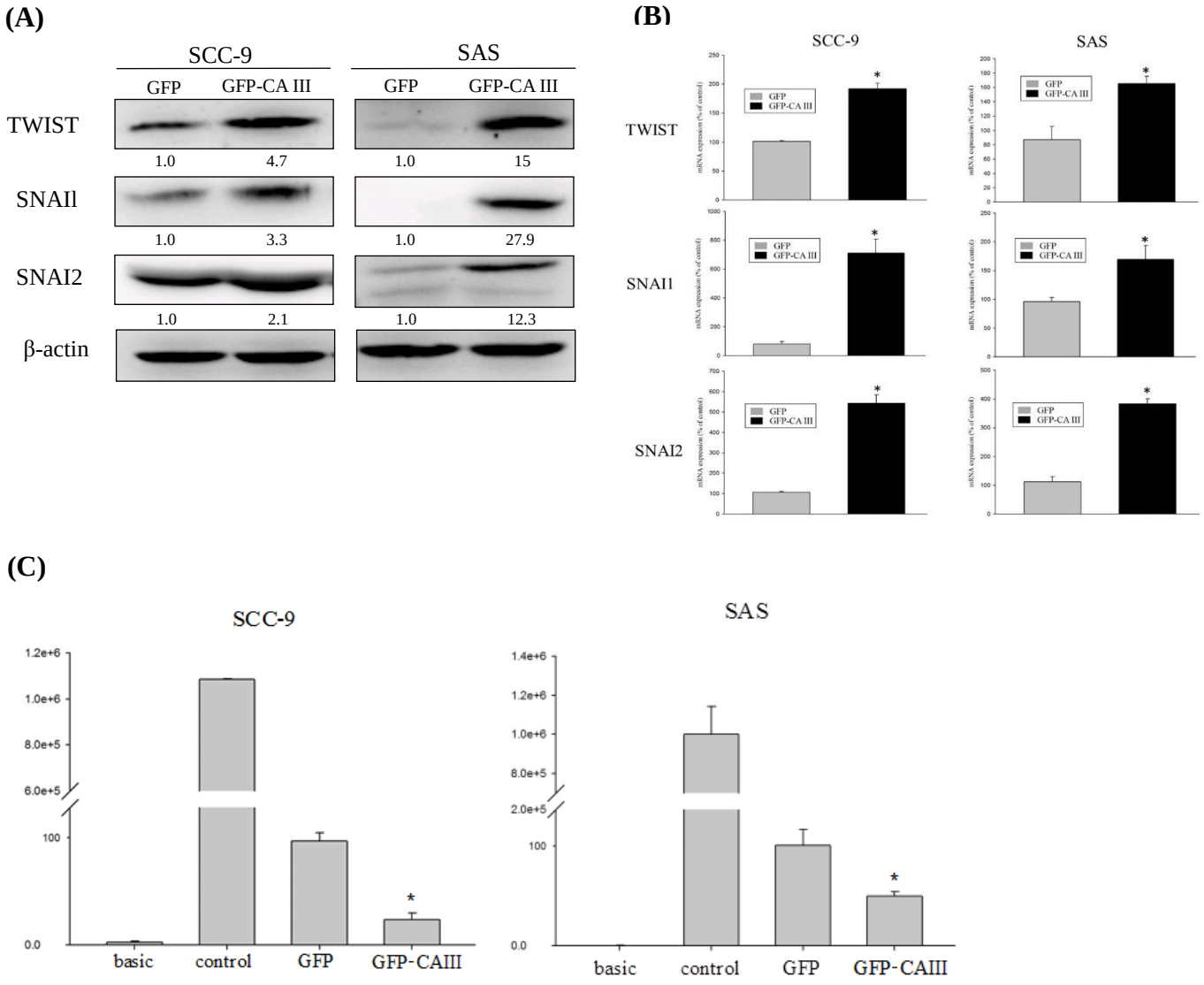


Figure 5. ECM related transcription factors twist, snail1 and snail2 effected under CA III overexpressed system. (A) E-cadherin related transcription factors twist, snail1 and snail2 were highly expression since CA III overexpressed in both SCC-9 and SAS cell lines. (B) Also, the mRNA expression were significant higher compared to the GFP control group (p<0.05) in SCC-9 and SAS cell lines. (C) Luciferase assay used to reconfirm the affinity of E-cadherin promoter transcription binding sites, using β- galactosidase as internal control. Under CA III overexpressed system, the binding affinity of E-cadherin promoter binding sites were significant decrease compared to GFP control group (p<0.05) in both SCC-9 and SAS cell lines.

Figure 6

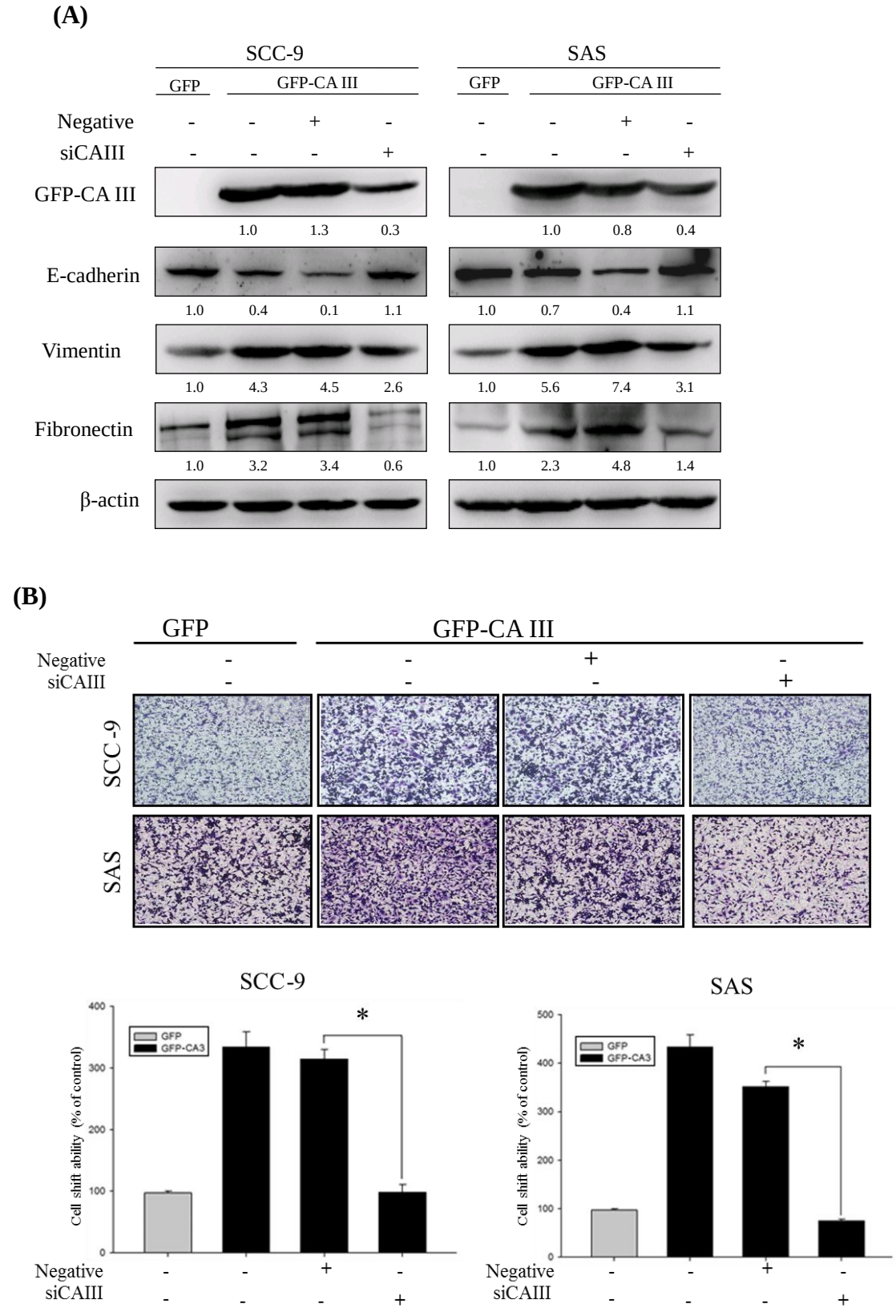

(A)
SCC-9
SAS
GFP
GFP-CA III
GFP
GFP-CA III
Negative
siCAIII
GFP-CA III
1.0 1.3 0.3
1.0 0.8 0.4
E-cadherin
1.0 0.4 0.1 1.1
1.0 0.7 0.4 1.1
Vimentin
1.0 4.3 4.5 2.6
1.0 5.6 7.4 3.1
Fibronectin
1.0 3.2 3.4 0.6
1.0 2.3 4.8 1.4
β-actin
(B)
GFP
GFP-CA III
Negative
siCAIII
SCC-9
SAS
SCC-9
SAS
Cell shift ability (% of control)
GFP
GFP-CA3
*
Negative
siCAIII

Figure 6. Using CA III siRNA to declare the affections under CA III overexpressed system, such protein expression and cell shift abilities. (A) The decreased E-cadherin protein expression by CA III were recovered after treated with CA III siRNA (SCC-9: 0.1 versus 1.1; SAS: 0.4 versus 1.1). Also, increased vimentin and fibernectin protein expressions were inhibited by CA III siRNA (vimentin: 4.5 versus 2.6, 7.4 versus 3.1; fibronectin: 3.4 versus 0.6, 4.8 versus 1.4). (B) The raised cell shift abilities by CA III were decreased after treated with CA III siRNA ($p<0.05$) in both SCC-9 and SAS cell lines.

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

105 年 10 月 17 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	2016/07/09~2016/07/12 英國曼徹斯特 (Manchester, UK)	本會核定 補助文號	
會議名稱	（中文）第 24 屆歐洲癌症學會 （英文）24th Biennial Congress of the European Association for Cancer Research		
發表論文題目	（中文）Melatonin 抑制口腔癌轉移的機制探討 （英文）Melatonin regulates oral cancer cell migration by suppressing histone acetylation on the CBP/P300 gene		

報告內容應包括下列各項：

一、參加會議經過

7/9 抵達英國曼徹斯特開會地點，7/9 早上前往曼徹斯特會議中心報到及領取大會議程及摘要手冊，並於 7/10 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。

二、與會心得

本人的研究主題主要是 Melatonin 抑制人類口腔癌轉移及基質金屬蛋白水解酶-9 的機制探討，褪黑激素(Melatonin)是由腦部松果體(Pineal gland)合成並分泌出來的一種激素，褪黑激素也具有「抗氧化劑」能力，可以抑制癌症細胞，協助改善憂鬱症，增強免疫功能。而我們發現褪黑激素可以透過 Erk 訊息傳遞路徑使 MMP-9 表現下降，進而抑制口腔癌的轉移。在會議上，許多學者對褪黑激素有高度的興趣，尤其可以應用在抑制口腔癌的轉移。而本次大會的主題除了涵蓋人類不同癌症的基礎研究之外，還加入臨床治療。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

科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/17

科技部補助計畫	計畫名稱: 細胞質中碳酸酐異構酶在口腔癌致癌過程的表現及其調控轉移相關因子的機制探討
	計畫主持人: 楊順發
	計畫編號: 102-2314-B-040-008-MY3　　學門領域: 牙醫學

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：楊順發	**計畫編號：**102-2314-B-040-008-MY3
計畫名稱：細胞質中碳酸酐異構酶在口腔癌致癌過程的表現及其調控轉移相關因子的機制探討	

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			0	篇	
		研討會論文			3		
		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	1		
		博士生	1		
		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。）					

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）
   上皮細胞間質化（epithelial-mesenchymal transition, EMT）是一種上皮細胞轉化成間質細胞的過程，是癌症轉移的主要機制之一。近年有多篇文獻證實 Cytosolic CA 中的 CAIII 參與蛋白質的硫化作用同時提供細胞抗氧化的角色，而這些抗氧化的能力可能對腫瘤轉移與侵襲相關。然而對 CAIII 在口腔癌的發展與轉移的過程中許多機制仍不清楚，本研究試探討 CAIII 對口腔癌轉移與 EMT 之間的關係並其詳細機制為何。結果發現由 CAIII 所造成細胞侵襲與轉移可能是透過影響 FAK/Src 路徑進而造成EMT 引起，此發現未來可當作口腔癌治療轉移的輔助療法。

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）