行政院國家科學委員會專題研究計畫 成果報告

熱休克蛋白27與其客戶蛋白在乳癌幹細胞的角色
研究成果報告(精簡版)

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中文摘要： 熱休克蛋白通常在細胞遭遇環境壓力時被大量表現，其作用為維持細胞內蛋白質構形的正確性，以使細胞能維持正常生理功能，然而在許多癌症發現熱休克蛋白通常也過度表現，並與疾病進程有相關性。癌症幹細胞為癌症組織中具有高度致癌性的一小群細胞，被認為是起始腫瘤組織的關鍵細胞，並與癌症的轉移、抗藥性及復發具有極高的相關性。在乳癌研究中已知熱休克蛋白 27(Hsp27)的過度表現與乳癌細胞的侵犯性與抗藥性有關，但對於 Hsp27 在乳癌幹細胞之維持仍不清楚。本研究發現 Hsp27 與其磷酸化形式蛋白在乳癌幹細胞中過度表達，並藉由干擾 RNA 抑制 Hsp27 表現後，能夠抑制乳癌幹細胞的自我更新以及細胞移動能力。此外，植物多酚類化合物槲黃素(quercetin)也可以藉由抑制 Hsp27 來抑制乳癌幹細胞的自我更新。以干擾 RNA 抑制乳癌幹細胞內 Hsp27 後，細胞內之上皮間質轉換過程發生反轉現象，其中 snail 轉錄因子與細胞骨架蛋白 vimentin 明顯受到抑制，而細胞黏著蛋白 E-cadherin 表現增加。我們還發現，抑制 Hsp27 的表現能夠降低乳癌幹細胞內核因子 Kappa B 的活化，此現象能藉由抑制核因子 Kappa B 上游抑制因子 I kappa B alpha 獲得反轉。我們進一步探討 Hsp27 對於乳癌幹細胞抵抗熱休克蛋白 90(hsp90)抑制劑的影響。我們發現 Hsp90alpha 在乳癌幹細胞中過度表現，而 Hsp90 抑制劑 Geldanamycin (GA)能降低乳癌細胞株中乳癌幹細胞的比例，但低劑量 Hsp90 抑制劑則效用不佳，並同時伴隨 Hsp27 與 Hsp70 蛋白的增加，因此我們假設低劑量 Hsp90 所誘導的 Hsp27 表現是小果不佳的原因。當以干擾 RNA 或熱休克蛋白抑制劑處理乳癌幹細胞時，的確能增加細胞對於 GA 的敏感性。總結本研究的成果，我們發現 Hsp27 能藉由調控上皮間質轉換、核因子 Kappa B 活性以及抗藥性，參與乳癌幹細胞的維持，並且設計以 Hsp27 為目標的藥物將是未來乳癌治療的潛力藥物之一。

中文關鍵詞： 乳癌、乳癌幹細胞、熱休克蛋白 27、熱休克蛋白 90、核因子 Kappa B、上皮間質轉換

英文摘要： Heat shock proteins (HSPs) are normally induced under environmental stress to serve as chaperone for maintenance of correct protein folding but they are often overexpressed in many cancers. The expression of Hsp27 is associated with cell migration and drug resistance of breast cancer cells. Breast cancer stem cells (BCSCs) have been identified as a subpopulation of breast cancer cells with markers of high

intracellular aldehyde dehydrogenase activity (ALDH+). Here we investigate the involvement of Hsp27 in the maintenance of BCSC. We found Hsp27 and its phosphorylation were increased in ALDH+ BCSCs in comparison with ALDH- non-BCSCs. Knockdown of Hsp27 in breast cancer cells decreased characters of BCSCs, such as mammosphere formation, cell migration and the in vivo CSC frequency. Treatment of quercetin, a plant flavonoid, could also inhibit ALDH+ cells and it be reversed by overexpression of Hsp27. Knockdown of Hsp27 also suppressed signatures of epithelial-mesenchymal transition (EMT) such as decreasing snail and vimentin and increasing E-cadherin. Furthermore, knockdown of Hsp27 decreased the activity of NF-kB in ALDH+ BCSCs and it could be reversed by IkBa knockdown. We also found that the expression of Hsp90a was increased in ALDH+ human breast cancer cells. Hsp90 inhibitor Geldanamycin (GA) could suppress ALDH+ breast cancer cells in a dose dependent manner. We found an insufficiently inhibitory effect of low dose GA treatment and it was correlated with the upregulation of Hsp27 and Hsp70. Co-treatment with HSP inhibitors and GA displayed a synergistic effect in targeting ALDH+ BCSCs. With siRNA mediated gene silencing, we found that knockdown of Hsp27 could mimic the effect of HSP inhibitors to potentiate the BCSC targeting effect of GA. In conclusion, our data suggest that Hsp27 regulates EMT process, NF-kB activity and drug resistance to contribute the maintenance of BCSCs. Targeting Hsp27 may be considered as a novel strategy in breast cancer therapy.

英文關鍵詞： breast cancer； breast cancer stem cells； Hsp27； Hsp90； NF-kappa B； epithelial-mesenchymal transition

中文摘要

熱休克蛋白通常在細胞遭遇環境壓力時被大量表現，其作用為維持細胞內蛋白質構形的正確性，以使細胞能維持正常生理功能，然而在許多癌症發現熱休克蛋白通常也過度表現，並與疾病進程有相關性。癌症幹細胞為癌症組織中具有高度致癌性的一小群細胞，被認為是起始腫瘤組織的關鍵細胞，並與癌症的轉移、抗藥性及復發具有極高的相關性。在乳癌研究中已知熱休克蛋白 27(Hsp27)的過度表現與乳癌細胞的侵犯性與抗藥性有關，但對於 Hsp27 在乳癌幹細胞之維持仍不清楚。本研究發現 Hsp27 與其磷酸化形式蛋白在乳癌幹細胞中過度表達，並藉由干擾 RNA 抑制 Hsp27 表現後，能夠抑制乳癌幹細胞的自我更新以及細胞移動能力。此外，植物多酚類化合物槲黃素(quercetin)也可以藉由抑制 Hsp27 來抑制乳癌幹細胞的自我更新。以干擾 RNA 抑制乳癌幹細胞內 Hsp27 後，細胞內之上皮間質轉換過程發生反轉現象，其中 snail 轉錄因子與細胞骨架蛋白 vimentin 明顯受到抑制，而細胞黏著蛋白 E-cadherin 表現增加。我們還發現，抑制 Hsp27 的表現能夠降低乳癌幹細胞內核因子 Kappa B 的活化，此現象能藉由抑制核因子 Kappa B 上游抑制因子 I kappa B alpha 獲得反轉。我們進一步探討 Hsp27 對於乳癌幹細胞抵抗熱休克蛋白 90(hsp90)抑制劑的影響。我們發現 Hsp90alpha 在乳癌幹細胞中過度表現，而 Hsp90 抑制劑 Geldanamycin (GA)能降低乳癌細胞株中乳癌幹細胞的比例，但低劑量 Hsp90 抑制劑則效用不佳，並同時伴隨 Hsp27 與 Hsp70 蛋白的增加，因此我們假設低劑量 Hsp90 所誘導的 Hsp27 表現是小果不佳的原因。當以干擾 RNA 或熱休克蛋白抑制劑處理乳癌幹細胞時，的確能增加細胞對於 GA 的敏感性。總結本研究的成果，我們發現 Hsp27 能藉由調控上皮間質轉換、核因子 Kappa B 活性以及抗藥性，參與乳癌幹細胞的維持，並且設計以 Hsp27 為目標的藥物將是未來乳癌治療的潛力藥物之一。

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Heat shock proteins (HSPs) are normally induced under environmental stress to serve as chaperone for maintenance of correct protein folding but they are often overexpressed in many cancers. The expression of Hsp27 is associated with cell migration and drug resistance of breast cancer cells. Breast cancer stem cells (BCSCs) have been identified as a subpopulation of breast cancer cells with markers of high intracellular aldehyde dehydrogenase activity (ALDH+). Here we investigate the involvement of Hsp27 in the maintenance of BCSC. We found Hsp27 and its phosphorylation were increased in ALDH+ BCSCs in comparison with ALDH- non-BCSCs. Knockdown of Hsp27 in breast cancer cells decreased characters of BCSCs, such as mammosphere formation, cell migration and the in vivo CSC frequency. Treatment of

quercetin, a plant flavonoid, could also inhibit ALDH+ cells and it be reversed by overexpression of Hsp27. Knockdown of Hsp27 also suppressed signatures of epithelial-mesenchymal transition (EMT) such as decreasing snail and vimentin and increasing E-cadherin. Furthermore, knockdown of Hsp27 decreased the activity of NF-kB in ALDH+ BCSCs and it could be reversed by IkBa knockdown. We also found that the expression of Hsp90a was increased in ALDH+ human breast cancer cells. Hsp90 inhibitor Geldanamycin (GA) could suppress ALDH+ breast cancer cells in a dose dependent manner. We found an insufficiently inhibitory effect of low dose GA treatment and it was correlated with the upregulation of Hsp27 and Hsp70. Co-treatment with HSP inhibitors and GA displayed a synergistic effect in targeting ALDH+ BCSCs. With siRNA mediated gene silencing, we found that knockdown of Hsp27 could mimic the effect of HSP inhibitors to potentiate the BCSC targeting effect of GA. In conclusion, our data suggest that Hsp27 regulates EMT process, NF-kB activity and drug resistance to contribute the maintenance of BCSCs. Targeting Hsp27 may be considered as a novel strategy in breast cancer therapy.

一、前言與文獻探討

Heat shock proteins (Hsp) are a group of proteins that were first discovered under heat shock or other chemical stimulus in a wide range of species and function as molecular chaperones that could interact with their substrates to shift the balance from denatured protein conformation toward functional conformation. Besides their chaperone function, Hsps have been reported to be overexpressed in various of cancers and display a correlation with patients' survival or response to therapy in specific cancer types and may serve as novel therapeutic targets. Hsps are widely known about their cytoprotection functions in cancer cells. The mechanisms include their molecular chaperone activity, anti-apoptosis function and influence of the stability of client proteins. Hsp27 binds with cytochrome c to inhibit apotosis. Hsp70 and Hsp90 binds to apoptotic protease activating factor 1 to inhibit the activation of caspases and apoptosis. Hsp27 has been found to contribute to the malignant properties of cancer cells including increased tumorigenicity, treatment resistance and apoptosis inhibition. In breast cancer, Hsp27 has been reported as a risk factor of malignant progression in benign proliferating breast lesions and its expression could help to differentiate benign and malignant breast lesions in fine needle aspirate. Hsp27 has been reported to be associated with drug resistance and cell mobility properties of breast cancer.

Cancer stem cells are emerging targets in cancer research which are a particular subset of cancer cells responsible for tumorigenesis, chemoresistance and metastasis. In breast cancer, breast cancer stem cells (BCSCs) have been identified as cells with surface marker of CD24-CD44+ or high intracellular aldehyde dehydrogenase activity (ALDH+). Recently, Hsp27 has been proven to contribute in the drug resistance property of lung cancer stem cells. The expression of Hsp27 was increased in lung CSCs treated with cisplatin/gemcitabine. Combination of chemotherapy with a plant flavonoid compound quercetin, that can inhibit Hsp27 expression, could suppress the tumor growth as well as the expression of stemness genes including Oct4, Nanog and Sox2. Quercetin could also sensitize epigallocathechin gallate to inhibit the spheroid formation, cell survival and invasion of CD44+CD133+ prostate cancer stem cells, although the detailed molecular mechanisms remains unknown.

二、研究目的

Although Hsp27 is involved in chemoresistance and invasion phenotypes of breast cancer cell lines and has been demonstrated to participate in the drug resistance of lung CSCs, the involvement of Hsp27 in BCSCs is not fully understood. In this project, we would like to investigate the role of Hsp27 in BCSC characters, especially in the self-renewal, tumorigenicity, epithelial-mesenchymal transition and drug resistance.

三、研究方法

1. **Mice and *in vivo* breast cancer animal model** NOD/SC*ID and* BALB/c female mice (6-12-weeks-old) are purchased from National Laboratory Animal Center (Taipei, Taiwan) and subsequently maintained in the Animal Center of Chung Shan Medical University (Taichung, Taiwan). Mice will receive treatment of etoposide (30mg/kg) for 5 days before transplantation with tumor cells.
2. **Plasmids, siRNAs and transfection** Full length construct of Hsp27 will be cloned from AS-B145 cDNA and inserted into pDsRed-Express-C1 vector (Evrogen) with BglII/EcoRI restriction enzyme site.

The Hsp27 specific siRNA oligos or negative control siRNA oligos will be purchased from Santa Cruz Biotechnologies, Inc. MetafectenePro or MetafecteneSI transfection reagent will be used for DNA or siRNA transfection following manufacture's protocol (Biontex).

3. **Cell culture** AS-B145 and AS-B244 breast cancer cells which derived from BC0145 or BC0244 xenograft human breast cancer cells will be provided by Dr. Alice L. Yu (Genomics Research Center, Academia Sinica, Taipei, Taiwan), and will be cultured in MEMα supplemented with 10% fetal bovine serum, bovine insulin (0.1 mg/mL), sodium pyruvate (1 mM), and Glutamax (2 mM). The cells will be maintained in a 5% CO_2 air humidif$_{i}$ed atmosphere at 37 ℃. Human breast cancer cell line, MDA-MB-231, will obtain from ATCC (Manassas, USA) and culture as ATCC's recommendation.
4. **Aldefluor assay** Aldefluor assay kit is purchased from StemCell Technologies, Inc. (Vancouver, BC, Canada) and used following manufacture's recommendation. Briefly, 1×10^5 cells will be ^{s}uspended in 50μl of assay buffer and added Aldefluor to final concentration of 1μM. For ALDH1 inhibitor control, DEAB will be added to final concentration of 150μM. Cells will be then incubated at 37°C for 45min 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™, BD Biosciencs) by comparing the fluorescence intensity of DEAB treated sample and these cells will be represented as cells with high ALDH activity (ALDH+ cells).
5. **Mammosphere culture** Cells will be harvested from monolayer culture or collected by FACS sorting and prepared as density of 1×10^4 cells/ml in DMEM/F^{1}2 medium contain 0.5% methylcellulose, 0.4% bovine serum albumin, 10ng/ml EGF, 10ng/ml bFGF, 5μg/ml insulin, 1μM hydrocortisone and 4μg/ml heparin. 2ml of cell solution will be seeded into wells of ultralow attachment 6-well-plate (Corning) and incubated for 7 days. For secondary spheres, the cells will be collected from accutase treated primary spheres and seeded as density of 2500 cells/ml.
6. **Migration/invasion assay** Cell migration/invasion assay will be conducted by Oris Universal Cell Migration Assembly kit (Platypus Technologies, LCC) following manufacture's protocol. Briefly, 5×10^4 cells/well/100μl will be loaded into stopper loaded wells and incubated overnight to permit cell attachment. To start cell migration, the stoppers will be removed, wash wells gently with PBS, add complete cell culture medium and incubate for 16-24h. Pictures of wells will be captured with inverted microscopy after fixation and stain with 0.5% crystal violet/50% EtOH. Data will be analyzed with ImageJ software.
7. **Western blot** Cells will be washed with 1X PBS and lysed in RIPA lysis buffer containing NP-40. 25 microgram of extracted protein will be separated by using SDS-PAGE and transferred to the PVDF membrane (Immobilon-P, Millipore). The membrane will be then incubated with primary antibodies (Hsp27 and Hsp90a purchased from Stressgen; Akt, p-Akt^{ser473}, IκBα, p-IκBα, E-cadherin, vimentin, Twist, Snail purchased from Santa Cruz Biotechnology Inc.; GAPDH was purchased from MDBio Inc.) at 4℃ overnight. Peroxidase-conjugated anti-rabbit anti-mouse IgG (Cell Signaling Technology, Inc.) or anti-goat Ig (Sana Cruz Biotechnology, Inc.) will be used as secondary antibodies. Developed

chemiluminescence signals from catalyzed ECL substrate (Pierce Biotechnology) will be detected by Luminescence-Image Analyzer (Fujifilm Life Science).

8. **NF-kB reporter assay** The luciferase based NF-κB reporter vector was obtained from Stratagene. The assay was conducted with dual reporter assay system. Briefly, NF-κB vector was co-transfected with reference *Renilla* luciferase vector (Promega, Madison, WI, USA) as ratio of 10:1. After transfection for 48h, cells was lysed by passive lysis buffer (Promega) and luciferase activity was detected with Beetle-Juice (for firefly luciferase (FLuc)) and Gaussia-Juice (for Renilla luciferase (RLuc)) substrates (PJK GmbH, Kleinblittersdorf, Germany) and luminescence was counted with luminescence reader (Promega). The results of FLuc count were normalized with RLuc, which represented the transfection efficiency of each sample.

四、結果

I. Upregulation of Hsp27, p-Hsp27^{Ser82} and Hsp90a in breast cancer stem cells

With ALDH activity, proteins extracted from ALDH- or ALDH+ cells of BC0145 or BC0244 xenografts were subjected to mitogen activated protein kinase (MAPK) antibody array and results indicated that the phosphorylation of Hsp27, which may result from p38 MAPK activity, was increased in ALDH+ BCSCs from BC0145 or BC0244 xenograft cells (Fig. 1A). We also used western blot to check the level of total Hsp27 protein between ALDH- and ALDH+ AS-B244 cells, which derived from ALDH+ BC0244 xenograft cells. As shown in Fig. 1B, total protein level of Hsp27 was higher in ALDH+ cells than in ALDH- cells. We also detected the expression of Hsp90a in ALDH+/ALDH- cells of AS-B244 and results indicated that Hsp90a was upregulated in ALDH+ cells (Fig. 1C). These results indicate that Hsp27, p-Hsp27^{ser82} and Hsp90a are upregulated in BCSCs.

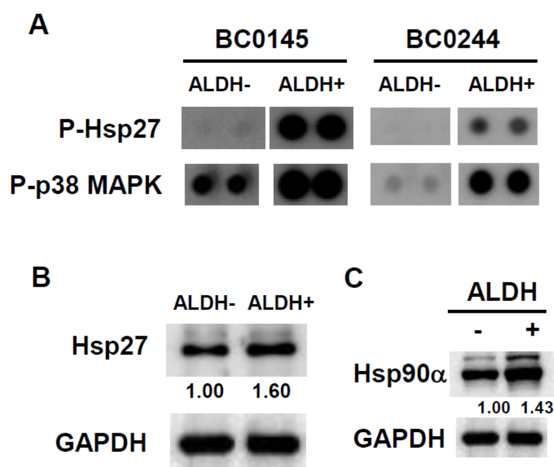
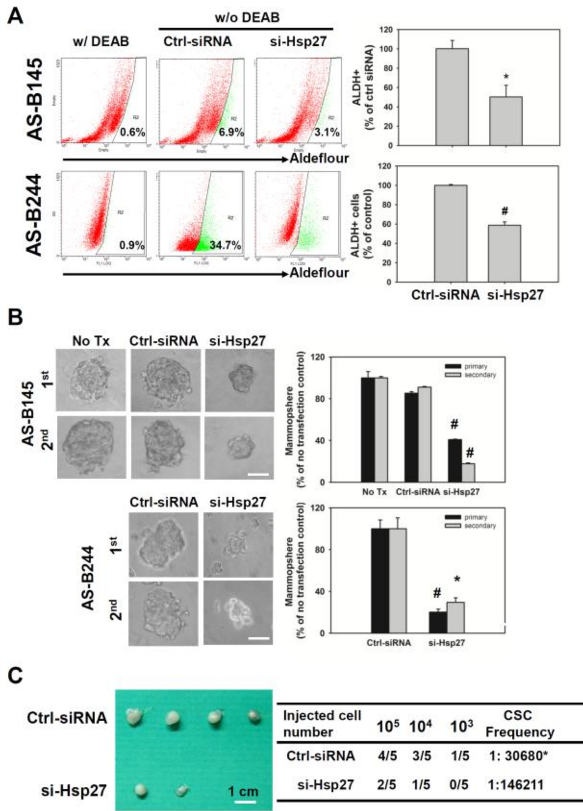


Fig.1. The expression of Hsp27 p-Hsp27^{ser82} and Hsp90α in BCSCs.

II. Hsp27 determines the maintenance and epithelial-mensenchymal transition of breast cancer stem cells

We next investigated the role of Hsp27 in maintenance of BCSCs by siRNA mediated gene silence of Hsp27 expression. After transfection with Hsp27 specific siRNA, the population of ALDH+ cells in AS-B145 or AS-B244 cells was significantly decreased when compared with cells transfected with negative control siRNA (Fig. 2A). Other than ALDH+ population of cells, the number of mammospheres as well as the size of formed spheres in AS-B145 or AS-B244 cells were also decreased (Fig. 2B). We further examined if

Hsp27 involves in the tumorigenicity of BCSCs. As shown in Fig. 2C, 10^5 negative control siRNA transfected AS-B145 sphere cells formed tumors in 4 out of 5 mice but 10^5 Hsp27 knockdown cells only formed tumors in 2 out of 5 mice at Day 44 (Fig2C, left panel). The CSC frequency of Hsp27 knockdown AS-B145 sphere cells was significantly decreased when compared with negative control siRNA groups (1:30680 versus 1:146211, p=0.0206). In addition to RNA interference, we also used quercetin, a plant flavonoid compound which has been reported to suppress the protein level of Hsp27, to treat AS-B145 and AS-B244 cells. Quercetin inhibited the expression of Hsp27 protein (Fig. 3A) as well as the population of ALDH+ cells (Fig. 3B) in both AS-B145 and AS-B244 cells in a dose dependent manner (Fig. 3C). In order to confirm if the inhibition effect of quercetin is mediated by downregulation of Hsp27, we next overexpressed Hsp27 in AS-B145 cells and examined the ALDH+ population under quercetin treatment. As shown in Fig. 3D and 3E, the inhibitory effect of quercetin could be reversed by overexpression of Hsp27 in AS-B145 cells. We next tested if quercetin also inhibits the self-renewal of BCSCs by mammosphere formation assay. The size and number of primary and secondary mammopsheres in AS-B145 (Fig. 4A) and AS-B244 (Fig. 4B) was suppressed by quercetin in a dose dependent manner. EMT is an important character of cancer stem cells. We next examined if Hsp27 mediates EMT features of BCSCs. With wound healing based cell migration assay, the cell migration ability of ALDH+ AS-B244, AS-B145, MDA-MB-231 and Sca-1+ 4T1 cells was inhibited by quercetin treatment in a dose dependent manner (Fig, 5A). By siRNA mediated knockdown of Hsp27, the cell migration capacity of AS-B145, MDA-MB-231 or ALDH+ AS-B244 cells was also inhibited in comparison with negative control siRNA (Fig. 5B). We also investigated if Hsp27 pathway also regulates EMT related molecular signatures. With western blot analysis, knockdown of Hsp27 in AS-B145 or ALDH+ AS-B244 cells decreased the expression of snail and vimentin and increased the expression of E-cadherin (Fig. 5C). These results indicate that Hsp27 may regulate self-renewal of BCSCs through manipulating EMT process.



Injected cell number	10^5	10^4	10^3	CSC Frequency
Ctrl-siRNA	4/5	3/5	1/5	1: 30680*
si-Hsp27	2/5	1/5	0/5	1:146211

Fig.2. Knockdown of Hsp27 inhibits BCSC features.

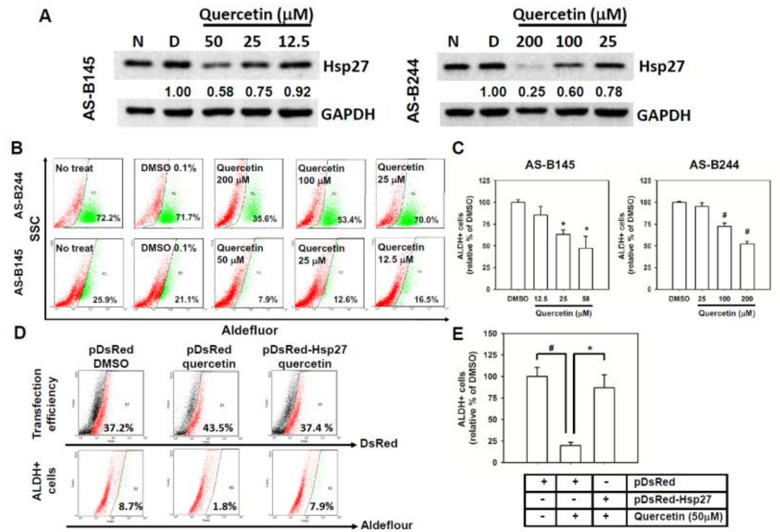


Fig. 3. Quercetin suppresses BCSCs through inhibition of Hsp27.

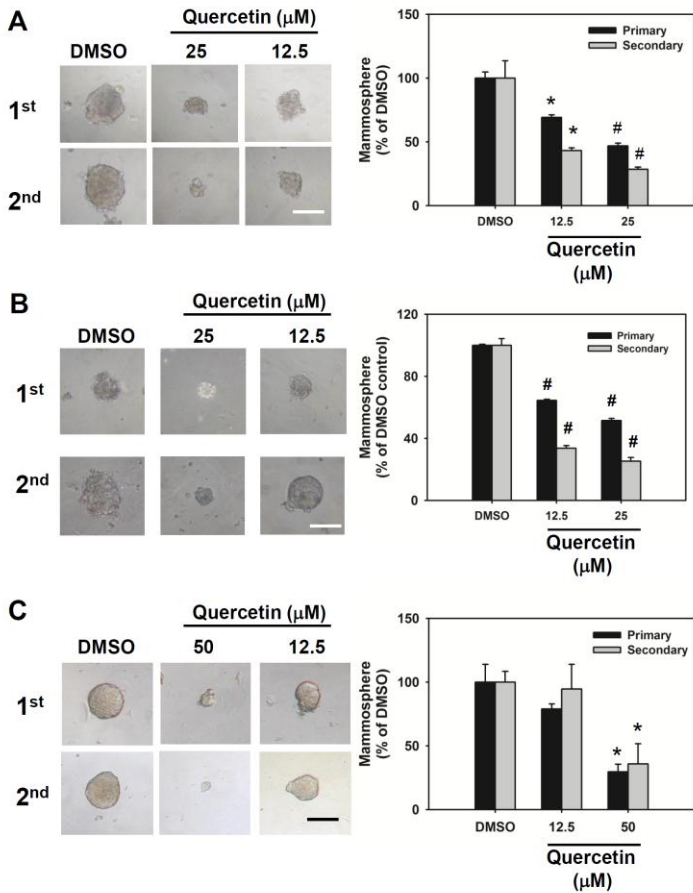


Fig.4. Quercetin inhibits self-renawal of BCSCs.

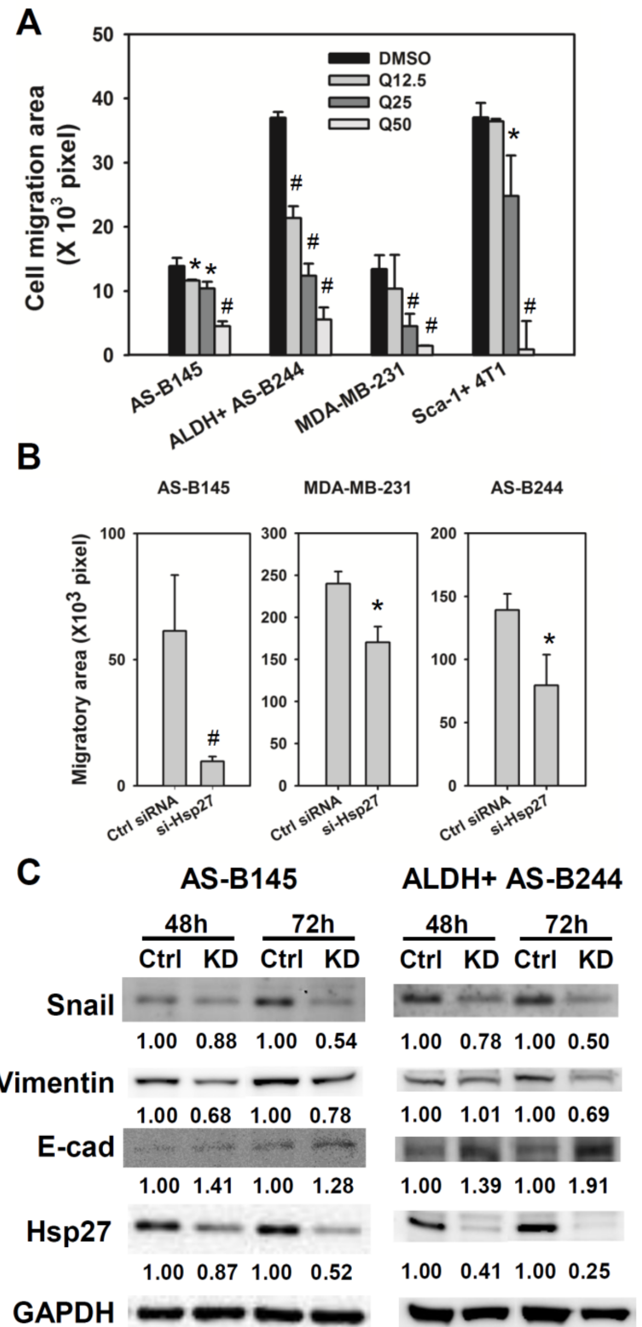


Fig.5. Inhibition of Hsp27 suppresses EMT of BCSCs

III. Hsp27 contributes to NF-κB activation in breast cancer stem cells

We next examined if Hsp27 regulates NF-κB activity in BCSCs. By siRNA mediated knockdown of Hsp27, the expression of IκBα was increased in both AS-B145 and ALDH+ AS-B244 cells and its phosphorylation was decreased (Fig. 6A). The nuclear translocation of NF-κB was also inhibited in both AS-B145 and ALDH+ AS-B244 cells when knockdown of Hsp27 (Fig. 6B). In the meantime, we also observed that Hsp27 could enter into nucleus (Fig. 6B). With luciferase based reporter assay, the NF-κB activity was decreased in ALDH+ AS-B244 and AS-B145 cells when knockdown of Hsp27 (Fig. 6C). We next used NF-κB inhibitors to examine their effects of BCSCs. In the presence of JSH-23, a NF-κB inhibitor which inhibits the nuclear translocation of NF-κB, the ALDH+ population of AS-B145 and AS-B244 cells was suppressed in a dose dependent manner (Fig. 6D). We further examined if the inhibitory effect of ALDH+ cells by Hsp27 knockdown could be diminished by restoring the activation of NF-κB. The increased IκBα, which was

caused by knockdown of Hsp27, was suppressed by knockdown of IκBα (Fig. 7A) and the NF-κB activity could be restored in Hsp27 knockdown AS-B145 or AS-B244 cells (Fig. 7B). The inhibitory effect of ALDH+ cells by Hsp27 knockdown could be reversed by knockdown of IκBα in both AS-B145 and AS-B244 cells (Fig. 7C and 7D). These results suggest that Hsp27 regulates the maintenance of BCSCs through NF-κB activity.

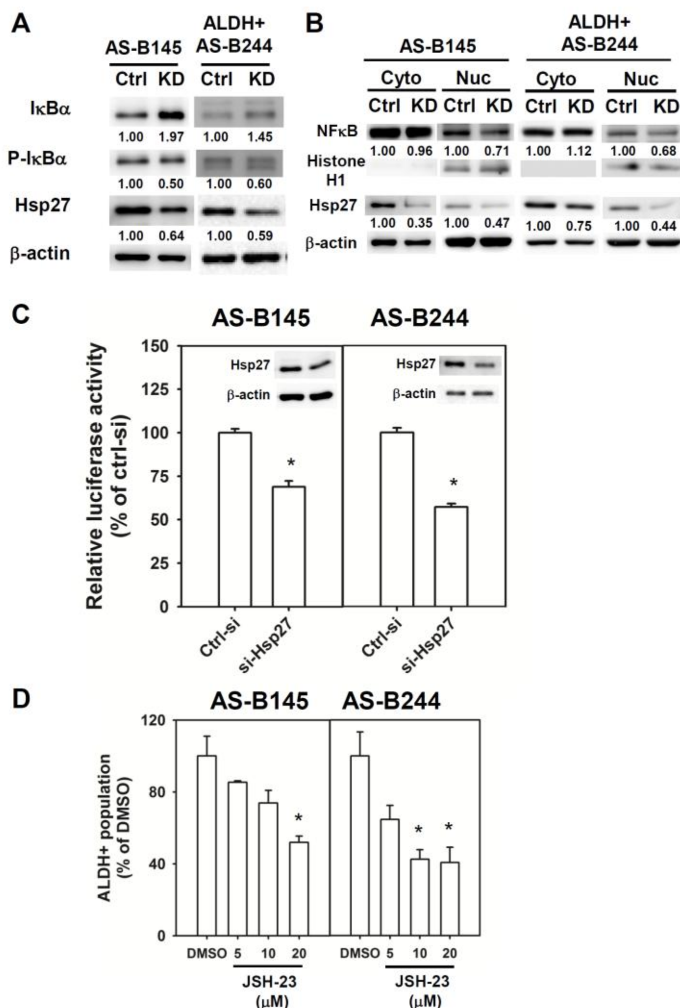


Fig.6. Knockdown of Hsp27 influences NF-κB Activation in BCSCs.

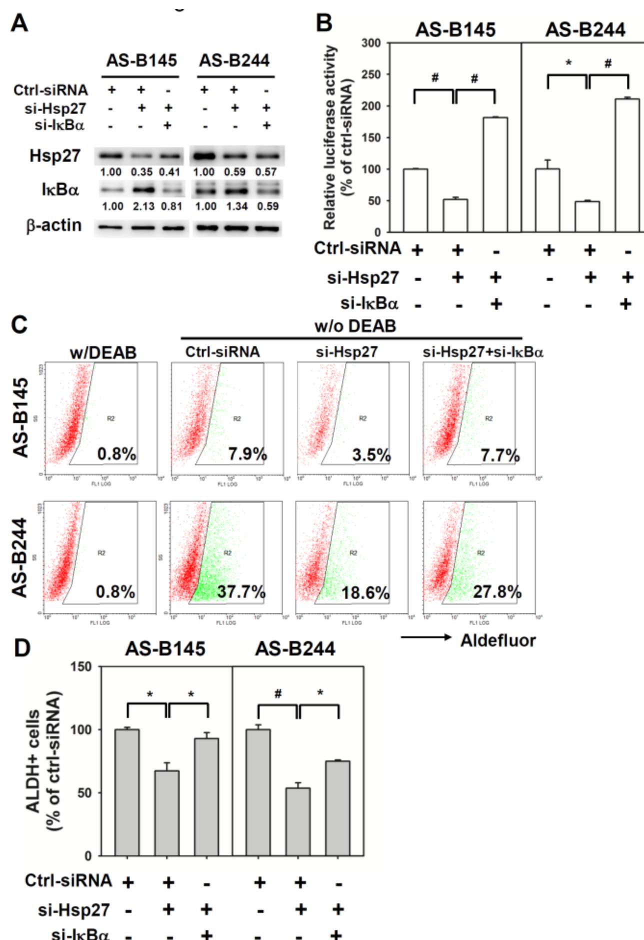


Fig.7. Restoring NF-kB activity abolishes the suppressive effect of Hsp27 siRNA.

IV. HSP inhibitors or knockdown of Hsp27 sensitized ALDH+ BCSCs toward Hsp90 inhibitors

We next examined the effect of Hsp90 inhibitors, GA and 17-DMAG, in targeting ALDH+ BCSCs. With ALDEFLUOR assay, the ALDH+ population of both AS-B145 and AS-B244 human breast cancer cells could be suppressed by GA treatment in a dose dependent manner (Fig. 8A and 8B). We next examined if treatment of Hsp90 inhibitors at non-sufficient dose induces Hsp27 or Hsp70 expression and protect BCSCs from inhibition of Hsp90 inhibitors. GA or 17-DMAG could induce Hsp27 or Hsp70 expression both mammosphere cells from AS-B145 and AS-B244 breast cancer cells (Fig.8C and 8D). It suggested that the induction of Hsp27 or Hsp70 may cause resistance of low dose treatment of Hsp90 inhibitors in ALDH+ BCSCs. By co-treatment of HSP inhibitors (quercetin or KNK437), there was a synergistic effect in the inhibition of ALDH+ cells when combination of quercetin or KNK437 with GA (Fig.9). We next examined if knockdown of Hsp27 expression could potentiate ALDH+ BCSCs to the suppressive effect of GA. Knockdown of Hsp27 expression in AS-B244 cells with specific siRNA caused 20.3% reduction in ALDH+

population and 63.7% reduction when combination with 40nM of GA (Fig. 10A). By western blot analysis, knockdown of Hsp27 caused down-regulation of Hsp70 and HSF-1 protein expression. When Hsp27 knockdown AS-B244 cells were treated with GA, the protein expression of Hsp70 and HSF-1 were reversed to the level of negative control siRNA transfected and DMSO treated cells (Fig. 10B). It also suggests that Hsp27, but not Hsp70, plays the protection role of ALDH+ BCSCs in resistant to low dose of GA treatment.

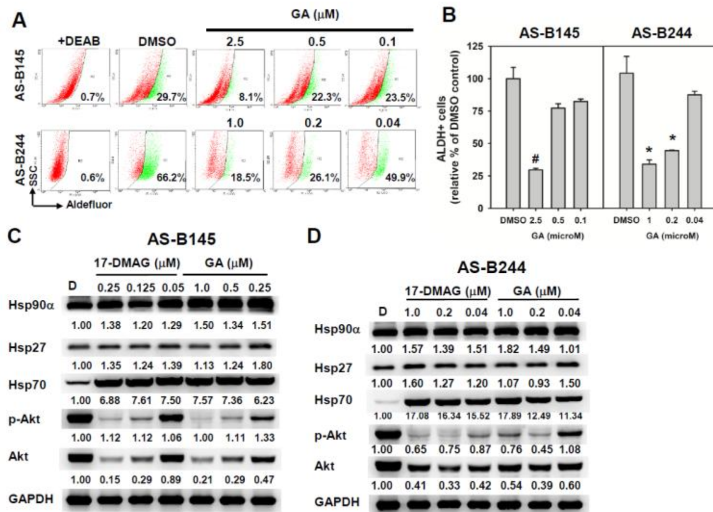


Fig.8. Hsp90 inhibitors suppress BCSCs.

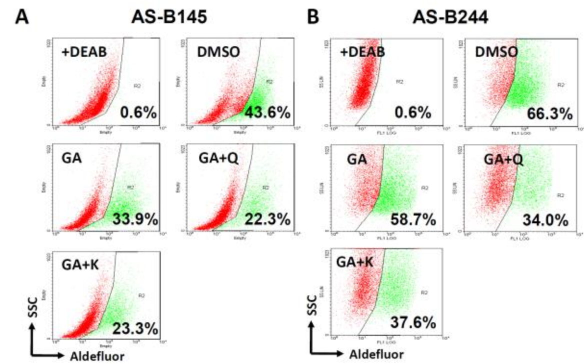


Fig.9. Combination of HSP inhibitors sensitizes BCSCs toward inhibition of GA.

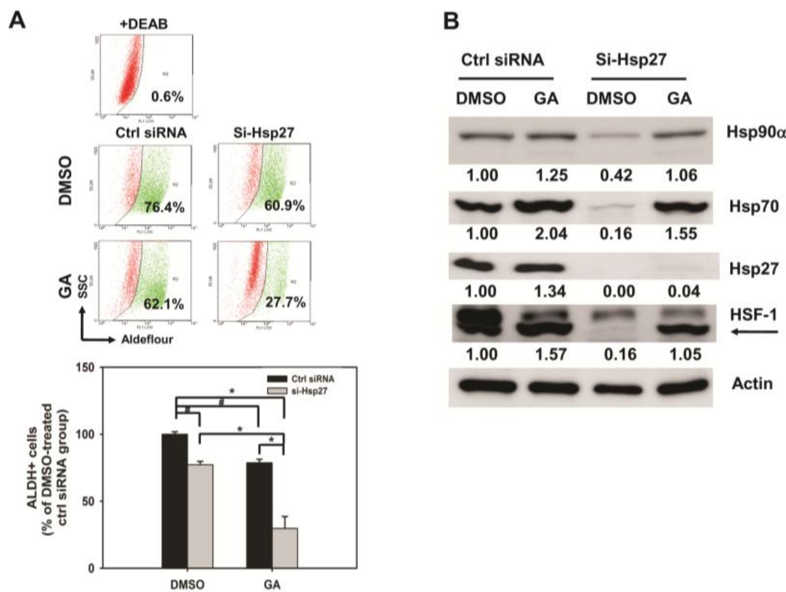


Fig.10. Knockdown of Hsp27 sensitizes BCSCs toward inhibition of GA.

五、討論與建議

本計畫原為 3 年期計畫，可惜最終只獲得一年補助，在有限的經費與時間中，我們仍舊繳出不錯的成績，產出兩篇 SCI 收錄之期刊，其中一篇發表在 Breast Cancer Research(IF=5.25)，是乳癌研究領域中的優秀期刊，並且在一年間已獲得 12 次引用(Google Scholar 資料)以及被下載次數超過 4000 次。希望未來國科會在計畫審查上能更重視研究計畫的未來性與前瞻性，讓這一類被國際重視的研究能夠獲得多年期補助，以期能夠完成所有計畫目標。

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：101 年 10 月 23 日

<table>
<tr><td>計畫編號</td><td colspan="3">NSC100－2314－B－040－013－</td></tr>
<tr><td>計畫名稱</td><td colspan="3">熱休克蛋白 27 與其客戶蛋白在乳癌幹細胞的角色</td></tr>
<tr><td>出國人員姓名</td><td>張文瑋</td><td>服務機構及職稱</td><td>中山醫學大學生物醫學科學系
副教授</td></tr>
<tr><td>會議時間</td><td>100 年 11 月 11 日至
100 年 11 月 13 日</td><td>會議地點</td><td>中國北京</td></tr>
<tr><td>會議名稱</td><td colspan="3">(中文)第四屆世界再生醫學與幹細胞會議
(英文)BIT's 4th Annual World Congress of Regenerative Medicine & Stem Cell</td></tr>
<tr><td>發表論文題目</td><td colspan="3">(中文)熱休克蛋白 27 藉由調控上皮-間質轉換以及核因子 kappa B 促進乳癌幹細胞的維持
(英文)Hsp27 participates in the maintenance of breast cancer stem cells through regulation of epithelial-mesenchymal transition and nuclear factor-kappa B</td></tr>
</table>

一、參加會議經過

本人於 2011.08.22 收到大會邀請信件，2011.08.23 同意接受邀請為會議講員，並於 2011.11.10 出發前往北京國際會議中心參加會議，並於 2011.11.13 日返抵台灣

二、與會心得

本會議為中國細胞分子學會委託BIT會議專辦公司舉辦之國際再生醫學暨幹細胞學

術研討會，由 BIT 公司以電子郵件方式邀請相關領域研究人員或產業界人士參加，皆為口頭發表論文的方式進行，並全程提供食宿，這類型會議目前除中國大陸外，加拿大與美國也有類似會議專辦公司承接各領域國際研討會，與過去多由各領域學會主辦之研討會稍有差異，主要以口頭報告為主，少了看板論文的部分，但由於包含餐會，多了與各研究人員或產業界人士交流的機會，不失為可以獲得人脈與知識的方式。其中，中央研究院幹細胞實驗室主持人游正博特聘研究員發表新穎的胚胎幹細胞及癌症幹細胞分子標記最為讓我印象深刻，主要原因除與我本身領域相同外，與游教授本是舊識也是一個。而北京國際會議中心鄰近京奧會場，讓人除了參加會議外，也可趁隙前往參觀。

三、考察參觀活動(無是項活動者略)

無

四、建議

這種模式的國際會議近來連學術界頂尖期刊 Cell 也開始採用，如果台灣採用同樣形式，委託會議專辦公司，在各種領域邀請國內外著名學者，主辦各領域相關國際會議，並配合相關台灣特色觀光行程，不但有助於提升國內學術水準，更能讓台灣國際能見度提高，有助於國內觀光產業的活絡。

五、攜回資料名稱及內容

1. 會議手冊一本(內含會議議程及論文摘要)

2. 大會紀念電腦提包一只

六、其他

BIT Life Sciences' 4th Annual Congress of

Regenerative Medicine & Stem Cell-2011

Theme: Smart Healing, Eternal Beauty and Longevity
Time: November 11-13, 2011
Venue: Beijing, China
Website: http://www.bitlifesciences.com/rmsc2011/default.asp

Official Letter

Dear Wen-Wei Chang,

BIT Life Sciences and Organizing Committee are organizing the 4th **Annual Congress of Regenerative Medicine & Stem Cell-2011** (RMSC-2011), which is designed to offer a platform for promoting international exchanges and strengthening cooperation in science and technology involved in regenerative medicine and stem cell, which offered excellent opportunities for participants to get the lasted research progress, seek cooperation and gain the new ideas through the communicate with international experts. The congress with a **theme** of "**Smart Healing, Eternal Beauty and Longevity**" will be held during November11-13, 2011 in Beijing, China. On behalf of the Organizing Committee, we are pleased to inform you that we have accepted your abstract and welcome you to give a speech at **S217: Circulating Tumor Cell (CTC) and Cancer Stem Cell** in our conference.

Regenerative Medicine & Stem Cell has a perspective to form a biotechnology industry chain with the development of regenerative medicine industry as the center, which will be one of the high-tech industries with the huge potential in 21 century. Through RMSC-2011, we hope to make some contributions to the development of Regenerative Medicine & Stem Cell!

The program will cover the following topics:

Forum 1: Outlook on Global Stem Cell and Tissue Engineering Projects

Forum 2: Basic Sciences and Innovative Technologies

Forum 3: Cell, Tissue and Biomaterial Development

Forum 4: Drug Discovery and Clinical Translation

Forum 5: Anti-aging, Cosmetic and Non-Medical Applications

Forum 6: Bioprocessing of Engineered Tissues and Stem Cells

Forum 7: Business Development

Forum 8: Young Scientist Research

Workshop & Training Courses

Poster Sessions and Exposition

Undoubtedly, the venue of RMSC-2011, Beijing, is an extremely attractive site for an international meeting, the excellent confusion of ancientness and modernness from China will amaze your experience for this visit!

For further details about the conference, please visit the meeting website at: http://www.bitlifesciences.com/rmsc2011/default.asp.

Sincerely yours,

Xiaodan Mei, Ph.D.
Executive Chair of Regenerative Medicine & Stem Cell-2011
East Area, F11, Building 1,
Dalian Ascendas IT Park,
1 Hui Xian Yuan,
Dalian Hi-tech Industrial Zone,
LN 116025, China
CC: Laura Chen E-mail: Laura@bitlifesciences.com

Title: Hsp27 participates in the maintenance of breast cancer stem cells through regulation of epithelial-mesenchymal transition and nuclear factor-kappa B

Wen-Wei Chang*, Hsiu-Huan Wang, Hui-Mei Hong, Alice L. Yu, Hsiang-Pu Feng
Assistant Professor
Chung Shan Medical University
Taiwan

Abstract

Heat shock proteins (HSPs) are normally induced under environmental stress to serve as chaperone for maintenance of correct protein folding but they are often overexpressed in many cancers including breast cancer. The expression of Hsp27 is associated with cell migration and drug resistance of breast cancer cells. Breast cancer stem cells (BCSCs) have been identified as a subpopulation of breast cancer cells with markers of high intracellular aldehyde dehydrogenase activity (ALDH+) and proved to be associated with radiation resistance and metastasis. However, the involvement of Hsp27 in the maintenance of BCSC is largely unknown. Here we found that Hsp27 and its phosphorylation were increased in ALDH+ BCSCs in comparison with ALDH- non-BCSCs with antibody array and western blot analysis. Knockdown of Hsp27 in breast cancer cells decreased characters of BCSCs, such as ALDH+ population, mammosphere formation, cell migration and the *in vivo* CSC frequency. Treatment of quercetin, a plant flavonoid inhibitor of Hsp27, could also inhibit ALDH+ cells and it be reversed by overexpression of Hsp27. Knockdown of Hsp27 also suppressed signatures of epithelial-mesenchymal transition (EMT), such as decreasing the expression of snail and vimentin and increasing the expression of E-cadherin. Furthermore, knockdown of Hsp27 decreased the nuclear translocation as well as the activity of NF-κB in ALDH+ BCSCs which resulted from increasing expression of IκBα and it could be reversed by restoring the activation of NF-κB by IκBα knockdown. In conclusion, our data suggest that Hsp27 regulates EMT process and NF-κB activity to contribute the maintenance of BCSCs. Targeting Hsp27 may be considered as a novel strategy in breast cancer therapy.

Key Words:

Biography

Wen-Wei Chang, male, cancer biologist, graduated from Institute of Basic Medical Sciences, National Cheng Kung University (Taiwan) in 2006. He worked as a post-doctoral fellow in Genomics Research Center, Academia Sinica (Taiwan) 2006-2009 and became an Assistant Professor in School of Biomedical Sciences, Chung Shan Medical University (Taiwan) from 2009 to present. His major interests are to understand the signal transduction in the maintenance of cancer stem cells and to discover anti-cancer stem cell agents from natural compounds.

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/23

<table>
<tr><td rowspan="3">國科會補助計畫</td><td>計畫名稱: 熱休克蛋白27與其客戶蛋白在乳癌幹細胞的角色</td></tr>
<tr><td>計畫主持人: 張文瑋</td></tr>
<tr><td>計畫編號: 100-2314-B-040-013- 學門領域: 幹細胞/再生生物醫學</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
</table>

100 年度專題研究計畫研究成果彙整表

計畫主持人：張文瑋	計畫編號：100-2314-B-040-013-
計畫名稱：熱休克蛋白 27 與其客戶蛋白在乳癌幹細胞的角色	

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	1	1	100%		國科會成果報告(精簡版)
		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	0	0	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	2	2	100%	篇	2011 年發表於 Breast Cancer Research 期刊論文已被引用 12 次(Google Scholar 資料)，並原文已被下載超過 4000 次
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		受邀至第四屆世界再生醫學與幹細胞會議進行口頭論文發表
		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。)		本研究之成果已發表在兩本 SCI 收錄之國際期刊，其中發表在 Breast Cancer Research 之文章截至 2012.10.23 已被引用 12 次(資料來自於 Google 學術搜尋引擎)，在原始期刊網站之統計，本文章已經被下載 4357 次，具有極高的引用率，顯示此研究成果對於乳癌幹細胞研究領域有所貢獻。此外，另一成果發表於 Biochimie 後，收到相關論文投稿審稿要求，為國外研究人員看到本實驗室成果後，進一步以臨床檢體驗證，顯示該研究成果的確具有臨床意義，也再次說明本實驗室研究主題極具國際能見度。					

	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：

   原計畫為申請三年，但最終只獲得一年補助，在有限經費與時間下，僅能完成部分研究目標，但仍有相當的研究產出，期望未來能獲得更多的經費支持

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）

   本研究之成果已發表在兩本 SCI 收錄之國際期刊，其中發表在 Breast Cancer Research 之文章截至 2012.10.23 已被引用 12 次(資料來自於 Google 學術搜尋引擎)，在原始期刊網站之統計，本文章已經被下載 4357 次，具有極高的引用率，顯示此研究成果對於乳癌幹細胞研究領域有所貢獻。此外，另一成果發表於 Biochimie 後，即收到相關論文投稿審稿要求，為國外研究人員看到本實驗室成果後，進一步以臨床檢體驗證，顯示該研究成果的確具有臨床意義，也再次說明本實驗室研究主題極具國際能見度。