科技部補助專題研究計畫成果報告
期末報告

熱休克蛋白27與其磷酸化對於乳癌幹細胞之維持的影響(第3年)

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 103-2314-B-040-015-MY3
執 行 期 間 ： 105年08月01日至106年07月31日
執 行 單 位 ： 中山醫學大學生物醫學科學學系（所）

計 畫 主 持 人： 張文瑋
共 同 主 持 人： 張為超
計畫參與人員： 學士級-專任助理：王惠琳
碩士班研究生-兼任助理：張庭瑜
碩士班研究生-兼任助理：陳裕益

報 告 附 件 ： 出席國際學術會議心得報告

中 華 民 國 106 年 10 月 29 日

中 文 摘 要 ： 熱休克蛋白(heat shock proteins, Hsps)的功能主要為維持細胞內蛋白質穩定正確結構，這些熱休克蛋白通常在細胞遭受環境壓力時會大量表現。Hsps也被發現在許多癌症組織以及癌細胞中有過度表現的現象，在乳癌細胞中，Hsp27的表現與細胞的移動侵襲以及抗藥性能力有關。近年來在乳癌研究中發現存在一群獨特的乳癌幹細胞，具有起始腫瘤生成以及分化的能力，並且具有抗藥性以及轉移的特性，這些特別的細胞可利用螢光分子標記以流式細胞儀進行細胞分選或以乳腺小球(mammosphere)培養取得。目前這群乳癌幹細胞被認為是重要的治療標的。在過去的研究中，我們先前實驗已證實乳癌幹細胞具有較高量的Hsp27以及其磷酸化態表現，並已證明Hsp27可透過調節上皮-間質轉換以及核因子B的活性，進而調節乳癌幹細胞的自我更新與致癌性(Wei L, et al. Breast Cancer Res. 2011. 13:R101)。我們也證明IGF-1R訊息路徑可調控乳癌幹細胞的自我更新(Chang WW. et al., Breast Cancer Res. 2013. 15:R39)。在本計畫第一年中，我們發現Hsp27之磷酸化是乳癌幹細胞維持自我更新的重要關鍵。在第二年計畫中，我們進一步發現魚針草內酯(ovatodiolide)可誘導SMAD Specific E3 Ubiquitin Protein Ligase 2，進而抑制乳癌幹細內Hsp27的表現，達到抑制乳癌幹細胞自我更新的效果，此成果已發表於Toxins期刊(Lu KT, et al., Toxins 2016. 8(5):127)。同時，我們也發現乳癌細胞過度表現野生型Hsp27時能增加細胞內FAK的表現，而Hsp27之磷酸化死亡型則不具這樣的現象，而Hsp27磷酸化死亡突變型無法與FAK進行交互作用，顯示Hsp27的磷酸化可能具有調節乳癌細胞內FAK表現的功能。在第三年計畫中，我們利用帶有myc-tag的Hsp27相關慢病毒載體建立穩定表現Hsp27野生型、磷酸化死亡型及磷酸化模擬型之乳癌細胞株，並結合免疫沉澱法與蛋白質體技術，發現XRCC1或MeCP2與Hsp27的交互作用會受到Hsp27磷酸化狀態的影響，並且XRCC1或MeCP2在乳癌細胞內的表現會因為過度表現Hsp27磷酸化死亡型而降低；此外，當乳癌細胞過度表現Hsp27磷酸化死亡型時，其癌幹細胞活性下降，並同時使其活體致癌力降低;利用斑馬魚異體腫瘤模式，我們也發現當BT-474乳癌細胞表現Hsp27磷酸化模擬型時，其轉移能力明顯增加，顯示Hsp27的磷酸化能導致乳癌細胞轉移。從此三年期計畫，我們證明Hsp27磷酸化確實影響乳癌幹細胞維持，而XRCC1或MeCP2可能是關鍵因子，但詳細機制有待未來繼續研究。

中 文 關 鍵 詞 ： 乳癌幹細胞；熱休克蛋白27; 磷酸化; 自我更新; 蛋白質交互作用; 癌症轉移

英 文 摘 要 ： Heat shock proteins (Hsps) are a group of proteins which express under environmental stress to serve as a chaperone for maintenance of corrected protein folding. Hsps have been reported to be overexpressed in many cancers including breast cancer. Breast cancer stem cells (BCSCs) could be enriched by fluorescence-based cell sorting or by mammosphere cultivation. These particular breast cancer cells have been proved to be associated with radiation resistance and metastasis. Recently, we have demonstrated that Hsp27 plays a role in the maintenance, tumorigenicity

and drug resistance of BCSCs (Wei L, et al. Breast Cancer Res. 2011. 13:R101.; Lee CH, et al. Biochimie. 2012. 94:1382). We also found that Hsp27 phosphorylation was increased in ALDH+ BCSCs. We also have demonstrated that IGF-1R is a novel marker for BCSCs and its signal regulates self-renewal of BCSCs (Chang WW. et al., Breast Cancer Res. 2013. 15:R39). In the first year of project, we discovered that the phosphorylation of Hsp27 is the key in the maintenance of BCSCs. In the second year of this project, we found that ovatodiolide could suppress Hsp27 expression through the upregulation of SMAD Specific E3 Ubiquitin Protein Ligase 2 to inhibit the self-renewal capability of breast cancer stem cells. These works have been published in Toxins (Lu KT, et al., Toxins 2016. 8(5):127). We also found that the overexpression of wildtype Hsp27, but not phosphor-dead mutant form, upregulated FAK expression in breast cancer cells. Furthermore, the interaction between Hsp27 and FAK was lost when the phosphorylation sites of Hsp27 was mutated. In the last year of project, we used lentiviral transduction to overexpress myc-tagged Hsp27 constructs to investigate the Hsp27 interacting proteins associated with its phosphorylation status and found that XRCC1 or MeCP2 was the potential targets. The expression of XRCC1 or MeCP2 was down-regulated in breast cancer cells with Hsp27 phosphor-dead overexpression. We also demonstrated that the self-renewal capability of breast cancer cells with Hsp27 phosphor-dead overexpression was diminished, as well as the in vivo tumorigenicity. Using zebrafish as a tumor xenotransplatation model, we found that BT-474 cells with Hsp27 phosphor-mimic Hsp27 overexpression obviously increased their metastatic capability. From this three-year-grant, we clearly demonstrated that the phosphorylation of Hsp27 is important for the maintenance and tumorigenicity of BCSCs which may involves in the interaction with XRCC1 or MeCP2. The detail mechanisms of Hsp27 phosphorylation and the involving interacting proteins remains to be further investigated in the future.

英文關鍵詞：Breast cancer stem cells; heat shock protein 27; phosphorylation; self-renewal; metastasis; protein-protein interaction

中文摘要

熱休克蛋白(heat shock proteins, Hsps)的功能主要為維持細胞內蛋白質穩定正確結構，這些熱休克蛋白通常在細胞遭受環境壓力時會大量表現。Hsps 也被發現在許多癌症組織以及癌細胞中有過度表現的現象，在乳癌細胞中，Hsp27 的表現與細胞的移動侵襲以及抗藥性能力有關。近年來在乳癌研究中發現存在一群獨特的乳癌幹細胞，具有起始腫瘤生成以及分化的能力，並且具有抗藥性以及轉移的特性，這些特別的細胞可利用螢光分子標記以流式細胞儀進行細胞分選或以乳腺小球(mammosphere)培養取得。目前這群乳癌幹細胞被認為是重要的治療標的。在過去的研究中，我們先前實驗已證實乳癌幹細胞具有較高量的 Hsp27 以及其磷酸化態表現，並已證明 Hsp27 可透過調節上皮-間質轉換以及核因子κB 的活性，進而調節乳癌幹細胞的自我更新與致癌性(**Wei L, et al. Breast Cancer Res. 2011. 13:R101**)。我們也證明 IGF-1R 訊息路徑可調控乳癌幹細胞的自我更新(**Chang WW. et al., Breast Cancer Res. 2013. 15:R39**)。在本計畫第一年中，我們發現 Hsp27 之磷酸化是乳癌幹細胞維持自我更新的重要關鍵。在第二年計畫中，我們進一步發現魚針草內酯(ovatodiolide)可誘導 SMAD Specific E3 Ubiquitin Protein Ligase 2，進而抑制乳癌幹細內 Hsp27 的表現，達到抑制乳癌幹細胞自我更新的效果，此成果已發表於 Toxins 期刊(**Lu KT, et al., Toxins 2016. 8(5):127**)。同時，我們也發現乳癌細胞過度表現野生型 Hsp27 時能增加細胞內 FAK 的表現，而 Hsp27 之磷酸化死亡型則不具這樣的現象，而 Hsp27 磷酸化死亡突變型無法與 FAK 進行交互作用，顯示 Hsp27 的磷酸化可能具有調節乳癌細胞內 FAK 表現的功能。在第三年計畫中，我們利用帶有 myc-tag 的 Hsp27 相關慢病毒載體建立穩定表現 Hsp27 野生型、磷酸化死亡型及磷酸化模擬型之乳癌細胞株，並結合免疫沉澱法與蛋白質體技術，發現 XRCC1 或 MeCP2 與 Hsp27 的交互作用會受到 Hsp27 磷酸化狀態的影響，並且 XRCC1 或 MeCP2 在乳癌細胞內的表現會因為過度表現 Hsp27 磷酸化死亡型而降低；此外，當乳癌細胞過度表現 Hsp27 磷酸化死亡型時，其癌幹細胞活性下降，並同時使其活體致癌力降低；利用斑馬魚異體腫瘤模式，我們也發現當 BT-474 乳癌細胞表現 Hsp27 磷酸化模擬型時，其轉移能力明顯增加，顯示 Hsp27 的磷酸化能導致乳癌細胞轉移。從此三年期計畫，我們證明 Hsp27 磷酸化確實影響乳癌幹細胞維持，而 XRCC1 或 MeCP2 可能是關鍵因子，但詳細機制有待未來繼續研究。

英文摘要

Heat shock proteins (Hsps) are a group of proteins which express under environmental stress to serve as a chaperone for maintenance of corrected protein folding. Hsps have been reported to be overexpressed in many cancers including breast cancer. Breast cancer stem cells (BCSCs) could be enriched by fluorescence-based cell sorting or by mammosphere cultivation. These particular breast cancer cells have been proved to be associated with radiation resistance and metastasis. Recently, we have demonstrated that Hsp27 plays a role in the maintenance, tumorigenicity and drug resistance of BCSCs (Wei L, et al. Breast Cancer Res. 2011. 13:R101.; Lee CH, et al. Biochimie. 2012. 94:1382). We also found that Hsp27 phosphorylation was increased in ALDH+ BCSCs. We also have demonstrated that IGF-1R is a novel marker for BCSCs and its signal regulates self-renewal of BCSCs (**Chang WW. et al., Breast Cancer Res. 2013. 15:R39**). In the first year of project, we discovered that the phosphorylation of Hsp27 is the key in the maintenance of BCSCs. In the second year of this project, we found that ovatodiolide could suppress Hsp27 expression through the upregulation of SMAD Specific E3 Ubiquitin Protein Ligase 2 to inhibit the self-renewal capability of breast cancer stem cells. These works have been published in *Toxins* (**Lu KT, et al., Toxins 2016. 8(5):127**). We also found that the overexpression of wildtype Hsp27, but not phosphor-dead mutant form, upregulated FAK expression in breast cancer cells. Furthermore, the interaction between Hsp27 and FAK was lost when the phosphorylation sites of Hsp27 was mutated. In the last year of project, we used lentiviral transduction to overexpress myc-tagged Hsp27 constructs to investigate the Hsp27 interacting proteins associated to its phosphorylation status and found that XRCC1 or MeCP2 was the potential targets. The expression of XRCC1 or MeCP2 was down-regulated in breast cancer cells with Hsp27 phosphor-dead overexpression. We also demonstrated that the self-renewal capability of breast cancer cells with Hsp27 phosphor-dead overexpression was diminished, as well as the in vivo tumorigenicity. Using zebrafish as a tumor xenotransplatation model, we found that BT-474 cells with Hsp27 phosphor-mimic Hsp27 overexpression obviously increased their metastatic capability. From this three-year-grant, we clearly demonstrated that the phosphorylation of Hsp27 is important for the maintenance and tumorigenicity of BCSCs which may involves in the interaction with XRCC1 or MeCP2. The detail mechanisms of Hsp27 phosphorylation and the involving interacting proteins remains to be further investigated in the future.

I. Introduction

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 [1]. 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 [2, 3].The most studied Hsps are Hsp27, Hsp70 and Hsp90. Hsp70 and Hsp90 are large Hsps which are ATP-dependent chaperones and Hsp27 belongs to small heat shock proteins which are ATP-independent fashion. Hsps are widely known about their cytoprotection functions in cancer cells [4]. The mechanisms include their molecular chaperone activity, anti-apoptosis function and influence of the stability of client proteins [5]. A lot of Hsp27 client proteins have been reported previously [6]. For example, Hsp27 binds with cytochrome c to inhibit apoptosis. Hsp27 has been found to contribute to the malignant properties of cancer cells including increased tumorigenicity, treatment resistance and apoptosis inhibition [7]. In breast cancer, Hsp27 has been reported as a risk factor of malignant progression in benign proliferating breast lesions [8] and its expression could help to differentiate benign and malignant breast lesions in fine needle aspirate [9]. Our previous study demonstrated that knockdown of Hsp27 in breast cancer cells/ALDH+ breast cancer stem cells could decrease snail expression, a transcriptional repressor of E-cadherin, to inhibit epithelial-mesenchymal transition (EMT) [10]. Cancers consist of varieties of cell types such as cancer cells themselves, stroma cells, endothelial cells, immune cells, etc. and are thought as tissues with heterogeneity[11]. There are also phenotypic and functional diversity among cancer cells. In the past two decades, the identification of cancer stem cells (CSCs) and the power of these cells in establishment of new tumors when experimental implant in animal hosts[12], CSCs have got many attentions in the field of cancer research. The definitions of CSCs are a sub-population of cancer cells with the properties of tumor initiation and their capability of asymmetric division to propagate CSCs themselves and the differentiated daughter cells to form the bulk of the tumor[13], which is similar to the normal stem cells to differentiate into multiple cell types within a tissue or organ. It also has been widely demonstrated that CSCs hijack the cellular mechanisms in the maintenance of self-renewal property of normal stem cells to maintain the high tumorigenicity of these particular cancer cells[14]. As the increasing relapse rate of cancers toward current chemo- or radiotherapy and the link between CSCs and drug resistance of cancers, to target CSCs have been considered as the key for successful cancer treatment[15]. In breast cancer, CSCs could be identified with surface markers $CD24^{-}CD44^{+}$[16], intracellular aldehyde dehydrogenase (ALDH) activity[17] or mammospheres that proliferate as spheroids in a non-adherence and serum-free condition[18, 19].

II. Results

A. Ovatodiolide Inhibits Breast Cancer Stem/Progenitor Cells through SMURF2-Mediated Downregulation of Hsp27

I. Ovatodiolide Inhibited Self-Renewal Capability of BCSCs

Ovatodiolide (Ova) is a macrocyclic diterpenoid compound extracted from *Anisomeles indica* (L.) Kuntze [20] with activities of anti-inflammation [21], anti–*Helicobacter pylori* [22], dermatological whitening [23],

and anti-neoplasm [24-27].We applied the mammosphere assay to evaluate the anti-self-renewal activity of ovatodiolide (Ova). AS-B145 or BT-474 cells were cultivated into primary mammospheres in the presence of Ova at the concentration of 1 or 4 μM, which was below the IC_{50} value in the proliferation inhibition effect, and the self-renewal capability of primary spheres was determined by the formation of secondary mammospheres without Ova treatment. As shown in Figure 1, Ova dose-dependently inhibited the formation of the secondary mammosphere of AS-B145 (Figure 1A) and BT-474 (Figure 1B). The CD24-CD44+ BCSCs were also analyzed in AS-B145 or BT-474 sphere cells. After treatment of Ova at a concentration of 4 μM, the population of CD24-CD44+ cells in mammospheres of AS-B145 (Figure 1C) or BT-474 (Figure 1D) was decreased (from 99.8% to 48.5% for AS-B145 and from 87.1% to 29.9% for BT-474). From these results, Ova displayed an anti-self-renewal activity in BCSCs.

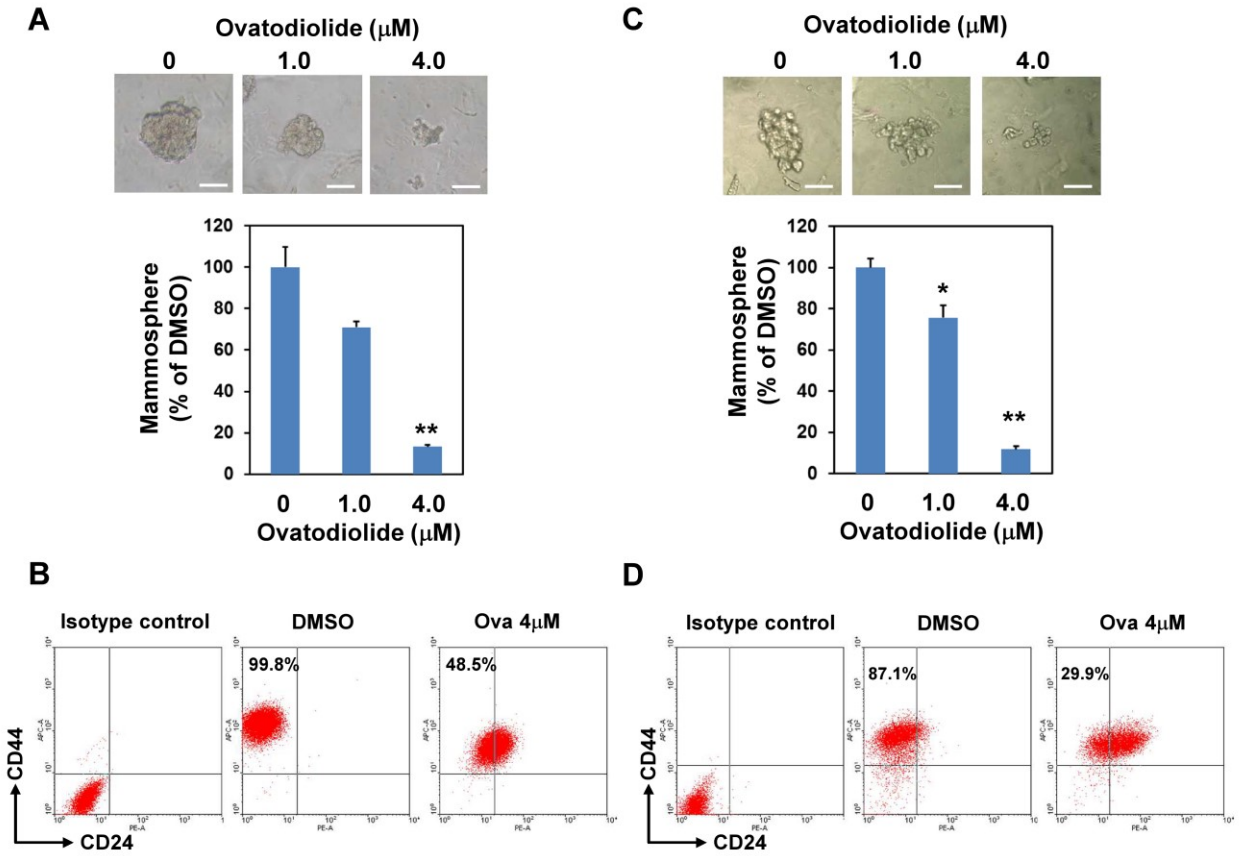


Figure 1. Ovatodiolide suppresses the self-renewal property of BCSCs. AS-B145 (A) or BT-474 (B) cells were seeded into ultralow attachment in a six-well plate under 0.1% DMSO or different concentrations of ovatodiolide (1 or 4 μM) for seven days and the formed primary mammospheres were collected and dissociated into a single cell suspension. The same number of dissociated primary sphere cells was used to evaluate the effect of ovatodiolide on the self-renewal property of BCSCs by secondary mammosphere formation without treatment of ovatodiolide (n = 3 for each treatment). The experiments were repeated two times and results from a representative experiment were presented. Data were presented as relative percentage of DMSO control. Scale bar = 50 μm. *, $p < 0.05$; **, $p < 0.01$. The mammosphere cells of AS-B145 (C) or BT-474 (D) were harvested and dissociated into a single-cell suspension. CD24-CD44+ cells were analyzed by flow cytometry.

II. Ovatodiolide Downregulated the Expression of Stemness Genes and Hsp27 but Upregulated SMURF2 Expression

We next examined the effect of Ova on the expression of stemness genes. With Western blot analysis, Ova dose-dependently inhibited the expression of Oct4 (Figure 2C) and Nanog (Figure 2D) in mammosphere cells derived from AS-B145 (Figure 2A) or BT-474 (Figure 2B) and the inhibitory effect was significantly observed at a concentration of 4 μM. We previously demonstrated that Hsp27 regulated the self-renewal and tumorigenicity of BCSCs through modulating EMT and NF-κB activity [10]. The effect of Ova in Hsp27 expression in mammosphere cells was examined. As shown in Figure 3, Ova dose-dependently downregulated Hsp27 expression (Figure 2E) in mammosphere cells derived from AS-B145 (Figure 3A) or BT-474 (Figure 3B). However, the mRNA expression of Hsp27 in mammosphere cells of AS-B145 or BT-474 was not inhibited by Ova treatment. A previous report indicated that SMURF2 mediated the ubiquitin-dependent degradation of Hsp27 in A549 lung cancer cells [28]. We further examined the expression of SMURF2 in mammosphere cells after Ova treatment and results revealed that Ova dose-dependently upregulated SMURF2 expression (Figure 2F) in AS-B145 (Figure 2A) or BT-474 (Figure 2B).

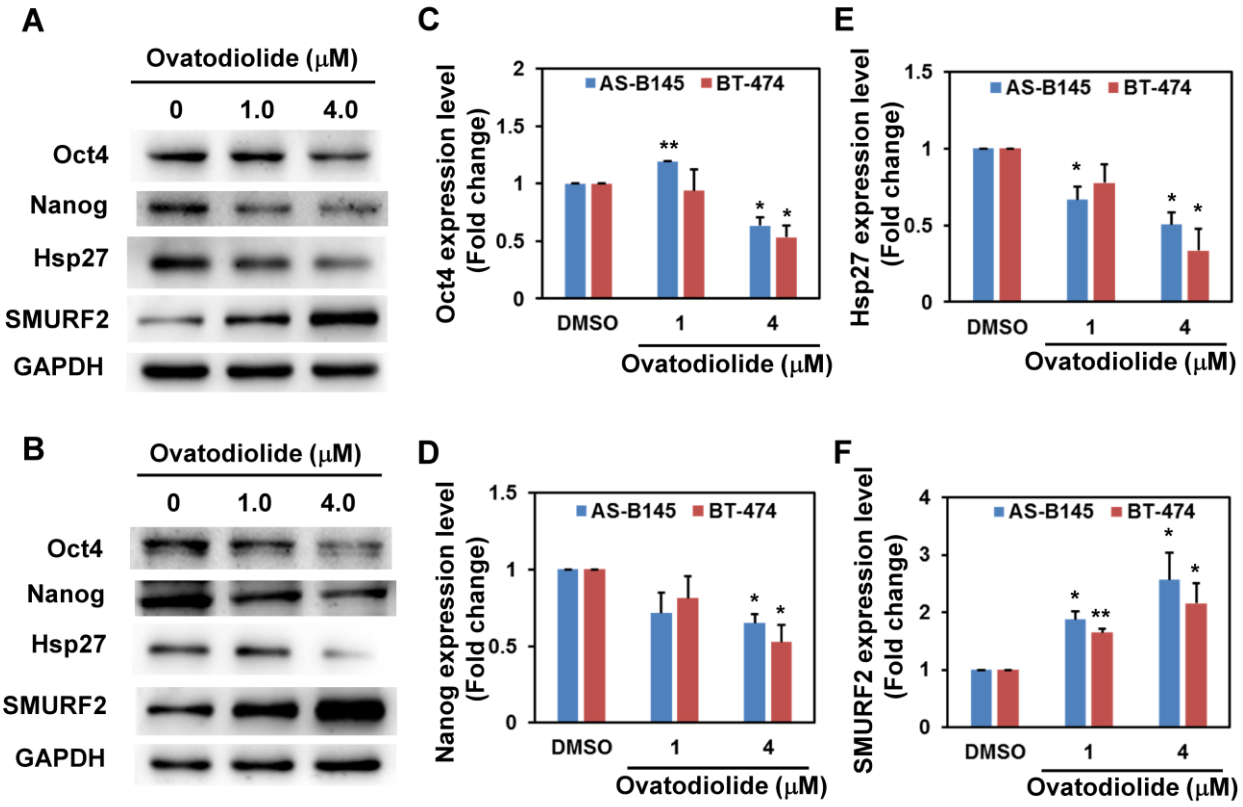


Figure 2. The change of protein expression in ovatodiolide-treated BCSCs. BCSCs were first enriched by primary mammosphere cultivation from AS-B145 (A) or BT-474 (B), dissociated into a single-cell suspension, and treated with different concentrations of ovatodiolide (0, 1, 4 μM) for 72 h (n = 2 for each treatment). The expressions of Oct4, Nanog, Hsp27 and SMURF2 were determined by Western blot. The quantification results of Oct4 (C), Nanog (D), Hsp27 (E) and SMURF2 (F) were determined by Image J software. The experiments were repeated three times and results from two representative experiments were used for quantifications. * $p < 0.05$; ** $p < 0.01$.

III. Overexpression of Hsp27 or Knockdown of SMURF2 Alleviated the Inhibitory Effect of Ovatodiolide

We next examined if overexpression of Hsp27 could alleviate the inhibitory effect of Ova on the mammosphere formation capability of AS-B145 cells. Overexpression of Hsp27 in AS-B145 or BT-474 cells was performed by lentivirus-mediated gene delivery and confirmed by Western blot (Figure 3A). Exogenous Hsp27 expression in AS-B145 (Figure 3B,C) or BT-474 (Figure 3D,E) significantly diminished the suppressive activity of Ova on mammosphere formation at a concentration of 4 μM ($p = 2.9\times10^{-6}$ for AS-B145 and $p = 1.6\times10^{-4}$ for BT-474). We further performed the knockdown of SMURF2 in AS-B145 or BT-474 cells by lentivirus delivery of two independent shRNA clones and both clones efficiently knocked down the mRNA expression of SMURF2 (Figure 4A). The inhibitory effect of Ova in mammosphere formation was alleviated in both sh-SMURF2 clone-transduced AS-B145 or BT-474 cells (Figure 4B,C). We also analyzed the protein expression of Hsp27 and SMURF2 in shRNA-carrying lentviruse-transduced AS-B145 or BT-474 mammosphere cells after Ova treatment. As shown in Figure 4D, these two sh-SMURF2 lentiviruses efficiently knocked down SMURF2 protein expression in AS-B145 and BT-474 mammosphere cells and alleviated the inhibitory effect of Ova in Hsp27 protein expression (Figure 4D). These results suggest the suppressive effect of Ova in the self-renewal of BCSCs through the SMURF2-mediated downregulation of Hsp27 expression.

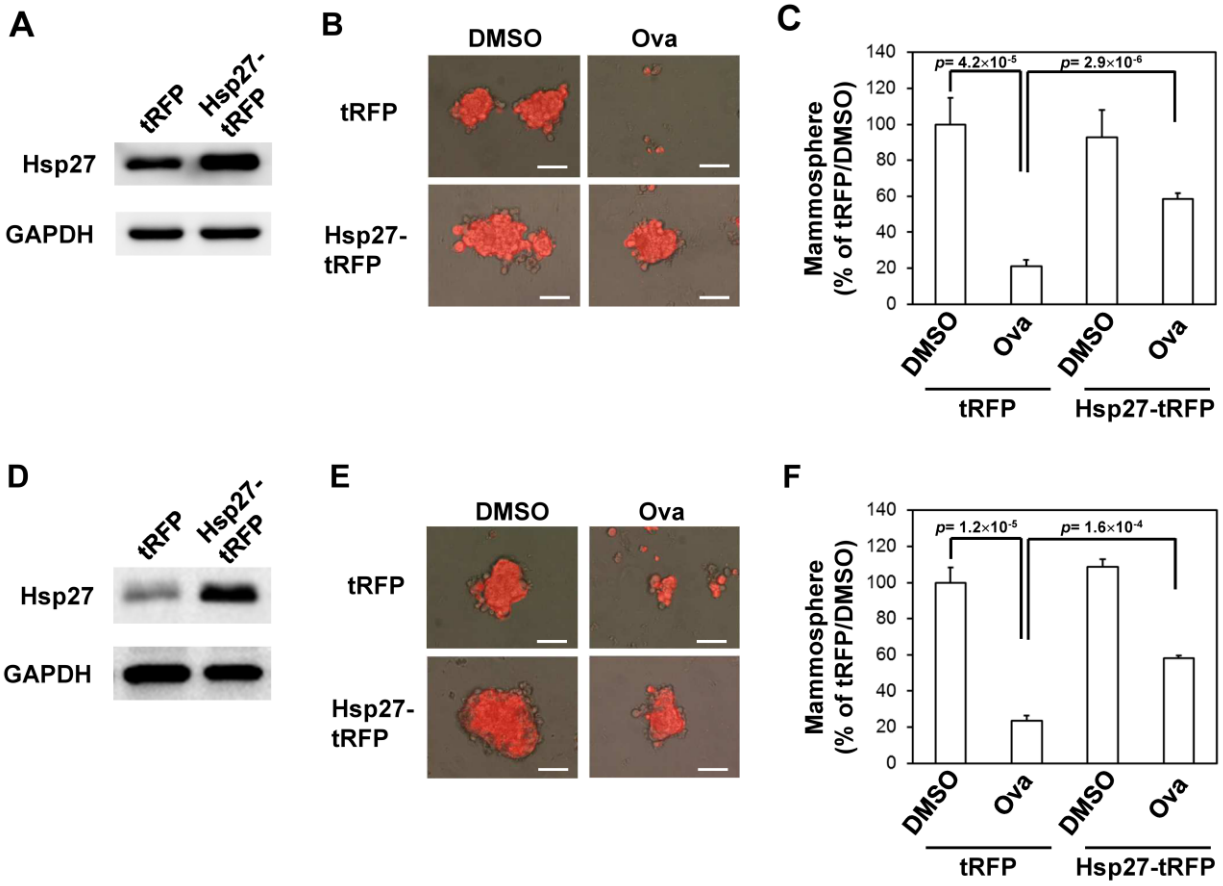


Figure 3. Overexpression of Hsp27 diminishes the inhibitory effect of ovatodiolide on the self-renewal capability of BCSCs. AS-B145 or BT-474 cells were transduced with tRFP or Hsp27-tRFP lentivirus and selected by 20 μg/mL blasticidin S for one week. The overexpression of Hsp27 was confirmed by Western blot (A for AS-B145 and D for BT-474). BCSCs were first enriched by primary mammosphere cultivation

from tRFP- or Hsp27-overexpressed cells, dissociated into a single-cell suspension, and underwent secondary mammosphere formation under treatment with 4 μM ovatodiolide (Ova) or 0.1% DMSO (n = 3 for each treatment). Formed mammospheres were pictured (B for AS-B145 and E for BT-474) and were counted at Day 7 and displayed as the relative percentage of the DMSO group (C for AS-B145 and F for BT-474). The experiments were repeated two times and results from a representative experiment were presented. Scale bar = 50 μm.

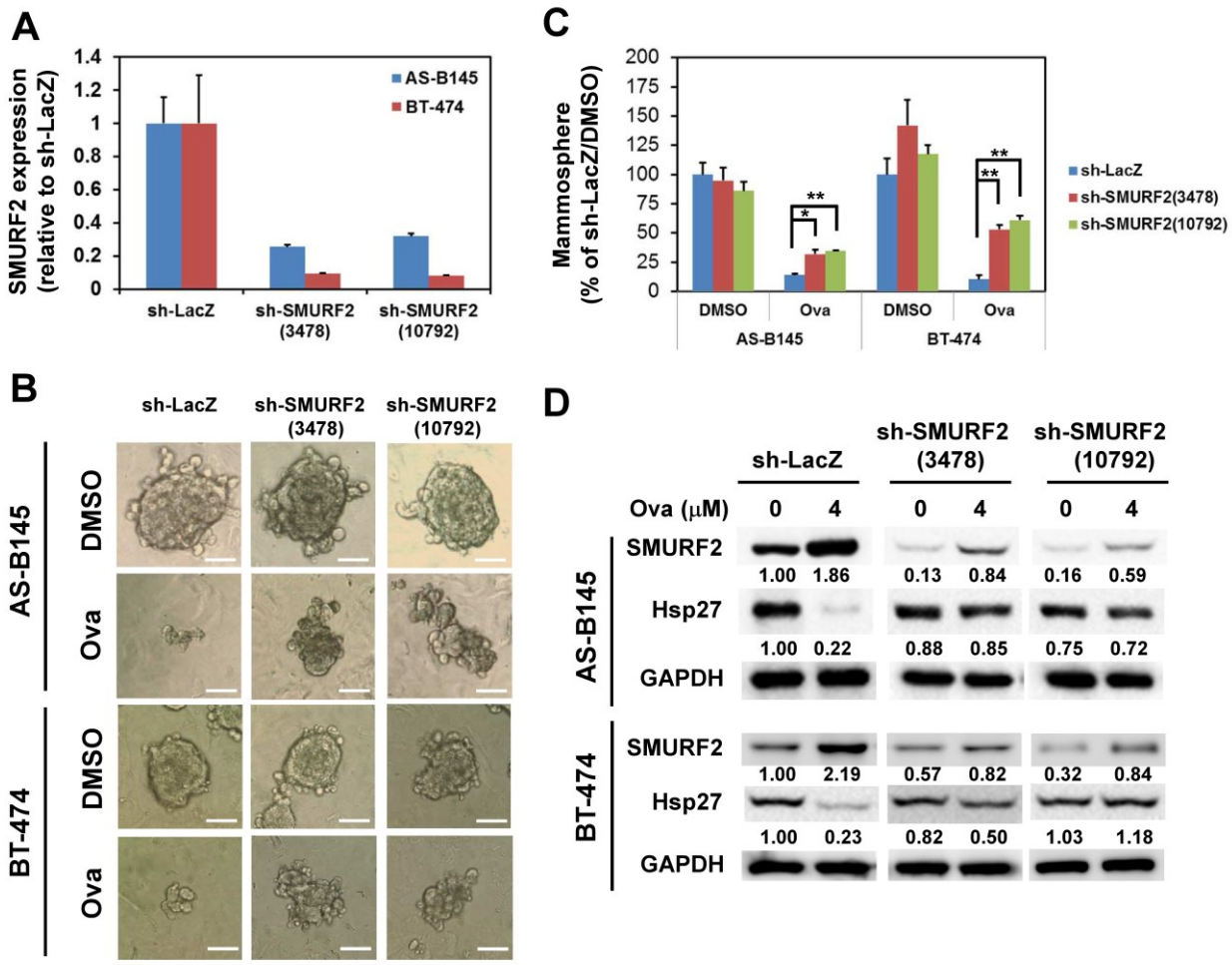


Figure 4. Knockdown of SMURF2 alleviates the suppressive effect of ovatodiolide on the self-renewal capability of BCSCs. AS-B145 or BT-474 cells were transduced with shellacs, sh-SMURF2(3478), or sh-SMURF2(10792) carrying lentivirus and selected by 2 μg/mL puromycin for three days. The knockdown efficiency was determined by qRT-PCR (**A**). After puromycin selection, the surviving cells were first cultured for primary mammosphere formation. The self-renewal capability of primary mammospheres under 4 μM ovatodiolide (Ova) or 0.1% DMSO was determined by the formation of secondary mammospheres (n = 3 for each treatment). Formed secondary mammospheres were pictured (**B**) and were counted at Day 7 and displayed as the relative percentage of the DMSO-treated sh-LacZ group (**C**). Scale bar = 50 μm. *, $p < 0.05$; **, $p < 0.01$. The expression of Hsp27 or SMURF2 was further determined by Western blot (**D**). The experiments were repeated two times and results from a representative experiment were presented.

B. Hsp27 phosphorylation regulates the expression of FAK in breast cancer cells

We successfully established breast cancer cells with stable expression of myc-tagged wildtype Hsp27

(wtHsp27) or phosphor-dead Hsp27 (Hsp27A) in MDA-MB-231 human triple negative breast cancer cells. We next investigated the effect of Hsp27 phosphorylation on the expression of FAK in MDA-MB-231 breast cancer cells. Overexpression of wtHsp27, but not Hsp27A which is the phosphor-dead mutant form of Hsp27, regulated FAK expression (Fig. 5A). We further found that the Hsp27 phosphor-dead mutant of Hsp27 lack the interaction activity with FAK (Fig. 5B).

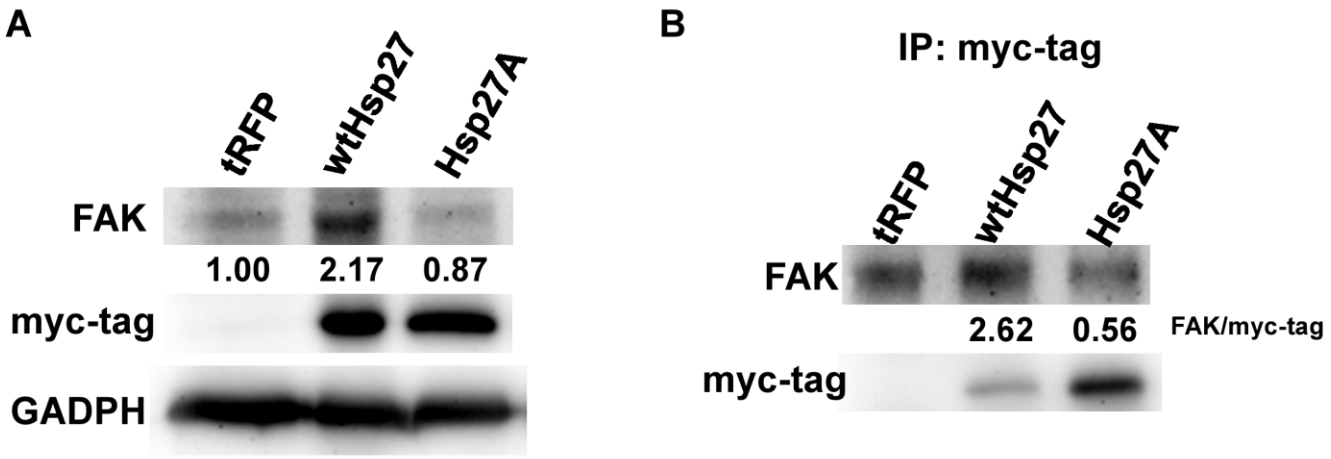


Figure 5. Hsp27 phosphorylation regulates the expression of FAK in breast cancer cells. MDA-MB-231 human breast cancer cells were transduction with myc-tagged wildtype Hsp27 (wtHsp27) or phosphor-dead Hsp27A mutant (Hsp27A) lentivirus and selected with 20 μg/ml blasticidin S for at least 1 week. (A) Total cell proteins were separated by 12% SDS-PAGE and transferred onto PVDF membrane. The expression of myc-tagged wtHsp27 or Hsp27A or FAK protein was detected by anti-myc-tag or anti-FAK antibody. GAPDH was used as protein loading control. (B) The total proteins of tRFP, wtHsp27, or Hsp27A transduced cells were used for immunoprecipitation of myc-tagged proteins and the expression of FAK within pull-down proteins was detected by anti-FAK antibody.

C. Seeking the interacting proteins regarding Hsp27 phosphorylation

We successfully used lentiviral transduction methods to establish human breast cancer cells (MDA-MB-231, Hs578t or AS-B244) with overexpression of myc-tagged Hsp27 phosphorylation mutants (Fig. 5). By sepharose beads immunoprecipitation method, we pulled down the myc-tagged Hsp27 proteins by anti-myc antibody and performed mass spectrometry (MS) analysis with the help of the Co-PI, Dr. Wen-Chao Chang in China Medical University. We used the selection criteria: the identified proteins with expression level more than 1 when performed a comparison between Hsp27D and WTHsp27 (27D/WT >1) and less than 1 when a comparison between Hsp27A and WtHsp27 (27A/WT<1), there were 28 proteins being identified (Table 1). Two proteins, XRCC1 and MeCP2, got our attention because of the association with cancer progression [29, 30] or resistance to treatment [31, 32]. By western blot analysis, we found that the expression of MeCP2 or XRCC1 in MDA-MB-231 cells was decreased when cells overexpressed Hsp27A, the phosphor-dead mutant form (Fig, 6).

Table 1. The mass spectrometry analysis of putative interacting proteins with phosphorylated Hsp27.

27D/WT >1 and 27A/WT <1

Gene Name	Avg-27A	Avg-27D	Avg-wt	Avg-tRFP	27A/wt	27D/wt	tRFP/wt
S100A8	1270500	3805200	1894050	3784000	0.67	2.01	2.00
TGM1	11271	173600	19042	34737	0.59	9.12	1.82
PYGB	0	5054	2165	204975	0.00	2.33	94.66
SPRR2E	0	530915	235180	0	0.00	2.26	0.00
S100A14	0	1644200	624050	1209230	0.00	2.63	1.94
POM121	34403	452945	36477	0	0.94	12.42	
PAXIP1	12769	333430	38031	0	0.34	8.77	
MPG	25454	135606	100750	0	0.25	1.35	
TERF2	4025	226022	16603	0	0.24	13.61	
KIF18B	2627	276610	13356	0	0.20	20.71	
PPP2R2B	3126	262760	21295	0	0.15	12.34	
TTC9C	0	311535	280795	0	0.00	1.11	
S100A13	0	228085	137482	0	0.00	1.66	
XRCC1	0	193085	109470	0	0.00	1.76	
CTDSPL2	0	528665	233025	0	0.00	2.27	
TGM1	0	81416	33809	0	0.00	2.41	
SLC1A5	0	162585	67330	0	0.00	2.41	
ZNF280C	0	63805	20674	0	0.00	3.09	
MECP2	0	204040	58395	0	0.00	3.49	
MYH3	0	82622	15341	0	0.00	5.39	
TMEM209	0	425265	53020	0	0.00	8.02	
BAZ2A	0	203120	24639	0	0.00	8.24	
AHDC1	0	32744	3042	0	0.00	10.76	
MORC2	0	80415	675	0	0.00	119.05	
RLF	0	32412	2922	1334	0.00	11.09	
HIST1H2BN			1605440	0	0.87	3.26	0.00
HIST1H2BO			172970	0	0.83	6.98	0.00
FN1			66010	0	0.78	3.19	0.00
PLEC			3583	1731	0.78	3.23	0.48
EEF2			7959	10231	0.29	2.75	1.29
SNRPA			61825	119620	0.00	2.15	1.93
UBAP2L			12890	13211	0.00	2.27	1.02

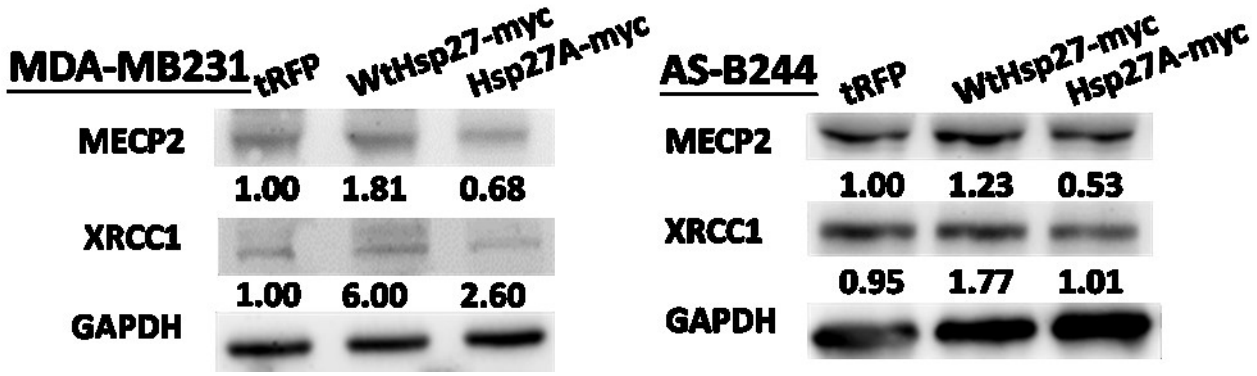


Fig. 6. The expression of MeCP2 or XRCC1 in human breast cancer cells with overexpression of different Hsp27 constructs. MDA-MB-231 or AS-B244 human breast cancer cells were transduced with lentivirus carrying WTHsp27 or Hsp27A and selected with blasticidin S for 5 days. The survived cells were harvested and the expression of MeCP2 or XRCC1 in total cellular proteins was detected with western blot anaylsis.

D. The phosphorylation status of Hsp27 within human breast cancer cells interferes their self-renewal capability and tumorigenicity

We next examined if Hsp27 phosphorylation status will contribute to the self-renewal capability and in vivo tumorigenicity of human breast cancer cells. The mammosphere cultivation was used for determination of the self-renewal capability of breast cancer cells and results showed that the self-renewal capability of triple negative breast cancer cells was decreased when cells overexpressed Hsp27 phosphor-dead mutant form of Hsp27 (Fig. 7). We also checked the expression of several genes involving in self-renewal of BCSCs and results revealed that the overexpression of WtHsp27 increased mRNA expression of BMI1 which was not in those with Hsp27A overexpression (Fig. 8A). The expression of IGF1R (Fig. 8B) or ZEB1 (Fig. 8C) was increased in cells with Hsp27D overexpression while it was decreased in those with Hsp27A overexpression. We next injected the MDA-MB-231 cells with the overexpression of different Hsp27 constructs into mammary fatpads of NOD/SCID mice to determine their in vivo tumorigenicity. From Figure 9, the tumor size at Day 91 of MDA-MB-231 cells with overexpression of Hsp27A was significantly lower than those with WtHsp27 or Hsp27D overexpression (Fig. 9). These results clearly demonstrated that the phosphorylation status of Hsp27 in human breast cancer cells interferes the maintenance of breast cancer stem cells and their tumorigenicity.

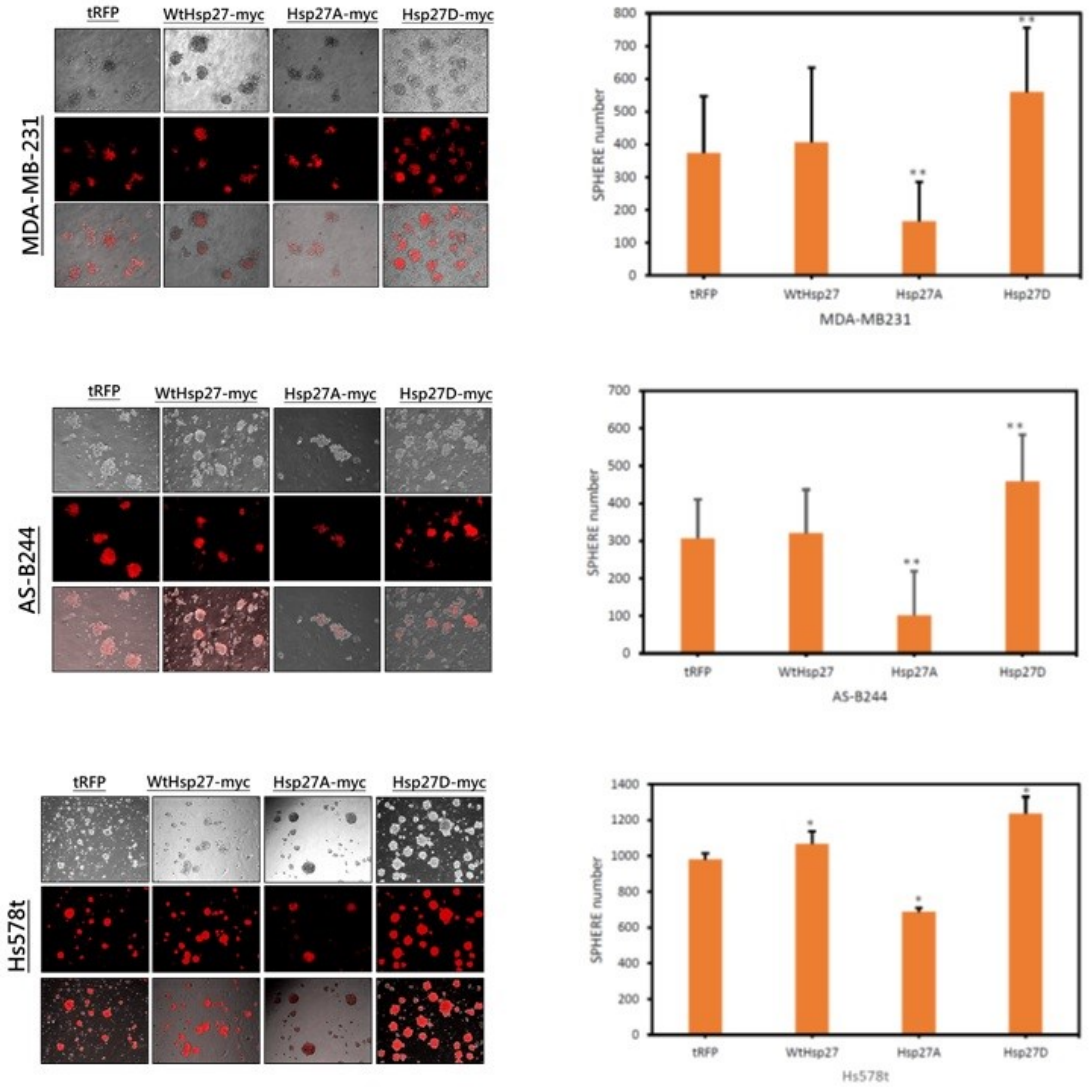


Fig. 7. The self-renewal capability of human triple negative breast cancer cells was decreased when overexpressed with Hsp27 phosphor-dead mutant. MDA-MB-231, AS-B244 or Hs578t human triple negative breast cancer cells were transduced with lentivirus carrying tRFP or different Hsp27 constructs (WtHsp27, Hsp27A or Hsp27D) and selected by blasticidin S for 5 days. The survived cells were harvested and performed mammosphere cultivation at a density of 2500 cells/2ml/well in 6-well-plate with ultralow attachment surface for 7 days. The formed mammospheres were pictured and counted at Day 7. *, $p < 0.05$; **, $p < 0.01$.

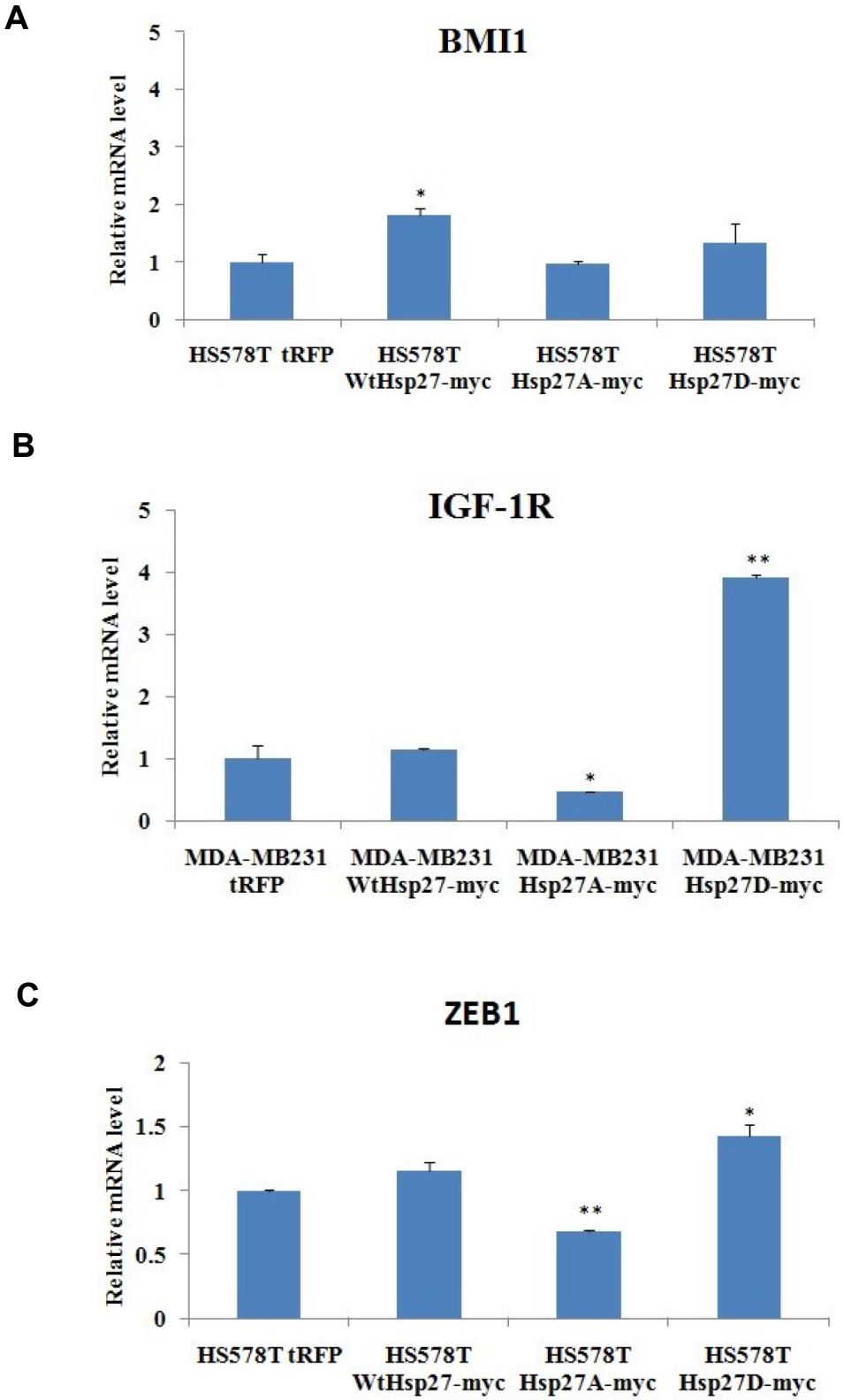


Fig. 7. Hsp27 phosphorylation status in human breast cancer cells changes the expression of genes involved in self-renewal of BCSCs. MDA-MB-231 or Hs578t cells were transduced with lentivirus carrying tRFP or different Hsp27 constructs (WtHsp27, Hsp27A or Hsp27D) and selected for 5 days. The survived cells were then performed mammosphere cultivation for 5 days and the mRNA expression of BMI1 (A), IGF1R (B) or ZEB1 (C) was determined by SYBR Grenn-based real-time RT-PCR. *, $p < 0.05$; **, $p < 0.01$.

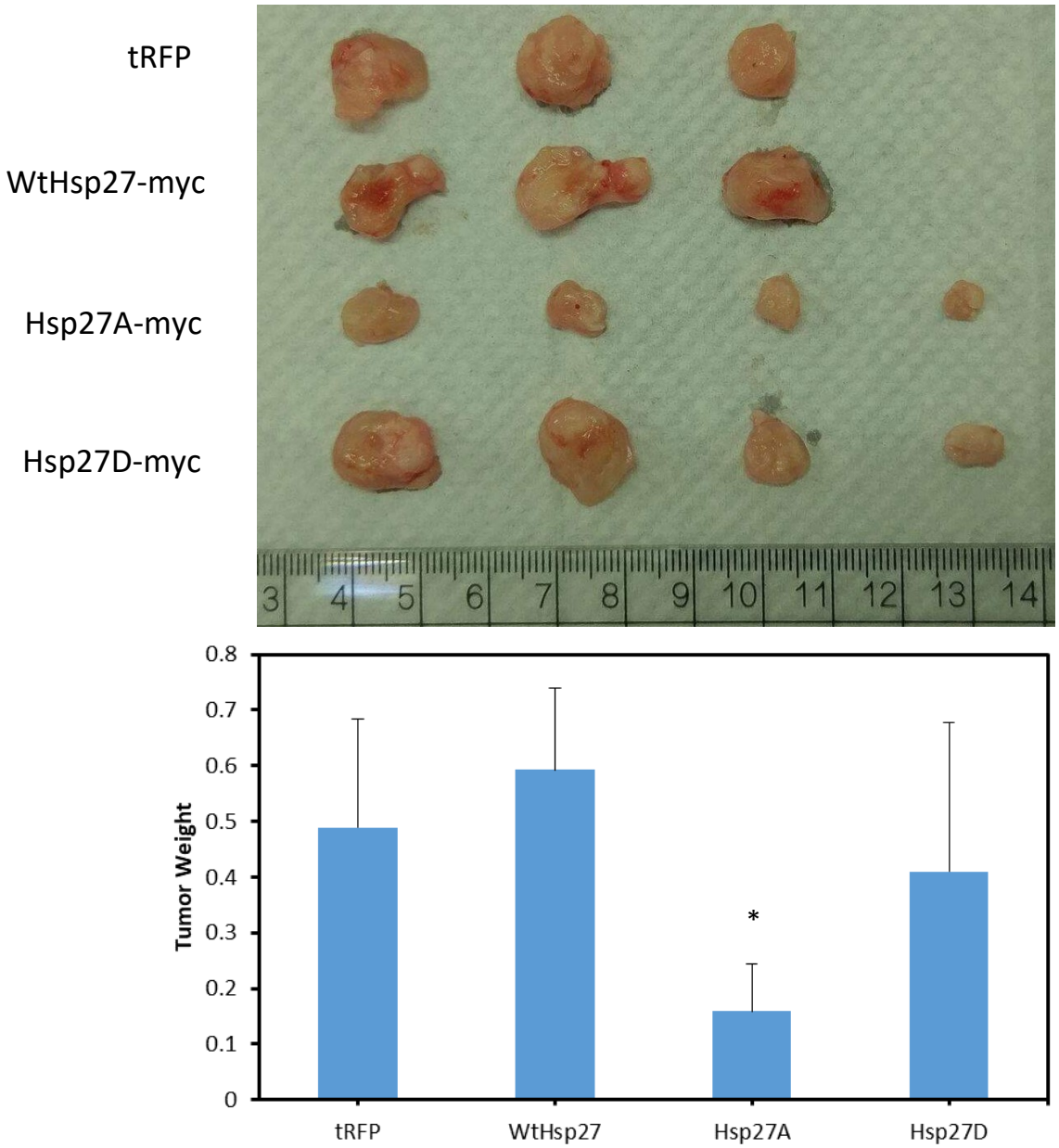


Fig. 9. The phosphorylation status of Hsp27 interferes the tumorigenicity of MDA-MB-231 cells. MDA-MB-231 cells were transduced with lentivirus carrying different forms of Hsp27 constructs and selected with blastcidin S for 5 days. The survived cells were harvested and 1×106 cells were mixed with 2.5 mg/ml Matrigel to inject into mammary fatpads of NOD/SCID mice for tumor growth. The formed tumors were taken out at Day 91 after injection.

E. The phosphorylation status of Hsp27 within human breast cancer cells interferes their metastatic potential

Zebrafish has been used for establishment of xenotransplantation model for cancer research field (PMID: 28277839). With the cooperation with Professor Jiann-Jou Yang in our department, we first demonstrated that HeLa cells, a human cervical cancer cell line, with tRFP red fluorescent protein expression was retained in yolk sac for 24 h after injection into embryo of zebrafish at yolk sac (Fig. 10A), but the injected embryos were dead after 24 hours when cultured at 28°C. After modification of culture temperature from 28°C to 37°C by gradient heating, the embryos could survive for 5 days. We next tested the metastatic potential of

BT-474 cells with overexpression of tRFP or different Hsp27 constructs by injection into yolk sac of Tg(*fli1:EGFP*) zebrafish, the model of green fluorescent blood vessels. The Hsp27D overexpressd BT-474 cells displayed a greater extravasation behavior than WtHsp27 counterparts and were observed to metastasize into intestine at Day 3 post injection (Fig. 10B). The tumor nodules were also observed at Day 5 post injection only in embryos injected with Hsp27D cells (Fig. 10B). Our data reveal that Hsp27 phosphorylation in human breast cancer cells enhances the metastatic potential *in vivo*.

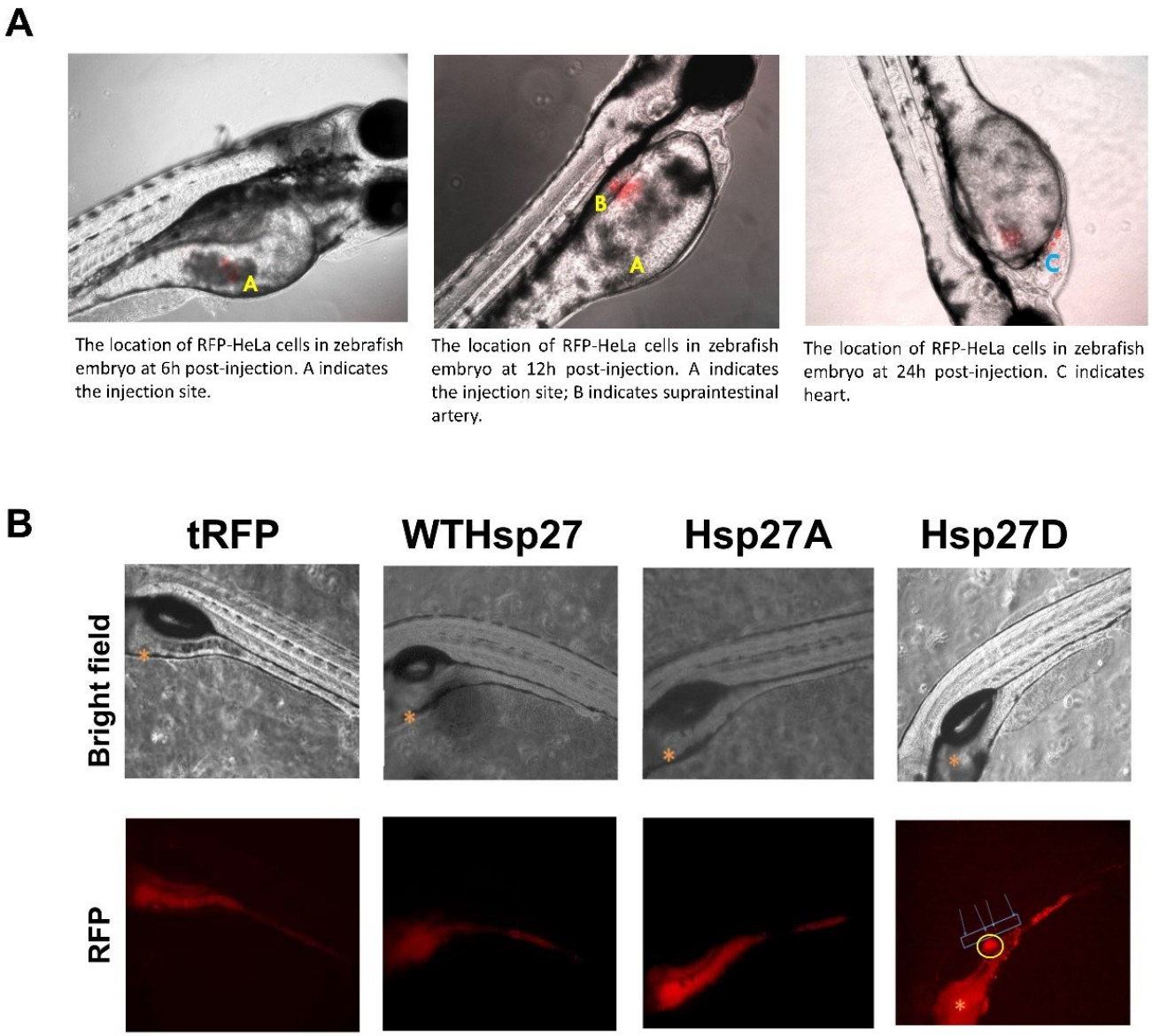


Fig. 10. Using human cancer cells xenotransplantation zebrafish model to demonstrate the metastatic enhancement function of phosphorylated Hsp27. (A) The demonstration of xenotransplation zebrafish model by injection of tRFP overexpressed HeLa cells into yolk sac of zebrafish for 6, 12, or 24h. (B) The Hsp27 with tRFP or different Hsp27 constructs (WtHsp27, Hsp27A or HSp27D) overexpression BT-474 cells were injected into yolk sac with a number of 150 cells in 2.3 nl PBS and observed the red fluorescence signal at Day 5 post injection. Star symbol (*) indicated the injection site, arrows indicated cells with extravasation and yellow circle indicated a potential established tumor mass.

F. Breast cancer cells with surface Hsp27 expression display a great activity in the formation of

mammosphere

We next examined that if there were surface Hsp27 (sHsp27) expressing cells with in AS-B244 breast cancer cells. By con-staining with anti-Hsp27-PE and anti-Hsp70-FITC antibodies, there was 3.4% of sHsp27+/sHsp70- cells, 1.6% of sHsp27-/sHp70+ cells and 1.1% of sHsp27+/sHsp70+ cells (Fig. 12A) We further sorted sHsp27+/sHsp70- and sHsp27-/sHsp70+ cells by FACS cell sorter and analyzed their self-renewal capacity by mammopshere cultivation. From Fig. 12B, we found that sHsp27+/sHsp70- cells displayed a greater mammosphere forming capability in comparison with sHsp27-/sHsp70+ cells. We next examined if sHsp27+/CD44+ breast cancer cells display a greater tumorigencity than those with sHp27-/CD44+ cells. When injection of 10^4 cells with sHsp27+/CD44+ or sHsp27-/CD44+ MDA-MB-231 cells into mammary fatpads of NOD/SCID mice, cells with sHsp27+/CD44+ cells formed bigger tumors than sHsp27-/CD44+ counterparts (Fig. 12C). The expression of stemness genes, such as Nanog, Oct4 or Sox2, was upregulated in tumors derived from sHsp27+/CD44+ cells (Fig. 12C). It suggests that sHsp27+/CD44+ cells are high tumorigenic within breast cancer stem cells.

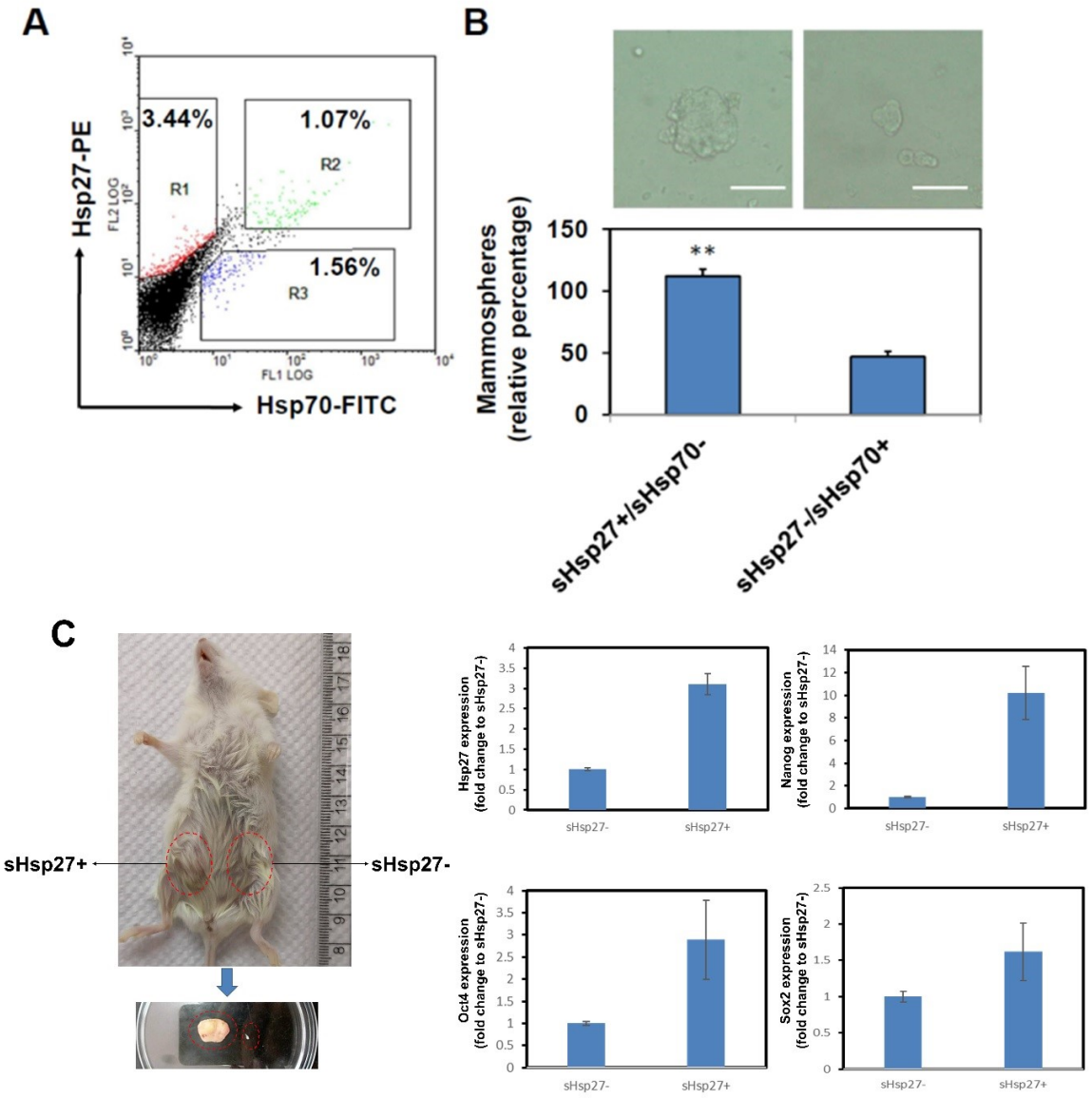


Fig. 12. Surface Hsp27 on human breast cancer cells serves as a highly tumorigenic marker for BCSCs. (A) AS-B244 breast cancer cells were stained with anti-Hsp27-PE and anti-Hsp70-FITC antibodies

at 4°C for 30 mins and the fluorescence signals were collected with flow cytometry. (B) sHsp27+/sHsp70- or sHsp27-/sHsp70+ cells were sorted from AS-B244 cells by FACS cell sorter and 1×10^4 sorted cells were used for mammopshere cultivation to determine their self-renewal capability. **, $p<0.01$. (C) MDA-MB-231 cells were stained with anti-Hsp27-FITC and anti-CD44-APC antibody at 4°C for 30 mins on ice and performed FACS sorting. 1×10^4 sorted cells were suspended at 50μl of 2.5mg/ml Matrigel and injected into mammary fatpads for tumor growth. The formed tumors were taken at 6 months and the expression of Hsp27, Nanog, Oct4 or Sox2 was determined by SYBR Green based real-time RT-PCR.

References

1. Georgopoulos C, Welch WJ: **Role of the major heat shock proteins as molecular chaperones**. *Annu Rev Cell Biol* 1993, **9**:601-634.
2. Soo ET, Yip GW, Lwin ZM, Kumar SD, Bay BH: **Heat shock proteins as novel therapeutic targets in cancer**. *In Vivo* 2008, **22**(3):311-315.
3. Ciocca DR, Arrigo AP, Calderwood SK: **Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update**. *Archives of toxicology* 2013, **87**(1):19-48.
4. Didelot C, Schmitt E, Brunet M, Maingret L, Parcellier A, Garrido C: **Heat shock proteins: endogenous modulators of apoptotic cell death**. *Handb Exp Pharmacol* 2006(172):171-198.
5. Gaëtan Jego AH, Renaud Seigneuric, Carmen Garrido **Targeting heat shock proteins in cancer**. *Cancer letters* 2010.
6. Arrigo AP: **The cellular "networking" of mammalian Hsp27 and its functions in the control of protein folding, redox state and apoptosis**. *Advances in experimental medicine and biology* 2007, **594**:14-26.
7. Parcellier A, Schmitt E, Brunet M, Hammann A, Solary E, Garrido C: **Small heat shock proteins HSP27 and alphaB-crystallin: cytoprotective and oncogenic functions**. *Antioxid Redox Signal* 2005, **7**(3-4):404-413.
8. O'Neill PA, Shaaban AM, West CR, Dodson A, Jarvis C, Moore P, Davies MP, Sibson DR, Foster CS: **Increased risk of malignant progression in benign proliferating breast lesions defined by expression of heat shock protein 27**. *British journal of cancer* 2004, **90**(1):182-188.
9. Mlynarczyk-Liszka J, Maksymiuk B, Ponikiewska D, Krzyzowska-Gruca S, Lange D, Krawczyk Z, Malusecka E: **HSP27 diagnostic utility in the fine needle aspirate of breast. Correlation with progesterone and estrogen receptors**. *Neoplasma* 2009, **56**(4):357-360.
10. Wei L, Liu TT, Wang HH, Hong HM, Yu AL, Feng HP, Chang WW: **Hsp27 participates in the maintenance of breast cancer stem cells through regulation of epithelial-mesenchymal transition and nuclear factor-kappaB**. *Breast cancer research : BCR* 2011, **13**(5):R101.
11. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation**. *Cell* 2011, **144**(5):646-674.
12. Hermann PC, Bhaskar S, Cioffi M, Heeschen C: **Cancer stem cells in solid tumors**. *Seminars in cancer biology* 2010, **20**(2):77-84.
13. Pattabiraman DR, Weinberg RA: **Tackling the cancer stem cells - what challenges do they pose?** *Nature reviews Drug discovery* 2014, **13**(7):497-512.

14. Ajani JA, Song S, Hochster HS, Steinberg IB: **Cancer Stem Cells: The Promise and the Potential**. *Seminars in oncology* 2015, **42 Suppl 1**:S3-S17.
15. Maccalli C, De Maria R: **Cancer stem cells: perspectives for therapeutic targeting**. *Cancer immunology, immunotherapy : CII* 2015, **64**(1):91-97.
16. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF: **Prospective identification of tumorigenic breast cancer cells**. *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**(7):3983-3988.
17. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S *et al*: **ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome**. *Cell stem cell* 2007, **1**(5):555-567.
18. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG: **Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties**. *Cancer research* 2005, **65**(13):5506-5511.
19. Saadin K, White IM: **Breast cancer stem cell enrichment and isolation by mammosphere culture and its potential diagnostic applications**. *Expert review of molecular diagnostics* 2013, **13**(1):49-60.
20. Arisawa M, Nimura M, Fujita A, Hayashi T, Morita N, Koshimura S: **Biological active macrocyclic diterpenoids from chinese drug "Fang Feng Cao"; II. Derivatives of ovatodiolids and their cytotoxity**. *Planta Med* 1986(4):297-299.
21. Rao YK, Chen YC, Fang SH, Lai CH, Geethangili M, Lee CC, Tzeng YM: **Ovatodiolide inhibits the maturation of allergen-induced bone marrow-derived dendritic cells and induction of Th2 cell differentiation**. *Int Immunopharmacol* 2013, **17**(3):617-624.
22. Lien HM, Wang CY, Chang HY, Huang CL, Peng MT, Sing YT, Chen CC, Lai CH: **Bioevaluation of Anisomeles indica extracts and their inhibitory effects on Helicobacter pylori-mediated inflammation**. *J Ethnopharmacol* 2013, **145**(1):397-401.
23. Huang HC, Lien HM, Ke HJ, Chang LL, Chen CC, Chang TM: **Antioxidative characteristics of Anisomeles indica extract and inhibitory effect of ovatodiolide on melanogenesis**. *Int J Mol Sci* 2012, **13**(5):6220-6235.
24. Liao YF, Rao YK, Tzeng YM: **Aqueous extract of Anisomeles indica and its purified compound exerts anti-metastatic activity through inhibition of NF-kappaB/AP-1-dependent MMP-9 activation in human breast cancer MCF-7 cells**. *Food Chem Toxicol* 2012, **50**(8):2930-2936.
25. Lin KL, Tsai PC, Hsieh CY, Chang LS, Lin SR: **Antimetastatic effect and mechanism of ovatodiolide in MDA-MB-231 human breast cancer cells**. *Chemico-biological interactions* 2011, **194**(2-3):148-158.
26. Bamodu OA, Huang WC, Tzeng DT, Wu A, Wang LS, Yeh CT, Chao TY: **Ovatodiolide sensitizes aggressive breast cancer cells to doxorubicin, eliminates their cancer stem cell-like phenotype, and reduces doxorubicin-associated toxicity**. *Cancer letters* 2015, **364**(2):125-134.
27. Ho JY, Hsu RJ, Wu CL, Chang WL, Cha TL, Yu DS, Yu CP: **Ovatodiolide Targets beta -Catenin Signaling in Suppressing Tumorigenesis and Overcoming Drug Resistance in Renal Cell Carcinoma**. *Evid Based Complement Alternat Med* 2013, **2013**:161628.
28. Sun Y, Zhou M, Fu D, Xu B, Fang T, Ma Y, Chen J, Zhang J: **Ubiquitination of heat shock protein 27 is mediated by its interaction with Smad ubiquitination regulatory factor 2 in A549 cells**. *Exp Lung*

Res 2011, **37**(9):568-573.

29. Zhao L, Liu Y, Tong D, Qin Y, Yang J, Xue M, Du N, Liu L, Guo B, Hou N *et al*: **MeCP2 Promotes Gastric Cancer Progression Through Regulating FOXF1/Wnt5a/beta-Catenin and MYOD1/Caspase-3 Signaling Pathways**. *EBioMedicine* 2017, **16**:87-100.
30. Liu Y, Jin X, Li Y, Ruan Y, Lu Y, Yang M, Lin D, Song P, Guo Y, Zhao S *et al*: **DNA methylation of claudin-6 promotes breast cancer cell migration and invasion by recruiting MeCP2 and deacetylating H3Ac and H4Ac**. *Journal of experimental & clinical cancer research : CR* 2016, **35**(1):120.
31. Xu W, Wang S, Chen Q, Zhang Y, Ni P, Wu X, Zhang J, Qiang F, Li A, Roe OD *et al*: **TXNL1-XRCC1 pathway regulates cisplatin-induced cell death and contributes to resistance in human gastric cancer**. *Cell Death Dis* 2014, **5**:e1055.
32. Abdel-Fatah T, Sultana R, Abbotts R, Hawkes C, Seedhouse C, Chan S, Madhusudan S: **Clinicopathological and functional significance of XRCC1 expression in ovarian cancer**. *International journal of cancer Journal international du cancer* 2013, **132**(12):2778-2786.

Publications contributed by the fund of this project

1. Lee YC, Yu CC, Lan C, Lee CH, Lee HT, Luo YL, Wang PH, **Chang WW.** Plasminogen activator inhibitor-1 regulates tumor initiating cell properties in head and neck cancers. *Head&Neck*. 2016. 38: E895–E904. (Corresponding author, IF=2.641, R/C= **4/43** in Otorhinolaryngology)
2. Tu DG, Yu Y, Lee CH, Kuo YL, Lu YC, Tu CW, **ChangWW**. **2015**. Hinokitiol inhibits vasculogenic mimicry activity of breast cancer stem/prgenitor cells through proteasome-mediated degradation of epidermal growth factor receptor. *Oncology Letters*. 2016. 11: 2934-2940 (Corresponding author. IF=1.554, R/C=172/211 in Oncology)
3. Lu KT, Wang BY, Chi WY, Chang-Chien J, Yang JJ, Lee HT, Tzeng YM, Chang WW. Ovatodiolide Inhibits Breast Cancer Stem/Progenitor Cells through SMURF2-Mediated Downregulation of Hsp27. Toxins (Basel). 2016. 8(5). pii: E127. (Corresponding author, IF=**3.03,** R/C= **35/117** in Toxicology)
4. Lee CH, Yu CC, Wang BY, Chang WW. Tumorsphere as an effective in vitro platform for screening anti-cancer stem cell drugs. *Oncotarget*. 2016. 12;7(2):1215-26. (Corresponding author, IF=**5.168,** R/C=44/217 in Oncology)
5. Chang YC, Lin CW, Yu CC, Wang BY, Huang YH, Hsieh YC, Kuo YL, Chang WW. Resveratrol suppresses myofibroblast activity of human buccal mucosal fibroblasts through the epigenetic inhibition of ZEB1 expression. *Oncotarget*. 2016. 15;7(11):12137-49. (Corresponding author, IF=**5.168,** R/C=44/217 in Oncology)
6. Chen SM, Wang BY, Lee CH, Lee HT, Li JJ, Hong GC, Hung YC, Chien PJ, Chang YC, Hsu LS, Chang WW. Hinokitiol up-regulates miR-494-3p to suppress BMI1 expression and inhibits self-renewal of breast cancer stem/progenitor cells. *Oncotarget*. 2017; 8:76057-76068. (Corresponding author, IF=**5.168,** R/C=44/217 in Oncology)
7. Lee YC, Chang WW, Chen YY, Tsai YH, Chou YH, Tseng HC, Chen HL, Wu CC, Chang-Chien J, Lee HT, Yang HF, Wang BY. Hsp90α Mediates BMI1 Expression in Breast Cancer Stem/Progenitor Cells through Facilitating Nuclear Translocation of c-Myc and EZH2. *Int J Mol Sci*. 2017. 18(9). pii: E1986.

(Corresponding author, IF=**3.226,** R/C=**28/149** in Chemical physics and physical chemistry)

附件六

科技部補助專題研究計畫出席國際學術會議心得報告

日期： 105 年 10 月 29 日

計畫編號	MOST103－2314－B－040－015－MY3		
計畫名稱	熱休克蛋白 27 與其磷酸化對於乳癌幹細胞之維持的影響		
出國人員 姓名	張文瑋	服務機構 及職稱	中山醫學大學生物醫學科學系/ 教授
會議時間	105 年 10 月 6 日 至 105 年 10 月 8 日	會議地點	新加坡
會議名稱	(中文)第九屆斑馬魚疾病模式會議 (英文)The 9th Zebrafish Disease Model Conference		
發表題目	(中文)熱休克蛋白 27 的磷酸化增強乳癌細胞在斑馬魚異種移植模式中的轉移能力 (英文)Phosphorylation of heat shock protein 27 enhances metastatic potential of breast cancer cells in a zebrafish model		

一、 參加會議經過

摘要在 2016/08/25 收到大會看板論文接受函，於 105/10/05 自桃園機場出發前往新加坡參加會議，於 105/10/08 返抵台灣

二、 與會心得

以斑馬魚作為癌症研究模式已經在斑馬魚研究圈引起重視，包含以轉殖基因魚誘發斑馬魚產生腫瘤(黑色素瘤)，而斑馬魚已建立出免疫缺陷魚，使得病人腫

瘤異體移植(patient derived xenografts) 可以在斑馬魚中建立，進行個人化藥物篩選平台。而在癌症基礎研究上，配合斑馬魚中早已使用許久的各式細胞螢光魚，如各式免疫細胞螢光魚，以斑馬魚作為癌症活體模式更能作為癌症細胞與腫瘤微環境的研究平台。

三、 發表論文全文或摘要

Heat shock protein 27 (Hsp27) is a molecular chaperon and is overexpressed in a variety of cancers including breast cancer and we previously demonstrated its role in the maintenance and migratory capability of breast cancer stem cells (BCSCs). We also found that the phosphorylation of Hsp27 regulated the vasculogenic mimicry activity of BCSCs, which contributes to tumor vascularization. Here we established a zebrafish model to further investigate the role of Hsp27 phosphorylation in the metastatic potential of BT-474 human breast cancer cell line. After injection of tRFP expressing BT-474 cells with overexpression of wildtype Hsp27 (WTHsp27) or phosphor-mimic mutant (Hsp27D) into yolk sac of 3 day-post-fertilization Tg(fli1:EGFP) zebrafish embryos, the injected embryos were maintained from 28 C to 37 C by gradient heating and traced the red fluorescence signals for 5 days. The Hsp27D cells displayed a greater extravasation behavior than WTHsp27 counterparts and were observed to metastasize into intestine at Day 3 post injection. The tumor nodules were also observed at Day 5 post injection only in embryos injected with Hsp27D cells. Our data suggest that Hsp27 phosphorylation plays a promoting role in breast cancer metastasis in a Tg(fli1:EGFP) zebrafish model. Targeting Hsp27 phosphorylation may be considered as a potential strategy in fighting breast cancer metastasis.

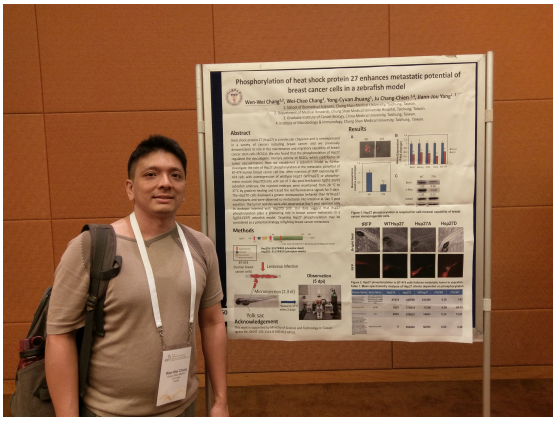
四、 建議

國內已有斑馬魚研究的社群，並在國家衛生研究院設立了斑馬魚研究服務中心，建議該中心可引進免疫缺陷斑馬魚，讓國內有興趣以斑馬魚作為癌症研究的學者可以快速進入此研究領域，以趕上國際潮流。

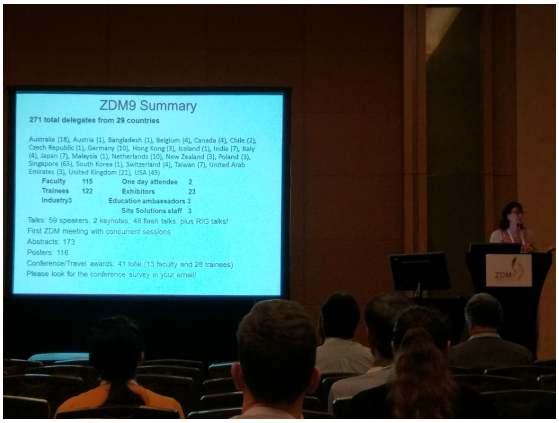
五、 攜回資料名稱及內容

攜回資料名稱及內容（附件：與會手冊封面、論文暨海報發表時程等影本） 手冊一本，與會名牌一只

六、 其他


Phosphorylation of heat shock protein 27 enhances metastatic potential of breast cancer cells in a zebrafish model
Abstract
Results
Methods
Leishmania infection
Observation
Acknowledgment


#ZDM9


ZDM9 Summary
271 total delegates from 29 countries
Australia (18), Austria (1), Bangladesh (1), Belgium (4), Canada (4), Chile (2), Czech Republic (1), Germany (10), Hong Kong (3), Iceland (1), India (7), Italy (4), Japan (7), Malaysia (1), Netherlands (10), New Zealand (3), Poland (3), Singapore (63), South Korea (1), Switzerland (4), Taiwan (7), United Arab Emirates (3), United Kingdom (21), USA (45)
Faculty 115
Trainees 122
Industry2
One day attendee 2
Exhibitors 23
Education ambassadors 3
Site Solutions staff 3
Talks: 59 speakers, 2 keynotes, 46 flash talks, plus RIG talks!
First ZDM meeting with concurrent sessions
Abstracts: 173
Posters: 116
Conference/Travel awards: 41 total (13 faculty and 28 trainees)
Please look for the conference survey in your email!

103年度專題研究計畫成果彙整表

計畫主持人：張文瑋	計畫編號：103-2314-B-040-015-MY3
計畫名稱：熱休克蛋白27與其磷酸化對於乳癌幹細胞之維持的影響	

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			1		The effect of Hsp27 phosphorylation in its signaling network within breast cancer cells. Ting-Yu Chang (張庭瑜), Wen-Wei Chang (張文瑋) and Wei-Chao Chang (張為超). 第32屆生物醫學聯合學術年會（台北國防醫學院）
		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			7	篇	1. Lee YC, Yu CC, Lan C, Lee CH, Lee HT, Luo YL, Wang PH, Chang WW. Plasminogen activator inhibitor-1 regulates tumor initiating cell properties in head and neck cancers. Head&Neck. 2016. 38: E895－E904. (Corresponding author, IF=2.641, R/C= 4/43 in Otorhinolaryngology) 2. Tu DG, Yu Y, Lee CH, Kuo YL, Lu YC, Tu CW, ChangWW. 2015. Hinokitiol inhibits vasculogenic mimicry activity of breast cancer stem/prgenitor cells through

					proteasome-mediated degradation of epidermal growth factor receptor. Oncology Letters. 2016. 11: 2934-2940 (Corresponding author. IF=1.554, R/C=172/211 in Oncology) 3. Lu KT, Wang BY, Chi WY, Chang-Chien J, Yang JJ, Lee HT, Tzeng YM, Chang WW. Ovatodiolide Inhibits Breast Cancer Stem/Progenitor Cells through SMURF2-Mediated Downregulation of Hsp27. Toxins (Basel). 2016. 8(5). pii: E127. (Corresponding author, IF=3.03, R/C= 35/117 in Toxicology) 4. Lee CH, Yu CC, Wang BY, Chang WW. Tumorsphere as an effective in vitro platform for screening anti-cancer stem cell drugs. Oncotarget. 2016. 12;7(2):1215-26. (Corresponding author, IF=5.168, R/C=44/217 in Oncology) 5. Chang YC, Lin CW, Yu CC, Wang BY, Huang YH, Hsieh YC, Kuo YL, Chang WW. Resveratrol suppresses myofibroblast activity of human buccal mucosal fibroblasts through the epigenetic inhibition of ZEB1 expression. Oncotarget. 2016. 15;7(11):12137-49. (Corresponding author, IF=5.168, R/C=44/217 in Oncology) 6. Chen SM, Wang BY, Lee CH, Lee HT, Li JJ, Hong GC, Hung YC, Chien PJ, Chang YC, Hsu LS, Chang WW. Hinokitiol up-regulates miR-494-3p to suppress BMI1 expression and inhibits self-renewal of breast cancer stem/progenitor cells. Oncotarget. 2017; 8:76057-76068. (Corresponding author, IF=5.168, R/C=44/217 in Oncology) 7. Lee YC, Chang WW, Chen YY, Tsai YH, Chou YH, Tseng HC, Chen HL, Wu CC, Chang-Chien J, Lee HT, Yang HF, Wang BY. Hsp90α Mediates BMI1 Expression in Breast Cancer Stem/Progenitor Cells through Facilitating Nuclear Translocation of c-Myc and EZH2. Int J Mol Sci. 2017. 18(9). pii: E1986. (Corresponding author, IF=3.226, R/C=28/149 in Chemical physics and

					physical chemistry)
		研討會論文	4		1. Chang WW, Lee YC, Lan C, Yu CC. Tiplaxtinin (PAI-039) suppresses tumor initiating cells in head and neck cancer through downregulation of Sox2. The Japanese Society of Medical Oncology 2015 Annual Meeting. Sapporo. Japan. (Poster Presentation, No. P2-9-6) 2. Chang WW, Chang-Chien J, Yang JJ Establishment of a zebrafish model for the study of metastatic process of human cancer cells. 8th Zebrafish Disease Models Conference. 2015. Boston. USA. (Poster presentation. No. 136) 3. Chang WW, Tsai YH, Huang YH. 17-Demethoxygeldanamycin inhibits self-renewal of breast cancer stem cells through EZH2/c-Myc/Bmi1 pathway. ESMO Asia Congress 2015. Singapore. Poster presentation (No. 3P). 4. Chang WW, Chang WC, Jhuang YC, Chang-Chien J, Yang JJ. Phosphorylation of heat shock protein 27 enhances metastatic potential of breast cancer cells in a zebrafish model. 9th Zebrafish Disease Models Conference. 2016. Singapore. (Poster presentation, No. 060).
		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	
		專利權 發明專利 已獲得	0		
		專利權 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	2		張庭瑜、陳裕益
		博士生	0		
		博士後研究員	0		
		專任助理	1		王惠琳(學士級)
	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：
   本研究成果已經證明Hsp27的磷酸化狀態確實影響乳癌幹細胞的自我更新、活體致癌力以及轉移，但詳細分子機制仍未完全釐清，有待日後申請新的計畫來繼續完成。

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）
   由本計畫支持之論文發表共7篇，其中包含3篇IF>5；一篇排名<10%；一篇排名排名<20%

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）
   本研究證明Hsp27的磷酸化狀態影響乳癌幹細胞的致癌力以及轉移能力，並發現魚針草內酯化合物Ovatodiolide能透過抑制Hsp27達到抑制乳癌幹細胞自我更新的功效，這些成果有助於發展以Hsp27之磷酸化作為乳癌病人癒後診斷的依據及標靶Hsp27作為乳癌治療藥物的學理依據。

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）