科技部補助專題研究計畫報告

免疫檢查哨分子在癌幹細胞之非免疫調節活性的功能性探討(第3年)

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報 告 附 件 ： 出席國際學術會議心得報告

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

中 華 民 國 109 年 10 月 14 日

中 文 摘 要 ： 癌幹細胞在癌組織中具有起始腫瘤、抗藥性及造成癌症轉移等特性，是目前癌症藥物開發亟需標靶的癌細胞群。免疫檢查哨分子programmed cell death receptor ligand-1 (PD-L1)可透過與T 細胞表面的PD-1 結合，造成腫瘤浸潤T 細胞無法活化來抑制抗腫瘤免疫力。PD-1/PD-L1訊號在癌細胞中被發現能增加乳癌細胞的上皮間質轉換特性或造成骨髓瘤細胞的抗藥性。Indoleamine 2,3-dioxygenase 1 (IDO1)能藉由代謝腫瘤微環境中的tryptophan 為kynurenine，造成浸潤T 細胞無法增生，抑制腫瘤浸潤T 細胞功能。IDO1 被報導能調節細胞週期，造成癌細胞的放射線抗性。這些研究顯示免疫檢查哨分子可能對癌細胞或癌幹細胞具有非免疫調節的功能，導致癌症惡化，但其機制尚未被清楚證明。本研究的目標包含兩大部分: (1) IDO1 及tryptophan 代謝物對子宮頸癌癌幹細胞的影響；(2) PD-L1 反向訊息對癌幹細胞自我更新的影響。在第一部份的成果，我們發現子宮頸癌幹細胞內之IDO1表現較非癌幹細胞群提升，而子宮頸癌細胞暴露放射線後亦可觀察到IDO1蛋白及其活性的增加。進一步利用專一性RNA干擾或小分子藥物INCB-0234360，我們發現子宮頸癌細胞內IDO1的抑制導致細胞增加對放射線的敏感性；而處理kynurenine則使癌細胞對放射線敏感性下降。在分子機制上，我們證實IDO1在子宮頸癌幹細胞的增加與Notch1的活化有關，使用-secretase小分子抑制劑Ro-4929097，則可抑制子宮頸癌幹細胞內IDO1的表現。此外，我們還發現IDO1的訊息傳遞亦可調節Notch1的表現，在子宮頸癌幹細胞內抑制IDO1表現可造成Notch1活性下降，而此現象與aryl hydrocarbon receptor nuclear translocator結合至Notch1啟動子有關。我們也首次在活體腫瘤模式中證實，INCB-0234360具有放射增敏的功效，此部分成果證實在子宮頸癌幹細胞內存在著IDO1與Notch1之間的相互調節機制，以致使子宮頸癌幹細胞對放射線治療產生抗性。在第二部分的成果，我們首先發現在PD-L1高度表達的SCC4口腔癌細胞中，PD1-Fc重組蛋白可刺激其癌幹細胞自我更新及癌症幹姓(Cancer stemness)相關基因的表達。透過RNA測序分析，我們發現PD1-Fc的刺激增加了SCC4細胞內G2/M細胞週期等訊號路徑相關基因的表現。從The Cancer Genome Atlas資料庫中頭頸部癌的數據，我們也發現PD-L1的mRNA表現與這些訊息路徑中相關基因呈現正相關，顯示在口腔癌細胞表面表現的PD-L1可對癌細胞傳遞訊息，進而增加細胞增生以及癌幹細胞活性。

中 文 關 鍵 詞 ： 吲哚胺-吡咯2，3-二加氧酶, Notch1, 子宮頸癌, 放射線抗性, 細胞程式死亡-配體1, 口腔癌, 癌幹細胞

英 文 摘 要 ： Cancer stem cells (CSCs) are known to participate in tumor initiation, drug resistance, and metastasis in cancers. Targeting CSCs is considered as the key for the development of anti-cancer drugs. Programmed cell death receptor ligand-1 (PD-L1) is one of the immune checkpoint molecules that can inhibit anti-tumor immunity by binding to PD-1 on the surface of T cells and causes the inactivation of tumor-infiltrating T cells. PD-1/PD-L1 signals have been found in cancer cells to increase the epithelial-mesenchymal transition characteristics of breast cancer

cells or cause drug resistance in myeloma cells. Indoleamine 2,3-dioxygenase 1 (IDO1) can metabolize tryptophan to kynurenine followed by the suppression of T cell activation in tumor microenvironemnt. IDO1 has been reported to regulate the cell cycle and cause radiation resistance in cancer cells. These studies have shown that immune checkpoint molecules may have non-immune modulation functions on cancer cells or CSCs, leading to cancer progression, but the underlying molecular mechanisms have not been clearly proven. The specific aims of this study include two parts: (1) the effect of IDO1 and tryptophan metabolites on cervical CSCs; (2) the effect of PD-L1 reverse signal on the self-renewal of CSCs. In the first part, we found that the IDO1 expression in cervical CSCs was higher than that of non-CSCs, and the increase in IDO1 protein and its activity could also be observed in cervical cancer cells after exposure to radiation. Using the RNA interference or INCB-0234360, the small molecule inhibitor of IDO1, we found that the inhibition of IDO1 in cervical cancer cells led to an increase in radiosensitivity and the treatment of kynurenine increased the radioresistance. In the molecular mechanisms, we confirmed that the activation of Notch1 mediated the increase of IDO1 in cervical CSCs. The treatment of Ro-4929097, the small molecule inhibitor of gamma-secretase, inhibited the expression of IDO1 in cervical CSCs. Besides, we also found that the inhibition of IDO1 in cervical CSCs caused a decrease in Notch1 activity, which was mediated by the binding of aryl hydrocarbon receptor nuclear translocator to the Notch1 promoter. We also confirmed for the first time in the xenograft tumor model that INCB-0234360 displayed a radiosensitization effect. These results confirm a reciprocal regulation mechanism between IDO1 and Notch1 in cervical CSCs to participate in their radioresistance. In the second part, we first found that the treatment of PD-1-Fc recombinant protein to the PD-L1 highly expressing SCC4 oral cancer cells stimulated the self-renewal of CSCs and the expression of genes involving in cancer stemness. Through RNA sequencing analysis, we found that PD1-Fc stimulation increased the G2/M cell cycle, TGF-beta, and Wnt/beta-catenin related genes in SCC4 cells. The analysis of HNSCC dataset in The Cancer Genome Atlas database, we also found that the mRNA expression of PD-L1 positively correlates with genes associated with EMT or cancer stemness, such as SERPINE1/TGFB1/TGFBR1/CTNNB1/MYC/NANOG. Our data suggest that PD-L1 displayed on the surface of oral cancer cells could transduce signals to increase cell proliferation and CSC activity.

英文關鍵詞：Indoleamine 2,3-dioxygenase 1, cervical cancer, Notch1, radioresistance, Programmed cell death receptor ligand-1, oral cancer, cancer stem cells

科技部補助專題研究計畫報告

免疫檢查哨分子在癌幹細胞之非免疫調節活性的功能性探討

報告類別：□進度報告

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計畫主持人：張文瑋

共同主持人：

計畫參與人員：王惠琳、黃彥翔、李玉齡

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□執行國際合作與移地研究心得報告

■出席國際學術會議心得報告

□出國參訪及考察心得報告

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(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

中　華　民　國　109 年 10 月 15 日

一、中文摘要

癌幹細胞在癌組織中具有起始腫瘤、抗藥性及造成癌症轉移等特性，是目前癌症藥物開發亟需標靶的癌細胞群。免疫檢查哨分子 programmed cell death receptor ligand-1 (PD-L1)可透過與 T 細胞表面的 PD-1 結合，造成腫瘤浸潤 T 細胞無法活化來抑制抗腫瘤免疫力。PD-1/PD-L1 訊號在癌細胞中被發現能增加乳癌細胞的上皮間質轉換特性或造成骨髓瘤細胞的抗藥性。Indoleamine 2,3-dioxygenase 1 (IDO1)能藉由代謝腫瘤微環境中的 tryptophan 為 kynurenine，造成浸潤 T 細胞無法增生，抑制腫瘤浸潤 T 細胞功能。IDO1 被報導能調節細胞週期，造成癌細胞的放射線抗性。這些研究顯示免疫檢查哨分子可能對癌細胞或癌幹細胞具有非免疫調節的功能，導致癌症惡化，但其機制尚未被清楚證明。本研究的目標包含兩大部分: (1) IDO1 及 tryptophan 代謝物對子宮頸癌癌幹細胞的影響；(2) PD-L1 反向訊息對癌幹細胞自我更新的影響。在第一部份的成果，我們發現子宮頸癌幹細胞內之 IDO1 表現較非癌幹細胞群提升，而子宮頸癌細胞暴露放射線後亦可觀察到 IDO1 蛋白及其活性的增加。進一步利用專一性 RNA 干擾或小分子藥物 INCB-0234360，我們發現子宮頸癌細胞內 IDO1 的抑制導致細胞增加對放射線的敏感性；而處理 kynurenine 則使癌細胞對放射線敏感性下降。在分子機制上，我們證實 IDO1 在子宮頸癌幹細胞的增加與 Notch1 的活化有關，使用γ-secretase 小分子抑制劑 Ro-4929097，則可抑制子宮頸癌幹細胞內 IDO1 的表現。此外，我們還發現 IDO1 的訊息傳遞亦可調節 Notch1 的表現，在子宮頸癌幹細胞內抑制 IDO1 表現可造成 Notch1 活性下降，而此現象與 aryl hydrocarbon receptor nuclear translocator 結合至 Notch1 啟動子有關。我們也首次在活體腫瘤模式中證實，INCB-0234360 具有放射增敏的功效，此部分成果證實在子宮頸癌幹細胞內存在著 IDO1 與 Notch1 之間的相互調節機制，以致使子宮頸癌幹細胞對放射線治療產生抗性。在第二部分的成果，我們首先發現在 PD-L1 高度表達的 SCC4 口腔癌細胞中，PD1-Fc 重組蛋白可刺激其癌幹細胞自我更新及癌症幹姓(Cancer stemness)相關基因的表達。透過 RNA 測序分析，我們發現 PD1-Fc 的刺激增加了 SCC4 細胞內 G2/M 細胞週期等訊號路徑相關基因的表現。從 The Cancer Genome Atlas 資料庫中頭頸部癌的數據，我們也發現 PD-L1 的 mRNA 表現與這些訊息路徑中相關基因呈現正相關，顯示在口腔癌細胞表面表現的 PD-L1 可對癌細胞傳遞訊息，進而增加細胞增生以及癌幹細胞活性。

關鍵詞: 吲哚胺-吡咯 2，3-二加氧酶, Notch1, 子宮頸癌, 放射線抗性, 細胞程式死亡-配體 1, 口腔癌, 癌幹細胞

二、英文摘要

Cancer stem cells (CSCs) are known to participate in tumor initiation, drug resistance, and metastasis in cancers. Targeting CSCs is considered as the key for the development of anti-cancer drugs. Programmed cell death receptor ligand-1 (PD-L1) is one of the immune checkpoint molecules that can inhibit anti-tumor immunity by binding to PD-1 on the surface of T cells and causes the inactivation of tumor-infiltrating T cells. PD-1/PD-L1 signals have been found in cancer cells to increase the epithelial-mesenchymal transition characteristics of breast cancer cells or cause drug resistance in myeloma cells. Indoleamine 2,3-dioxygenase 1 (IDO1) can metabolize tryptophan to kynurenine followed by the suppression of T cell activation in tumor microenvironemnt. IDO1 has been reported to regulate the cell cycle and cause radiation resistance in cancer cells. These studies have shown that immune checkpoint molecules may have non-immune modulation functions on cancer cells or CSCs, leading to cancer progression, but the underlying molecular mechanisms have not been clearly proven. The specific aims of this study include two parts: (1) the effect of IDO1 and tryptophan metabolites on cervical CSCs; (2) the effect of PD-L1 reverse signal on the self-renewal of CSCs. In the first part, we found that the IDO1 expression in cervical CSCs was higher than that of non-CSCs, and the increase in IDO1 protein and its activity could also be observed in cervical cancer cells after exposure to radiation. Using the RNA interference or INCB-0234360, the small molecule inhibitor of IDO1, we found that the inhibition of IDO1 in cervical cancer cells led to an increase in radiosensitivity and the treatment of kynurenine increased the radioresistance. In the molecular mechanisms, we confirmed that the activation of Notch1 mediated the increase of IDO1 in cervical CSCs. The treatment of Ro-4929097, the small molecule inhibitor of gamma-secretase, inhibited the expression of IDO1 in cervical CSCs. Besides, we also found that the inhibition of IDO1 in cervical CSCs caused a decrease in Notch1 activity, which was mediated by the binding of aryl hydrocarbon receptor nuclear translocator to the Notch1 promoter. We also confirmed for the first time in the xenograft tumor model that INCB-0234360 displayed a radiosensitization effect. These results confirm a reciprocal regulation mechanism between IDO1 and Notch1 in cervical CSCs to participate in their radioresistance. In the second part, we first found that the treatment of PD-1-Fc recombinant protein to the PD-L1 highly expressing SCC4 oral cancer cells stimulated the self-renewal of CSCs and the expression of genes involving in cancer stemness. Through RNA sequencing analysis, we found that PD1-Fc stimulation increased the G2/M cell cycle in SCC4 cells. The analysis of HNSCC dataset in The Cancer Genome Atlas database, we also found that the mRNA expression of PD-L1 positively correlates with genes associated with EMT or cancer stemness. Our data suggest that PD-L1 displayed on the surface of oral cancer cells could transduce signals to increase cell proliferation and CSC activity.

Keywords: Indoleamine 2,3-dioxygenase 1, cervical cancer, Notch1, radioresistance, Programmed cell death receptor ligand-1, oral cancer, cancer stem cells

三、前言

免疫檢查哨分子對於癌細胞非免疫相關的活性

近年來對於免疫檢查哨分子在癌症發展過程的了解，除了用以抑制抗腫瘤免疫力外，尚有非免疫調節相關的活性。在小鼠乳癌細胞 4T1 以 shRNA 抑制 Ido-1 表現，發現 Ido-1 低表現的 4T1 細胞在活體腫瘤生長與轉移能力都受到明顯抑制[1]；在人類癌細胞株中則發現，IDO-1 的表現與癌細胞的抗化療藥物或抗放射線表型有關，可能由於 IDO-1 可調控細胞週期 [1]。此外，在大腸癌研究中則發現，tryptophan 代謝物 kynurenine 或 quinolinic acid 能活化大腸癌細胞內β-catenin，促使癌細胞的增生[2]。PD-L1 則在屬於 claudin-low 人類乳癌細胞株中具有較高的表現，而高度表現 PD-L1 的人類乳癌細胞同時具有 CD24-CD44+之乳癌幹細胞標記，以 TGF-β1 使乳癌細胞進行上皮間質轉換，則能同時誘導乳癌細胞表現乳癌幹細胞標記與增加 PD-L1 的表現[3]。PD1/PD-L1 訊號已經知道對於樹突細胞能夠傳遞反向訊息(reverse signal)，當利用 soluble PD1 對表現 PD-L1 的樹突細胞進行刺激，發現能促使樹突細胞呈現免疫抑制的表現型，包含使樹突細胞處於較不成熟狀態以及增加免疫抑制細胞激素 IL-10 的表現[4]。這樣的反向訊息在這兩年中也被發現可能存在於腫瘤細胞中，例如在小鼠黑色素瘤細胞則發現，癌細胞能同時表現 PD-1/PD-L1，而癌細胞間相互刺激活化的 PD-1/PD-L1 訊號能透過 mTOR 訊號增強小鼠黑色素瘤活體腫瘤生長，而 anti-PD-1 抗體則能有效拮抗此 PD-1/PD-L1 對癌細胞之細胞內生長訊號刺激[5]。在多發性骨髓瘤(multiple myeloma, MM)細胞中利用 PD1-Fc 重組蛋白進行刺激，則能活化 PI3K/Akt 訊息，進而促使 MM 細胞對化療藥物產生抗藥性[6]。這些研究顯示，免疫檢查哨分子訊息除調控腫瘤的免疫逃脫外，更可能直接提供癌細胞朝向更惡性發展的訊息，但免疫檢查哨分子是否影響癌幹細胞特性，包含自我更新、抗藥性及轉移特徵，與其中可能參與的訊息傳遞路徑，則仍未完全清楚。

四、研究目的

本研究的目的為探討兩個免疫檢查哨分子，IDO1 與 PD-L1，在癌症幹細胞的抗放射線或自我更新活性中所扮演的角色，以及其背後所參與的分子機轉，我們相信本研究的成果將有助於以標靶免疫檢查哨藥物作為控制癌症幹細胞自我更新的手段之一，具有其臨床應用價值。

本研究的目標包含兩大部分: (1) IDO1 及 tryptophan 代謝物對子宮頸癌癌幹細胞的影響；(2) PD-L1 反向訊息對癌幹細胞自我更新的影響。

五、研究成果

IDO1 及 tryptophan 代謝物對子宮頸癌癌幹細胞的影響:

IDO1 is Upregulated in Cervical CSCs and Cerivcal Cancer Cells after Exposure of Radiation

Using Gene Expression Profiling Interactive Analysis webtool (GEPIA, http://gepia.cancer-pku.cn/index.html) to analyze the data from The Cancer Genome Atlas (TCGA), the expression of *IDO1* mRNA was significantly

increased in cervical cancer tissues when compared to normal cervical tissues (Fig. 1A). To explore the potential function of IDO1 in CSC behavior, we firstly examined its expression between two cultivation methods of conventional 2-dimensioanl (2-D) or tumorsphere, a cell culture based method to enrich CSCs [7,8], and found that IDO1 protein expression was upregulated in cervical tumorspheres from HeLa or SiHa cervical cancer cells (Fig. 1B). In addition to protein level, the IDO1 activity, which was determined by the conversion of kynurenine from tryptophan using Ehlrich reagent, was also increased in HeLa or SiHa tumorspheres in comparison to 2-D cultured cells (Fig. 1C). Radioresistance is one of the features of CSCs including cervical cancers [9,10]. We examined the expression of IDO1 in HeLa or SiHa cells after 2 Gy radiation treatment and resulted showed that IDO1 protein level was increased by radiation stimulation (Fig. 1D). The suppressive effect to the proliferation of Jurkat T cells with the conditional media collected from irradiated HeLa or SiHa cells was significantly enhanced in comparison to non-irradiated cells (Fig. 1E) supporting the observations of the IDO1 upregulation in irradiated cervical cancer cells. These data clearly demonstrate that IDO1 activity is elevated in cervical CSCs or irradiated cervical cells and it also suggests that IDO1 activity may involve in the radiation response of cervical CSCs.

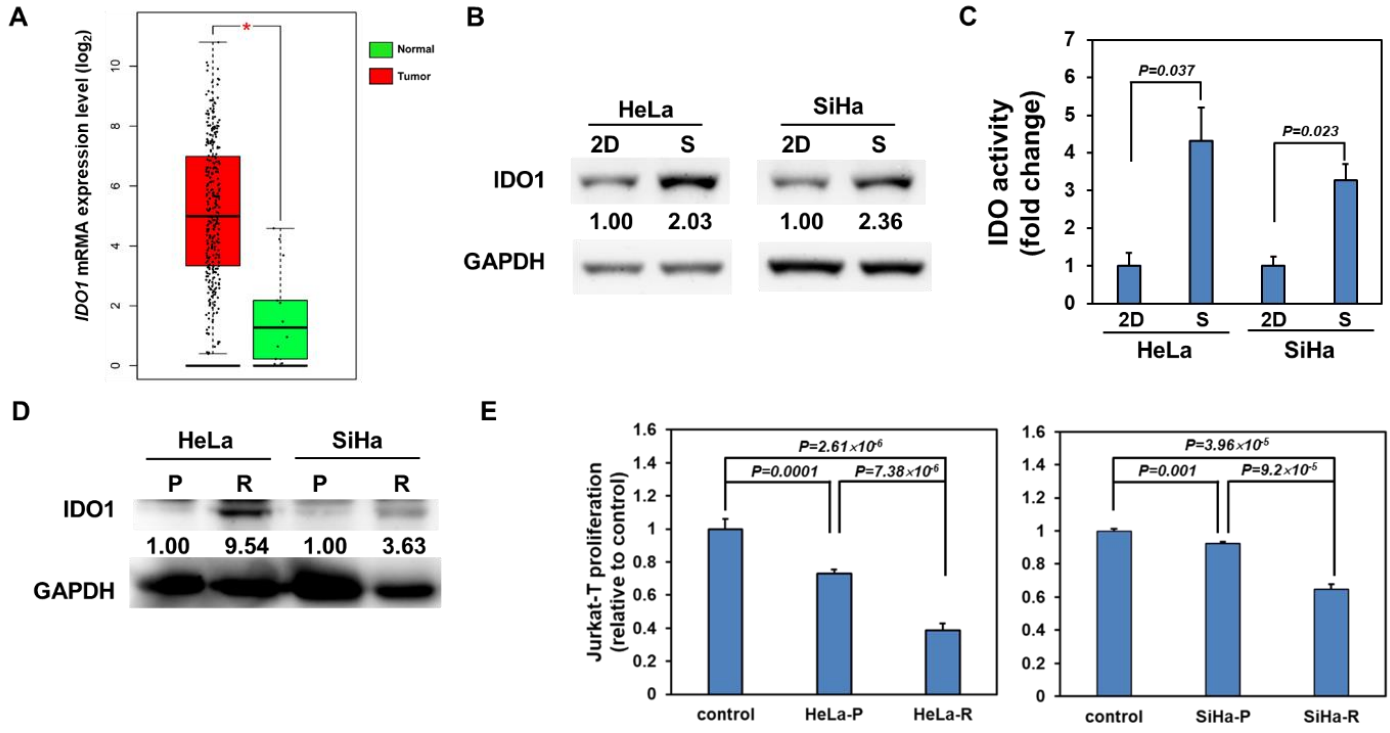


Figure 1. **IDO1 is upregulated in cervical CSCs and irradiated cervical cancer cells.** (A) The expression levels of *IDO1* mRNA among normal cervical or cervical cancer tissues were analyzed by GEPIA website using the data of TCGA. *, p< 0.01. (B, C) The total cell proteins were harvested from HeLa or SiHa cells under 2-dimensional culture (2D) or tumorsphere culture (S). The IDO1 protein expression was determined by western blot (B). The inserted numbers indicated the relative expression level of S in comparison to 2D. The IDO1 activity was determined by the conversion of kynurenine from tryptophan using Ehlrich reagent and measured the absorbance at 492 nm (C). The data were presented as fold change to 2D group. (D) The total cell proteins were harvested from parental (P) or irradiated (R) HeLa or SiHa cells and the IDO1 protein expression was determined by western blot. The inserted numbers indicated the relative expression level of R in comparison to P. (E) The culture supernatant of parental (P) or

irradiated (R) HeLa or SiHa cells was mixed with RPMI-1640 medium as a ratio of 1:1 and used for examining the T cell suppression effect by measuring the proliferation of Jurkat T cells. Control indicated the mixture of fresh DMEM medium and RPMI-1640 medium.

Notch1 Activation Contributes to the Increased IDO1 Expression in Cervical CSCs

It is known that the activation of Notch1 contributes to the radioresistance in several cancer types [11-14]. We firstly observed that the expression of intracellular domain of Notch (NICD), the activation form of Notch1, was increased in tumorspheres derived from HeLa or SiHa cells (Fig. 2A). We next examine the role of Notch1 in IDO1 expression of cervical CSCs. With lentiviral delivery of Notch1 specific shRNAs to inhibit NICD expression, the IDO1 protein level was downregulated (Fig. 2B). We also found that the kynurenine concentration in culture supernatant of HeLa or SiHa tumorspheres was significantly reduced after knockdown of Notch1 (Fig. 2C). In addition to RNA interference, we also used Ro-4929097, a γ-secretase inhibitor, to inhibit Notch1 activation in HeLa or SiHa tumorspheres and found that the IDO1 expression was also downregulated (Fig. 2D). The activated NICD is known to translocate into nucleus to turn on the downstream target genes through the association with RBPJ (Recombination signal binding protein for immunoglobulin kappa J region)/CSL (CBF1/Suppressor of Hairless/LAG-1) transcription factor complex [15]. From the analysis of IDO1 promoter on the Eukaryotic Promoter Database (EPD, https://epd.epfl.ch//index.php), there was a putative RBPJ/CSL binding site of (-TGTGGGAA-) at -790 upstream or +140 downstream from transcription start site (TSS) (Fig. 2E). With chromatin immunoprecipitation (ChIP) method by using an anti-Notch1 antibody to pull-down chromatins followed by detection of IDO1 promoter with qPCR analysis, we further found that the treatment of Ro-4929097 strongly suppressed the binding of NICD to the IDO1 promoter at the +140 site, one of the putative RBPJ/CSL binding sites, in both HeLa or SiHa tumorspheres (Fig. 2E). We also confirmed that the knockdown of Notch1 in HeLa or SiHa tumorspheres increased their sensitivity to radiation treatment (Fig. 3). The SERs for FS of 0.5 in Notch1 knockdown cells were 2.19 or 1.48 for HeLa tumorsphere cells and 1.53 or 1.83 for SiHa tumorsphere cells (Fig. 3). These results suggest that Notch1 activation positively regulates the upregulation of IDO1 in cervical CSCs.

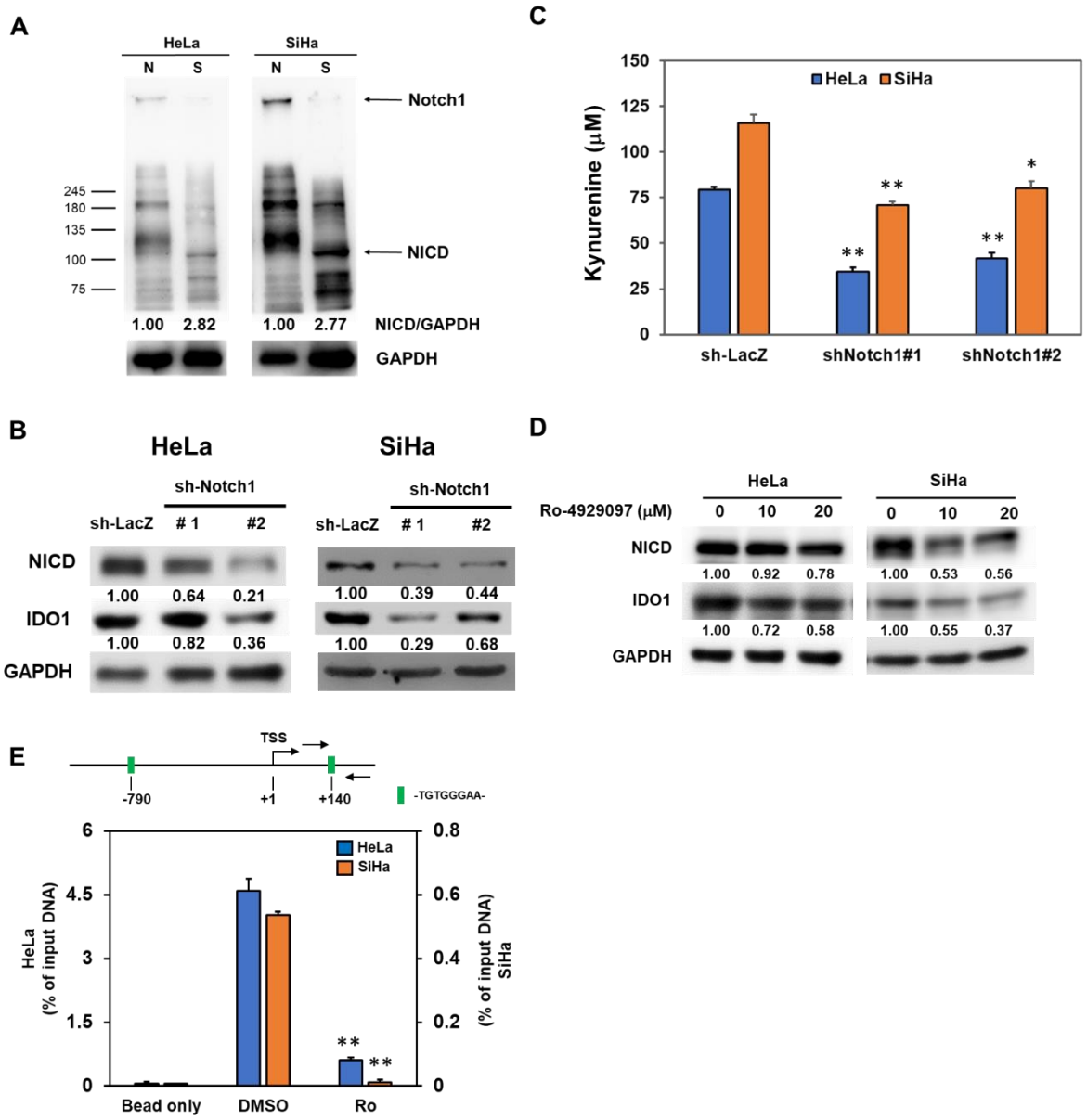
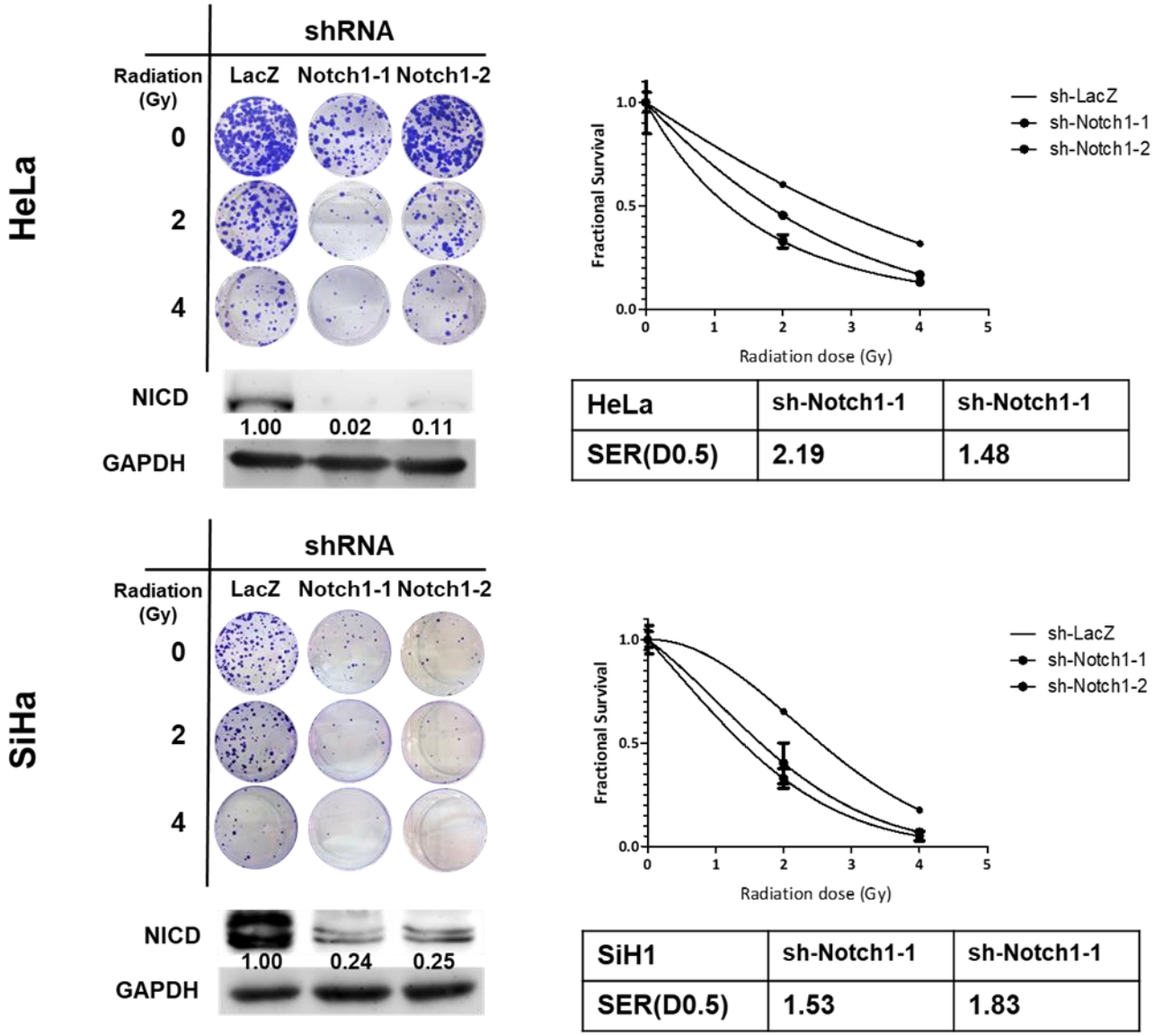


Figure 2. **Notch1 activation in cervical CSCs participates to the IDO1 induction.** (A) Total cell lysates were collected from conventional adherent culture (N) or tumorspheres (S) from HeLa or SiHa cells and the expression of Notch1 and NICD was determined by western blot. (B, C, D) Tumorspheres from HeLa or SiHa cells were dissociated into single cell suspension and were transduced with lentiviruses carrying Notch1 specific shRNAs (sh-Notch1#1 or sh-Notch1#2) followed by puromycin selection. The survived cells were collected and detected the expression of NICD or IDO1 by western blot (B). The insert numbers indicated the relative expression level in comparison to sh-LacZ transduced cells. Kynurenine concentration in culture supernatant was determined by Ehrlich's reagent (C). *, $p< 0.05$; **, $p< 0.01$. Tumorsphere cells were treated with Ro-4929097 as the indicated concentration and detected the expression of NICD or IDO1 by western blot (D). The insert numbers indicated the relative expression level in comparison to 0.1% DMSO control (labeled as 0 μM). (E) The putative binding site of RBPJ/CSL (-TGTGGGAA-) in IDO1 promoter was analyzed by EPD website and the binding of NICD to the RBPJ/CSL binding site of IDO1 promoter at +140 site in HeLa or SiHa tumorsphere cells was detected by ChIP method with an anti-Notch1 antibody and quantitated by qPCR method. Data were presented as percentage of input DNA. **, $p< 0.01$. All the experiments were repeated for three times and data from one experiment were presented.



HeLa	sh-Notch1-1	sh-Notch1-1
SER(D0.5)	2.19	1.48

SiH1	sh-Notch1-1	sh-Notch1-1
SER(D0.5)	1.53	1.83

Figure 3. **Knockdown of Notch1 in cervical CSCs enhances the efficacy of radiation treatment.** HeLa or SiHa tumorsphere cells were transduced with lentiviruses carrying Notch1 specific shRNAs (sh-Notch1-1 or sh-Notch1-2) followed by puromycin selection. The survived cells were collected and performed radiation treatment followed by clonogenic assay. The data were presented as fractional survival and the SER for an estimated fractional survival as 0.5 (D0.5) (SER(D0.5)) was calculated by D0.5(sh-LacZ)/D0.5(sh-Notch1). All the experiments were repeated for three times and data from one experiment were presented.

INCB-024360 could serve as a radiosensitizer in vivo

Due to the observations of that the inhibition of IDO1 activity enhanced the radiosensitivity of cervical CSCs (Fig. 3), we hypothesize that the IDO1 inhibitors could function as radiosensitizers for helping the radiation therapy in cervical cancer. After subcutaneous injection of SiHa tumorsphere cells into the back skin of nude mice to form xenograft tumors, we examined the radiosensitizer potential of INCB-024360 with an injection dose of 50 mg/kg at the day before radiation treatment. The final tumor sizes and weights in the group of INCB-024360 pre-injection plus radiation were the smallest than radiation alone group (Fig. 4A and 4B). The expression of Ki-67, the proliferation marker, was significantly decreased whereas the expression of phosphor-γH2Ax^{ser139}, the marker for DNA damage, was significantly increased in the group of INCB-024360 pre-injection plus radiation when compared to radiation alone group (Fig. 4C and 4D). These results suggest that INCB-024360 is a potential radiosensitizer to cervical cancer.

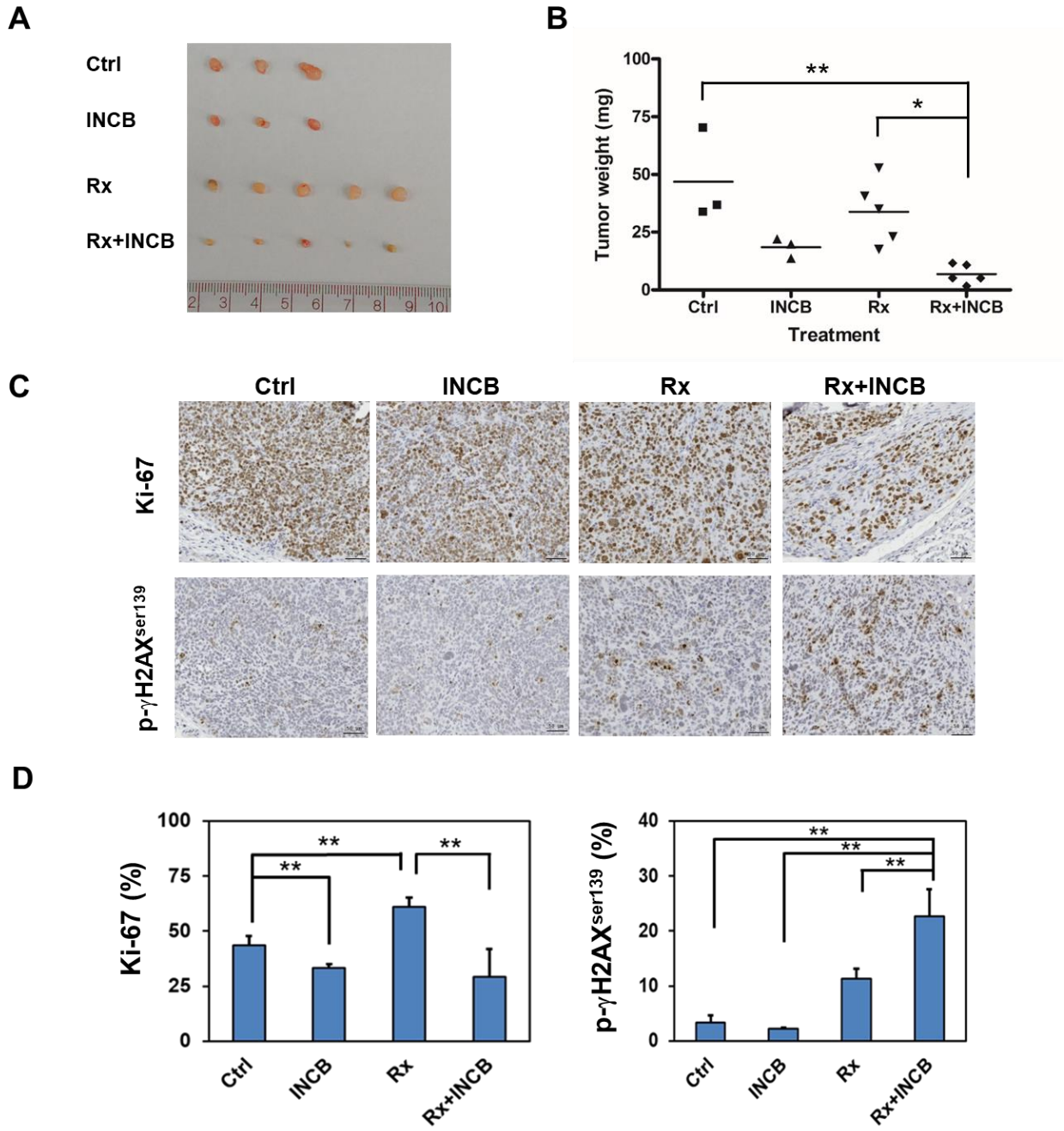


Figure 4. **IDO1 inhibitor enhances the efficacy of radiotherapy in vivo.** 1×10^5 of SiHa tumorsphere cells were subcutaneously injected to nude mice for tumor growth. After tumor volume reached 50 mm3, the mice were divided into four groups of non-treated

(Ctrl), INCB-024360 treated (INCB), radiotherapy (Rx), or INCB-024360 plus radiotherapy (INCB+Rx). For INCB or INCB+Rx group, mice were injected once with 50 mg/kg INCB-024360 intraperitoneally before radiotherapy. For Rx or INCB+Rx group, mice received 2 Gy radiation per day for total 10 Gy. Mice were sacrificed at Day 30 after the last radiation treatment and the xenografted tumors were taken out for picturing (A) and weighting (B). The expression of Ki-67 or p-γH2AX^{ser139} was determined by paraffin section followed by immunohistochemistrical staining (C). The inserted bars indicated 50 μm. The quantification results were performed by TissueFAX software (D). *, $p< 0.05$; **, $p< 0.01$. The experiments were repeated for two times and data from one experiment were presented.

結論

In the present study, we demonstrated that IDO1 expression was upregulated in cervical CSCs or cervical cancer cells after irradiation. Inhibition of IDO1 by RNA interference or INCB-024360 treatment increased the radiosensitivity of cervical CSCs indicating that IDO1 expression protects cervical CSCs from radiation treatment. We also found that the increased IDO1 in cervical CSCs was mediated by Notch1 activation through the direct binding of NICD to IDO1 promoter. In addition, IDO1 also regulated Notch1 expression through the binding of AhR/ARNT to Notch1 promoter. The treatment of INCB-024360 in SiHa xenograft model significantly enhanced the efficacy of radiotherapy. Furthermore, kynurenine enhanced the self-renewal capability of cervical cancer cells. These results suggest that IDO1 inhibitors are potentially developed as radiosensitizers for future cervical cancer therapy.

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六、論文發表

I. 期刊論文

1. Low HY, Lee YC, Lee YJ, Wang HL, Chen YI, Chien PJ, Li ST, **Chang WW**. Reciprocal Regulation Between Indoleamine 2,3-Dioxigenase 1 and Notch1 Involved in Radiation Response of Cervical Cancer Stem Cells. *Cancers (Basel)*. 2020. 12(6):1547.
2. Shen HT, Chien PJ, Chen SH, Sheu GT, Jan MS, Wang BY*, **Chang WW***. BMI1-Mediated Pemetrexed Resistance in Non-Small Cell Lung Cancer Cells Is Associated with Increased SP1 Activation and Cancer Stemness. *Cancers (Basel)*. 2020. 12(8):2069. *Contributed equally.
3. Lin LC, Lee HT, Chien PJ, Huang YH, Chang MY, Lee YC*, **Chang WW***. NAD(P)H:quinone oxidoreductase 1 determines radiosensitivity of triple negative breast cancer cells and is controlled by long non-coding RNA NEAT1. *Int J Med Sci.* 2020. 17(14):2214-2224. *Contributed equally.

II. 研討會論文

1. Shen HT, Wang HL, Chen YI, Jan MS, **Chang WW***. Interplay between Indoleamine 2,3-dioxigenase 1 and Notch1 in radiation response of cervical cancer stem cells. The 25th Biennial Congress of the European Association for Cancer Research (EACR25). 2018. Amsterdam, Netherlands. (Poster presentation) *, presenter.
2. Wang HL*, Chen YI, **Chang WW**. Interplay between Indoleamine 2,3-dioxigenase 1 and Notch1 in radiation response of cervical cancer stem cells. 2018 Taiwan Society for Stem Cell Research Annual Meeting. Maoli. (Poster Presentation, No.P8-07). *, presenter.
3. Wang HL, Chen YI, **Chang WW***. Reciprocal regulation of Indoleamine 2,3-dioxygenase 1 and Notch1 in radiation responses of cervical cancer stem cells. The 78th Annual Meeting of the Japanese Cancer Association, Kyoto, Japan. Sep 26-28, 2019. (Poster No. P-3264, Travel Grant Award awardee) *, presenter.
4. Dong JR*, Chen SM, **Chang WW**. Anti-Uterine fibroids activity of Strobilanthes Cripus water extracts is mediated by autophagy and reactive oxygen species induced G1 arrest. The 42rd Annual Meeting of The Molecular Biology Society of Japan (MBSJ). Fukuoka, Japan. (Poster No. 1P-0252) *, presenter.

MOST106-2314-B-040-024-MY3

出席國際會議心得報告(108 年度)

出差人	張文瑋(計畫主持人)	系所	生物醫學科學系(所)
會議日期	2019/09/26-28	會議地點	日本京都
會議名稱	The Molecular Biology Society of Japan 2019 Annual Meeting		
發表論文主題	Reciprocal regulation of Indoleamine 2,3-dioxigenase 1 and Notch1 in radiation response of cervical cancer stem cells		
心得報告			

1. 本次會議報告論文獲得大會之 Travel Grant Award 肯定

2. 本次會議中，以 single cell RNA analysis 分析癌症治療效果評估的講題不少，包含免疫檢查哨抑制劑使用前後的腫瘤內免疫細胞種類變化、癌症組織內腫瘤細胞/免疫細胞種類變化等，顯示單細胞定序分析未來可會是研究以及臨床精準醫療的主流方向之一。

3. 日本學術研討會在海報論文報告的特色為，有分區以及分別設置座長主持，並讓每篇海報論文報告者逐一報告，達到確實的研討會交流，其做法值得國內會議參考。

MOST106-2314-B-040-024-MY3
出席國際會議心得報告(108 年度)

出差人	董俊仁(碩士級兼任助理)	系所	生物醫學科學系(所)
會議日期	2019/12/3-6	會議地點	日本福岡
會議名稱	The Molecular Biology Society of Japan 2019 Annual Meeting		
發表論文主題	Anti-Uterine fibroids activity of Strobilanthes Cripus water extract is mediated by autophagy and reactive oxygen species induced G1 arrest		

心得報告

Intratumoral Heterogeneity Confers Drug Resistance in Cancer
Yusuke Yamamoto (National Cancer Center Research Institute)

講者建立卵巢癌病理組織的初代細胞培養，進行分析後證明癌細胞的 copy number 歧異度較大。後續以紫杉醇處理細胞，發現在某些細胞對於其有較高的抗性，便將紫杉醇處理後有抗性的細胞分離出 48 個不同的 single cell 擴增的細胞株，分析後發現大部分的細胞株皆有較高表現量之黃體酮受體，並進一步證明黃體酮受體之化學抑制劑可以降低這些細胞株對於紫杉醇的抗藥性。另外講者使用 MCF7 乳癌細胞株進行 single cell 的分離、基因體擴增及分析，發現 miR-27b 在某些細胞內表現量會下降，且 miR-27b 的 knockdown 會造成癌細胞細胞週期的停滯，並傾向進行上皮¬—間質轉換 (EMT)，幹性相關的基因表現量亦會上升；而 miR-27b 可能藉由靶向 LEF1，抑制 EMT 的進行而導致抗藥性的降低。講者利用腫瘤內細胞的異質性，進行藥物抗性篩選後分析單一細胞對於藥物抗性所做出的反應，將有助於改善現有抗癌藥物的使用方法。

Regulation of immune responses by extracellular vesicles
Toshiro Moroishi1,2,3 (1.Dep. of Mol. Enzymol., Kumamoto Univ., 2.CMHA, Kumamoto Univ., 3.PRESTO, JST)

作為細胞之間的溝通方法，外泌體(extracellular vesicles, EV)是近期非常熱門的研究主題，但其生理及分泌機制尚未明瞭。Hippo 訊號傳導路徑是細胞內的重要訊息路徑，會受到壓力、細胞間的接觸及 TGFb-Wnt 訊號的調控，Hippo 的活化會增加細胞的增生並使癌症趨向惡性；相反地，Hippo 的突變會導致癌細胞生長的抑制。講者首先使用螢光標定 EV，發現 CD63 及 CD11c 的抗體處理會使 EV 的分泌量降低，而 Hippo 訊號的抑制會改變 EV 的特徵與性質。接著，講者發現 Hippo 的突變會使樹突細胞活化，並且增加對 T 細胞的抗原呈現，也會使細胞內的核酸結合蛋白表現量增加，並藉由 Toll-Like Recptors 的核酸偵測系統增加 T 細胞的免疫反應。講者證實 EV 的

分泌及生理狀態可以由細胞內的訊息路徑所改變，且可以造成免疫系統的強烈反應，日後將會對 EV 及免疫系統活化的詳細機轉進行詳細的探討。

106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：106-2314-B-040-024-MY3		
計畫名稱：免疫檢查哨分子在癌幹細胞之非免疫調節活性的功能性探討					
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	1		Wang HL*, Chen YI, Chang WW. Interplay between Indoleamine 2,3-dioxigenase 1 and Notch1 in radiation response of cervical cancer stem cells. 2018 Taiwan Society for Stem Cell Research Annual Meeting. Maoli. (Poster Presentation, No.P8-07). *, presenter.
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	3	篇	1. Low HY, Lee YC, Lee YJ, Wang HL, Chen YI, Chien PJ, Li ST, Chang WW. Reciprocal Regulation Between Indoleamine 2,3-Dioxigenase 1 and Notch1 Involved in Radiation Response of Cervical Cancer Stem Cells. Cancers (Basel). 2020. 12(6):1547. 2. Shen HT, Chien PJ, Chen SH, Sheu GT, Jan MS, Wang BY*, Chang WW*. BMI1-Mediated Pemetrexed Resistance in Non-Small Cell Lung Cancer Cells Is Associated with Increased SP1 Activation and Cancer Stemness. Cancers (Basel). 2020. 12(8):2069. *Contributed equally. 3. Lin LC, Lee HT, Chien PJ, Huang YH, Chang MY, Lee YC*, Chang WW*. NAD(P)H:quinone oxidoreductase 1 determines radiosensitivity of triple negative breast cancer cells and is controlled by long non-coding RNA NEAT1. Int J Med Sci. 2020. 17(14):2214-2224. *Contributed equally.
		研討會論文	3		1. Shen HT, Wang HL, Chen YI, Jan MS, Chang WW*. Interplay between

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		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	10	人次	黃彥翔、洪冠琦、李玉齡、董俊仁、陳仕宏、蔡育昕、王彥智、鍾譯萱、張毓庭、葉育真
		碩士生	4		張文馨、李玉齡、董俊仁、陳仕宏
		博士生	0		
		博士級研究人員	0		
		專任人員	1		王惠琳(106/08-109/03)
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	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。）	