

參加美國細胞生物學會 2018 年會心得

錢宗良

今年美國細胞生物學會(ASCB)與歐洲分子生物學會(EMBO)在美國聖地牙哥 San Diego Convention Center 共同舉辦 2018 年大會 (圖一)。上次來訪聖地牙哥是 2014 年公務行程參加美國生技大展(BIO USA)，久別重逢這氣候宜人且景觀美麗的海港城市。本次參加主要以海報論文展現尚未發表的研究成果“Neural Differentiation of Glioblastoma Cell Lines by Glial Fibrillary Acidic Protein Promoter Driven HSVtk/Ganciclovir Suicide System”，此研究主要由碩士班畢業生駱為家同學完成之論文，駱同學於今年九月進入美國加州洛杉磯分校(UCLA)攻讀博士，惟因剛適應新環境且課業繁重，並未能共同參與這次年會。

本年度開幕 Keynote 演講，邀請到美國德州大學小兒醫學研究中心主任 Dr. Sean Morrison 以“Niches for Stem Cells in Bone Marrow”為題，介紹他一系列的研究成果，針對血液腫瘤與幹細胞的關係，非常深入地以分子生物學及細胞生物學技術解答許多問題，其中利用代謝體學技術發現造血的幹細胞對於維生素 C 的主要成分 Ascorbate，有極重要的需求。因此，在抵抗血液腫瘤的觀點而言，營養學中的維生素 C，除稱之“抗壞血酸”外，最新的研究亦有降低血液腫瘤發生的功能。

在 Dr. Morrison 演講完後，緊接的是大會安排的 Opening Night Reception，在會場中碰到同校生化所張明富教授及該所畢業校友，目前任教於中國醫藥大學呂郁蕙副教授等人。接著在會場舉辦之 International Research and Training Exchange Fair，我們特別去捧由臺大研發長李芳仁教授負責的臺灣攤位 (圖二)。由李教授安排由他的博士後研究員及研究生組成的服務團隊，成功行銷臺灣在細胞生物學及分子生物學的研究能量。

本年度值得特別注意的主題是“Machine Learning in Cell Biology”利用系統生物學所建立的數據資料利用人工智慧推斷細胞可能的行為或形態變化。此主題有別於傳統細胞生物學之固定研究模式，個人覺得是今年也是往後細胞生物學發展的重點趨勢。此外，本年度除傳統細胞形態觀察、紀錄的影像設備外，許多顯微鏡大廠，逐步發展 3D 影像之建構技術，從過去 Confocal Images，到目前可利用電子顯微鏡把更細緻的細胞內結構詳細呈現出來，當然在細胞到組織之巨觀結構而言，亦可藉由高速電腦運算重建起更連續完整的組織甚至器官影像。這些細胞生物學的最新進展，是過去 10 年 20 年前在研究方法技術設備有限的情況下，幾乎是夢想。而今，在財力雄厚的研究單位，技術設備均已不再是問題，惟有進一步可努力的地方，就是人才培訓與尋找細胞生物學尚未解決問題的答案！

由於我的海報論文被安排在最後一天的時段 (圖三)。因此，很難得三天的海

報論文發表均能有機會進一步瞭解。本年度的海報數量，如陳列廠商攤位一樣增加許多。參加年會的臺灣學者專家亦可藉由海報發表時段多做交流。本年度美國細胞生物學會成功地與 EMBO 結合(圖四)，在美國西岸生技產業大城聖地牙哥舉辦大會，就規模而言與 2016 年我參加舊金山的年會相比，廠商的贊助多許多。而各國參與的學者專家亦增加不少。對於單一學會辛苦舉辦年會，若能策略上找到可合辦之國際夥伴學會，相信這是一個成功雙贏的合作案例。

筆者參與美國細胞生物學年會，平均兩年參加一次。在此特別感謝科技部的計畫支持(圖五)，得以參加學習國際學術發展趨勢並可以親身觀察比較近年國際上基礎生物學研究之變化。由於各國科研計畫經費均有調整，對於從事基礎研究的學者愈來愈辛苦，所幸在年輕一輩對於生命科學的好奇與求知慾驅動下，仍有許多人願意投入。相對的各國在發展基礎研究的投資，則出現類似“軍備競賽”的趨勢，像美國頂尖的學術研究中心其軟、硬體的設備投入，加上原有傑出的資深研究人員熱心教育提攜後進，造就美國在全球人才競逐上仍居於領先之地位。

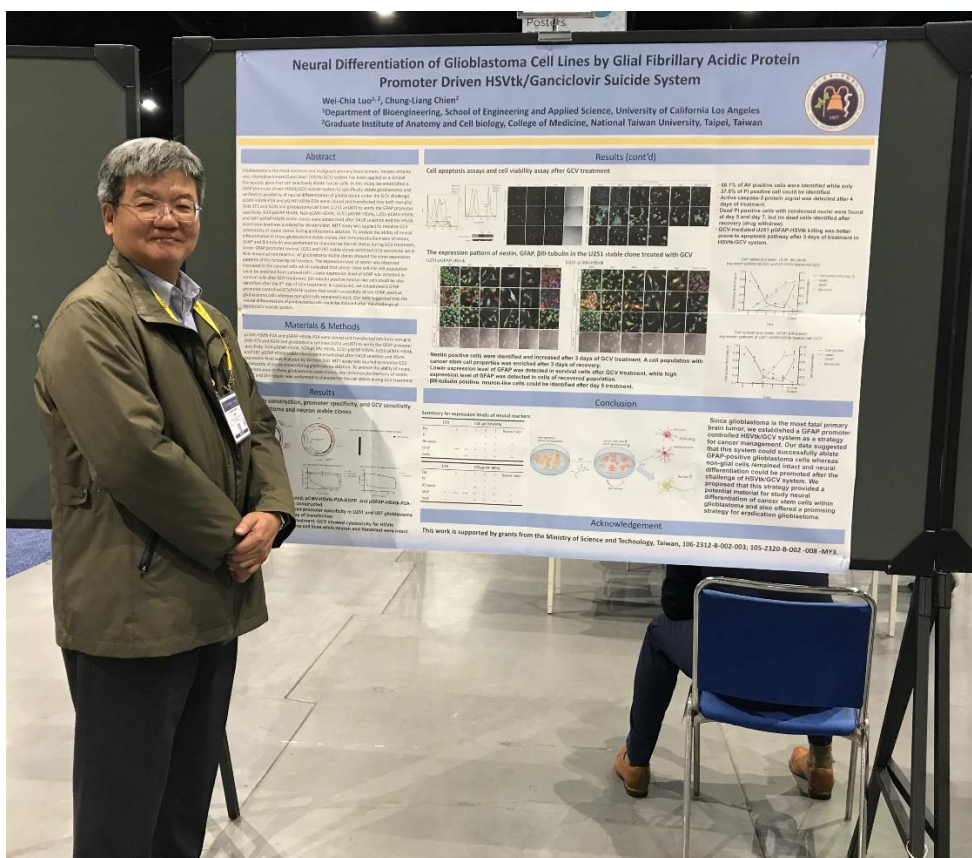
臺灣近兩年對於基礎研究發展，特別是生命科學領域有著茫然不知方向的情況。雖然，臺灣的學術就業市場有限，卻已培養許多相關領域的人才，年輕一輩為求未來發展，人才勢必外流。對於年輕學子能到美國或其他國家發展，也未必不是件壞事。期許這些出國進修工作的臺灣學子，能如同三、四十年前學有所成的前輩，在世界各地均能擁有一片天地，一旦有機會仍能回臺貢獻所學。



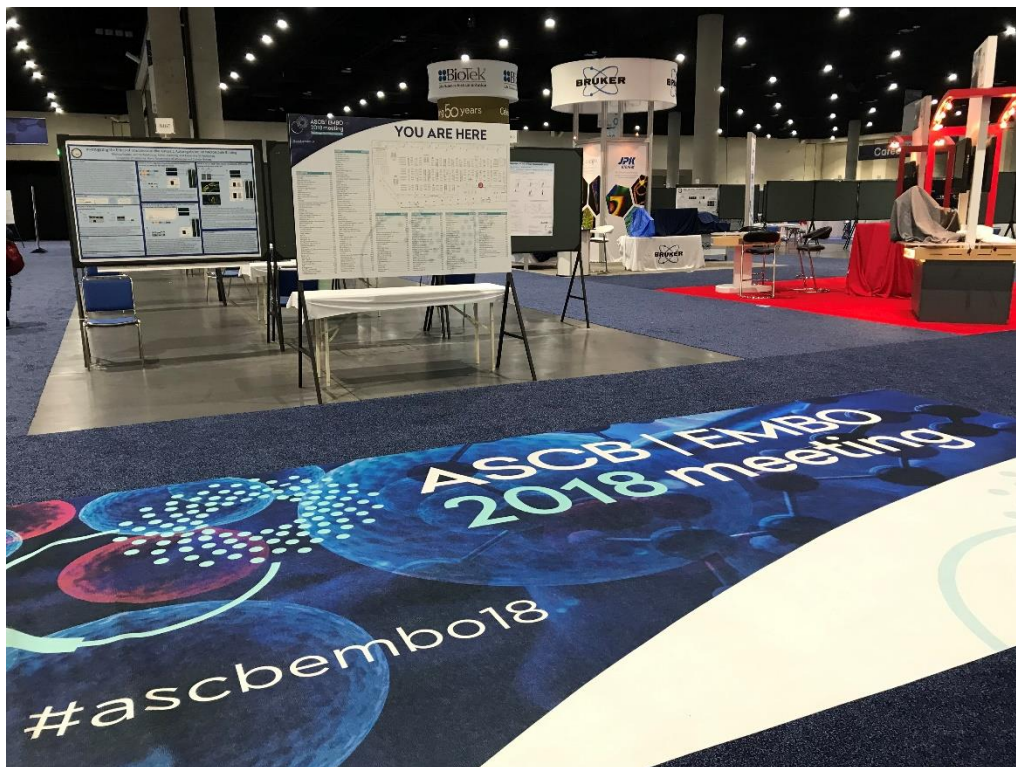
圖一、ASCB/ EMBO 2018 Meeting 在 San Diego Convention Center 舉行



圖二、筆者與張明富教授及李芳仁教授等人於 International Research and Training Exchange Fair 會場合影



圖三、筆者於海報發表演場



圖四、ASCB/ EMBO 2018 Meeting 展場入口

Neural Differentiation of Glioblastoma Cell Lines by Glial Fibrillary Acidic Protein Promoter Driven HSVtk/Ganciclovir Suicide System

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Abstract

Glioblastoma is the most common and malignant primary brain tumors. Inverse oncoprotein thymidine kinase/ganciclovir (HSVtk/GCV) system has been applied as a clinical therapeutic gene that can selectively ablate cancer cells. In this study, we established a therapeutic gene that can selectively ablate cancer cells. In this study, we established a therapeutic gene that can selectively ablate cancer cells. In this study, we established a therapeutic gene that can selectively ablate cancer cells.

Materials & Methods

pCMV-HSVtk-P2A and pGFP-HSVtk-P2A were cloned and transfected into both non-glioblastoma (N1A and N2A) and glioblastoma cell lines (U251 and U87) to verify the GFP promoter specificity. N1A-pGFP-HSVtk, N2A-pGFP-HSVtk, U251-pGFP-HSVtk, U87-pGFP-HSVtk, and U87-pGFP-HSVtk stable clones were established after G412 selection and the HSVtk expression level was analyzed by Western blot. MTT assay was applied to monitor GCV cytotoxicity of stable clones during glioblastoma ablation. To analyze the ability of neural differentiation in these glioblastoma stable clones, the immunocytochemistry of nestin, GFAP, and β -tubulin was performed to characterize the cell status during GCV treatment.

Results

Plasmids construction, promoter specificity, and GCV sensitivity of glioblastoma and neuron stable clones

Results (cont'd)

Cell apoptosis assays and cell viability assay after GCV treatment

88.1% of AV positive cells were identified while only 37.8% of P4 positive cell could be identified. Active caspase-3 protein signal was detected after 4 days of treatment. Dead P4 positive cells with condensed nuclei were found at day 5 and day 7, but no dead cells identified after recovery (drug withdrawal). GCV-mediated U251-pGFP-HSVtk killing was better proven to apoptosis pathway after 3 days of treatment in HSVtk/GCV system.

The expression pattern of nestin, GFAP, β -tubulin in the U251 stable clone treated with GCV

Nestin positive cells were identified and increased after 3 days of GCV treatment. A cell population with cancer stem cell properties was enriched after 3 days of recovery. Lower expression level of GFAP was detected in survival cells after GCV treatment, while high expression level of GFAP was detected in cells of recovered population. β -tubulin positive, neuron-like cells could be identified after day 5 treatment.

Conclusion

Since glioblastoma is the most fatal primary brain tumor, we established a GFP promoter controlled HSVtk/GCV system as a strategy for cancer management. Our data suggested that this system could successfully ablate GFAP-positive glioblastoma cells whereas non-glioblastoma cells remained intact and neural differentiation could be promoted after the challenge of HSVtk/GCV system. We proposed that this strategy provided a differentiation of cancer stem cells within glioblastoma and also offered a promising strategy for eradication glioblastoma.

Acknowledgement

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