# 建立抗癌症轉移與美白活性模式及其機制探討

# Study on the establishment of the bioassay models and its mechanism on antimetastasis and depigmentation

## 中文摘要

本實驗建立抗癌症轉移與美白活性的篩選平台，並針對一系列由天然物衍生而成的結構類似化合物進行金屬基質蛋白酵素-2 (MMP-2)與 MMP-9 和酪胺酸酵素活性檢測與機制探討。

在抗癌症轉移方面，藉由明膠 zymography 法、西方墨點法與反轉錄聚合酵素連鎖反應發現化合物 DH 可藉由降低 MMP-2 與 MMP-9 基因和蛋白表現量來抑制 MMP-2 與 MMP-9 活性，但其結構類似物之對照化合物 D 則不具此活性；由纖維蛋白 (fibrin)酵素電泳法與酵素分析得知 DH 也能藉由抑制尿激酵素型纖維溶解酵素激活物 (urokinase-type plasminogen activator, uPA)酵素活性（IC50 為 13.724 mM）。在劃痕試驗（IC50 為 1.75 mM）、群落擴散試驗與跨膜實驗（IC50 為 1.262 mM）上，DH 具有濃度相關的抑制 HT 1080 細胞株之遷移和侵襲；細胞貼附試驗也發現 DH 能降低 HT 1080 對細胞外間質的貼附力。於活體試驗中，以 B16-F10 為轉移細胞株，經由尾靜脈注射一次，經 3 週後，可成功誘導成肺轉移瘤。每天一次腹腔注射 DH (50 mg/kg/day)顯著下降肺轉移現象，且與對照組比較，DH 可降低因癌症轉移引起之病理現象（如：體重減輕、脫毛與後肢行走無力等）。故推論 DH 可能經抑制 uPA 酵素活性以阻斷 pro-MMPs 被轉換成活化型 MMPs、及抑制 MMP-2 與 MMP-9 基因表現量，造成 MMP-2 與 MMP-9 活性降低，而來阻止癌細胞的遷移、侵襲和與細胞外間質的貼附，最終達到抗轉移之效果。

而美白活性方面，以商品酪胺酸以商品酪胺酸酵素（香菇來源）進行實驗，當基質為酪胺酸時，發現 DH 與 EH 具濃度相關抑制效果，IC50 分別為 5.697 與 5.256 mM，且其相對於基質酪胺酸之抑制型態皆為非競爭型抑制，而其結構類似物之對照化合物 D 與 E 則不具此活性；但當基質為多巴時則皆不具此活性；利用原態 PAGE 電泳活性染色（基質為酪胺酸）亦發現抑制酪胺酸酵素結果，但 EH 在基質為多巴時也能下降酪胺酸酵素活性。以 B16-F10 細胞株進行相關美白活性探討，EH 在無顯著毒性之下，於有無 alpha-MSH 存在時皆可降低細胞的黑色素生成量與其酪胺酸酵素活性；利用西方墨點法發現其可下降酪胺酸酵素、TRP-1、TRP-2 與 MITF 之蛋白質表現量；且於 IBMX（cAMP 活化劑）存在下，EH 依然對黑色素生成有抑制現象；而外加 PD98059（ERK 抑制劑），並不會抑制 EH 對黑色素生成之下降趨勢，故推測 EH 抑制酪胺酸酵素可能經由影響 cAMP 的增加所造成黑色素生成上升的相關訊息傳導路徑。同時，於細胞處理 IBMX 之下，EH 對於酪胺酸酵素、MITF、CREB、p-CREB 與 PKA (PKA catalytic subunit)之蛋白質表現量皆有下降現象，並降低 p-CREB/CREB 的比率；且會減少 cAMP 的表現。故 EH 可能藉由降低 cAMP 表現以降低 PKA 的活化，以及下降 CREB 之蛋白質表現量，因而降低 CREB 被磷酸化形成 p-CREB，進而減少 MITF 與酪胺酸酵素蛋白質表現量，最終達到降低酪胺酸酵素活性與黑色素生成量之效果。

## 英文摘要

The goal of this study is to establish the bioassay models on antimetastasis and

depigmentation. We screen the effects of similar structure of synthetic products on matrix metalloproteinase (MMP)-2, MMP-9 and tyrosinase activities in cell models and further investigate their possible mechanisms.

For antimetastasis, DH suppresses the expression of gene and protein levels in MMP-2 and MMP-9, and then decreases their corresponding activities in HT 1080 as the results of RT-PCR assay, western blot assay and gelatin-SDS zymography, however, the control synthetic product, D, has no effects as above-mentioned. From the results of fibrin-SDS zymography assay, it is found that DH could suppress the uPA (urokinase-type plasminogen activator) activity (IC50 is 13.724 mM). DH also exhibits the dose-dependently inhibitory activities against migration and invasion of HT 1080 in wound healing assay (IC50 is 1.75 mM), the transwell assay (IC50 is 1.262 mM) and colony dispersion assay. Result of cell adhesion assay shows that DH could inhibit cell from adhering to extracellular matrix (ECM). In animal experiment, the B16-F10 cell line is used to establish the metastatic model in C57BL/6 mice. The intraperitoneal administration of 50 mg/kg/day DH once a day for 3-weeks is found to decrease the number of metastatic nodules and lung weight, and elevated the survival rate of mice comparing with control group. Furthermore, DH could suppress the pathologic phenomena (ex. The body weight loss, molt, and the lacking strength of limbs). These results demonstrate that DH might effectively inhibit tumor metastasis in vitro and in vivo. It is proposed that the inhibition of cell adhesion and mobility by suppressing the MMP-2 and MMP-9 activities. Moreover, the down-regulations of MMP-2 and MMP-9 gene expression and the inhibition of activation of pro-MMPs to MMPs by suppressing the uPA activity may be involved in the possible molecular mechanism.

At the depigmentation assay, DH and EH show dose-dependently inhibitory activities against mushroom tyrosinase, and the IC50 is 5.697 and 5.256 mM, respectively, and show noncompetitively inhibitory modes in the present of tyrosine, but not DOPA. The control synthetic products, D and E, have no inhibitory effects on tyrosinase. Using tyrosinase activity staining in PAGE gels, both DH and EH show dose-dependently inhibitory pattern in the presence of tyrosine, while, EH also could suppress tyrosinase activity in the presence of DOPA. Using B16-F10 cell lines to investigate the possible mechanism of depigmentation, it is found that EH could decrease melanin content and tyrosinase activity in dose-dependent patterns with or without alpha-MSH, and suppress tyrosinase, TRP-1, TRP-2 and MITF protein expression in western blot assay. EH is found to inhibit melanin content in the presence of IBMX (cAMP activator), but is failed to inhibit the reduced melanin contents in the presence of PD98059 (ERK inhibitor). Therefore, it is proposed that the EH may inhibit tyrosinase activity in a cAMP-dependent pathway. Furthermore, EH could suppress tyrosinase, MITF, CREB, PKA (PKA catalytic subunit) protein expression levels, p-CREB/CREB ratio, and cAMP levels. These depigmentation results reveal that EH suppresses the activation of PKA by decreasing cAMP levels, and decreases the expression of protein levels in CREB, and then decreases the phosphorylation of CREB, the MITF and tyrosinase protein expressions, and finally suppresses tyrosinase activity and melanin contents.