

# 不同型G蛋白在藥劑誘導K562細胞分化下所扮演調合者角色 = G-Proteins play concerted roles in chemical induced K562 cell ...

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## 摘要

人類慢性骨髓性白血病細胞株 (chronic myeloid leukemia cell line, CML) K562, 於不同之誘導劑, 如Hemin、HMBA、sodium butyrate及DMSO等刺激下, 可使K562血癌細胞分化成具有功能性之紅血球細胞。而異三元體鳥糞嘌呤核苷酸結合蛋白 (heterotrimeric guanine nucleotide binding proteins), 簡稱G 蛋白 (G-protein), 其位於細胞內雙層膜內部, 為膜上之周邊蛋白 (peripheral protein) 的一種, 是負責接受細胞膜上受體 (G protein coupled receptor, GPCR) 所耦合 (couple) 之訊號後, 以放大 (amplification) 訊號並活化作用體 (effector), 切換 (switch) 之傳遞路徑。G蛋白於訊息傳遞中扮演著最重要之一環, 於荷爾蒙 (hormones) 的調控、細胞之生長、分化 (differentiation) 及發育 (development) 上, 為極具重要之角色。而G蛋白是由 $\beta$  和  $\gamma$  三種次單元 (subunit) 所組合而成, 而其中G蛋白 $\alpha$  侖堇璠落脛蛄TPase之活性, 能夠水解 (hydrolysis) GTP成GDP。而依據蛋白質功能性與同源性的不同, 可分為四大家族: Gs、Gi / o、Gq / 11和G12 / 13。本研究使用K562人類血癌細胞株作為模式細胞, 利用Hemin、HMBA以及黃耆 (Huangqi) 來誘導細胞進行分化, 以合成  $\alpha$ -球蛋白 (globin) 或  $\beta$ -球蛋白作為分析指標, 並以半定量反轉錄 $\beta$ -聚合 $\beta$ 連鎖反應 (semi-quantitative RT-PCR) 分析不同誘導劑誘導後不同家族之G蛋白 $\alpha$  侖堇璠落脛蛄變化。從試驗中觀察到四種基因為GNAS isoform (GNAS和GNASS)、G  $\alpha$  i2、G  $\alpha$  11以及G  $\alpha$  11 pseudo gene, 其中: (1) G  $\alpha$  i2於HMBA誘導後表現量顯著高於控制組, 其表現量高出一倍之多 ( $P < 0.05$ ); (2) 經黃耆誘導後, GNAS表現量與控制組相較明顯之增加許多, 而另一方面添加黃耆後之GNAS的short form表現量則較控制組的少 ( $P < 0.05$ ); 而將GNAS isoform基因片段分別轉染至K562細胞中並以黃耆加以誘導, 發現經GNAS form轉染後, 細胞中  $\alpha$ -球蛋白表現量顯著高於GNAS short form之  $\alpha$ -球蛋白表現量 ( $P < 0.05$ )。因此本實驗室提出一種假說, 在經由黃耆誘導K562細胞中, G蛋白 $\alpha$  侖堇璠落脛蛄NAS form會將訊號傳遞給作用體腺核酸環化 $\beta$  (adenylyl cyclase, AC), 增加cAMP含量, 因而活化PKA; 再經由PKA路徑活化下游因子以產生  $\alpha$ -球蛋白; (3) 此外由於本研究所選殖之G  $\alpha$  11, 也選殖到G  $\alpha$  11 pseudogene, 經由酶素Xho I剪切確認發現, 經誘導劑誘導後均會表現G  $\alpha$  11 pseudogene, 而G  $\alpha$  11在誘導劑誘導下, 表現量均顯著高於控制組 ( $P < 0.05$ ), 此意味G  $\alpha$  11與G  $\alpha$  11 pseudogene是否會互相競爭而抑制了G  $\alpha$  11之功能, 此發現值得更進一步研究探討其原因及調控機制。以上不同之G蛋白 $\alpha$  侖堇璠落脛蛄T息傳遞調控機制尚未完全明瞭, 仍需更進一步之研究, 尤其是中藥黃耆對於血液疾病之治療極具幫助, 若能完全了解其分化機制, 或許能讓紅血球相關疾病患者, 可藉此得到治癒或減緩病情的機會。 關鍵字: G蛋白, 黃耆, GNAS form,  $\alpha$ -球蛋白

關鍵詞: G蛋白; 黃耆; GNAS form;  $\alpha$ -球蛋白

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