

# 孤兒 G 蛋白偶聯受體56於 K562 細胞之功能分析 = Functional analysis of G-protein coupled receptor 56 in K562 cell

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## 摘要

megakaryocyte G蛋白偶聯受體 ( G protein-coupled receptor, GPCR ) 為細胞表面受器中之最大家族，在人類基因組中有多於1%的基因編碼此類受體。當GPCR接受如香味、風味等刺激後，將細胞外信號轉變為影響生物體生理及細胞反應訊息。GPCR56是GPCR的家族新發現成員之一。對照其他正常之組織，在幾個癌症組織中，GPCR56的表現相對的較高，推測GPCR56為潛在致癌因子。但在黑色素瘤細胞的研究中，卻有相反的研究結果。增強GPCR56表現可抑制黑色素瘤轉移和腫瘤成長，而減少GPCR56也提高腫瘤進展。因此，GPCR56在癌化過程之角色仍然未明。由於GPCR56的表現在K562血癌細胞高於血癌患者達四倍之多，推測降低GPCR56的表現有益於血癌細胞發展或GPCR56與分化為功能性血球有關。本實驗以過量表現GPCR56在K562細胞中，並且以西方墨點轉漬法證明蛋白質之表現性。以及利用RNA干擾方式 ( RNA interference ) 來導致GPCR56在細胞內靜默。經分析GPCR56之表現不影響細胞生長，而且巨核型細胞數量增加；在Hemin存在下，紅血球  $\alpha$ -珠蛋白微幅增加，而  $\beta$ -珠蛋白微幅減少；本篇利用RT-PCR檢測顆粒細胞granulocyte ( CD13、CD33 ) ；單核細胞monocyte ( CD14、CD68 ) ；巨核細胞megakaryocyte ( CD41、CD61 ) 等細胞分化族群 ( cluster of differentiation ; CD marker ) 之表現。其中，CD33、CD41及CD61是增加的；而CD13、CD14及CD68不變。綜合以上結果，推論GPCR56可能參與或領導K562細胞走向巨核細胞。 [ 英文摘要 ]

關鍵詞：G蛋白偶聯受體；單核細胞；顆粒細胞

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## 參考文獻

- 62 - 參考文獻 1. 涂慧珠。(2006)。GNAS、Gi、Gq蛋白經由Huangqi、Hemin和HMBA誘導K562細胞分化中所扮演之角色。大葉大學分子生物科技研究所碩士論文。第1-39頁。 2. Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Ogreid, D., Doskeland, S. O., Jahnsen, T., and Cho-Chung, Y. S. (1988) Selective modulation of protein kinase isozymes by the site-selective analog 8-chloroadenosine 3',5'-cyclic monophosphate provides a biological means for control of human colon cancer cell growth, *Proc Natl Acad Sci U S A* 85, 6319-6322. 3. Andersson, L. C., Nilsson, K., and Gahmberg, C. G. (1979) K562--a human erythroleukemic cell line, *Int J Cancer* 23, 143-147. 4. Baron, F., Storb, R., and Little, M. T. (2003) Hematopoietic cell transplantation: five decades of progress, *Arch Med Res* 34, 528-544. 5. Baselga, J., and Arribas, J. (2004) Treating cancer's kinase 'addiction', *Nat Med* 10, 786-787. 6. Bianchi, N., Osti, F., Rutigliano, C., Corradini, F. G., Borsetti, E., Tomassetti, M., Mischiati, C., Feriotto, G., and Gambari, R. (1999) The DNA-binding drugs mithramycin and chromomycin are powerful inducers of erythroid differentiation of human K562 cells, *Br J Haematol* 104, 258-265. 7. Bixby, D. L., and Talpaz, M. (2008) Efficacy of various doses and schedules of second-generation tyrosine kinase inhibitors, *Clin Lymphoma Myeloma* 8 Suppl 3, S95-S106. 8. Bjarnadottir, T. K., Fredriksson, R., Hoglund, P. J., Gloriam, D. E., Lagerstrom, M. C., and Schioth, H. B. (2004) The human and mouse repertoire of the adhesion family of G-protein-coupled receptors, *Genomics* 84, 23-33. 9. Blaker, M., Schmitz, M., Gocht, A., Burghardt, S., Schulz, M., Broring, D. C., - 63 - Pace, A., Greten, H., and De Weerth, A. (2004) Differential expression of somatostatin receptor subtypes in hepatocellular carcinomas, *J Hepatol* 41, 112- 118. 10. Bockaert, J., and Pin, J. P. (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success, *EMBO J* 18, 1723-1729. 11. Bohler, T., Schutz, M., Budde, K., Neumayer, H. H., and Waiser, J. (2007) Differential effects of single dose FTY720 on CD62L+ B-cells in stable renal allograft recipients, *Int Immunopharmacol* 7, 88-95. 12. Borgatti, R., Marelli, S., Bernardini, L., Novelli, A., Cavallini, A., Tonelli, A., Bassi, M. T., and Dallapiccola, B. (2009) Bilateral frontoparietal polymicrogyria (BFPP) syndrome secondary to a 16q12.1-q21 chromosome deletion involving GPR56 gene, *Clin Genet* 76, 573-576. 13. Boylan, M. O., Athanassiou, M., Houle, B., Wang, Y., and Zarbl, H. (1996) Activation of tumor suppressor genes in nontumorigenic revertants of the HeLa cervical carcinoma cell line, *Cell Growth Differ* 7, 725-735. 14. Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug, *Nat Rev Drug Discov* 1, 493-502. 15. Chavez-Gonzalez, M. A., Ayala-Sanchez, M., and Mayani, H. (2009) [Chronic myeloid leukemia in the 21st century: biology and treatment], *Rev Invest Clin* 61, 221-232. 16. Chen, Z., Hu, M., and Shivdasani, R. A. (2007) Expression analysis of primary mouse megakaryocyte differentiation and its application in identifying stage-specific molecular markers and a novel transcriptional target of NF-E2, *Blood* 109, 1451-1459. 17. Colvin, G. A., and Eifenbein, G. J. (2003) The latest treatment advances for acute myelogenous leukemia, *Med Health R I* 86, 243-246. 18. Cortes, J. (2004) Natural history and staging of chronic myelogenous leukemia, - 64 - *Hematol Oncol Clin North Am* 18, 569-584, viii. 19. Dabasi, G., Hauser, P., Kertesz, G. P., Balazs, G., Karadi, Z., Constantin, T., Bognar, L., Klekner, A., Schuler, D., and Garami, M. (2007) [Imaging of pediatric brain tumors using somatostatin analogue 111In-DTPA-D-Phe1- octreotide], *Magy Onkol* 51, 229-234. 20. Dai, Y., Rahmani, M., Corey, S. J., Dent, P., and Grant, S. (2004) A Bcr/Abl- independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2, *J Biol Chem* 279, 34227-34239. 21. Delgado, M. D., Quincoces, A. F., Gomez-Casares, M. T., Martinez, C. A., Cuadrado, M. A., Richard, C., and Leon, J. (1992) Differential expression of ras protooncogenes during in vitro differentiation of human erythroleukemia cells, *Cancer Res* 52, 5979-5984. 22. Donato, N. J., Wu, J. Y., Stapley, J., Gallick, G., Lin, H., Arlinghaus, R., and Talpaz, M. (2003) BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571, *Blood* 101, 690-698. 23. Drexler, H. G., MacLeod, R. A., and Uphoff, C. C. (1999) Leukemia cell lines: in vitro models for the study of Philadelphia chromosome-positive leukemia, *Leuk Res* 23, 207-215. 24. Druker, B. J. (2008) Translation of the Philadelphia chromosome into therapy for CML, *Blood* 112, 4808-4817. 25. D'Souza, U. M., Russ, C., Tahir, E., Mill, J., McGuffin, P., Asherson, P. J., and Craig, I. W. (2004) Functional effects of a tandem duplication polymorphism in the 5'flanking region of the DRD4 gene, *Biol Psychiatry* 56, 691-697. 26. Ebert, M. H., and Kagen, M. S. (1948) Chronic myelogenous leukemia with purpura, *Arch Derm Syphilol* 57, 458. 27. Erdstein, A. A., Daas, P., Bradstock, K. F., Robinson, T., and Hertzberg, M. S. (2004) Tuberculosis in allogeneic stem cell transplant recipients: still a problem - 65 - in the 21st century, *Transpl Infect Dis* 6, 142-146. 28. Eszlinger, M., Krohn, K., Beck, M., Kipling, D., Forbes-Robertson, S., Lauter, J., Toenjes, A., Wynford-Thomas, D., and Paschke, R. (2006) Comparison of differential gene expression of hot and cold thyroid nodules with

primary epithelial cell culture models by investigation of co-regulated gene sets, *Biochim Biophys Acta* 1763, 263-271. 29. Faderl, S., Talpaz, M., Estrov, Z., O'Brien, S., Kurzrock, R., and Kantarjian, H. M. (1999) The biology of chronic myeloid leukemia, *N Engl J Med* 341, 164- 172. 30. Fattal, O., Van Dop, C., Chen, F., Chang, J. T., Zoltan, T. B., Wetzel, G. T., and Klitzner, T. S. (1995) Steady-state mRNA levels of G protein subunits in developing rabbit myocardium, *Biochem Mol Med* 56, 108-114. 31. Fibach, E., Prasanna, P., Rodgers, G. P., and Samid, D. (1993) Enhanced fetal hemoglobin production by phenylacetate and 4-phenylbutyrate in erythroid precursors derived from normal donors and patients with sickle cell anemia and beta-thalassemia, *Blood* 82, 2203-2209. 32. Fraser, C. M., and Lee, N. H. (1995) Regulation of muscarinic receptor expression by changes in mRNA stability, *Life Sci* 56, 899-906. 33. Frazer, R., Irvine, A. E., and McMullin, M. F. (2007) Chronic Myeloid Leukaemia in The 21st Century, *Ulster Med J* 76, 8-17. 34. Gambari, R., and Fibach, E. (2007) Medicinal chemistry of fetal hemoglobin inducers for treatment of beta-thalassemia, *Curr Med Chem* 14, 199-212. 35. George, S. R., O'Dowd, B. F., and Lee, S. P. (2002) G-protein-coupled receptor oligomerization and its potential for drug discovery, *Nat Rev Drug Discov* 1, 808- 820. 36. Gilliland, D. G., Jordan, C. T., and Felix, C. A. (2004) The molecular basis of leukemia, *Hematology Am Soc Hematol Educ Program*, 80-97. 37. Goldman, J. (2003) Chronic myeloid leukemia--past, present, and future, *Semin - 66 - Hematol* 40, 1-3. 38. Goldman, J. M. (2007) Advances in CML, *Clin Adv Hematol Oncol* 5, 270-272, 292. 39. Goldman, J. M., and Druker, B. J. (2001) Chronic myeloid leukemia: current treatment options, *Blood* 98, 2039-2042. 40. Goldman, J. M., and Melo, J. V. (2003) Chronic myeloid leukemia--advances in biology and new approaches to treatment, *N Engl J Med* 349, 1451-1464. 41. Gratwohl, A., Baldomero, H., Frauendorfer, K., and Urbano-Ispizua, A. (2006) EBMT activity survey 2004 and changes in disease indication over the past 15 years, *Bone Marrow Transplant* 37, 1069-1085. 42. Grigg, A., and Hughes, T. (2006) Role of allogeneic stem cell transplantation for adult chronic myeloid leukemia in the imatinib era, *Biol Blood Marrow Transplant* 12, 795-807. 43. Hait, W. N., Choudhury, S., Srimatkandada, S., and Murren, J. R. (1993) Sensitivity of K562 human chronic myelogenous leukemia blast cells transfected with a human multidrug resistance cDNA to cytotoxic drugs and differentiating agents, *J C*