Resveratrol induces differentiation and apoptosis in human leukemic cells

沈美伶、廖慧芬 博士 ; 謝淳仁 博士

E-mail: 9318413@mail.dyu.edu.tw

ABSTRACT

Cancer is a severe problem in human health. The method to treat cancer, such as chemotherapy, radiotherapy, and surgical treatment, caused worse side effect in cancer patients. Recently, research on the induction of apoptosis and differentiation in tumor cells were considered as a new strategy with low toxicity and more effective. Natural products like resveratrol may correspond with this concept. Resveratrol (trans-3,5,4 ’ - trihydroxystilbene) is a phytoalexin found in grapes and other herbs that has anticarcinogenic, anti-inflammatory, antiplatelet, antioxidant and profungicidic effects. In the present study, we investigated the effects of resveratrol on differentiation and apoptotic processes in human leukemic U937, HL-60, NB4 and K562 cell lines. Cytotoxicity of resveratrol on these four cells showed a dose- and time-dependent increase. Induction of apoptosis in resveratrol-treated U937, HL-60 and NB4 cells was demonstrated by the appearance of apoptotic body. However, resveratrol-treated K562 cells showed neither apoptotic body nor sub-G1 peak by flow cytometric assay. In addition, morphological observation, functional changes by Hb stain and cell surface antigens expression showed the erythropoiesis of K562 cells were induced by resveratrol.

Keywords : Resveratrol ; Leukemia ; Apoptosis ; Differentiation

Table of Contents

目錄 封面內頁 簽名頁 授權書…….iii 中文摘要…….v 英文摘要…….vii 誌謝…….viii 目錄…….…ix 圖目錄…….xiii 表目錄…….xv 附錄圖表…….xvi 縮寫表…….xvii 第一章　緒論 第二章 文獻回顧 2.1癌症…….…..3 2.1.1 一般癌症形成的主要原因…….3 2.1.2 癌症治療…….4 2.1.3 白血病分類與治療…….5 2.2細胞週期…….5 2.3 細胞凋亡與壞死……..7 2.3.1細胞壞死（Necrosis）……7 2.3.2細胞凋亡（Apoptosis）……8 2.3.3細胞凋亡與壞死之生物化學特徵…….8 2.4 細胞分化…….…10 2.5細胞分化誘導及評估……..11 2.6人類白血病細胞株簡介……..12 2.7 MAPK簡介…….…14 2.7.1 ERK1/2 —MAPK……..…...15 2.7.2 p38 —MAPKs……..15 2.7.3 JNK —MAPKs簡介……15 2.8各種抑制劑簡介…….…16 2.8.1 JNK抑制劑SP600125……..16 2.8.2 ERK抑制劑PD98059……17 2.8.3 p38-MAPK抑制劑SB203580……..…17 2.8.4 Serine/Threonine kinas抑制劑 5-iodotubercidin……..17 2.9白藜蘆醇 (Resveratrol，簡稱RES)……..18 第三章　材料與方法 3.1 實驗材料……..……..20 3.1.1人類白血病細胞株……..20 3.1.2 藥品……..20 3.1.3單株抗體……..……..21 3.1.4實驗用試劑……..21 3.1.5各種抑制劑……..22 3.2實驗儀器…….……..22 3.3實驗方法…….……..23 3.3.1細胞株冷凍、解凍與培養……..23 3.3.2細胞毒性 (Cytotoxicity) 分析…….…...24 3.3.3細胞週期分析……..25 3.3.4細胞凋亡之分析……..25 3.3.5 RES合併STI571對K562細胞之影響 分析……26 3.3.6細胞分化作用之分析……..28 3.3.7細胞表面抗原的測定……..29 3.3.8各種抑制劑的處理及評估……..30 第四章　結果 4.1 RES對人類白血病細胞之生長抑制活性…….32 4.2 RES對誘導人類白血病細胞凋亡之能力…….32 4.3 RES對K562細胞週期之影響……..….33 4.4 RES對誘導人類白血病細胞分化之活性…….33 4.5 RES誘導人類白血病細胞產生過氧化物之 活性……34 4.6 RES誘導人類白血病細胞產生吞噬能力之 活性……..……..34 4.7 RES誘導K562細胞紅血球分化之活性.…….35 4.8 RES對K562細胞表面抗原表現的影響….. ...35 4.9 RES與STI571誘導K562細胞分化之能力…36 4.10 RES誘導MAPK對K562細胞之生理活性 影響……..37 第五章 討論 第六章 結論 6.1 總結…….…41 6.2 重要性…….42 圖目錄 圖1 RES對NB4細胞之增殖抑制影響……..……..55 圖2 RES對U937細胞之增殖抑制影響……..56 圖3 RES對HL-60細胞之增殖抑制影響……..….…...57 圖4 RES對K562細胞之增殖抑制影響……..58 圖5 RES誘導NB4

細胞之形態變化……...59 圖6 RES誘導U937細胞之形態變化…….……60 圖7 RES誘導HL-60細胞之形態變化…….…...61 圖8 RES誘導K562細胞之形態變化……..62 圖9 RES誘導NB4細胞之形態變化……...63 圖10 RES誘導U937細胞之形態變化…….…...64 圖11 RES誘導HL-60細胞之形態變化……..65 圖12 RES誘導K562細胞之形態變化……66 圖13 RES誘導人類血癌細胞凋亡之能力……..…….67 圖14 RES誘導K562細胞表現sub-G1之能力分析……...68 圖15 RES誘導K562細胞表現sub-G1之能力分析……...69 圖16 RES誘導NB4細胞產生過氧化物之作用…….……70 圖17 RES誘導U937細胞產生過氧化物之作用……71 圖18 RES誘導HL-60細胞產生過氧化物之作用….…….72 圖19 RES誘導K562細胞產生過氧化物之作用……73 圖20 RES誘導NB4細胞產生吞噬作用之能力……74 圖21 RES誘導U937細胞產生吞噬作用之能力……75 圖22 RES誘導HL-60細胞產生吞噬作用之能力….…….76 圖23 RES誘導K562細胞產生吞噬作用之能力……77 圖24 RES誘導K562細胞產生HB之能力……....78 圖25 K562細胞經RES處理後之細胞表面抗原表現… ..79 圖26 RES合併STI571之抑制作用分析……80 圖27 RES合併STI571對K562誘導凋亡作用…..……81 圖28 K562細胞經處理2天後之細胞週期之變化…… 82 圖29 NBT還原分析RES合併STI571誘導K562細胞 產生過氧化物之作用……83 圖30 Hb染色分析RES合併STI571誘導K562細胞 分化之作用……84 圖31 Hb染色分析RES合併STI571誘導K562細胞 分化之細胞比率……85 圖32 Hb染色分析RES合併PD98059誘導K562細胞 分化之作用……86 圖33 Hb染色分析RES合併SB203580誘導K562細胞 分化之作用……87 圖34 Hb染色分析RES合併5-iodotubercidin誘導K562 細胞分化之作用……88 表目錄 表1. 血癌細胞HL-60、U937、K562之比較…….89 表2. 細胞分化評估……....90 附錄圖表 圖表1. 細胞凋亡與壞死……..91 圖表2. 造血作用……..92 圖表3. MAPK 途徑…….93 圖表4. 抑制劑結構……94 圖表5. resveratrol之結構……95 圖表6. RES生理活性……..96 圖表7. RES抗癌相關機制……..97 圖表8、RES抗癌相關機制……98 圖表9、RES抗癌相關機制……99 縮寫表(Abbreviations) RES：Reveratrol GPA：Glycophorin A AML：Acute myeloid leukemia, ALL：Acute lymphoblastic leukemia APL：Acute promyelocytic leukemin cell line CML：Chronic myeloid leukema cell line, NSE：Nonspecific esterase NBT：Nitroblue tetrazolium MAPK：Mitogen-activated protein kinase ERK：Extracellular signal-regulated kinase JNK：c-jun NH2-terminal kinase SAPK：Stress-activated protein kinase MEK：Mitogen/extracellular-regulated kinase Hb：Haemoglobin

REFERENCES

1. 林勝豐（2002）認識癌症。高醫醫訊（22）1-6。 2. 程竹青（1999）膳食與癌症。食品工業月刊（31）12-31。 3. Alexander S., Lia W., Francesca L. S., Rachel M., and Jan A. L. (2002) Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. Mol. Microbiol. 43:883-894. 4. Anthony W. O., Lijun T., Anthony E. B., Dorothy R. S., Anjili A., and Liu J. R. (2004) Resveratrol-induced autophagocytosis in ovarian cancer cells. Cancer Res. 64:696-703. 5. Ashkenazi A., and Dixit V. M. (1998) Death Receptors: Signaling and Modulation. Science 281:1305-1308. 6. Baehner R. L., Nathan D. G. (1968) Quantitative nitroblue tetrazolium test in chronic granulomatous disease. New Engl. J. Med. 278:971-976. 7. Benz E. J., Murnane M. J., Tonkonow B. L., Berman B. W., Mazur E. M., Cavallesco C., Jenko T., Snyder E. L., Forget B. G., and Hoffman. R. (1980) Embryonic-fetal erythroid characteristics of human leukemic cell line. Proc. Natl. Acad. Sci. U.S.A. 77:3509-3513. 8. Berttelli A. A., Givoannini L., Giannessi D., Migliori M., Bernini W, Fregoni M. and Bertelli A. (1995) Antiplatelet activity of cis-resveratrol. Drug Exp. Clin. Res. 17:1-3. 9. Birnie G.D. (1988) The HL-60 cell line: a model system for studying human myeloid cell differentiation. Br. J. Cancer 58:41-45. 10. Bryan A. B. and John B. (2001). Review : Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. Cell Growth & Differ. 12:397-408. 11. Cellier M. F. M., Taimi M., Chateau M. T., Cannat A., and Marti J. (1993) Thermal stress as an inducer of differentiation of U937 cells. Leukemia Res. 17:649-656. 12. Chang L., and Karin M. (2001) Mammalian MAP kinase signalling cascades. Nature 410:37-40. 13. Chen N., Nomura M., She Q. B., Ma W. Y., Bode A. M., Wang L., Flavell R. A., and Dong Z. (2001) .Suppression of skin tumorigenesis in c-Jun NH(2)-terminal kinase-2-deficient mice. Cancer Res. 61:3908-3912. 14. Chenfei Y., Yuzuru M., Jiyan Z., Jing L., Fangming T., Truc N. B., Jialing X., and Anning L. (2004). JNK suppresses apoptosis via phosphorylation of the proapoptotic Bcl-2 family protein BAD. Mol. Cell 13:329-340. 15. Christer S., and Kenneth N. (1976) Establishment and characterization of a human histocytic lymphoma cell line (U937). Int. J. Cancer 17:565-577. 16. Ci Y., Zhang C., and Feng J. (1998) Progress in analytical methods of apoptosis. Progress in Chemistry 4:1-11. 17. Clement M.V., Hirpara J.L., Chawdhury S.H. and Pervaiz S. (1998) Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cell. Blood 92::996-1002. 18. Collins S. J., Gallo R. C.,Gallagher R. E. (1977) Continuous growth and differentiation of homan myeloid leukamic cell in suspension culture. Nature 270:347-349. 19. Collins S. J., Ruscetti F. W., Gallagher R.E., and Gallo R. C. (1979) Normal functional characteristics of HL-60 after induction of Differentiation by DMSO. J. Exp. Med. 149:969-974. 20. Davis R. J. (2000). Signal transduction by the JNK group of MAPK kinas. Cell 103:239-252. 21. Degli-Esposti M. (1999) To die or not to die--the quest of the TRAIL receptors. J. Llukocyte. Biology 65:535-542. 22. Eitan F. Aliza T. and Eliezer A. R. (1983). Spontaneous commitment of murine erythroleukemic

cells to terminal differentiation.Cancer Research 43:4136-4141. 23. Friend C., Scher W., Holland J. G., and Sato T. (1971). Proc. Nat. Acad. Sci. 68:378-382. 24. Gallagher R., Collins S., Trujillo J., McCredie M., Ahearn M., Tsai S., Metzqar R., Aulakh G., Ting R., Ruscetti F., and Gallo R. (1979) Characterization of the continuous, differenntiating cell line (HL-60) from a patient with acute promyelocytic leukemia. Blood 54:713-733. 25. Gary L., Johnson and Razvan Lapadat (2002).Mitogen-Activated Protein KinasePathways Mediated by ERK, JNK, and p38 Protein Kinases Science 298(6):1911-1912. 26. Goyert S. M., Ferrero E. M., Semeritis S. V., Winchester R. J., Silber J., and Mattison A. C. (1986) Biochemistry and expression of myelomonocytic antigens. J. Immunol. 137:3909-3914. 27. Grana X., and Reddy E. P. (1995). Cell cycle control in manmalian cells: role of cyclins, cyclin dependent kinases(CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene 11:211-219. 28. Green D. R., and Reed J. C. (1998) Mitochondria and apoptosis. Science 281:1309-1312. 29. Hans G. D. and Jun M. (1994) Hematopoietic cell lines. Altas of human tumor cell lines 213-250. 30. Hass R., Meinhardt G., Hadam M., and Bartels H. （1994）Characterization of human TUR leukemia cell: continued cell cycle progression in the presence of phorbol ester is associated with resistance to apoptosis. Eur. J. Cell Biol. 65:408-416. 31. Helene F. D., Olivier G., Maria M. M., Joseph V., Francis B., Christian B., Maryse D., Denis T., Jean P. K., Gerald M., Josy R., and Djavad M. (2002) Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells. Carcinogenesis 23(8):1327-1333. 32. Horita M., Andreu E. J., Benito A., Arbona C., Sauz C., Benet I., Prosper F., and Fernandez-Luna J. L. (2000). Blockade of the Bcr-Abl kinase activity induces apoptosis of chromic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xl. J Exp Med 191(6):977-984. 33. Hunter T., and Pines J. (1994). Cyclins and cancer. ＩＩ: cyclin D and CDK inhibitors come of age. Cell 79:573-582. 34. Itaru M., Akira K., Hirokazu T., Junko S., Sachiko E., Naoko M., Koichi N., Masayuki Y., and Yuzuru K. (2000) Biologic significance of GATA-1 activities in Ras-mediated megakaryocytic differentiation of hematopoietic cell lines. Blood 96:2440-2450. 35. James M. O., and Andrew R. H. (2004). P38 MAP kinase: a convergence point in cancer therapy. TRENOS in Mol. Medicine 10(3):125-129. 36. Jang M., Cai L., Udeani G. O., Slowing K. V., Thomas C. F., Beecher C. W. W., Fong H. H. S., Farnsworth N. R., Kinghorn A. D., Mehta R G., Moon R. C., and John M. P. （1997）Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275:218-220. 37. Johnson G. L., and Lapadat R. (2002). Science 298: 1911-1912. 38. Julie, G. Hugues, M. and Ghanem A.(2002). Commentary: A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. Carcinogenesis 22(8):1111-1117. 39. Kamijo R., Takeda K., Nagumo M., Konno K. (1990) Effects of combinations of transforming growth factor-b and tumor necrosis factor on induction of differentiation of human myelogenous leukemic cell lines. J. Immunol. 144:1311-1316. 40. Kashif A. A., Marie V. C., Ismail M. H., and Shazib P. (2004) Resveratrol inhibits drug-induced apoptosis in human leukemia cell by creating an intracellular milieu nonpermissive for death execution. Cancer research 64:1452-1459. 41. Kerr J. F. R., Winterford C. M., and Harmon B. V. (1994) Apoptosis. Cancer 73:2013-2016. 42. Koeffler H. P., and Golde G. W. (1980) Human myeloid leukemia cell lines: a review. Blood 56:344-350. 43. Kumar V., Cotran R. S., Robbins S. L., and Perkins J. A. (1997) Basic Pathology (6th ed.). Philadelphia:aunder. 4-20,132-174. 44. Lanotte M., Martin-Thouvenin V., Najman S., Ballerini P., Valensi F., Bergen R. (1991) NB4, a maturation inducible cell line with t(15;17) marker isolated from a acute promyelocytic leukemia (M3). Blood 77:1080-1087. 45. Lee K. H., Chang M. Y., Ahn J. I., Yu D. H., Jung S. S., Choi J. H., Noh Y. H., Lee Y. S., and Ahn M. J.（2002）Differential gene expression in retinoic acid-induced differentiation of acute promyelocytic leukemia cell, NB4 and HL-60 cells. Biochem. Biophys. Res. Commun. 296:1125-1133. 46. Lee J., Lee C., Trevor J. B., and Alan P. F., (1999) Protein Kinase C Activity Is Necessary for Bcr-Abl-mediated Resistance to Drug-induced Apoptosis. J. Biol. Chem. 274: 3927-3930. 47. Li Y. Y., Liang N. C., Jiang L., Wu T., and Lin X. Y. (2000) Dimethylamiloride-induced differentiation of HL-60 cells. Acta Pharmacol. Sin. 21:445-449. 48. Lila P., Tero-Pekka A., Paivi N., Laura S., and Lea S. (1999) Differentiation lineage-specific expression of human heat shock transcription factor 2 .FASEB J. 13:1089-1098. 49. Lin A. (2003).Activation of the JNK signaling pathway: breaking the brake on apoptosis. Bioessays 25:1-8. 50. Lin J. K., and Tsai S. H. （1999）Chemoprevention of Cancer and Cardiovascular Disease by Resveratrol. Pro. Natl. Sci. Counc. 23:99-106. 51. Lozzio C. B., and Lozzio B. B. （1975）Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45:321-334. 52. Lozzio B. B., Lozzio C. B., Bamberger E. G., and Feliu A. S. (1981) A multipotential leukemia cell line (K562) of human origine. Proc. Soc. Exp. Biol. Med. 166:546-550. 53. Lucia A., Stivala M., Savio F., Carafoli P., Perucca L., Bianchi G., Maga L., Forti U. M., Pagnoni A., Albini E., Prosperi, and Vanio V. （2001）Specific structural determinanta are responsible for the antioxidation activity and cell cycle effects of resveratrol. J. Bio. Chem. 276:22586-22594. 54. Mauro M., Tatiano L., Pietro G., and Alessandro F. A. （1999）Resveratrol prevents apoptosis in K562 cell by inhibiting lipoxygenase and cyclooxygenase activity. Eur. J. Biochem. 265:27-34. 55. McConkey D. J. (1998) Biochemical determinants of apoptosis and necrosis. Toxicol. Let. 99:157-168. 56. Murray A., and Hut T.（1993） The cell cycle. W. H. Freeman, New York. 57. Nagata S., Nagase H., Kawane,K., Mukae N., and Fukuyama H. (2003) Degradation of chromosomal DNA during apoptosis. Cell Death Differ. 10:108-116. 58. Nicole R. M., and Alan P. F. (1997). Atypical Protein Kinase C ι Protects Human Leukemia Cells against Drug-induced Apoptosis. J. Biol. Chem. 272:27521-27524. 59. Nozawz K., Casino C. A., Hamel J. C., Fritzler M. J., and Chan E .K. L. (2002) Fragmentation of Golgi Complex and Golgi autoantigens during apoptosis and necrosis. Arthritis Res. 4:1-9. 60. Olsson I., Gullberg U., Ivhed I., and Nilsson K. (1990) Induction of differentiation of the human histiocytic lymphoma cell line U937 by 1,25-dihydroxycholecalciferol. Cancer Res. 43:5862-5867. 61. Paoletti P., Butti G., Knerich R., Gaetani P., and Assietti R. (1990) Chemotherapy for malignant of brain: a review of ten-years experience. Acta Neurochir. 103:35-46. 62. Paul D., Adly Y., Paul B. F., Michael P. H., and Steven G. (2003).MAPK Pathways in radiation responses. Oncogene. 22:5885-5896. 63. Ragione F. D., Cucciolla V., Borriello A., Pietra V. D., Racioppi L., Soldati G., Manna C., Galletti P., and Zappia V. (1998) Resveratrol arrests the cell division cycle at S/G2 phase transition. Biochem. Biophys. Res. Commun. 250:53-58. 64. Robert I. H., Samuel E. L.,

and Tbornas P. S. （1995） Principles and practice of hematology. Philadelphia: J.B. Lippincott. p439-574. 65. Rotondo S., Rajtar G., Manarini S., Celardo A., Rotillo D., de Gaetano G., Evangelista V., and Cerletti C. （1998） Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function. Br. J. Pharmacol. 123:1691-1699. 66. Sale S., Verschoyle1 R. D., Boocock1 D., Jones1 D. J. L., Wilsher N., Ruparelia K. C., Potter G. A., Farmer P. B., Steward W. P., and Gescher A. J. (2004) Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,40-tetramethoxystilbene British J. Cancer 90:736 — 744. 67. Seger R., and Krebs E. G. (1995). FASEB J. 9:726-735. 68. Seki T., Tsuji K., Hayato Y., Moritomo T., and Ariga T. (2000) Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. Cancer Let. 160:29-35. 69. Sen S., and Deincal C. M. (1992) Apoptosis Biochemical events and relevance to cancer chemotherapy. FEBS 307:122-127. 70. Stamenkovic I. (2000) Thyroid carcinoma cells are resistant to fas-mediated apoptosis but sensitive to tumon necrosis factor-related apoptosis-inducing ligand. Cancer Res. 60:4122-4129. 71. Stuart H. O., Ferenc I. H., and Philip L. (1975). Differentiation in Erytholeukemic cells and their somatic hybrids. Proc. Nat. Acad. Sci. 72(1):98-102. 72. Sundstrom C., and Nilsson K. (1976) Establishment and characterization of a human histocytic lymphoma cell line (U937). Int. J. Cancer 17:565. 73. Surh Y. J. (2003) ancer chemoprevention with dietary phytochemicals. nature review 3:768-780 74. Surth Y. J., Hurh Y. J., and Lee S. J. (1999) Resveratrol an antioxidant present in red wine, induced apoptosis in human promyelocytic leukemia (HL-60) cells. Cancer Let. 140:1-10. 75. Teiji W., and Josef M. P. (2004). Mitogen-activated protein kinase in apoptosis regulation. Oncogene 23:2838-2849. 76. Tibbles L. A., and Woodgett J. R. (1999). Cell. Mol. Life Sci. 55:1230-1254. 77. Wager R. E., Scotto L., and Assoian, R. K. (1994) Analysis of transforming growth factor beta 1 messenger RNA degradation the transcript-selective, 12-O-tetradecanoylphorbol-13- acetate- regulated ribonuclease system from U937 promonocytes. Cell Growth Differ. 5:117-124. 78. Wajant H., Pfizenmaier K., and Scheurich P. (2003) Tumor necrosis factor signaling. Cell Death Differ. 10:45-65. 79. Ways D. K., Qin W., Garris T. O., Chen J., Hao E., Cooper D. R., Usals S. J., Parker P. K., and Cook P. P. (1994) Effects of chronic phorobol ester trearment on protein kinese C activity, content, and gene expression in the human monoblastoid U937 cell. Cell Growth Differ. 5:161-.169. 80. Widmann C., Gibson S., Jarpe M. B., and Johnson G. L. (1999). Physiol. Rev. 79:143-180. 81. William F. W., Mark A. R., Anna E. S., Kari C., and Kevin N. D. (2004) Review: A kinetic approach towards understanding substrate interactions and the catalytic mechanism of the serine/threonine protein kinase ERK2: identifying a potential regulatory role for divalent magnesium. Biochimica et Biophysica Acta. 1697:81-87. 82. Woodgett J. R., Avruch J., and Kyriakis J. (1996). Cancer Surve. 27:127-138. 83. Yamamura M., Hayatsu H., Miyamae T., and Shimoyama Y. (1990) Heat production as a quantitative parameter for cell differentiation and cell function. Tokai. J. Exp. Clin. Med. 15(5):377-380.