Identification of an Active Compound from Wikstroemia indica C.A. Mey that Induced Apoptosis and Differentiation in Huma

蔡瑞騏、廖慧芬 ; 謝淳仁

E-mail: 9318470@mail.dyu.edu.tw

ABSTRACT

Wiktstroemia indica C. A. Mey (Thymelaceae), widely planted in the hillside of Taiwan and China, has been used in traditional Chinese medicine for the treatment of tumor, arthritis, tuberculosis, syphilis, and pertussis. In this study, we extracted the active components from the stem of W. indica, and identified their chemical structure and test the anti-leukemic activity. The active fraction (AF) from W. indica showed 80% of inhibition rate on human myeloid leukemic U937 cells at the concentration of 31.3 ng/ml. However, daphnoretin, a dicomurin compound from W. indica, only showed insignificant effect. Flow cytometric assay revealed that the cell cycle of AF-treated U937 cells was arrested at G0/G1 phase. AF (31.3 ng/ml) induced U937 cells to differentiate into monocytes and macrophages, accompanixiry with 75% of NBT-positive cells. In phagocytosis assay, 70% AF-treated U937 cells have the ability to swallow yeasts. AF also induced U937 cells to express the surface antigen CD11b. In addition, AF induces apoptosis in U937 cells in a dose- and time-dependent increase, including apoptotic body by morphological observation and sub-G1 phase by flow cytometric assay. The anti-proliferation and differentiation-induciry effect of AF was blocked by PD 98059, a MEK/ERK2 inhibitor, suggesting that the mechanisms by AF was through MAPK pathway. AF from W. indica may be a potential drug in anti-leukemia

Keywords : Wiktstroemia indica C. A. Mey ; Leukemia ; Differentiation ; Apoptosis ; Mitogen-activated protein kinase

Table of Contents

封面內頁 簽名頁 授權書 iii 中文摘要 iv 英文摘要 vi 誌謝 vii 目錄 viii 圖目錄 xii 表目錄 xiv 附錄目錄 xvi 縮寫表 xvii 第一章 緒論 1 第二章 文獻回顧 2 2.1 南嶺蕘花 (Wikstroemia indica C. A. Mey) 2 2.1.1 植株特徵 2 2.1.2 產地 2 2.1.3 採收加工 3 2.1.4 植物學分類 3 2.1.5 效用 3 2.1.6 毒性 3 2.1.7 已知成份 4 2.2 白血病 4 2.2.1 白血病之定義及分類 4 2.2.2 白血病之治療 5 2.2.3 關於誘導分化之研究 5 2.2.4 關於細胞凋亡之研究 9 2.3 MAPK之簡介 12 2.3.1 MAPK之簡介 12 2.3.2 ERK (extracedllular signal-regulated kinase) 13 2.3.3 JNK (c-Jun NH2-terminal kinase) 13 2.3.4 p38-MAPK 14 2.3.5 MAPK抑制劑 14 2.4 研究動機 15 第三章 材料與方法 16 3.1 儀器 16 3.2 化學藥劑及緩衝液 19 3.3 細胞 20 3.4 實驗方法 20 3.4.1 南嶺蕘花成份之分離及純化 20 3.4.2 細胞生長與分化之探討 24 3.4.3 細胞凋亡之探討 28 3.4.4 作用機制之探討 29 3.5 統計分析 30 第四章 結果 31 4.1 南嶺蕘花成份之分離純化 31 4.2 天然物活性成份AF與Daphnoretin誘導白血病細 胞分化之細胞生物學研究 32 4.2.1 AF與Daphnoretin抑制人類白血病細胞株之 生長 32 4.2.2 天然物AF對於U937細胞之細胞週期的影 響 33 4.2.3 天然物AF誘導U937細胞之形態學變化 33 4.2.4 天然物AF誘導U937細胞分化後對NBT之 還原能力 34 4.2.5 天然物AF誘導U937細胞分化後細胞吞噬 能力之評估 34 4.2.6 天然物AF誘導U937細胞分化後表現單核 球相關之表面抗原 34 4.2.7 天然物AF對於U937細胞之細胞週期的影 響 35 4.2.8 天然物AF對於U937細胞產生Sub-G1期的 評估 35 4.2.9 MAPK抑制劑對於天然物AF作用U937細 胞生長抑制的影響 36 4.2.10 MAPK抑制劑對於天然物AF作用U937細 胞之型態學變化 36 4.2.11 MAPK抑制劑對於天然物AF作用U937細 胞NBT還原能力的影響 37 第五章 討論 38 5.1 在抑制生長(anti-proliferation)方面 39 5.2 在誘導分化(differentiation induction)方面 39 5.3 在細胞凋亡(Apoptosis)方面 40 5.4 在MAPK訊息傳遞方面 40 第六章 結論 44 6.1 總結 44 6.2 未來展望 45 參考文獻 47 圖一. 南嶺蕘花之有機溶劑分離流程示意圖 58 圖二. 純化南嶺蕘花中具有抗白血病活性成分之流程示意圖 59 圖三. HPLC分析Daphnoretin 60 圖四. LC-MS分析Daphnoretin 61 圖五. 1H NMR分析圖譜 62 圖六. Daphnoretin化學結構圖 63 圖七. TLC分析AF與Daphnoretin 64 圖八. HPLC分析AF與Daphnoretin 65 圖九. 天然物AF與Daphnoretin抑制人類白血病U937細胞株之 生長作圖 66 圖十. 流式細胞儀分析U937細胞之細胞週期 67 圖十一. 天然物AF對於人類白血病U937細胞株細胞週期之影 響(G0/G1期) 68 圖十二. 天然物AF對於人類白血病U937細胞株細胞週期之影響(S期) 69 圖十三. 天然物AF對於人類白血病U937細胞株細胞週期之影 響(G2/M期) 70 圖十四. U937細胞於光學顯微鏡(1000X)下細胞型態之變化 71 圖十五. 天然物AF誘導人類白血病U937細胞株分化之形態變 化 72 圖十六. U937細胞於光學顯微鏡(1000X)下NBT反應之變化 73 圖十七. 天然物AF誘導人類白血病U937細胞株分化後NBT 反應之能力 74 圖十八. U937細胞於光學顯微鏡(1000X)下吞噬能力之影響 75 圖十九. 天然物AF誘導人類白血病U937細胞株分化後吞噬能 力之影響 76 圖二十. 流式細胞儀分析U937細胞之細胞表面抗原表現 77 圖二十一. 天然物AF誘導人類白血病U937細胞分化後表面抗原表現之影響 78 圖二十二. 凋亡小體於光學顯微鏡(1000X)下之型態 79 圖二十三. 流式細胞儀分析U937細胞之Sub-G1期分布 80 圖二十四. 天然物AF促進人類白血病U937細胞株Sub-G1期之 產生 81 圖二十五. MAPK抑制劑對於天然物AF促

進U937細胞株分化 之細胞型態變化(400X) 82 圖二十六. MAPK抑制劑對於天然物AF抑制U937細胞株生長 之影響 83 圖二十七. MAPK抑制劑對於天然物AF促進U937細胞株分化之 型態學變化 84 圖二十八. MAPK抑制劑對於天然物AF促進U937細胞株NBT 還原能力之影響 85 表一. 13C NMR分析Daphnoretin 86 表二. 1H NMR分析Daphnoretin 87 表三. 天然物AF抑制人類白血病U937細胞株之生長 88 表四. 天然物AF對於人類白血病U937細胞株細胞週期(G0/G1 期)之影響 89 表五. 天然物AF對於人類白血病U937細胞株細胞週期(S期)之 影響 90 表六. 天然物AF對於人類白血病U937細胞株細胞週期(G2/M 期)之影響 91 表七. 天然物AF誘導人類白血病U937細胞株分化之形態變化 92 表八. 天然物AF誘導人類白血病U937細胞株分化後NBT反應 及吞噬能力之評估 93 表九. 天然物AF誘導人類白血病U937細胞分化後表面抗原之 表現能力 94 表十. 天然物AF促進人類白血病U937細胞株Sub-G1期之產生 95 表十一. MAPK抑制劑對於天然物AF抑制U937細胞株生長之 影響 96 表十二. MAPK抑制劑對於天然物AF誘導U937細胞分化之型 態學變化 97 表十三. MAPK抑制劑對於天然物AF誘導U937細胞株NBT還 反應之影響 98 附錄一. 南嶺薷花(Wikstroemia indica C.A. Mey.)之原植物形態 圖 99 附錄二. 南嶺薷花(Wikstroemia indica C.A. Mey.)中已發現之組 成分 100 附錄三. 南嶺薷花 (Wikstroemia indica C.A. Mey.)已發現之成份 及其生物活性 101 附錄四. 急性白血病之分類 102 附錄五. 以型態學(FAB)分類之急性非淋巴球性白血病 103 附錄六. 細胞週期之示意圖 104 附錄七. MAPK signaling pathway 105 附錄八 MAPK signaling cascades . 106 附錄九. MAPK 抑制劑之結構 . 107 附錄十. MAPK抑制劑5-iodotubercidin及PD 98059於MAPK pathway中之抑制位置 . 108 附錄十一. MAPK抑制劑SB 203580 於MAPK pathway中之抑 制位置 109

REFERENCES

1.邱年永、張光雄 (1983) 原色臺灣藥用植物圖鑑，南天書局。 2.高木樹 (1981) 臺灣藥用植物手冊，南天書局。 3.歐潤芝 (1998) 臺灣中草藥圖鑑，三聯彩色印刷有限公司。 4.Ko FN, Chang YL, Ku YH, Lin YL and Teng CM (1993) Daphnoretin, a new protein kinase C activator isolated from Wikstroemia indica C. A. Mey. Biochem. J. 295:321-327. 5.Chen HC, Chou CK, Kuo YH and Yeh SF (1996) Identification of a protein kinase C (PKC) activator, daphnoretin, that suppresses hepatitis B virus gene expression in human hepatoma cells. Biochem. Pharmacol. 52:1025-1032. 6.Ke H, Hisayoshi K, Aijun D, Shigeo I and Xinsheng Y (2000) Antifungal, antimitotic and anti-HIV-1 agents from the roots of Wikstoemia indica. Planta Med. 66:564-567. 7.Kato A, Hashimoto Y and Kidokoro M (1979) (+)-Nortrachelogenin, a new pharmacologically active lignan from Wikstroemia indica. J. Nat. Prod. 42:159-162. 8.Lee KH, Tagahara K, Suzuki H, Wu RY, Haruna M, Hall IH, Huang HC, Ito K, IidaT and Lai JS (1981) Antitumor agents. 49 tricin, kaempferol-3-O-beta-D-glucopyranoside and (+)- nortrachelogenin, antileukemic principles from Wikstroemia indica. J. Nat. Prod. 44:530-534. 9.Tseng KF and Chao TY (1963) Studies on theflavonoids present in chinese drugs. IX. A new flavone gycoside isolated from Wikstroemia viridiflora. Yao Xue Xue Bao. 10:286-292. 10.Chen CC, Lin YC, Chen YP and Hsu HY (1981) A study on the constituents of Wistroemia Indica C. A. Mey. J. Taiwan Pharm. Assoc. 33:28-29. 11.Harrison (1995) Harrison ’ s principles of internal medicine. 12.Maxwell MW (1982) Clinical Hematology. 13.Sundstrom C and Nilsson K (1976) Establishment and characterization of a human histocytic lymphoma cell line U937. Int. J. Cancer 17:565-577. 14.Rabbins (1995) Pathology. 15.Olsson I and Breitman TR (1982) Induction of differentiation of the human histocytic lymphoma cell line U937 by retinoiz acid and cyclic adenosine 3 ’ ,5 ’ -monophosphate-inducing agents. Cancer Res. 42:3924-3927. 16.Hass R (1992) Retrodifferentiation- an alternative biological pathway in human leukemia cells. Eur. J. cell boil.58:1-11. 17.Yam LT, Li CY and Crosby WH (1971) Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 55:283-290. 18.Collins SJ, Ruscetti FW and Gallagher RE (1979) Normal functional characteristics of HL-60 after induction of differentiation by DMSO. J. Exp. 149:969-974. 19.Baehner RL and Nathan DG (1968) Quantitative nitrobule tetrazolium test in chornic granulomatous disease. New. Engl. J. 278:971-976. 20.Janeway Jr. CA and Travers P (1994) Immunobiology. 21.Ault KA and Springer T (1981) Cross-reaction of rat-anti-mouse phagocyte-specific monoclonal activity (anti-mac-1) with human monocytes and NK cells. J. Immunol. 126:359-364. 22.Goyert SM, Ferrero EM, Semeritis SV, Winchester RJ, Silber J and Mattison AC (1986) Biochemistry and expression of myelomonocytic antigens. J. Immunol. 137:3909-3914. 23.Wang QH, Xie Y, Fan HH, Gao L and Liu Y (2003) Effects of hexamethylene bisacetamide on cell cycle and expression of its regulatory proteins in HL-60 cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 11:480-484. 24.Shih CR, Wu J, Liu Y, Liang YC, Lin SY, Sheu MT and Lee WS (2004) Anti-proliferation effect of 5,5-diphenyl-2-thiohydantoin (DPTH) in human vascular endothelial cells. Biochem. Pharmacol. 67:67-75. 25.Chiu LC, Ooi VE and Wan JM (2001) Eicosapentaenoic acid modulates cyclin expression and arrests cell cycle progression in human leukemic K-562 cells. Int. J. Oncol 19:845-849. 26.Shao ZM, Wu J, Shen ZZ and Barsky SH (1998) Genistein inhibits both constitutive and EGF-stimulated invasion in ER-negative human breast carcinoma cell lines. Anticancer Res. 18:1435-1439. 27.Zeng XL and Tu ZG (2003) In vitro induction of differentiation by ginsenoside Rh2 in SMMC-7721 hepatocarcinoma cell line. Pharmacol. Toxicol. 93:275-283. 28.Kerr JF, Wyllie AH and Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26:239-257. 29.Reed JC (1999) Mechanisms of apoptosis avoidance in cancer. Curr. Opin. Oncol. 11:68-74. 30.Fraser A and Evan G (1996) A license to kill. Cell 85: 781-784. 31.Andrew HW (1997) Apoptosis and carcinogenesis. Eur. J. Cell Biol. 73: 189-197. 32.Leaver HA, Whittle IR, Wharton SB and Ironside JW (1998) Apoptosis in human primary brain tumours. Brit. J. Neurosurg. 12: 539-546. 33.Dzau VJ, Gibbons GH, Mann M and Braun-Dullaeus R (1997) Future horizons in cardiovascular molecular therapeutics. Am. J. Cardiol. 80: 331-391. 34.Borisenko GG, Matsura T, Liu SX, Tyurin VA, Jianfei J, Serinkan FB and Kagan VE (2003) Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic Jurkat cells-existence of a threshold. Arch. Biochem. Biophys. 413:41-52. 35.Iuvone T, Esposito G, Esposito R,

Santamaria R, Di Rosa M and Izzo AA (2004) Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. J. Neurochem. 89:134-141. 36.Blanc-Brude OP, Mesri M, Wall NR, Plescia J, Dohi T and Altieri DC (2003) Therapeutic targeting of the survivin pathway in cancer: initiation of mitochondrial apoptosis and suppression of tumor-associated angiogenesis. Clin. Cancer Res. 9:2683-2692. 37.Gupta S (2002) Molecular signaling in death receptor and mitochondrial pathways of apoptosis (Review). Int. J. Oncol. 22:15-20. 38.Reed JC (1995) Bcl-2 family protein: regulators of chemoresistance in cancer. Toxicol Lett. 82:155-158. 39.Han D, Chatterjee J, Early P, Pantazis EA and Wyche JH (1996) Isolation and characterization of an apoptosis-resistant variant of human leukemia HL-60 cells that has switched expression from Bcl-2 to Bcl-xL. Cancer Res. 56:1621-1628. 40.Reed JC (1997) Double identity for proteins of the Bcl-2 family. Nature 387:773-776. 41.Korsmeyer SJ (1999) Bcl-2 gene family and the regulation of programmed cell death. Cancer Res. 59: 1693-1700. 42.Herlaar E, Brawn Z (1999) p38 MAPK signaling cascades in inflammatory disease. Mol. Med. Today 5: 439-447. 43.Steeg PS (2003) Metastasis suppressors alter the signal transduction of cancer cells. Nat. Rev. Cancer 3:55-56. 44.Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathway mediated by ERK, JNK, and p38 protein kinase. Science 298: 1911-1912. 45.Teiji W, Josef M (2004) Mitogen-activated protein kinases in apoptosis regulation. Oncogene 23: 2838-2849. 46.Gallagher R, Collins S, TrujilloJ, McCredie M, Ahearn M, Tsai S, Metzqar R, Aulakh G, TingR, Ruscetti F and Gallo R (1979) Characterization of the continuous, differentiating cell line (HL-60) from a patient with acute promyelocytic leukemia. Blood 54:713-733. 47.Yen A, Browm D and Fishbaugh H (1987) Control of HL-60 monocytic differentiation. Exp. Cell Res. 168:247-254. 48.Denhardt DT (1996) Signal-transducing protein phosphorylation cascades mediated by Ras/Rho protein in the mammalian cell: the potential for multiplex signaling. Biochem. J. 318:729-747. 49.Takeda K, Iehiki T, Tokunou T, Lino N and Takeshita A (2001) 15-Deoxy-delta 12, 14-prostaglandin J2 and thiazolidinediones activate the MEK/ERK pathway through phosphatidylinositol 3-kinase in vascular smooth muscle cells. J. Biol. Chem. 276: 48950-48955. 50.Kawakami S, Arai G, Hayashi T, Fujii Y, Xia G, Kageyama Y and Kihara K (2002) PPAR gamma ligands suppress proliferation of human urothelial basal cells in vitro. J. Cell Physiol. 191: 310-319. 51.Miwa Y, Sasaguri T, Lnoue H, Taba Y, Lshida A and Abumiya T (2000) 15-deoxy-Delta (12, 14)-prostaglandin J2 induces G1 arrest and differentiation marker expression in vascular smooth muscle cells. Mol. Pharmacol. 58: 837-844. 52.Kawa S, Nikaido T, Unno H, Usuda N, Nakayama K and Kiyosawa K (2002) Growth inhibition and differentiation of pancreatic cancer cell lines by PPAR gamma ligand troglitazone. Pancreas 24: 1-7. 53.Wilmer WA, Dixon C, Lu L, Hilbelink T and Rovin BH (2001) A cyclopentenone prostaglandin activates mesangial MAP kinase independently of PPARgamma. Biochem. Biophys. Res. Commun. 281: 57-62. 54.Kim MS, LimWK, Cha JG, An NH, Yoo SJ, Park JH, Kim HM and Lee YM (2001) The activation of PI3-K and PKCzeta in PMA-induced differentiation of HL-60 cells. Cancer Lett. 171: 79-85. 55.Isshiki K, Haneda M, Koya D, Maeda S, Sugimoto T and Kikkawa R (2000) Thiazolidinedione compounds ameliorate glomerular dysfunction independent of their insulin-sensitizing action in diabetic rats. Diabetes 49: 1002-1032. 56.Huang WC, Chio CC, Chi KH, Wu HM and Lin WW (2002) Superoxide anion-dependent Raf/MEK/ERK activation by peroxisome proliferators activated receptor gamma agonists 15-deoxy-Delta (12, 14) prostaglandin J2, ciglitazone, and GW1929. Exp. Cell Res. 277: 192-200. 57.Daum G, Eisenmann-Tappe I, Fries HW, Troppmair J and Rapp UR (1994) The ins and outs of Raf kinases. Trends. Biochem. Sci. 19: 474-480. 58.Marais R, Light Y, Paterson HF and Marshall CJ (1995) Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. EMBO. J. 14: 3136-3145. 59.Hagemann C and Rapp UR (1999) Isotype-specific functions of Raf kinases. Exp. Cell Res. 253: 34-46. 60.Gold EJ, Mettelsmann RH, Itri LM, Gee T, Arlin Z, Kempin S, Clarkson B and Moore Mas (1983) Phase Iclinical trial of 13-cis-retinoic acid in myelodysplastic syndromes. Cancer Treat. Rep. 67:981-986. 61.Kieffler HP, Hirji K and Itri L (1985) In vivo and in vitro effents on human preleukemic and leukemic cells. Cancer Treat. Rep. 69:1399-1407. 62.Degos L, Castaigne S, Tilly H, Sigaux F and Daniel MT (1985) Treatment of leukemia with low-dose Ara-C: A study of 160 cases. Sem. Oncol (Suppl 3) 12:196-199. 63. http://www.nobel.se/medicine/laureates/2001/press.html 64. http://www.cellsignal.com/reference/pathway/MAPK_Cascade.asp 65. http://www.cellsignal.com/reference/pathway/MAPKERK.asp 66. http://www.cellsignal.com/reference/pathway/p38MAPK.asp