Shanxi Vocational Poly-tech College[1], Taiyuan, Department of Hematology[2], Changhai Hospital, The Second Military Medical University, Shanghai, China

Histone hyperacetylation is involved in the quercetin-induced human leukemia cell death

JIE JIA[1], JIE CHEN[2]

Received July 13, 2007, accepted August 13, 2007

Dr. Jie Chen, Department of Hematology, Changhai Hospital, The Second Military Medical University, Shanghai 200433, China;
jiechen73@yahoo.com.cn

Pharmazie: 63: 379–383 (2008) *doi: 10.1691/ph.2008.7693*

Quercetin (QU) is recognized as a promising anticancer drug, but its mechanism remains elusive. Here we found that QU induced human leukemia cell death in a dose-dependent manner. However, it did not show a dose-dependent inhibition on ROS generation (indicated by the level of malondialdehyde, MDA) in the same cells. QU showed similar antioxidant activity at concentrations of 50, 75 and 100 μM. Consistent with that, the antioxidant, *N*-acetyl-cysteine (NAC) could only further decrease the ROS generation and enhance the cell death triggered by QU at the concentrations less than 50 μM. These results indicate that an additional mechanism is involved in the anticancer activity of high concentrations of QU. When the effect of QU on histone acetylation was studied, QU induced significant histone hyperacetylation at 75 and 100 μM, indicating the possible involvement of histone hyperacetylation in the anticancer activity of high concentrations of QU. This conclusion was supported by the findings that when histone acetylation in the cells treated by QU was increased by different concentrations of TSA, the cell death was significantly enhanced. Our results thus provide the first evidence that QU can induce histone hyperacetylation and this induction of histone hyperacetylation may represent an unrevealed mechanism in its anticancer activity.

1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavone, QU) is one of the major dietary flavonoids, found in a broad range of fruits, vegetables and beverages such as tea and wine, with a daily intake in Western countries of 25–30 mg (Hollman et al. 1997; Morand et al. 1998), and has been found to show anticancer activity in different cancer cells and in animals (Chen et al. 2004a, b; Cipak et al. 2003; Kaneuchi et al. 2003; Liesveld et al. 2003; Chan et al. 2003; Lee et al. 2002; Mouria et al. 2002; Kuo et al. 2002; Feng et al. 2001; Kim et al. 2000; Kawaii et al. 1999; Kang et al. 1997; Uddin et al. 1995). Among the polyphenols, QU is one of the most potent antioxidants, as demonstrated in different *in vitro* and *in vivo* studies (Prior 2003; Duthie et al. 2000; Russo et al. 1999; Bors et al. 1990; Morand et al. 1998; Hollman et al. 1997), and this antioxidant activity is critically involved in its anticancer activity (Chen et al. 2004a, b; Duthie et al. 2000; Feng et al. 2001).

Histone acetylation contributes to form a transcriptionally competent environment by 'opening' chromatin and permit access of transcription factors to DNA (Fry et al. 2002; Grunstein 1997), whereas histone deacetylation contributes to a 'closed' chromatin state and transcriptional repression. The balance of histone acetylation and deacetylation favors hypoacetylation in tumor cells, while it is accurately maintained through a balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzyme activities in normal cells (Archer et al. 1999; Klochendler-Yeivin et al. 2001). Thus increasing the acetylation of histones in tumor cells through inhibiting the activity of HDAC has been suggested as an efficient strategy to combat cancer, and hence HDAC inhibitors are believed to be promising both as single anti-cancer agents and in combination therapies (Marks et al. 2000; Hendersona et al. 2003; Yamashita et al. 2003; Donadelli et al. 2003; Rosato et al. 2003; Marks et al. 2001; Kim et al. 2003). Increasing chemicals are found to exert their potent anticancer activity through promoting histone acetylation *in vivo* (Carey et al. 2006; Yamashita et al. 2003; Donadelli et al. 2003; Rosato et al. 2003; Marks et al. 2001), but no literature is presently available on whether regulation of histone acetylation is involved in the anticancer activity of QU. To address this question, the effect of QU on histone acetylation and cell death was studied in human leukemia HL-60 cells.

2. Investigations, results and discussion

2.1. Cytotoxicity of QU in human leukemia cells

Treating cells with QU significantly inhibited cell proliferation in a dose- (Fig. 1A) and time-dependent manner (Fig. 1B). And as the trypan blue exclusion assay showed, QU treatment also simultaneously increased the trypan blue-stained cells, indicating the loss of cell membrane integrity and cell death (Figs. 1C and D).

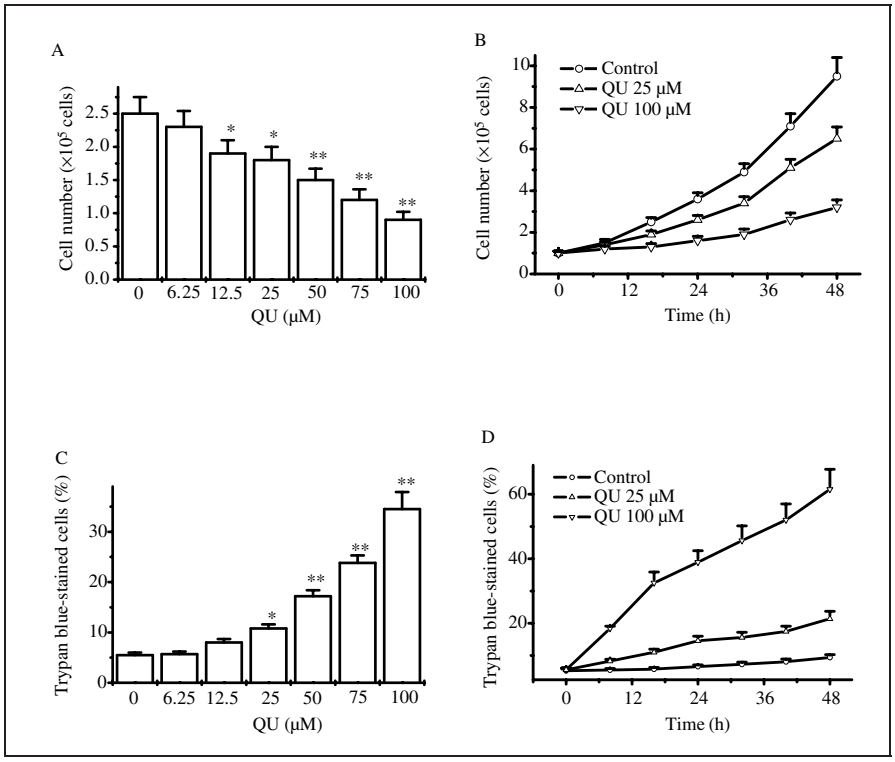

Fig. 1:
Effect of QU on the proliferation and viability of HL-60 cells. Cells were incubated with different concentrations of QU for 16 h in A and C; with 25 and 100 μM of QU for the indicated times in B and D. Then cell number (A, C) and trypan blue-stained cells (B, D) were measured. Means ± SD of three independent experiments was indicated, $n = 3 \times 3$ cultures per conditions, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group in A and C

2.2. Effect of QU on ROS generation and histone acetylation in human leukemia cells

Since ROS and histone hypoacetylation are critically involved in the different stages of carcinogenesis (Deshpande et al. 2002; Archer et al. 1999; Klochendler-Yeivin et al. 2001; Lehrmann et al. 2002), both antioxidants and HDAC inhibitors are thought to be promising anti-cancer agents (Kang et al. 1999, 2000; Prasad et al. 1999; Drisko et al. 2003; Conklin 2000; Kang et al. 2005; Marks et al. 2000; Hendersona et al. 2003; Yamashita et al. 2003; Donadelli et al. 2003; Rosato et al. 2003; Marks et al. 2001; Kim et al. 2003). QU is a naturally occurring strong antioxidant (Morand et al. 1998; Hollman et al. 1997; Duthie et al. 2000), and the antioxidant activity is believed to play important roles in the anticancer activity of QU (Chen et al. 2004a, 2004b; Duthie et al. 2000; Feng et al. 2001). Thus to test whether histone hyperacetylation is also one mechanism of QU to exert its anticancer activity, we determinded the level of ROS generation and the acetylation of histones in HL-60 cells treated by QU at the conditions where they exhibited cytotoxicity. According to the pre-

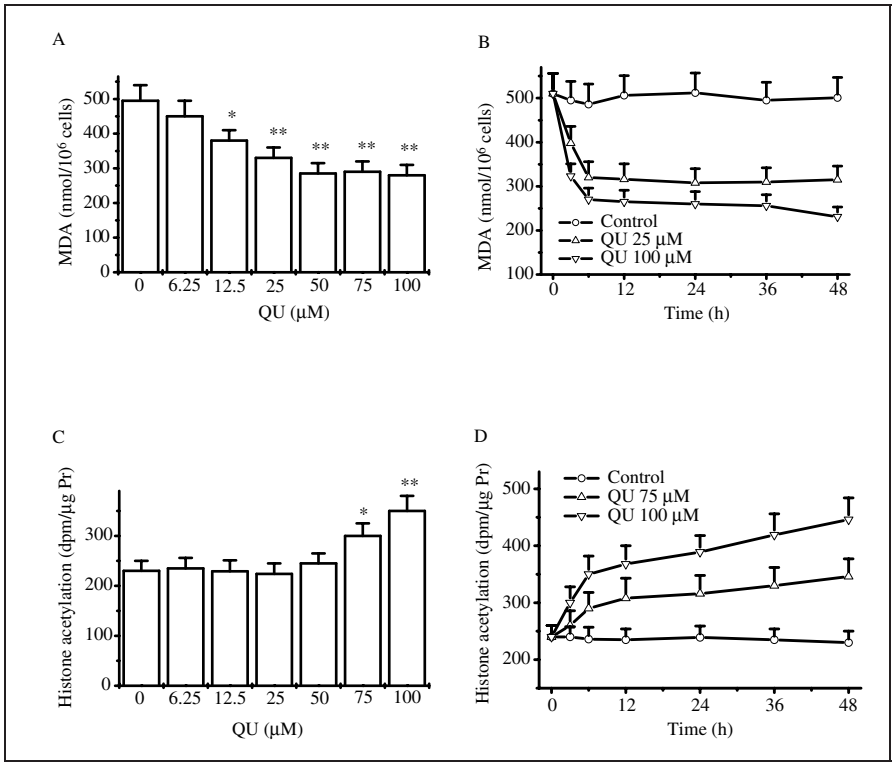

Fig. 2:
Effect of QU on lipid peroxidation and histone acetylation in HL-60 cells. Cells were incubated with different concentrations of QU for 16 h in A and C, or with 25 and 100 μM of QU in B and with 75 and 100 μM of QU in D for the indicated times. Lipid peroxidation (MDA content) and histone acetylation were measured. Means ± SD of three independent experiments was indicated, $n = 3 \times 3$ cultures per conditions, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the corresponding control group in A and C

vious report (Gutteridge et al. 1990), lipid peroxidation (LPO, as indicated by MDA content), resulted from the direct interaction between *in vivo* ROS and unsaturated fatty acid, was used as one representative of in vivo ROS accumulation. Figure 2A showed that treating cells with QU led to a marked decrease in the concentration of MDA. However, QU did not induce a dose-dependent MDA decrease in cells. QU induced similar MDA decrease at 50, 75 and 100 μM, although cell death gradually increased with the increase of its concentration. QU treatment caused a rapid, followed by a sustained decrease in the amount of MDA in HL-60 cells (Fig. 2B). When the state of histone acetylation was evaluated in differently treated cells, QU significantly increased the histone acetylation in HL-60 cells only at the concentrations of 75 and 100 μM (Fig. 2C). The induction of histone hyperacetylation by QU was further confirmed by the time-dependent assay where QU treatment caused a rapid, followed by a slow increase in the acetylation of histones in HL-60 cells (Fig. 2D). Collectively, these results firstly found that QU could also induce histone hyperacetylation, and this induction of histone hyperacetylation may represent one additional mechanism in its anticancer activity.

2.3. Effect of NAC and TSA on QU-induced ROS generation decrease, histone hyperacetylation and cell death

To test the above conclusion, the effect of N-acetylcysteine (NAC), a well known antioxidant and trichostatin (TSA), a well studied HDAC inhibitor (Yamashita et al. 2003; Donadelli et al. 2003; Rosato et al. 2003; Marks et al. 2001) on QU-induced ROS generation, histone acetylation and cell death was carefully determined. To our surprise, only in the cells treated by combination of 200 μM NAC with low concentrations of QU (at less than 50 μM), QU further decreased the MDA formation (Fig. 3A), showed no influence on the histone acetylation (Fig. 3B), and further enhanced the cell proliferation arrest and cell death (Figs. 3C and 3D). Combination of 500 μM NAC with low concentrations of QU obtained similar results (data not shown). And NAC itself at 200 and 500 μM did not induce significant cell proliferation arrest and cell death (Fig. 3 and additional data not shown). Combined with that QU showed dose-dependent activity to induce cell death but non dose-dependent activity to decrease the MDA formation any more at more than 50 μM, these data further confirmed the existence of additional mechanisms in the anticancer activity of high concentrations of QU. As we expected, 50 nM TSA showed no influence on the ROS generation (Fig. 4A), but significantly increased histone acetylation in the cells treated by QU (Fig. 4B), and significantly enhanced the cell proliferation arrest and cell death both in the presence and in the absence of QU (Figs. 4C and 4D). Combination of 20 nM TSA with QU obtained similar results, except that 20 nM TSA itself induced slight but not significant cell proliferation arrest and cell death (data not shown). These results strongly indicated the involvement of histone hyperacetylation in the cell death induced by QU.

In summary, although additional experiments are needed to clarify the detailed mechanism on the induction of QU on histone acetylation, our present study not only provided the first evidence that QU induces histone hyperacetylation in human leukemia cells, but also proved the involvement of this histone hyperacetylation induction in its anticancer activity. Considering the critical role of histone hypoacetylation in the different stages of carcinogenesis and the promising future of the agents being able to improving histone acetylation (Archer et al. 1999; Klochendler-Yeivin et al. 2001; Lehrmann et al. 2002), this study opened a new window to further understand the mechanism of QU's anticancer activity and evaluate its clinical potential.

3. Experimental

3.1. Reagents

Quercetin, trichostatin A, *N*-acetyl-cysteine, trypsin, trypan blue, 1,1,3,3-tetramethoxypropane and thiobarbituric acid were purchased from Sigma

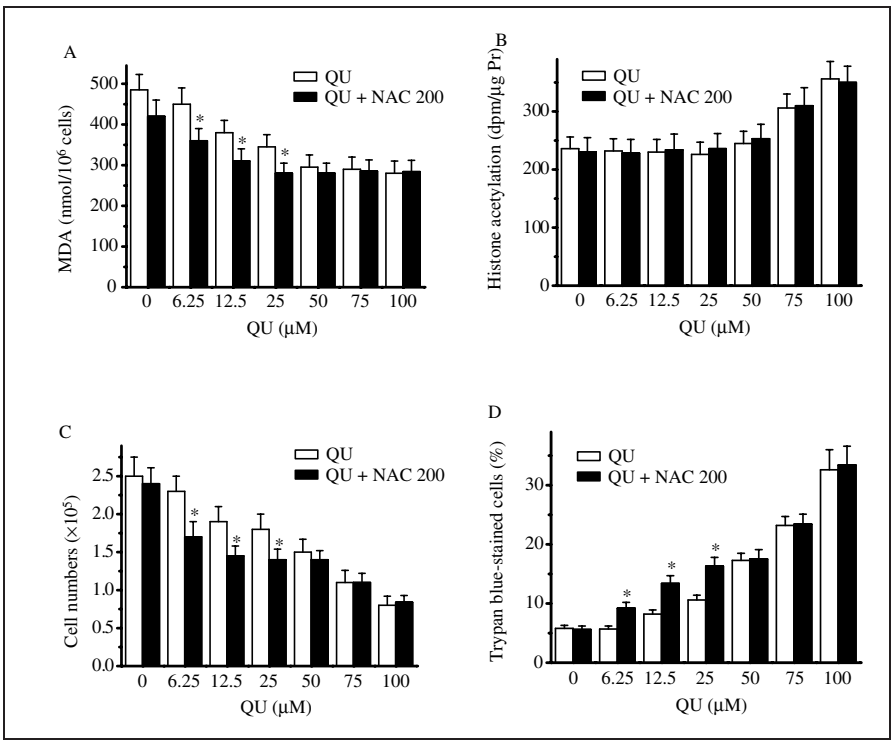

Fig. 3:
Effect of QU and NAC on lipid peroxidation, histone acetylation, cell proliferation arrest and cell viability in HL-60 cells. Cells were incubated with different concentrations of QU, or its combination with 200 μM NAC for 16 h, then lipid peroxidation (A), histone acetylation (B), cell proliferation arrest (C) and cell viability (D) were analyzed in different groups. Means ± SD of three independent experiments was indicated, $n = 3 \times 3$ cultures per conditions, $^{*}P < 0.05$ vs. the corresponding QU alone group. The effect of 500 μM NAC was also detected in these experiments, and similar results were obtained

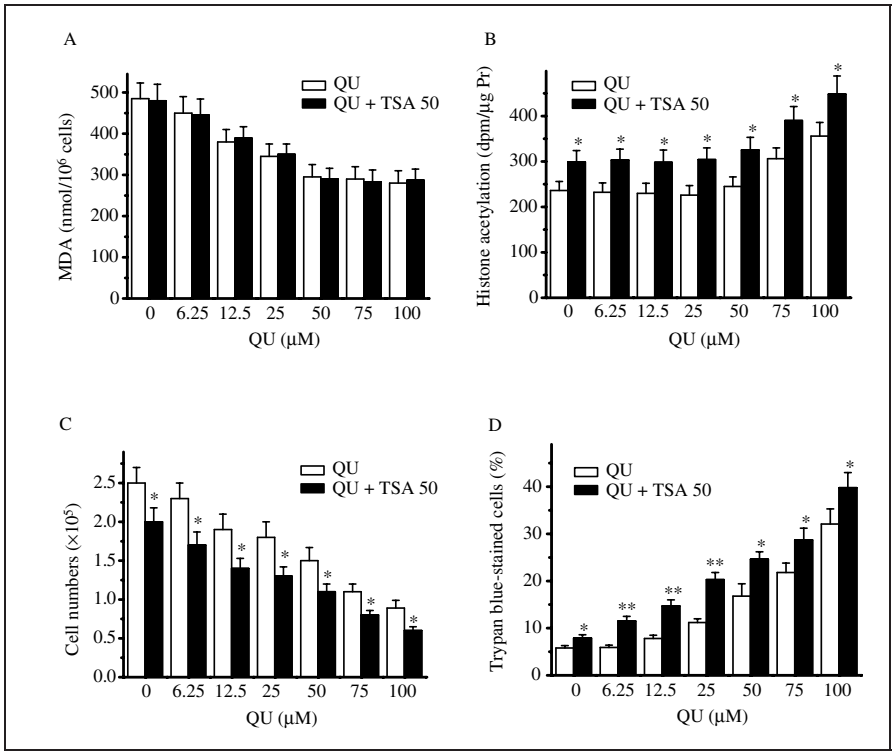

Fig. 4:
Effect of QU and TSA on lipid peroxidation, histone acetylation, cell proliferation arrest and cell viability in HL-60 cells. Cells were incubated with different concentrations of QU, or its combination with 50 nM TSA for 16 h, then lipid peroxidation (A), histone acetylation (B), cell proliferation arrest (C) and cell viability (D) were analyzed in different groups. Means ± SD of three independent experiments are displayed (means ± SD), $n = 3 \times 3$ cultures per conditions, *, ** indicates $p < 0.05$, $p < 0.01$ as compared with the corresponding QU alone group. The effect of 20 nM TSA was also detected in these experiments, and similar results were obtained

(Sigma, St. Louis, MO), RPMI-1640 was purchased from Gibco (Gibco, Santa Clara, CA). All other reagents are of analytical grade.

3.2. Cell culture and treatment

Human leukemia cells (HL-60) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (equivalent to 100 units/ml and 100 mg/ml, respectively) at 37 °C in a humidified atmosphere containing 5% CO_2. After culturing the cells (1×10^5 cells/ml) for 24 h, the culture medium was aspirated and replaced with new medium containing quercetin (QU), *N*-acetyl-cysteine (NAC) and/or trichostatin A (TSA) where indicated, after that, the different cultures were replaced with fresh medium containing the corresponding reagents every 24 h.

3.3. Determination of cell proliferation and viability

Cells at 1×10^5/ml were cultured for 24 h, then treated with QU and/or other agents at the indicated concentrations, three dishes for each of differently treated cells were collected every 8 h in the first 2 days, the total and dead cells were counted respectively by using the trypan blue stain exclusion method under a phase-contrast microscope.

3.4. The malondialdehyde (MDA) assay

After different treatment, HL-60 cells were collected and suspended in the lysis buffer (Kang et al. 2003). And the suspension was collected after pipetting the cell mixture up and down for 20 times. MDA amount in the obtained suspension was immediately evaluated using a colorimetric assay as described previously (Ohkawa et al. 1979). All the analyses were performed in triplicate.

3.5. Histone purification and histone acetylation assay

Cells were plated at a density of 2×10^5 cells/ml, exposed to QU, NAC and/or TSA as indicated in the presence of 5 μCi/ml [^{3}H] acetate (5.0 Ci/mmol) for the indicated times. Preparation of histones from HL-60 cells was done according to Cousens et al. (1979) with the following modifications: the washed cells were suspended in lysis buffer containing TSA (100 ng/ml) and PMSF (1 mM). After pipetting up and down 20 times, the nuclei were washed three times in the lysis buffer and once in 10 mM Tris and 13 mM EDTA (pH 7.4). The histones were extracted from the pellet in 0.4 N H_2SO_4. After centrifugation, the histones in the supernatant were collected by cold-acetone precipitation, air-dried, and ^{3}H-labelled histones were determined by liquid scintillation counting.

3.6. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA post-hoc Bonferroni), and $p < 0.05$ or 0.01 were denoted as * or **, respectively.

Acknowledgement: This research was supported by National Natural Science Foundation of China (30700333).

References

Archer SY, Hodin RA (1999) Histones acetylation and cancer. Curr Opin Gene Develop 9: 171–174.

Bors W, Heller W, Michel C, Saran M (1990) Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol 186: 343–355.

Carey N, La Thangue NB (2006) Histone deacetylase inhibitors: gathering pace. Curr Opin Pharmacol: 369–375.

Chan MM, Fong D, Soprano KJ, Holmes WF, Heverling H (2003) Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. J Cell Physiol 194: 63–70.

Chen J, Ou YX, Da WM, Kang JH (2004a) Coadjustment of quercetin and hydrogen peroxide: the role of ROS in the cytotoxicity of quercetin. Pharmazie 59: 155–158.

Chen J, Kang JH, Da WM, Ou YX (2004b) Combination with water-soluble antioxidants increases the anticancer activity of quercetin in human leukemia cells. Pharmazie 59: 859–863.

Cipak L, Rauko P, Miadokova E, Cipakova I, Novotny L (2003) Effects of flavonoids on cisplatin-induced apoptosis of HL-60 and L1210 leukemia cells. Leuk Res 27: 65–72.

Conklin KA (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer 37: 1–18.

Cousens LS, Gallwitz D, Alberts BM (1979) Different accessibilities in chromatin to histone acetylase. J Biol Chem 254: 1716–1723

Deshpande SS, Irani K (2002) Oxidant signalling in carcinogenesis: a commentary. Hum. Exp. Toxicol 21: 63–64.

Donadelli M, Costanzo C, Faggioli L, Scupoli MT, Moore PS, Bassi C, Scarpa A, Palmieri M (2003) Trichostatin A, an inhibitor of histone deacetylases, strongly suppresses growth of pancreatic adenocarcinoma cells. Mol Carcinogen 38: 59–69

Drisko JA, Chapman J, Hunter VJ (2003) The use of antioxidant therapies during chemotherapy. Gynecol Oncol 88: 434–439

Duthie GG, Duthie SJ, Kyle JAM (2000) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 13: 79–106.

Feng Q, Kumagai T, Torii Y, Nakamura Y, Osawa T, Uchida K (2001) Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. Free Radic Res 35: 779–788.

Fry CJ, Peterson CL (2002) Unlocking the gates to gene expression. Science 295: 1847–1848.

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389: 349–352.

Gutteridge JMC, Halliwell B (1990) The measurement and mechanism of lipid peroxidation in biological system. Trends Biochem Sci 15: 129–135.
Hendersona C, Brancolini C (2003) Apoptotic pathways activated by histone deacetylase inhibitors: implications for the drug-resistant phenotype. Drug Resist Updates 6: 247–256.
Hollman PC, Van Trijp JM, Mengelers MJ, de Vries JH, Katan MB (1997) Bioavailability of the dietary antioxidant flavonol quercetin in man. Cancer Lett 114: 139–40.
Kaneuchi M, Sasaki M, Tanaka Y, Sakuragi N, Fujimoto S, Dahiya R (2003) Quercetin regulates growth of Ishikawa cells through the suppression of EGF and cyclin D1. Int J Oncol 22: 159–164.
Kang J, Chen J, Shi Y, Jia J, Zhang Y (2005) Curcumin-induced histone hypoacetylation: the role of reactive oxygen species. Biochem Pharmacol 69: 1205–1213.
Kang J, Chen J, Zhang D, Da W, Ou Y (2004) Synergistic killing of human leukemia cells by antioxidants and trichostatin A. Cancer Chemother Pharmacol 54: 537–545.
Kang J, Shi Y, Zheng R (1999) Effects of ascorbic acid on human hepatoma cell proliferation and redifferentiation. Acta Pharmacol Sin 20: 1019–1024.
Kang JH, Shi YM, Zheng RL (2000) Effects of ascorbic acid and DL-alpha-tocopherol on human hepatoma cell proliferation and redifferentiation. Acta Pharmacol Sin 21: 348–352.
Kang J, Zhang Y, Chen J, Chen H, Lin C, Wang Q, Ou Y (2003) Nickel-induced histone hypoacetylation: the role of reactive oxygen species. Toxicol Sci 74: 279–286.
Kang TB, Liang NC (1997) Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. Biochem Pharmacol 54: 1013–1018.
Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M (1999) Effect of citrus flavonoids on HL-60 cell differentiation. Anticancer Res 19(2A): 1261–1269.
Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 63: 7291–7300.
Kim SY, Gao JJ, Kang HK (2000) Two flavonoids from the leaves of Morus alba induce differentiation of the human promyelocytic leukemia (HL-60) cell line. Biol Pharm Bull 23: 451–455.
Klochendler-Yeivin A, Yaniv M (2001) Chromatin modifiers and tumor suppression. Biochim Biophys Acta 1551: M1–M10.
Kuo HM, Ho HJ, Chao PD, Chung JG (2002) Quercetin glucuronides inhibited 2-aminofluorene acetylation in human acute myeloid HL-60 leukemia cells. Phytomedicine 9: 625–631.
Lee LT, Huang YT, Hwang JJ, Lee PP, Ke FC, Nair MP, Kanadaswam C, Lee MT (2002) Blockade of the epidermal growth factor receptor tyrosine kinase activity by quercetin and luteolin leads to growth inhibition and apoptosis of pancreatic tumor cells. Anticancer Res 22: 1615–1627.
Liesveld JL, Abboud CN, Lu C, McNair C, Menon A, Smith A, Rosell K, Rapoport AP (2003) Flavonoid effects on normal and leukemic cells. Leuk Res 27: 517–527.
Marks PA, Richon VM, Rifkind RA (2000) Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Nat Cancer Inst 92: 1210–1216.
Marks PA, Richon VM, Breslow R, Rifkind RA (2001) Histone deacetylase inhibitors as new cancer drugs. Curr Opin Oncol 13: 477–483.
Morand C, Crespy V, Manach C, Besson C, Demign'e C, R'em'esy C (1998) Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol 275: R212–9.
Mouria M, Gukovskaya AS, Jung Y, Buechler P, Hines OJ, Reber HA et al. (2002) Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis, Int J Cancer 98: 761–769.
Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358.
Prasad KN, Kumar A, Kochupillai V, Cole WC (1999) High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. J Am Coll Nutr 18: 13–25.
Prior RL (2003) Fruits and vegetables in the prevention of cellular oxidative damage, Am J Clin Nutr 78: 570S–578S.
Rosato RR, Grant S (2003) Histone deacetylase inhibitors in cancer therapy. Cancer Biol Ther 2: 30–37.
Russo M, Palumbo R, Tedesco I, Mazzarella G, Russo P, Iacomino G, et al. (1999) Quercetin and anti-CD95 (Fas/Apo1) enhance apoptosis in HPB-ALL cell line. FEBS Lett 462: 322–328.
Uddin S, Choudhry MA (1995) Quercetin, a bioflavonoid, inhibits the DNA synthesis of human leukemia cells. Biochem Mol Biol Int 36 (3): 545–550.
Wang PF, Kang JH, Zheng RL, Lu JF, Yang ZF, Jia ZJ, Gao JJ (1996) Scavenging effects of phenylpropanoid glycosides from Pedicularis on superoxide anion and hydroxyl radical by the spin trapping method. Biochem Pharmacol 51: 687–691.
Yamashita Y, Shimada M, Harimoto N, Rikimaru T, Shirabe K, Tanaka S, Sugimachi K (2003) Histone deacetylase inhibitor trichostatin A induces cell-cycle arrest/apoptosis and hepatocyte differentiation in human hepatoma cells. Int J Cancer 103: 572 –576.