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ORIGINAL ARTICLE

Fatty acids, inhibitors for the DNA binding of c-Myc/Max dimer, suppress proliferation and induce apoptosis of differentiated HL-60 human leukemia cell

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c-Myc is instrumental in the progression of Burkitt's lymphoma including HL-60 human leukemia cells. We tested fatty acids for their inhibitory effect on the DNA binding of c-Myc/Max dimeric proteins of human origin, prepared as recombinant proteins encompassing DNA binding (basic) and dimerization (HLHZip) domain, and found that those suppress proliferation and induce apoptosis of DMSO-differentiated HL-60 cells. The analyzed IC_{50} values of myristic acid, stearic acid, γ-linolenic acid, linoleic acid, linolenic acid and arachidonic acid by EMSA were 97(±3), 2.2(±1.2), 55(±5), 32(±2), 62(±12), 22(±2) μM for DNA binding of recombinant c-Myc/Max, respectively. According to the results shown by XTT assay, their influence on proliferation was quite different from the rank order of IC_{50}. Whereas the degree of influence of the unsaturated fatty acids on the proliferation of DMSO-differentiated HL-60 cells was similar, the influence of saturated fatty acids, stearic acid in particular, was very weak at same concentrations. In addition, we confirmed that these fatty acids have no influence on the expression of c-Myc in DMSO-differentiated HL-60 cells. Our experiments demonstrated that the inhibitors for the DNA binding of c-Myc/Max contribute to the downregulation of Myc-dependent proliferation and to the inducement of apoptosis, and serve as an exploration of potent new inhibitors.
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Introduction

The c-myc proto-oncogene encodes the c-Myc oncoprotein that exerts considerable control over transformation, differentiation, apoptosis, and cell cycle progression.[1–4] The Myc family proteins, including c-, N- and L-Myc, heterodimerize with the carboxy-terminal basic/helix–loop–helix/leucine zipper (bHLHZip) domain of Max protein.[5–8] Max–Max homodimer and Myc–Max heterodimers bind with a specific DNA sequence, CACGTG (E-box),[5,9,10] but the heterodimerization and DNA binding of Max are required for transcriptional activation of target genes by c-Myc, as well as its ability to promote proliferation, malignant transformation and apoptosis.[11,12] The *myc* gene is amplified in many tumors, particularly small-cell lung carcinoma, breast and cervical carcinomas.[13–15] Activated oncogenic c-Myc is instrumental in the progression of Burkitt's lymphoma[16] and its expression is elevated or deregulated in a wide range of other human cancers and is often associated with aggressive, poorly differentiated tumors.[17]

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The inhibition of proliferation and induction of apoptosis are regulated by a network of signaling pathways and transcription factors, which are possible targets for a rational tumor therapy.[18,19] For instance, some anticancer reagents cause cell death through interfering with the processes of the cell cycle [20] and some others cause cell death by apoptosis,[21,22] which plays an important role in the balance between cell replication and cell death. Leukemia, one of the most threatening hematological malignant cancers, has been found to be very sensitive to anticancer reagents which either block the process of the cell cycle or cause apoptosis.[23] This chemotherapy-sensitive property of leukemia entices researchers to look for more specific and potent drugs against it. As potent new drug candidates, natural compounds have been highlighted because of their low degree of cytotoxicity.

In this study, we prepared recombinant c-Myc and Max encompassing the DNA binding (basic) and dimerization (HLHZip) domain of human origin, and tested the inhibitory effect of fatty acids on the DNA binding of recombinant c-Myc/Max dimers. The inhibitory effect of fatty acids on the DNA binding step has been described in a previous study.[24] Also, we demonstrated that these fatty acids suppressed proliferation and induced apoptosis of HL-60 cells, whose differentiation had been stimulated by 1% DMSO,[25] with no influence on the expression of c-Myc.

Materials and methods

Construction of expression vector containing recombinant c-myc *and* max *gene*

Recombinant *c-myc* and *max* genes containing a DNA-binding basic domain, helix–loop–helix domain and leucine zipper domain (kindly provided by Dr Bruno Amati) were cloned into pET21a(+) vectors. The constructed vectors were transformed into *Escherichia coli* (*E. coli*) BL21(DE3) for the protein expression. The analyzed DNA sequences of recombinant c-myc and max were ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GGA TCC ACC ATG GGA AAT GTC AAG AGG CGA ACA CAC AAC GTC TTG GAG CGC CAG AGG AGG AAC GAG CTA AAA CGG AGC TTT TTT GCC CTG CGT GAC CAG ATC CCG GAG TTG GAA AAC AAT GAA AAG GCC CCC AAG GTA GTT ATC CTT AAA AAA GCC ACA GCA TAC ATC CTG TCC GTC CAA GCA GAG GAG CAA AAG CTC ATT TCT GAA GAG GAC TTG TTG CGG AAA CGA CGA GAA CAG TTG AAA CAC AAA CTT GAA CAG CTA CGG AAC TCT TGT GCG CTC GAG CAC CAC CAC CAC CAC CAC and ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGC GGA TCC ACC ATG GGA GAC AAA CGG GCT CAT CAT AAT GCA CTG GAA CGA AAA CGT AGG GAC CAC ATC AAA GAC AGC TTT CAC AGT TTG CGG GAC TCA GTC CCA TCA CTC CAA GGA GAG AAG GCA TCC CGG GCC CAA ATC CTA

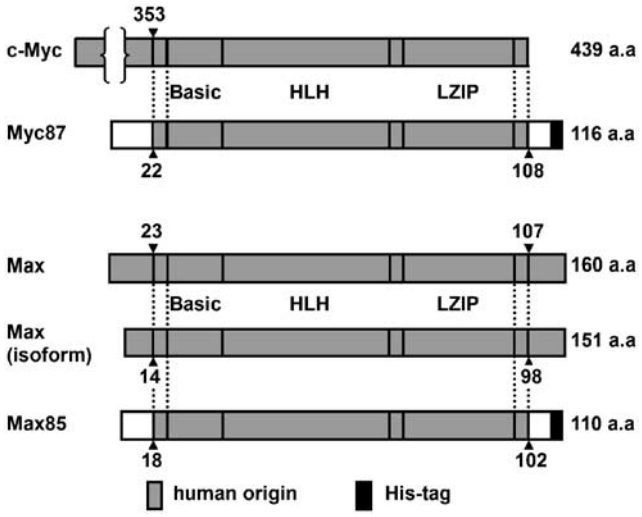

Figure 1 Schematic diagram of Myc87 and Max85. A His tag was attached to the C-terminal region of Myc87 and Max85. In all, 87 amino acids (22–108) of Myc87 and 85 amino acids (18–102) of Max85 are homological with human c-Myc and Max, respectively. The sequences of the bHLHZip regions of Myc87 and Max85, which are the kernel in the formation of DNA–Myc–Max complexes, are identical with those of human origin.

GAC AAA GCC ACA GAA TAT ATC CAG TAT ATG CGA AGG AAA AAC CAC ACA CAC CAG CAA GAT ATT GAC GAC CTC AAG CGG CAG AAT GCT CTT CTG GAG CAG CAA GTC CGT GCA CTG GAG AAG GCG AGG TCA CTC GAG CAC CAC CAC CAC CAC CAC, respectively. The protein sequences expected to be expressed are MASMT GGQQM GRGSE FGSTM GNVKR RTHNV LERQR RNELK RSFFA LRDQI PELEN NEKAP KVVIL KKATA YILSV QAEEQ KLISE EDLLR KRREQ LKHKL EQLRN SCALE HHHHH H and MASMT GGQQM GRGST MGDKR AHHNA LERKR RDHIK DSFHS LRDSV TSLQG EKASR AQILD KATEY IQYMR RKNHT HQQDI DDLKR QNALL EQQVR ALEKA RSLEH HHHHH, respectively. The underlined parts are homologous with human origins (Figure 1).

Preparation of recombinant c-Myc and Max proteins

Each colony containing recombinant human *c-myc* and *max* gene was grown in an LB culture media containing 0.1 mg ml^{-1} ampicillin at 37°C until their OD_{600} reached 0.6. Following incubation with 1 mM IPTG for 7 h, the cultures were harvested at 4°C by ultracentrifuge. The harvested cells including recombinant Max (Max85) and recombinant c-Myc (Myc87) were resuspended with Binding buffer solution (0.5 M NaCl, 20 mM Tris hydrochloride (pH 7.9) and 5 mM imidazole) and with Binding buffer solution containing 6 M urea, respectively. Since recombinant proteins were produced with a His-tag, they were easily purified using nickel-ion affinity chromatography (Novagen, Germany) at 4°C. Eluted buffer solutions containing Myc87 and Max85 were dialyzed into Binding buffer solution, and each Binding buffer solution containing proteins was diluted and dialyzed into TBS (Tris-buffered saline; 137 mM NaCl, 2.68 mM KCl and 10 mM Tris-HCl, pH adjusted to 7.4)

Preparation of HL-60 cell line and whole cell lysate

HL-60 cells was incubated in RPMI1640 (WelGENE, Korea) media supplemented with 20% fetal bovine serum (WelGENE, Korea) at 37°C in a 5% CO_2 humidified incubator. The whole cell proteins were obtained by lysing the cells on ice for 20 min in lysis buffer (50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 1% Nonidet P-40, 0.5 mM PMSF 50 μg/ml aprotinin, 10 μg/ml leupeptin, 50 μg/ml pepstatin, 0.4 mM sodium orthovanadate, 10 mM sodium fluoride, and 10 mM sodium pyrophosphate). The lysates were then sonicated for 30 s, and spun at 15 000 *g* for 10 min, and the supernatant was saved. The concentration of the total proteins was determined by the Bradford method.

Western blot analysis

Proteins and Western size markers (West-view, Elpis-Biotech, Korea) were electrophoresed in a 12% SDS-polyacryamide gel. The separated proteins were electrotransferred from the SDS-polyacryamide gel to a nitrocellulose membrane (Amersham Biosciences) for 2 h at 250 mA. The nitrocellulose membrane with the transferred proteins was then blocked with 5% skim milk in TBS-T (TBS, 0.1% Tween 20) overnight, and incubated with primary antibodies (200 ng ml^{-1}) at room temperature (RT) for 1 h. The primary antibodies were mouse monoclonal IgG_1 antibodies raised against a peptide corresponding to amino acids 408–439 within the C-terminal domain of c-Myc of human origin (Santa Cruz Biotechnology, CA, USA) for Myc87 and for c-Myc from HL-60 cell, and mouse monoclonal IgG_1 raised against a recombinant protein corresponding to amino acids 28–151 representing the C-terminal domain of Max21 of human origin (Santa Cruz Biotechnology, CA, USA) for Max85. The blots were washed four times with TBS-T, and incubated with HRP-conjugated anti-mouse IgG antibody (Santa Cruz Biotechnology, CA, USA) for 1 h. Immunoreactive bands were developed by ECL Western blotting detection reagents (iNtRON Biotechnology, Korea), and visualized on film through exposure for 1 min.

Preparation of fatty acid stock solution

Stearic acid, myristic acid, γ-linolenic acid, linoleic acid, linolenic acid and arachidonic acid were purchased from Sigma-Aldrich. Stock solutions were prepared in DMSO and their concentration was set to 10 mM.

Electrophoretic mobility shift assay (EMSA)

The proportions of protein–DNA complexes in each sample were determined by measurement of the intensity of bands on an autoradiograph generated by EMSA. Myc–Max consensus oligonucleotides (5′-dGGAAGCAGACCACGTGGTCTG CTTCC-3′, Santa Cruz Biotechnology) were labeled using [gamma-^{32}P] ATP (Amersham Biosciences) and T4 polynucleotide kinase (TaKaRa bio, Japan). Following incubation of the protein mixtures at RT for 5 min, the DMSO solution containing each fatty acid was added. After further incubation for 5 min, the labeled DNA was added. In order to achieve a state of equilibrium, we incubated the whole mixtures at RT for 10 min. The protein–DNA complexes were separated from the free DNA on a 6% polyacrylamide gel prepared and pre-electrophoresed in 0.5 × TBE buffer. Electrophoresis was performed in the 0.5 × TBE buffer at 100 V and 37°C for 1 h. Each band was visualized by autoradiography, and the intensities of bands were measured by image analysis software (TotalLab, NonLinear Dynamics, UK).

Cell proliferation assay

Maintained HL-60 cells were harvested by ultracentrifuge and resuspended with growth medium (RPMI1640 + 20% FBS).

Aliquots of 1×10^4 HL-60 cells in 100 μl were seeded per well. Fatty acids at a final concentration of 0–100 μM was added to each well, and the cells were incubated for 24 h. Cell viability was determined by XTT cell proliferation assay kit (Biological Industries, Israel). XTT (sodium 3′-1-(phenylaminocarbonyl)-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzene sulfonic acid), a yellow tetrazolium salt, was cleaved by the mitochondrial dehydrogenase in metabolically active cells to form an orange formazon dye. The absorbance of each sample was measured with a spectrophotometer (EMax plate reader, Molecular Devices) at a wavelength of 450 nm.

DNA fragmentation assay

The progress of apoptosis was detected by a DNA fragmentation assay in total genomic DNA. HL-60 cells treated with fatty acids were harvested by centrifugation and lysed in lysis buffer (2 mM EDTA, 100 mM Tris-HCl (pH 8.0), 0.8% SDS) on ice. The lysate was incubated with 5 mg/ml RNase A (Sigma-Aldrich) for 1 h at 37°C and then with 5 mg/ml proteinase K (Sigma-Aldrich) for 3 h at 50°C. After addition of 6 × Gel Loading buffer, the mixture was loaded into a 1.5% agarose gel followed by electrophoresis at 50 V for 1 h in 1 × TBE buffer and ethidium bromide staining.

Results

Recombinant c-Myc and Max proteins

Recombinant c-Myc (Myc87) and Max (Max85) were expressed in *E. coli* BL21 and were purified. The calculated molecular weights of Myc87 and Max85 are 13589.53 and 12865.37, respectively (http://us.expasy.org). The molecular weights measured by MALDI-TOF were 13353.97 for Myc87, and 12768.02 for Max85. The apparent molecular weights of Myc87 and Max85 on SDS-PAGE were about 16 kDa, and this reflects the anomalous electrophoretic mobility typical for recombinant c-Myc and Max. The homology of Myc87 and Max85 with human origin was verified by monoclonal antibodies (Figure 2).

Fatty acids inhibit DNA binding of Myc87/Max85 dimer

The inhibitory effect of fatty acids on the DNA binding of recombinant c-Myc/Max was confirmed by EMSA. Each fatty acid at a concentration of 0–100 μM was added to the mixture containing Myc87, Max85 and labeled DNA. The 50% inhibitory concentration (IC_{50}) was determined by analysis of its intensity on an autoradiograph using image analysis software. The analyzed IC_{50} values were 97($\pm$3) μM for myristic acid, 2.2($\pm$1.2) μM for stearic acid, 55($\pm$5) μM for γ-linolenic acid, 32($\pm$2) μM for linoleic acid, 62($\pm$12) μM for linolenic acid and 22($\pm$2) μM for arachidonic acid. The inhibitory effect of unsaturated fatty acids (γ-linolenic acid, linoleic acid, linolenic acid and arachidonic acid) for DNA binding was similar at a same concentration, but the effect of saturated fatty acids was differed greatly (Figure3).

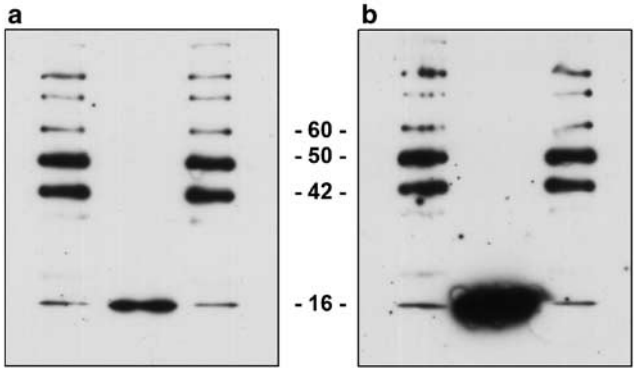

Figure 2 Western blotting analysis of purified Myc87 and Max85. The size marker proteins crossreacted with antibodies. Each recombinant protein was detected with mouse monoclonal antibodies and goat HRP-conjugated anti-mouse IgG antibody. Lane a, recombinant c-Myc (Myc87) dissolved in TBS; lane b, recombinant Max (Max85) dissolved in TBS.

Fatty acids suppress proliferation of HL-60 cells

HL-60 cells spontaneously differentiate and differentiation can be stimulated by dimethylsulfoxide (DMSO, 1–1.5%). We added 1 μl DMSO solutions containing each fatty acid to aliquots of 1×10^4 HL-60 cells in 100 μl growth media, and the final concentrations of fatty acids diluted into aliquots were 0, 1, 5, 10, 20, 30, 40, 50, 60, 80, 100 μM. Following incubation for 12 h, the cell viability in each well was determined by XTT assay. The rates of the proliferation of cells treated with stearic acid and with myristic acid remained at a consistently high level at all concentrations. As compared with cells treated with 1% DMSO, the viability of cells treated with 100 μM of myristic acid and stearic acid for 12 h was 47 and 73%. By contrast, the proliferation of cells was strongly inhibited by γ-linolenic acid, linoleic acid, linolenic acid and arachidonic acid. The relative viability of the cells treated with 100 μM of these unsaturated fatty acids was only 10–12%. However, the inhibitory effects of unsaturated fatty acids on HL-60 cell proliferation differed in the range of IC_{50}. In this range (20–60 μM), the inhibitory effect of linoleic acid and of arachidonic acid was stronger than that of γ-linolenic acid and of linolenic acid. This result is matched well with the order of IC_{50} values and shows that the inhibitory effect of fatty acids on the proliferation of HL-60 cells has a relation with its inhibitory effect on the DNA binding of c-Myc/Max (Figure 4).

Fatty acids induce apoptosis of HL-60 cells

The progress of apoptosis was detected by DNA fragmentation assay of the total genomic DNA. A total of 60 μl DMSO solutions containing each fatty acid were added to aliquots of 3×10^5 HL-60 cells in 6 ml, and the final concentrations of fatty acids diluted into aliquots were adjusted to 100 μM. In all, 3 ml of media were harvested from each aliquot before incubation and after incubation for 24 h, respectively. As shown in Figure 5, there is a difference of degree in the progress of apoptosis (Figure 5).

The degree of apoptosis in the media containing 100 μM of stearic acid and myristic acid was low relative to that in the media containing 100 μM of other unsaturated fatty acids. More precisely, the following order shows that in which fatty acid cells demonstrated the higher degree of apoptosis: linoleic acid $\approx$ linolenic acid $\approx$ arachidonic acid $>$ γ-linolenic acid $>$ stearic acid $>$ myristic acid. This order of degree is analogous with that of IC_{50}. Compared with the rate of cell proliferation, it is remarkable that the apoptosis of the cells treated with stearic acid makes rapid progress against myristic acid.

Fatty acids have no influence on the expression of c-Myc

To confirm the influence of fatty acids on the expression of c-Myc, we analyzed the amount of c-Myc protein in HL-60 cells

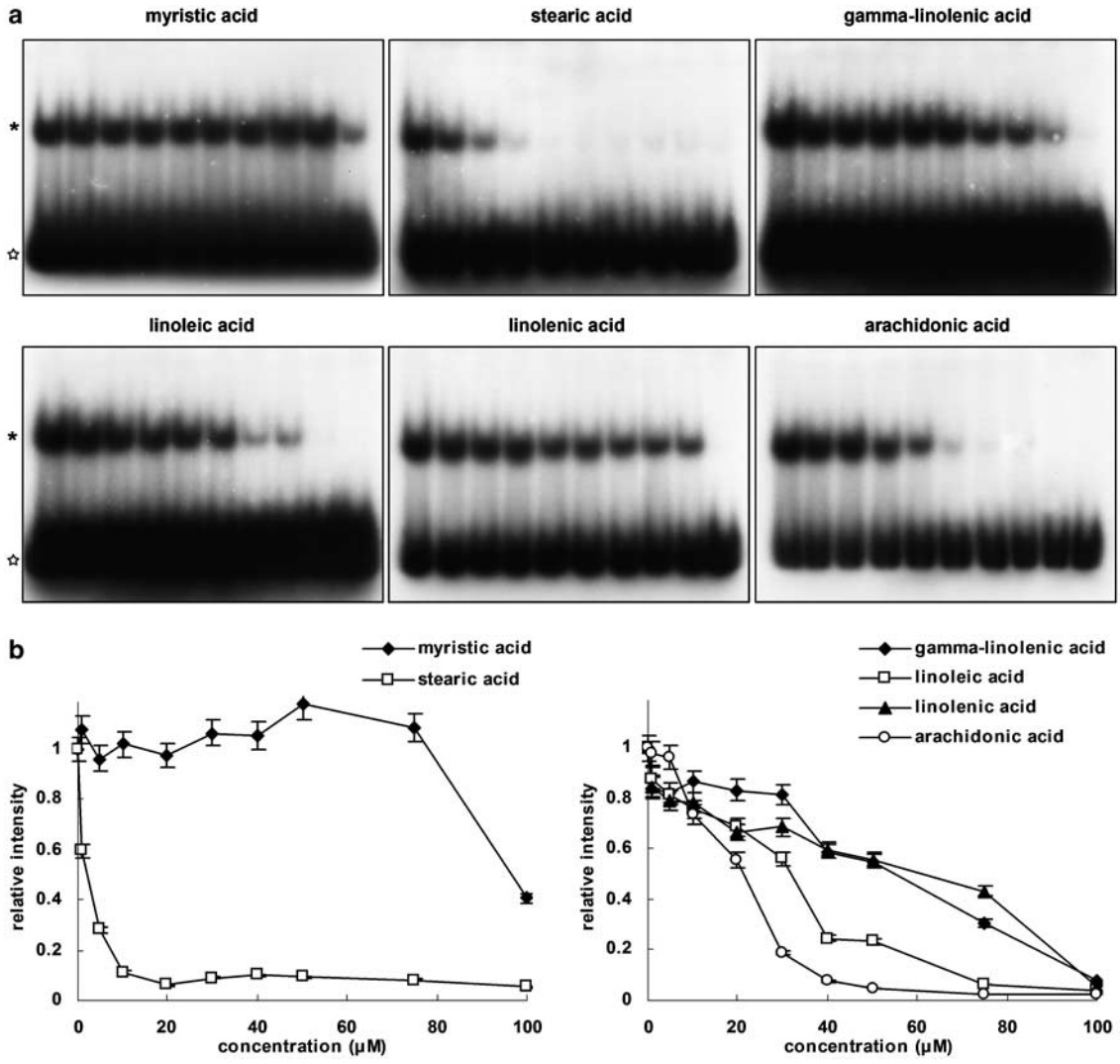

Figure 3 Inhibitory effect of fatty acids on DNA binding of Myc87/Max85 dimer. The IC_{50} values of each fatty acid were determined with EMSA. Myc87/Max85/DNA complexes were separated from the free DNA on a 6% polyacrylamide gel, and the intensity of each band on autoradiograph was measured by image analysis software. (**a**) Each fatty acid was added to a mixture containing Myc87/Max85 dimers, and the final concentration of each fatty acid in the mixture was 0 (line 1), 1 (line 2), 5 (line 3), 10 (line 4), 20 (line 5), 30 (line 6), 40 (line 7), 50 (line 8), 75 (line 9), and 100 μM (line 10). The complexes formed with Myc87/Max85/DNA (marked as asterisks (*)) decreased as the concentration of fatty acid increased gradually. The region marked as pentagrams (☆) was including free probe (labeled DNA) and unincorporated label ([λ-^{32}P]ATP). (**b**) The relative intensity of Myc87/Max85/DNA complexes was plotted versus the concentration of fatty acids. The value of relative intensity was determined by the intensity of each line 1 (DMSO control) for a standard.

treated with each fatty acid for 6 h. The amount of c-Myc in each aliquot was determined by Western blotting. The concentration of total protein in the each lysate was determined by the method of Bradford, and 60 μg of total protein extracted from the treated cells was loaded to each well of SDS-PAGE gel. There is no significant difference in the amount of c-Myc in each aliquot. This result demonstrates that fatty acids have no influence on the expression of c-Myc in DMSO-differentiated HL-60 cells (Figure 6).

Discussion

It has been reported that several fatty acids suppress the proliferation of differentiated HL-60. For instance, it was reported that blocking the metabolism of eicosapentaenoic acid and γ-linolenic acid by 5-lipoxygenase suppresses the proliferation and induced apoptosis of HL-60 cell,[26] and that when the activity of phospholipase A2, which participate in the metabolism of arachidonic acid, was blocked with a inhibitor, arachidonic acid suppresses the proliferation,[27] and induces apoptosis of HL-60 cell.[28] While the cause was not evident, or it has been considered that some fatty acids have influence on the metabolism or that the products from the metabolism of fatty acid suppress the proliferation, we hypothesized that the fatty acids suppressing proliferation of HL-60 cell may be the inhibitors for the DNA-binding step of the c-Myc/Max dimeric protein. We tested the inhibitory effect of fatty acids for the complexes formed with recombinant c-Myc and Max, and confirmed that the fatty acids which have a weak inhibitory effect have no influence on the proliferation of HL-60 cells. For instance, oleic acid has no effect as an inhibitor for the DNA-binding step of c-Myc/Max and did not suppress the proliferation of DMSO-differentiated HL-60 cells (data not shown).

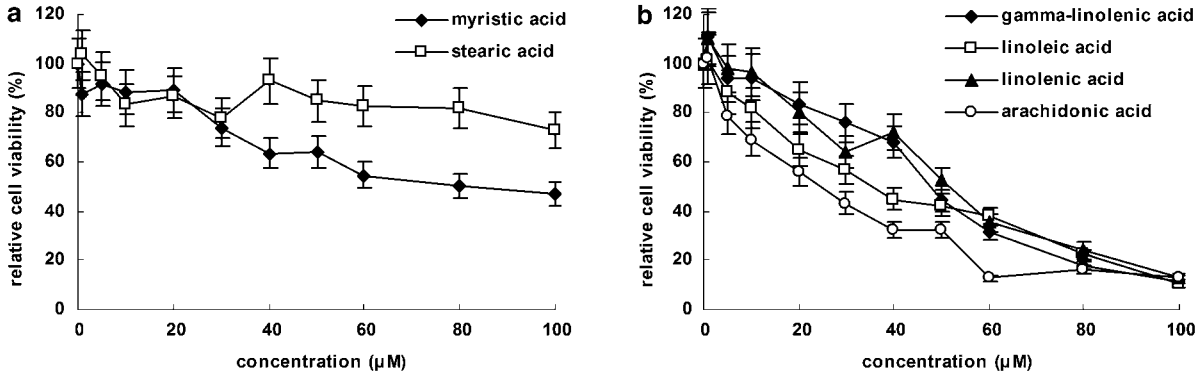

Figure 4 Inhibition of HL-60 cell proliferation with fatty acids. The inhibitory influence of fatty acid on the proliferation of DMSO-differentiated HL-60 cells was analyzed by XTT assay. Following incubation with fatty acids for 12 h, the XTT assay was performed. In all, 12 samples (including one blank) for each fatty acid was analyzed, and each sample contained 0, 1, 5, 10, 20, 30, 40, 50, 60, 80, 100 μM of fatty acid. Each fatty acid stock solution was prepared in DMSO, and the final concentration of DMSO in all samples was adjusted to 1%. Equal assay was performed three times, and the average values were plotted versus the concentration of fatty acids. (**a**) The relative cell viability in the media containing myristic acid and stearic acid. (**b**) The relative cell viability in the media containing γ-linolenic acid, linoleic acid, linolenic acid and arachidonic acid.

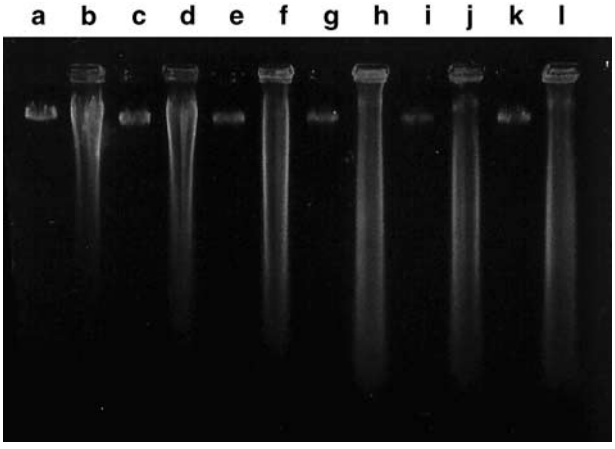

Figure 5 Induced apoptosis of HL-60 cells by fatty acids. The progress and the rate of apoptosis by fatty acids were detected with DNA fragmentation assay in total genomic DNA. DMSO solution containing 100 μM of each fatty acid was added to each growth media at a concentration of 1% and the spontaneous differentiation of HL-60 cells was stimulated. Cells in the media containing 100 μM of each fatty acid were harvested before incubation (**a**, **c**, **e**, **g**, **i**, **k**) and after incubation for 24 h (**b**, **d**, **f**, **h**, **j**, **l**). The fatty acids contained in the media were myristic acid (**a**, **b**), stearic acid (**c**, **d**), γ-linolenic acid (**e**, **f**), linoleic acid (**g**, **h**), linolenic acid (**i**, **j**) and arachidonic acid (**k**, **l**).

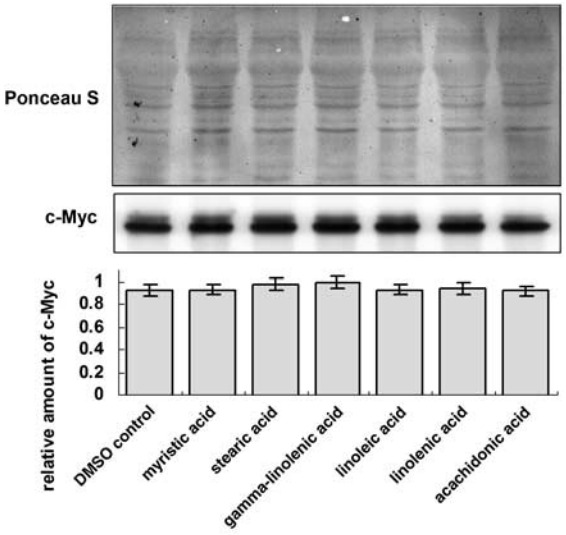

Figure 6 The amount of c-Myc protein in HL-60 cells treated with fatty acids. DMSO-differentiated HL-60 cells were incubated in the media containing 100 μM of each fatty acid, and were harvested after 6 h. The amount of c-Myc in the cells treated with DMSO (as control) and each fatty acid was measured by Western blot analysis. The concentration of total proteins loaded into each well was adjusted to 60 μg, and a nitrocellulose membrane stained with Ponceau S was shown to confirm an equal amount of protein loading. The relative amount of c-Myc was 0.93–1 with maximum value for a standard.

In our experiments, stearic acid, whose IC_{50} was measured as 2.2($\pm$1.2) μM, is a potent inhibitor for DNA binding of c-Myc/Max (Figure 3), but its influence on differentiated HL-60 was relatively weak. We performed many types of test enough to confirm the influence, but the results were the same every time. Ultimately, we concluded that the weak influence of stearic acid is caused by the rapid metabolism of saturated fatty acids. However, it is thought that the continuous oversupply of stearic acid induces the apoptosis of differentiated HL-60 cells (Figure 5).

In summary, we confirmed that the fatty acids, which inhibit the DNA binding of c-Myc/Max, suppress the proliferation and induce the apoptosis of differentiated HL-60, and we concluded that the inhibitors for the DNA binding of c-Myc/Max suppress the Myc-induced proliferation. In further studies, we will focus on what inhibits the DNA binding of c-Myc/Max specifically and potently.

Acknowledgements

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References

1 Henriksson M, Luscher B. Proteins of the Myc network: essential regulators of cell growth and differentiation. *Adv Cancer Res* 1996; **68**: 109–182.

2 Facchini LM, Penn LZ. The molecular role of Myc in growth and transformation: recent discoveries lead to new insights. *FASEB J* 1998; **12**: 633–651.
3 Prendergast GC. Mechanisms of apoptosis by c-Myc. *Oncogene* 1999; **18**: 2967–2987.
4 Iritani BM, Eisenman RN. c-Myc enhances protein synthesis and cell size during B lymphocyte development. *Proc Natl Acad Sci USA* 1999; **96**: 13180–13185.
5 Blackwood EM, Eisenman RN. Max: a helix–loop–helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 1991; **251**: 1211–1217.
6 Prendergast GC, Lawe D, Ziff EB. Association of Myn, the murine homolog of max, with c-Myc stimulates methylation-sensitive DNA binding and ras cotransformation. *Cell* 1991; **65**: 395–407.
7 Blackwood EM, Luscher B, Eisenman RN. Myc and Max associate *in vivo*. *Genes Dev* 1992; **6**: 71–80.
8 Wenzel A, Cziepluch C, Hamann U, Schurmann J, Schwab M. The N-Myc oncoprotein is associated *in vivo* with the phosphoprotein Max(p20/22) in human neuroblastoma cells. *EMBO J* 1991; **10**: 3703–3712.
9 Blackwell TK, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H. Sequence-specific DNA binding by the c-Myc protein. *Science* 1990; **250**: 1149–1151.
10 Kerkhoff E, Bister K, Klempnauer KH. Sequence-specific DNA binding by Myc proteins. *Proc Natl Acad Sci USA* 1991; **88**: 4323–4327.
11 Dang CV, Resar LM, Emison E, Kim S, Li Q, Prescott JE *et al*. Function of the c-Myc oncogenic transcription factor. *Exp Cell Res* 1999; **253**: 63–77.
12 Amati B, Littlewood TD, Evan GI, Land H. The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. *EMBO J* 1993; **12**: 5083–5087.
13 Guerin M, Barrois M, Terrier MJ, Spielmann M, Riou G. Overexpression of either c-myc or c-erbB-2/neu proto-oncogenes in human breast carcinomas: correlation with poor prognosis. *Oncogene Res* 1988; **3**: 21–31.
14 Mariani-Costantini R, Escot C, Theillet C, Gentile A, Merlo G, Lidereau R *et al*. *In situ* c-myc expression and genomic status of the c-myc locus in infiltrating ductal carcinomas of the breast. *Cancer Res* 1988; **48**: 199–205.
15 Spandidos DA, Field JK, Agnantis NJ, Evan GI, Moore JP. High levels of c-myc protein in human breast tumours determined by a sensitive ELISA technique. *Anticancer Res* 1989; **9**: 821–826.
16 Rabbitts TH, Hamlyn PH, Baer R. Altered nucleotide sequences of a translocated c-myc gene in Burkitt lymphoma. *Nature* 1983; **306**: 760–765.
17 Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999; **18**: 3004–3016.
18 Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; **267**: 1456–1462.
19 Kinloch RA, Treherne JM, Furness LM, Hajimohamadreza I. The pharmacology of apoptosis. *Trends Pharmacol Sci* 1999; **20**: 35–42.
20 Dirsch VM, Antlsperger DS, Hentze H, Vollmar AM. Ajoene, an experimental anti-leukemic drug: mechanism of cell death. *Leukemia* 2002; **16**: 74–83.
21 Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, Chevolleau S *et al*. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res* 2000; **60**: 1426–1433.
22 Klucar J, Al-Rubeai M. G2 cell cycle arrest and apoptosis are induced in Burkitt's lymphoma cells by the anticancer agent oracin. *FEBS Lett* 1997; **400**: 127–130.
23 Bieker R, Lerchenmuller C, Wehmeyer J, Serve HL, Mesters RM, Buchner T *et al*. Phase I study of liposomal daunorubicin in relapsed and refractory acute myeloid leukemia. *Oncol Rep* 2003; **10**: 915–920.
24 Jung KC, Rhee HS, Park CH, Yang CH. Determination of the dissociation constants for recombinant c-Myc, Max, and DNA complexes: the inhibitory effect of linoleic acid on the DNA-binding step. *Biochem Biophys Res Commun* 2005; **334**: 269–275.
25 Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. *Proc Natl Acad Sci USA* 1978; **75**: 2458–2462.
26 Gillis RC, Daley BJ, Enderson BL, Karlstad MD. Inhibition of 5-lipoxygenase induces cell death in anti-inflammatory fatty acid-treated HL-60 cells. *JPEN J Parenter Enteral Nutr* 2004; **28**: 308–314.
27 Liu Y, Levy R. Phospholipase A2 has a role in proliferation but not in differentiation of HL-60 cells. *Biochim Biophys Acta* 1997; **1355**: 270–280.
28 Gillis RC, Daley BJ, Enderson BL, Karlstad MD. Eicosapentaenoic acid and gamma-linolenic acid induce apoptosis in HL-60 cells. *J Surg Res* 2002; **107**: 145–153.