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Differential phosphorylation of mitogen-activated protein kinase families by Epidermal growth factor and Ultraviolet B irradiation in SV40-transformed human keratinocytes.

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Running title: Differential phosphorylation of MAP kinases in SVHK cells.

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[2]Abbreviations: EGF, epidermal growth factor; HB-EGF, heparin binding epidermal growth factor-like growth factor; TGF, transforming growth factor: SVHK, SV40-transformed human keratinocyte; MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; JNK, c-JUN N-terminal kinase; MEK, MAP/ERK kinase; MAPKK/MKK, MAP kinase kinase; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagles medium; UV, ultraviolet; ROS, reactive oxygen species.

Key words: extracellular signal-regulated protein kinase / p38 kinase / c-Jun N-terminal kinase / apoptosis

Abstract

SV-40 transformed human keratinocytes (SVHK cells) were stimulated with epidermal growth factor (EGF)[2] and ultraviolet B (UVB) irradiation. Following the stimulation, cell growth, apoptosis, and the activities of mitogen-activated protein (MAP) kinase families were analyzed. EGF (100ng/ml) increased SVHK cell number compared with control cells cultured in serum-free DMEM medium. The EGF-stimulated cells did not show DNA fragmentation. In contrast, UVB irradiation ($40mJ/cm^2$) markedly decreased viable cell number, that was accompanied with DNA fragmentation. EGF stimulated extracellular signal-regulated kinase (ERK) and stress-activated protein kinase/c-Jun N-terminal kinase (JNK). Following the EGF stimulation, phosphorylated ERK and JNK were detected by phospho-p42/44 MAP kinase antibody and phospho-SAPK/JNK antibody, respectively. On the other hand, UVB irradiation stimulated the phosphorylation of p38 and JNK but not of ERK. The stimulation of ERK and JNK induced by EGF was observed earlier than the stimulation of p38 and JNK induced by UVB. PD98059, a specific MAP kinase kinase (MAPKK) 1 (also referred to as MEK1) inhibitor, inhibited EGF-dependent cell proliferation, that was associated with the inhibition of ERK and JNK phosphorylation. In contrast, UVB-induced overall cell death was not significantly affected by PD98059, that inhibited phosphorylation of JNK but not of p38. PD98059, however, significantly augmented UVB-induced cell death earlier time points (30min - 2 h). These results indicate that ERK and JNK are activated following EGF stimulation, that might be associated with cell proliferation. On the other hand, UVB-induced apoptosis seems to be mostly associated with the activation of p38. JNK stimulation might provide an anti-apoptotic tonus during the UVB-induced, p38-associated SVHK cell death.

1. Introduction

During reepithlization, epidermal keratinocytes are exposed to various growth factors, among which are EGF family such as transforming growth factor (TGF)-α, heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, and epiregulin [1-3]. EGF receptor stimulation has been implicated as an autocrine stimulatory signal of cell growth [1,2,4]. The EGF-receptor-dependent signal transduction pathways include phosphatidylinositol pathway, leading to activation of protein kinase C and increase in intracellular Ca^{2+} concentration, as well as Ras-dependent pathway leading to MAP kinase activation [4,5].

Ultraviolet (UV) B irradiation induces various effects on keratinocytes, that include sunburn cell formation or apoptosis [6,7]. Although the molecular basis of UV irradiation has been suggested to be associated with reactive oxygen species (ROS) [8,9], the precise signal transduction mechanisms have not been clarified. We have shown that inhibitors of caspase 1 and 3 suppress the UVB-induced apoptosis of cultured human keratinocytes [10,11].

MAP kinase families, that include extracellular regulated kinase (ERK) 1, 2, p38, and stress-activated protein kinase/c-Jun N-terminal kinase (JNK), are essential signal transduction components, and are involved in various stimulatory or inhibitory signals [12-16]. The ERK is activated by various growth factors and phorbol esters, but are only weakly activated by inflammatory cytokines (tumor necrotic factor-α, interlleukin-1) and environmental stress. In contrast, JNK and p38 are strongly activated by the inflammatory cytokines and environmental stress, but are minimally activated by growth factors or phorbol esters. MAP kinases phosphorylate and regulate the activity of various enzymes and transcription factors including the EGF receptor, Rsk 90,

phospholipase A2, Elk-1, c-Myc, ATF2, and c-Jun. In keratinocytes, recent studies have shown that p38 activation is essential for UVB irradiation-induced apoptosis [17] and that the inhibition of ERK phosphorylation augments the UVB-induced apoptosis [9].

Despite that keratinocytes are under the dynamic control of cell proliferation and cell death to maintain the epidermal integrity, the precise controlling mechanism(s) remain to be determined. SV40-transformed human keratinocytes (SVHK cells) express SV40 virus-derived T cell antigen and, therefore, escape from p53-dependent inhibition of cell cycle progression or p53-dependent apoptosis [18]. Taking the advantage of the simplified nature of cell cycle control of SVHK cells, we investigated the effects of EGF and UVB-irradiation on cell proliferation and cell death, that were analyzed in terms of the activation of MAP kinase families in keratinocyte.

2. Materials and Methods

2.1. Materials

Anti-ERK antibody was obtained from Promega Co. (Madison, USA). Anti-phospho-ERK antibody was purchased from Transduction Laboratories (Kentucky, USA). Anti-p38, anti-phospho-p38, anti-phospho-JNK antibodies and PD98059 were from New England Biolads (Beverly, USA). Anti-JNK was from Santa Cruz Biotechnology Inc (California, USA). EGF was purchased from TOYOBO (Osaka, Japan) and used at 100ng/ml unless otherwise indicated. SB203580 were purchased from Calbiochem (San Diego, USA). Cell count reagent, SF, was purchased from Nakarai Chemicals Ltd. (Japan).

2.2. Cell culture

SV40-transformed human keratinocytes (SVHK cells) were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100mg/ml streptomycin at 37^{o}C in 5% CO_2. After the cells were grown to 60-70% confluence, they were incubated for 24 h in serum-free DMEM. Briefly 6X10$_5$ cells were treated with various reagents.

2.3. UVB irradiation and chemical treatment

Cells grown in 100mm culture dishes were washed twice with phosphate-buffered saline (PBS) (PH7.4) and exposed to UVB irradiation as described previously [10] by using Toshiba-Eizai Dermaray instrument (DMR-1, Tokyo Japan) with slight modifications. The source of UV irradiation was composed of five Toshiba tubes (FL-20-SE30, Tokyo Japan). The spectral output of UVB source was almost 280-320nm with an emission peak at 305nm. We measured radiation output energy (mJ / cm^2) by Toshiba radiometer (Tokyo Japan) at least twice for each experiment. All cells

were handled at room temperature and shielded from sunlight during the time of whole UVB treatment. The UVB doses used in this study correspond to less than 2-min exposure from the equipment.

2.4. Cell extraction

After treatment by various reagents for the indicated time, cells were washed twice by cold PBS (pH7.4) and scraped into cell extraction buffer containing 20mM Tris-HCl (pH7.5), 5mM EGTA, 0.5% NP-40, 50mM 2-glycerophosphate, 1mM phenylmethylsulfonyl fluoride, 2% aprotinin, 10mM DTT, 1mM Na_3VO_4. The extracts were centrifuged for 22,000Xg for 10min. The supernatant was designated as cell lysate. Protein concentration was measured by Lowry's method using bovine serum albumin as standard [19].

2.5. Western blot analysis

Western blot analysis was performed as previously described [10] with slight modification. Twelve to 20 μg of total protein was analyzed on 12% sodium dodecyl sulfate polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were incubated with anti-ERK, anti-phospho-ERK, anti-p38, anti-phospho-p38, anti-JNK, or anti-phospho-JNK, and positive spots were visualized with secondary Ab coupled to horseradish peroxidase, using enhanced chemiluminescence (ECL) (Amersham International plc, UK).

2.6. Analysis of DNA fragmentation

Under each conditions, cells were suspended in 100ml of 10mM Tris-HCl (pH7.4), 10mM EDTA, 0.5% Triton X-100 at 4^oC. The mixture was incubate for 10 min and centrifuged for 20 min at 22,000Xg. RNaseA was added and incubation was performed for 1 h. Proteinase K was added and incubation was performed for another 1

h. Then NaCl and isopropylalcohol was added at final concentrations of 0.5M and 50%, respectively. The whole mixture was centrifuged at 22,000Xg and the precipitate was collected by 10mM Tris-HCl (pH7.4) and 1mM EDTA. Total DNA was analyzed as previously described [10].

2.7. Cell viability assay

SVHK cells were seeded at 1 X 10^4 cells in 60mm dish. After the cells were grown to adequate confluence, cells were treated with various reagents or UVB. After the treatment, the cells were washed twice in cold PBS (pH7.4) and counted following the treatment with reagent SF. After 1 h incubation, the supernatant was collected and OD260nm was measured.

3. Results

3.1. EGF and UVB induced SVHK cell proliferation and cell death, respectively

EGF (100ng/ml) stimulated cell growth compared with serum-free cultured SVHK cells (Figure 1A). The effect was observed at 6 h and the maximal effect was observed at 18 h following the addition of EGF that increased cell number by nearly 2.5 fold (Figure 1A, open circle). At 30 h following PD98059 treatment, cell viability was only slightly decreased (80.8% of control). This was most likely induced by inhibition of basal ERK activity (data not shown). Consistent with our previous report [10], UVB-irradiation (40mJ/cm^2) markedly decreased SVHK cell number (Figure 1B), that was detected by 3 h following the irradiation; viable cells were hardly detected by 24 h (Figure 1B, open circle).

3.2. DNA fragmentation was induced by UVB but not by EGF

SVHK cells exposed to UVB (40mJ/cm^2) showed typical apoptotic morphology such as cell shrinkage and surface blebbing (not shown). Laddered oligonucleosomal DNA fragments (DNA ladder) were observed at 12 h following the irradiation (Figure 2 laneB40). On the other hand, EGF did not induce apoptosis (Figure 2 lane E). Higher dose of UVB irradiation (100 mJ/cm^2) also induced nuclear DNA ladders (Figure 2 laneB100). The sublethal UVB dose of 40mJ/cm^2 was used throughout the subsequent study, because it triggered more standardized stimulation of ERK, p38 and JNK in SVHK cells.

3.3. Phosphorylation of MAP kinases by EGF and UVB

Following EGF treatment, phosphorylated ERK was detected as early as 5 min, that remained up until 6 h (Figure 3A, upper panel). Anti-ERK antibody showed no remarkable change in total ERK. Anti-ERK antibody, however, revealed

electrophoretically retarded bands that represent the active, phosphorylated form of ERK (Figure 3A, lower panel). In contrast, moderate increase in phospho-ERK was detected at 30min that continued until 6 h following the UVB (40mJ/cm^2) irradiation (Figure 3B, upper panel). Higher doses (up to 100mJ/cm^2) of UVB irradiation did not result in any further increase in ERK phosphorylation of SVHK cells (data not shown).

EGF treatment resulted in a modest increase in p38 phosphorylation (Figure 3C, upper panel). The phosphorylation was transient reaching its peak by 5 min and returned to control level by 3 h. No remarkable change in total p38 amount was detected up until 12 h following the EGF treatment (Figure 3C, lower panel). In contrast, UVB (40mJ/cm^2) caused a delayed but significant increase in the phosphorylation of p38 (Figure 3D, upper panel). This phosphorylation was detected by 30 min and continued up until 12 h, suggesting that the UVB-induced p38 phosphorylation correlate with apoptosis but not with cell growth. No remarkable change in total p38 amount was detected following the UVB irradiation (Figure 3D, lower panel). Higher doses (up to 100mJ/cm^2) of UVB irradiation did not result in any further increase in p38 phosphorylation (data not shown).

Concomitant with the activation of ERK by EGF or p38 by UVB, two forms of JNK, p54 and p46, were activated. Time courses of JNK activation were similar to that of ERK activation by EGF (compare Figure 3E and Figure3A) and p38 activation by UVB (compare Figure 3F and Figure3D). EGF-dependent phosphorylation of JNK was earlier than UVB-dependent phosphorylation. No remarkable change in total JNK amount was detected by EGF or UVB (Figure 3E, F; lower panel). Higher doses (up to 100mJ/cm^2) of UVB irradiation did not result in any further increase in JNK phosphorylation in SVHK cells (data not shown).

3.4. Role of ERK and JNK in EGF-induced cell proliferation

We then investigated whether the activation of ERK was required for EGF-induced cell proliferation of SVHK cells. When SVHK cells were preincubated with MAP kinase kinase (MAPKK) 1 (also referred to as MEK1) inhibitor, PD98059, EGF-dependent ERK phosphorylation was significantly reduced. (Figure 4A). Retarded bands by anti-ERK antibody were not observed by PD98059 treatment. Under the condition, viable cell number was significantly decreased compared with EGF-treated SVHK cells (Figure 1A, closed circle), suggesting that ERK is essential for EGF-induced SVHK cell proliferation. No DNA fragmentation was detected by PD98059 treatment alone or EGF plus PD98059 treatment (Figure 5 lane 2 and 4). PD98059 also inhibited EGF-induced phosphorylation of JNK (Figure 4B), suggesting that EGF-dependent phosphorylation of JNK is closely associated with ERK phosphorylation.

3.5. Role of JNK in UVB-induced cell death

Since EGF-dependent ERK phosphorylation was associated with increased JNK phosphorylation, both of which were inhibited by PD98059, we investigated the effect of PD98059 on UVB-dependent JNK phosphorylation. PD98059 showed more marked cell death at earlier time points (30min - 2 h), that was accompanied by more evident DNA fragmentation (Figure 5 lane 5, compare with lane3). PD98059 did not stimulate UVB-induced cell death at late time points; no significant difference in viable cell number was detected at 3 h and thereafter following the irradiation (Figure 1B, closed circle). PD98059 did not affect UVB-induced p38-phosphorylation (Figure 6A), but significantly inhibited UVB-induced JNK phosphorylation (Figure 6B).

3.6. Role of p38 in UVB-induced cell death

To dissect the nature of UVB-induced apoptotic signaling pathways, effects of p38 inhibitor, SB203580, were analyzed. SB203580 was added to the culture medium 30 min prior to UVB irradiation. Surprisingly, no marked suppression of p38 was detected by SB203580 at the concentration range of 1-100μM (data not shown).

4. Discussion

Our results indicate that EGF stimulates cell proliferation, while UVB irradiation induces apoptosis of SVHK cells. The differential effects of EGF and UVB irradiation suggest that each signal transduction system is distinctive. Recently, MAP kinase families are suggested to play an important role on cell proliferation and cell death, both of which are closely associated with cell cycle [5]. In the present study, we demonstrated EGF-induced and UVB-induced selective phosphorylations of MAP kinases with distinctive time courses. EGF stimulated early and continuous phosphorylation of ERK and JNK, that was detected by 5 min, while UVB stimulated phosphorylation of p38 and JNK more slowly, that was detected by 30 min.

Stimulation of EGF receptor is known to induce cell proliferation [2,20]. This is associated with ERK activation at 5-60 min following the stimulation of EGF receptor [21,22], that is occasionally accompanied by JNK activation [23-25]. In SVHK cells, EGF activated ERK and JNK, that were associated with cell proliferation. Besides, the inhibitory effect of PD98059, the specific inhibitor of MAPKK1/MEK1 [26-28], on JNK phosphorylation suggests a close relation of ERK and JNK pathways in SVHK cells.

The role of ERK and JNK activation on cell survival and/or cell proliferation has been controversial. In mouse embryonic stem cells JNK and ERK are regulated by MAP kinase kinase kinase (MEKK) 1 that enhances cell survival following a stress response [29]. Previously, Rho GTPases, Rac and Cdc42, were found to promote cell cycle progression and activate JNK, possibly contributing to cell cycle progression [30]. More recently, vascular endothelial cell growth factor was shown to activate JNK through ERK activation resulting in endothelial cell proliferation [25]. The

EGF-dependent activation of ERK and JNK in SVHK cells is consistent with their results. The JNK pathway may be essential for EGF-induced SVHK cell proliferation. When SVHK cells were treated with PD98059, EGF-dependent phosphorylation of ERK and JNK was inhibited, that was associated with the inhibition of cell proliferation.

Isoforms of JNK, JNK1, JNK2 and JNK3, have been described with distinct substrate binding properties. JNK2 but not JNK1 is known to mediate the effects of EGF [23]. JNK3 on EGF-induced signal transduction remains unknown. The differential phosphorylation of JNK by EGF and UVB in the presence of PD98059 may reflect the distinctive roles of JNK isoforms in SVHK cells.

In many cells including keratinocytes, UVB irradiation-induced apoptosis depends on p38 [9,17,31]. Still UVB-induced activation of ERK and JNK has been reported in normal human keratinocytes and in HaCaT cells [24]. In COS1 cells, UVC activates JNK that binds to transactivation domain of c-Jun, playing an important role on tumor promotion [7]. In contrast, JNK1 stimulation is not essential for UV irradiation-dependent transactivation of c-Jun, but ERK2 (p42 MAP kinase) activation is required for increased c-Jun expression in NIH3T3 cells [32]. Our study demonstrated that UVB irradiation-induced apoptosis of SVHK cells is accompanied by a delayed phosphorylation of p38 and JNK with similar time courses. The results are in line with the previous reports showing that UV mediated apoptosis is associated with the activation of JNK in immortalized mouse keratinocyte cell line, C50 [33] and in HaCaT cells [17].

The significance of JNK on apoptosis remains unknown. Interestingly, although PD98059, the MAPKK/MEK 1 inhibitor, did not significantly alter overall cell

death after 3 h, it enhanced earlier cell death following UVB-irradiation (Figure 1B, closed circle). This was accompanied with more marked DNA fragmentation (Figure 5), and the early inhibition of JNK phosphorylation (Figure 6B). Thus JNK might play a role on cell survival in SVHK cells. We speculate that JNK stimulation might provide an anti-apoptotic tonus during the UVB-induced p38-associated cell death. Although the mechanism of JNK activation remains to be determined, both Ras-dependent and Ras-independent stimulation has been described [34]. Early activation of JNK and p38 is also known to regulate cell viability in response to tumor necrosis factor-α [35]. Peus et al reported that inhibition of ERK by PD98059 resulted in increased UVB-induced cell death [9]. ERK, however, was not significantly phosphorylated by UVB in SVHK cells.

The precise function of p38 on UVB-induced apoptosis remains to be determined. At least four p38 subfamilies (α, β, γ and δ) have been isolated. P38-α induces, while p38-β suppresses cell death [36]. SB203580 inhibits mainly α and β isoforms [37,38]. Because no significant suppression of p38 was detected by SB203580, γ and/or δ p38 isoforms may be predominantly expressed in SVHK cells. Leverkus et al showed that UV-induced apoptosis is mediated by Fas antigen-Fas ligand interaction in cultured normal human keratinocytes [39]. Furthermore, we recently found that UVB directly activates Fas antigen resulting in subsequent caspase cascade stimulation [11]. Fas-induced apoptosis involves the activation of MKK6 and/or MKK3 by ICE-like protease, where MKK3/6 activates CPP32-like protease(s) to promote cell death and also activates p38 signaling pathway [40,41]. Anti-Fas antibody and interferon γ stimulated p38 phosphorylation and induced cell death in SVHK cells (data not shown). No evidence, however, for the interaction of Fas and p38 phosphorylation in

UVB-induced apoptosis in SVHK cells is available at present.

Keratinocytes are exposed to various endogenous and exogenous stimuli, and cell death or cell growth would be expected by alteration of growth control signaling. Keratinocytes may escape from death signal through the activation of JNK and this might (at least in part) come from the sequential activation of ERK and MEK1. In cell proliferation, stimulation of JNK through ERK induces rescuing signal from cell death, while cell death occurs when the irreversible death signal by caspase(s) and/or p38-dependent pathway is stimulated even in the presence of JNK signal. Thus ERK, JNK, and p38, regulate cell death, survival, and growth, by using a combined activation of MAP kinase families in SVHK cells.

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Legends for Figures

Figure 1. Effects of EGF and UVB irradiation on SVHK cells with or without MEK1 inhibitor, PD98059 (50μM)

A; Effects of EGF on SVHK cells with or without PD98059. SVHK cells were incubated in serum-free DMEM for the indicated time. When cells were grown to 60-70% confluence, EGF (100ng/ml) was added (open circle). PD98059 was added at zero time (open square) or 1 hour prior to EGF addition (closed circle). Data are the means from at least two independent experiments. B; Effects of UVB on SVHK cells with or without PD98059. When cells were grown to 60-70% confluence, UVB ($40mJ/cm^2$) was irradiated (open circle). One hour prior to UVB irradiation, PD98059 was added (closed circle). Data are the means from at least two independent experiments.

Figure 2. DNA ladder formation by various stimulation

C (control); SVHK cells were cultured in serum-free DMEM for 24 h. E; EGF was added to the culture medium and the cells were incubated in serum-free DMEM for 24 h. UVB irradiation was performed and SVHK cells were incubated in serum-free DMEM for 12 h. B40 and B100 indicate irradiation doses at $40mJ/cm^2$ and $100mJ/cm^2$, respectively. M; multiples of 123-bp nucleosome-sized DNA fragment for molecular weight marker.

Figure 3. Phosphorylation of MAP kinases by EGF and UVB

SVHK cells were incubated for 24 h in serum-free DMEM before the treatments. A; EGF-induced ERK phosphorylation. Following the EGF (100ng/ml) treatment,

phosphorylated ERK was determined. Note less mobile phosphorylated ERK bands that were detected at 5 min and thereafter. B; ERK was little phosphorylated by UVB (40mJ/cm^2) irradiation. The retarded ERK bands are not obvious. C; P38 phosphorylation by EGF (100ng/ml). D; P38 phosphorylation by UVB irradiation (40mJ/cm^2). E; JNK phosphorylation by EGF. F; JNK phosphorylation by UVB irradiation. Note that time courses of EGF- and UVB-dependent JNK phosphorylation were considerably different. Representative experiments form three independent experiments are shown.

Figure 4. ERK and JNK phosphorylation by EGF with MEK1 inhibitor, PD98059

Cells were pretreated with 50μM PD98059 for 1 h before EGF addition. Other conditions are the same as in Figure 3.A: ERK phosphorylation, B:JNK phosphorylation.

Figure 5. DNA fragmentation of SVHK cells by EGF and UVB in the presence of PD98059

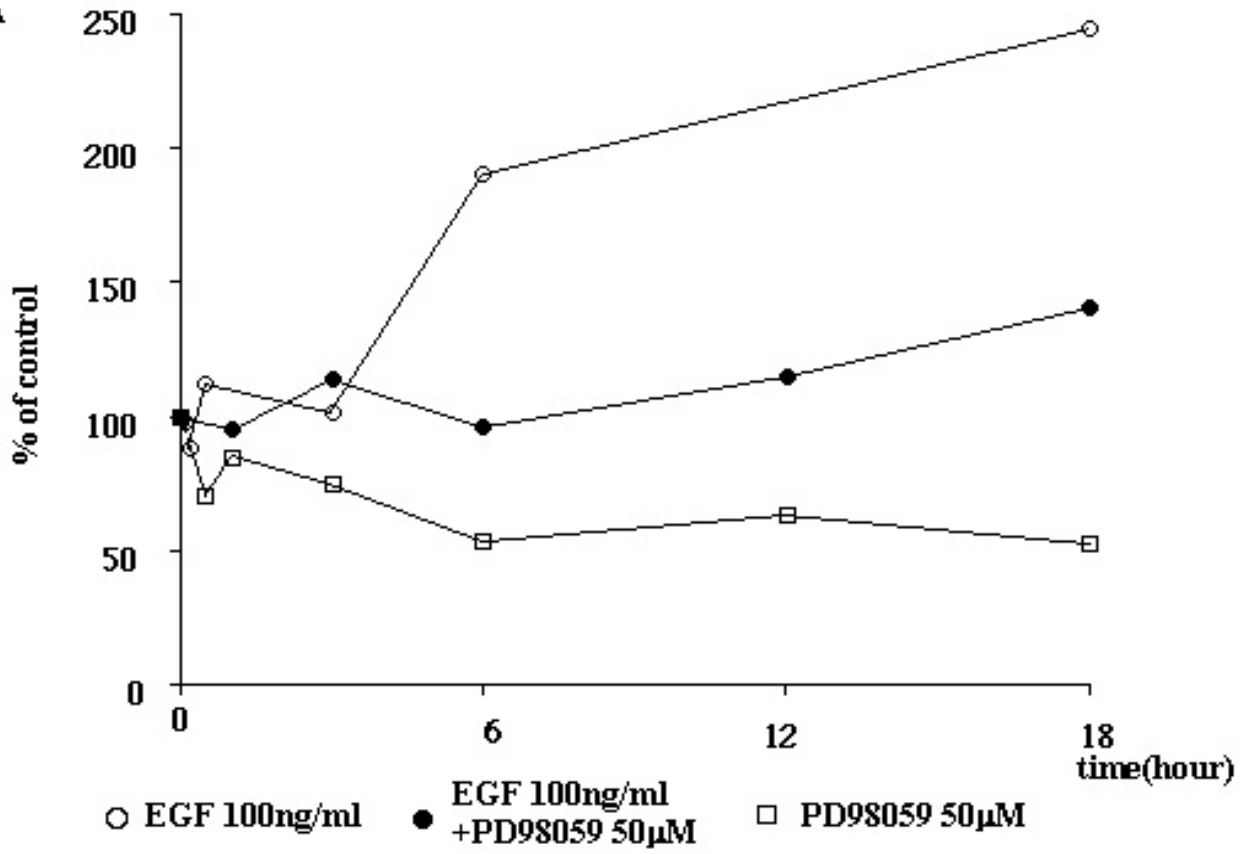
SVHK cells were treated with 50μM PD98059 only (PD), PD98059 plus 100ng/ml EGF (PD+EGF), PD98059 plus 40mJ/cm^2 UVB for 3 h (PD+UVB). UVB- and UVB plus PD98059-treated SVHK cells show the DNA fragmentation.

Figure 6. UVB-dependent p38 and JNK phosphorylation in the presence of MEK1inhibitor, PD98059

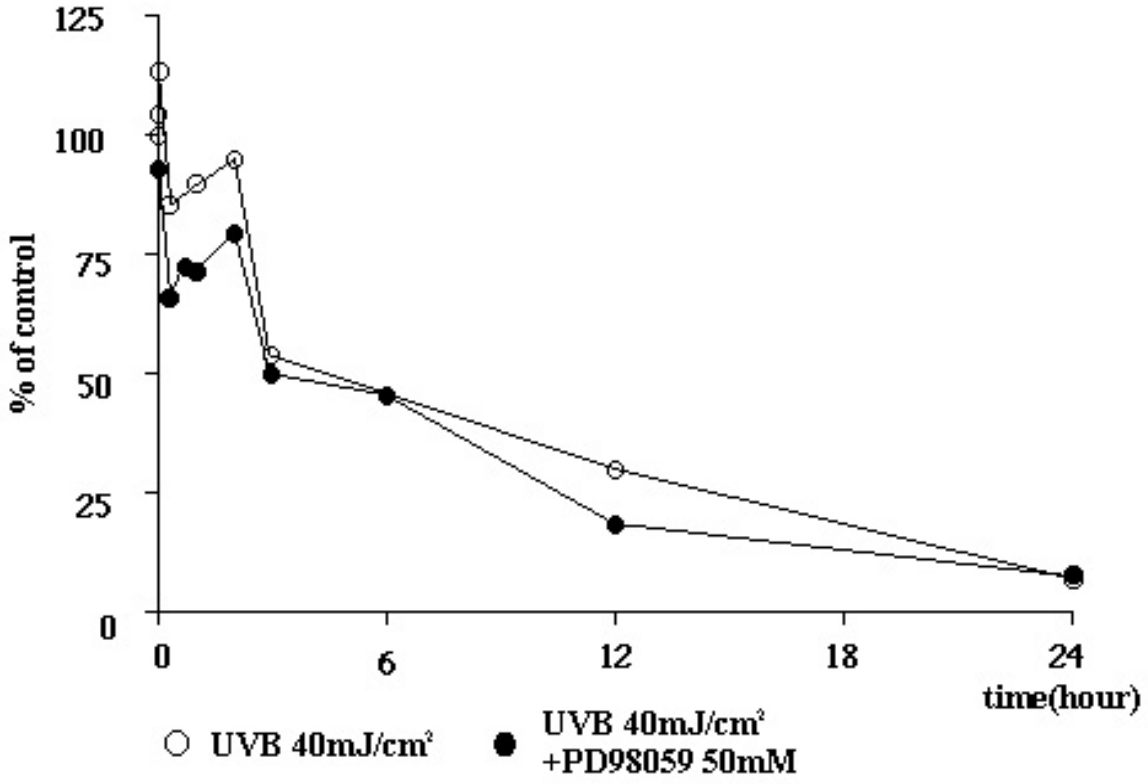
Cells were pretreated with PD98059 (50μM) for one hour before UVB irradiation (40mJ/cm^2). A: P38 phosphorylation, B: JNK phosphorylation. Other conditions are the

same as in Figure 3.

A


250
200
150
100
50
0
% of control
0
6
12
18
time(hour)
EGF 100ng/ml
EGF 100ng/ml
+PD98059 50μM
PD98059 50μM

B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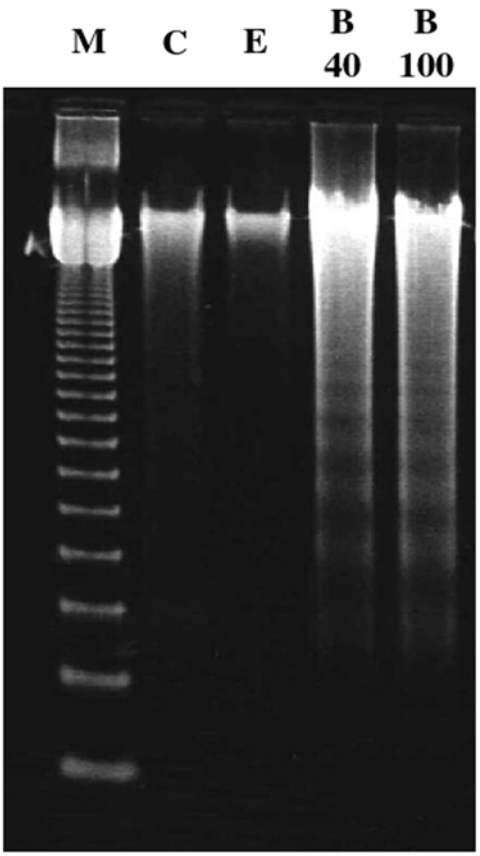
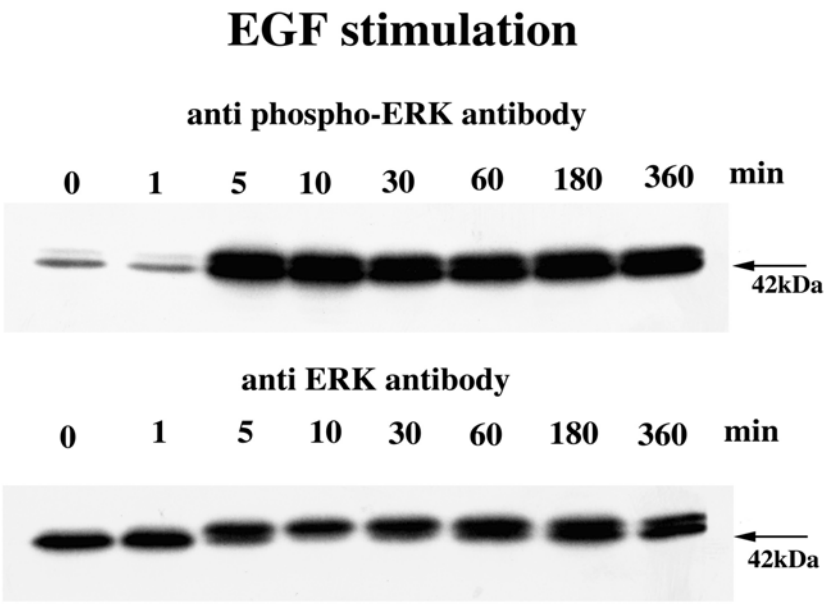
100
75
50
25
0
% of control
0
6
12
18
24
time(hour)
UVB 40mJ/cm²
UVB 40mJ/cm²
+PD98059 50mM


M
C
E
B
40
B
100


EGF stimulation
anti phospho-ERK antibody
0
1
5
10
30
60
180
360
min
42kDa
anti ERK antibody
0
1
5
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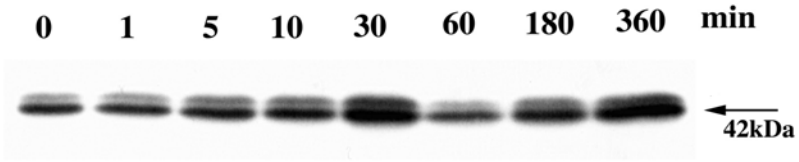
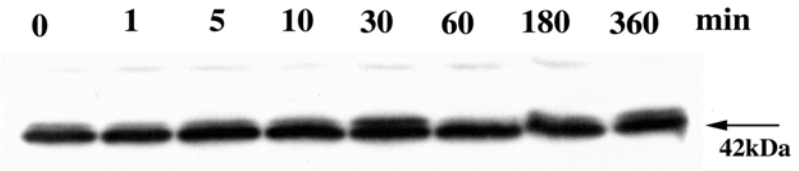
30
60
180
360
min
42kDa

UVB stimulation

anti phospho-ERK antibody

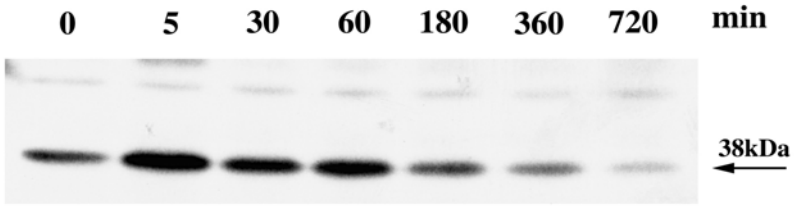


anti ERK antibody

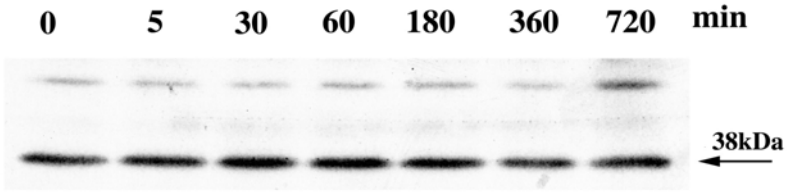


EGF stimulation

anti phospho-p38 antibody



anti p38 antibody



UVB stimulation

anti phospho-p38 antibody

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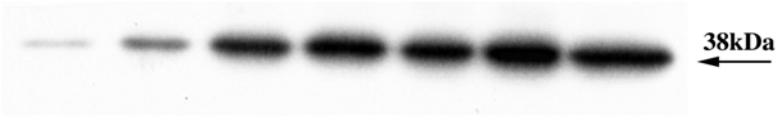


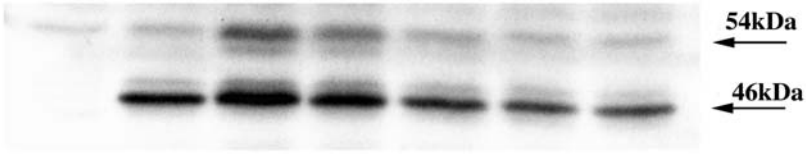
anti p38 antibody

0 5 30 60 180 360 720 min

EGF stimulation

anti phospho-JNK antibody

0 5 30 60 180 360 720 min



anti JNK antibody

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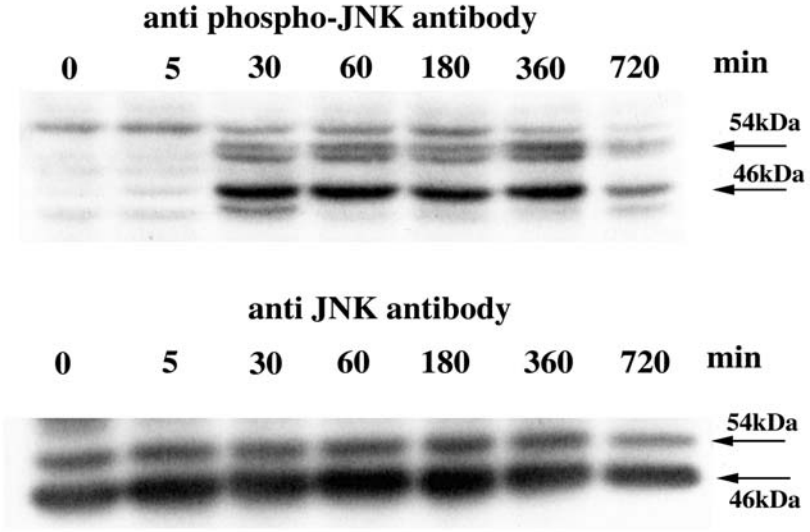
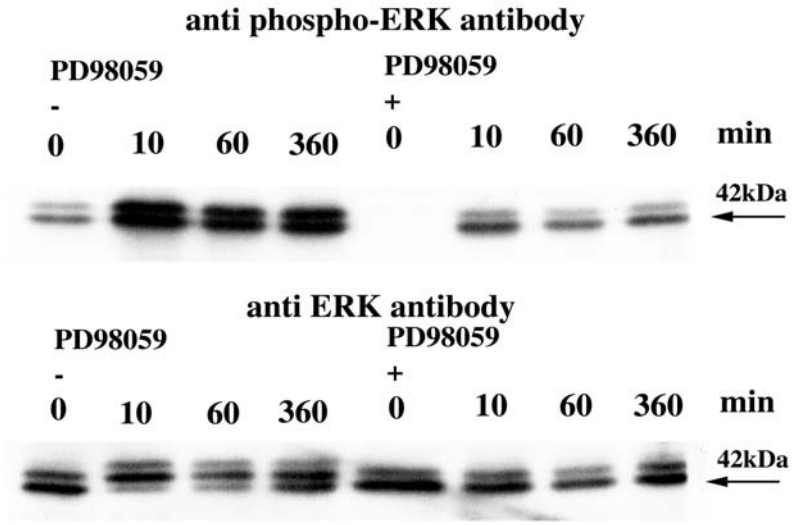
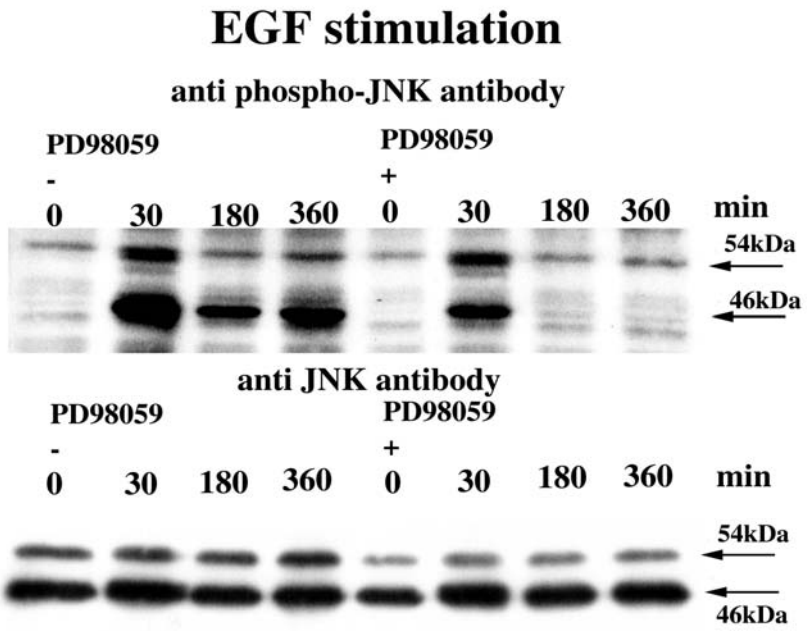
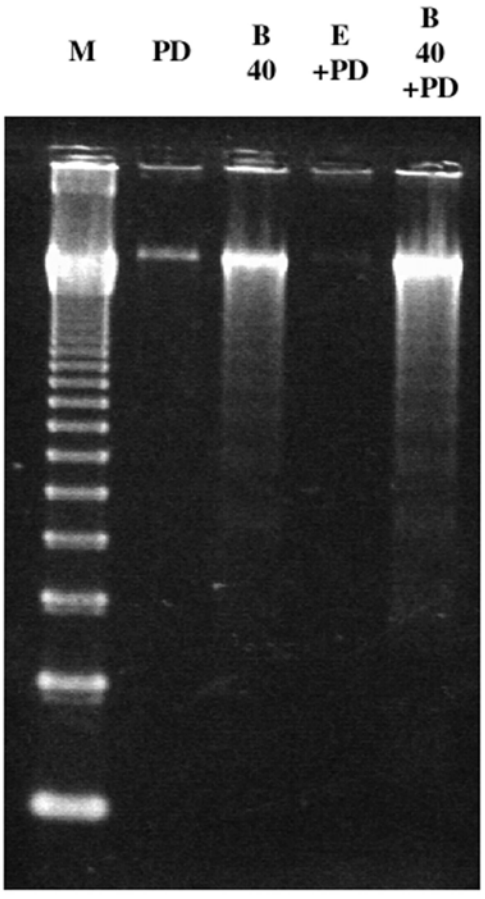
UVB stimulation



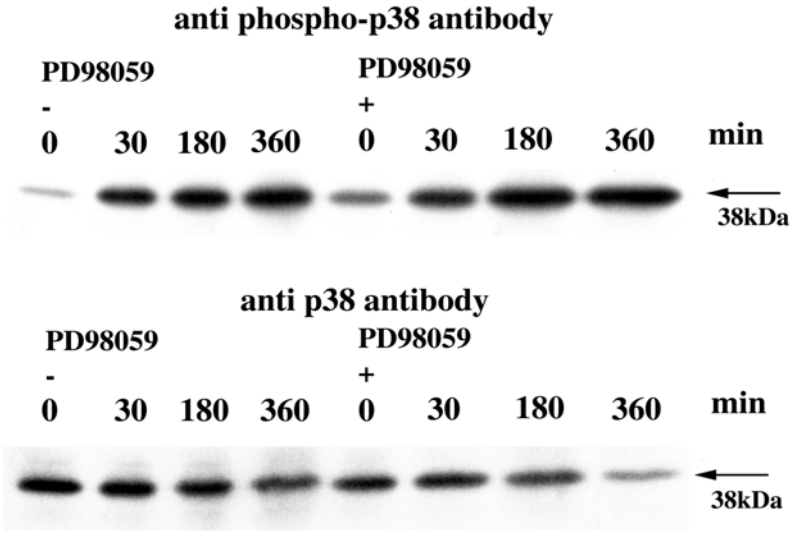
EGF stimulation




EGF stimulation
anti phospho-JNK antibody
PD98059
-
0 30 180 360
PD98059
+
0 30 180 360
min
54kDa
46kDa
anti JNK antibody
PD98059
-
0 30 180 360
PD98059
+
0 30 180 360
min
54kDa
46kDa


M
PD
B
40
E
+PD
B
40
+PD

UVB stimulation



UVB stimulation