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STAT1/2 IS INVOLVED IN THE INHIBITION OF CELL GROWTH INDUCED BY U0126 IN HELA CELLS

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Abstract – The mitogen-activated protein kinase (MAPK) signaling cascade plays an important role in cell life. Herein we show that small interfering RNAs targeting MAPK1 can inhibit HeLa cell growth and induce apoptosis along with up-regulation of signal transducers and activator 1 and 2 (STAT1/2). However, across-talk between the ras-raf-ERK1/2 signalling cascade and the JAK-STAT pathway remain largely unknown. Using MEK inhibitor U0126 and JAK-2 inhibitor AG490, we analyzed the relationship between ERK1/2 and STAT1/2 in HeLa cells. U0126 inhibited HeLa cell growth, arrested the cell cycle at G1/G0, and induced cell apoptosis, and AG490 partially reversed the effects of U0126. U0126 induced up-regulation of ERK1/2 and down-regulation of phosphorylated ERK1/2, increased STAT1 and STAT2 expression in a dose-dependent manner, and activated STAT1/2 via their phosphorylation. AG490 markedly inhibited the phosphorylation of STAT1 and STAT2 and slightly increased that of ERK1/2 inhibited by U0126. We suggest that STAT1/2 is involved in the inhibition of cell growth induced by U0126 in HeLa cells.

Key words U0126; AG490; ERK1/2; STAT1/2; HeLa cell; apoptosis.

INTRODUCTION

The mitogen-activated protein kinase (MAPK) signalling cascade involves membrane-to-nucleus signaling modules that are involved in multiple physiological processes. The cascade consists of three protein kinases: an MEK kinase (MEKK), a dual-specificity MAP kinase kinase (MAPKK or MEK), and a MAP kinase (MAPK)(29). Four major groups of MAPKs exist in mammalian cells: extracellular signal regulated kinase (ERK), c-jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK), p38, and extracellular signal regulated kinase-5 [ERK5, also called Big MAP kinase-1 (BMK1)] (2,6,10,20). ERK is activated mainly by mitogenic stimuli such as growth factors and hormones to induce cell proliferation. In most cell types, the mitogenic signal is relayed from the cytoplasm into the nucleus via nuclear translocation of the ubiquitously expressed p42/p44 isoforms, resulting in activation of a range of transcription factors such as Elk-1.

The RAS MAPK signaling pathway has long been viewed as an attractive pathway to use for anticancer therapies because of its central role in regulating the growth and survival of cells from a broad spectrum of human tumours (28). The ERK MAPKs pathway, which contains some proto-oncogenes and several other factors, has been examined in various human cancers (4,5,12,17,25,27,34,35). The inhibition of activity or down-regulation of ERK inhibits cell proliferation and induces apoptosis (3,14,21). Microarray analysis of MAPK p42 siRNA-treated HeLa cells demonstrated that MAPK p42 suppression was accompanied by overexpression of signal transducers and activators of transcription 1 and 2 (STAT1/2) and ISGs, which were regulated by STAT1/2(15). Inhibition of MEK was sufficient to trigger JAK-dependent STAT1 expression (22), suggesting the existence of cross-talk between the ras-raf-ERK1/2 signaling cascade and the JAK-STAT pathway.

STATs (signal transducers and activators of transcription) comprise a family of seven

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structurally and functionally related proteins: Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6. Among them, Stat1 and Stat2 have been shown to be involved in the IFNs signaling pathways (37). Recent studies have demonstrated that STAT-1 and STAT-2 play a key role in promoting apoptosis in a variety of cell types (7,9,11,). However, cross-talk between the ras-raf-ERK1/2 signaling cascade and the JAK-STAT pathway are largely unknown.

In the present study, we used U0126 and AG490, specific inhibitors of ERK1/2 and STATs, respectively, as a tool to explore cross-talk between the ras-raf-ERK1/2 signaling cascade and the JAK-STAT pathway in HeLa cells. Inhibition of U0126 on the cell growth was prevented by AG490, and U0126 partially induced apoptosis via the up-regulation or activation of STAT1/2.

MATERIALS AND METHODS

Drugs and reagents

U0126 and AG490 were purchased from Sigma Chemical Co. The Annexin V-FITC apoptosis detection kit was obtained from BD Bioscience Co, and the monoclonal antibody was bought from R&D SYSTEMS.

Cell culture

HeLa Cells (1.0×10^5 cells/mL) were cultured in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum containing 2.0 mmol/L glutamine and 20 µg penicillin-streptomycin/mL in 5% CO2 at 37 ℃.

Experimental design

Seven experimental conditions were tested: control; 10 µmol/L U0126; 15 µmol/L U0126; 20 µmol/L U0126; 15 µmol/LU0126 + 5 µmol/L AG490; 15 µmol/LU0126 + 10 µmol/L AG490; and 15 µmol/LU0126 + 15 µmol/L AG490.

MTT assay for cell viability

Cells (2×10^4 cells/well) were seeded into 96-well plates and incubated with the test substances for an indicated time at 37 ℃ in 5% CO2. Then, 20 µL/well of MTT solution (5 mg/mL) was added and the cells were incubated for another 4 h. Supernatants were removed and formazan crystals were dissolved in 200 µL of dimethylsulfoxide. Finally, optical density was determined at 490 nm using an POLARstar+OPTIMA (BMG Labtechnologies, Germany).

Flow cytometry analysis of the cell cycle

DNA content per duplicate was analyzed using a flow cytometer (BD). Adherent cells were harvested by brief trypsinization, washed with PBS, fixed in 70% ethanol, stained with 20 µg/mL propidium iodide (PI) containing 20 µg/mL RNase (DNase free) for 30 min, and analyzed by flow cytometry. The proportion of cells in the G0/G1, S, and G2/M stages were quantified.

Measurement of HeLa cell apoptosis by Annexin-V/PI staining

HeLa cells were treated with U0126 or U0126 plus AG490 at 37 ºC for 48 h, then harvested and washed twice with PBS. The cells were labeled by incubation with 5 µL FITC－Annexin V and 10 µL PI at 250 µg/ml for 10 min in the dark at room temperature. Cells then were washed with PBS and examined using flow cytometry. Quantification of apoptosis was determined by counting the number of cells stained by FITC-labeled Annexin V. The apoptosis of cells was detected using the Annexin V/PI Apoptosis Detection Kit by FACS. Early apoptotic cells were identified with PI negative and FITC Annexin V positive; cells that were in late apoptosis or already dead were both FITC Annexin V and PI positive.

Western blot

Cells were plated at 5×104 cells/tissue culture dish in six-well plates with RPMI-1640. After exposure to the inhibitor at different concentrations, the cells were washed with PBS and subsequently lysed in 200 µl of lysis buffer (150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 0.01% (w/v) sodium azide, 1% (v/v) NP-40, 1 mM phenylmethylsulfonyl fluoride). Insoluble material was removed by microcentrifugation at 13,000 rpm for 15 minutes at 4 ℃. Cell lysates (80 µg of protein/lane) were subjected to electrophoresis using 10% SDS polyacrylamide gels (SDS-PAGE). The proteins were transferred to a polyvinylidene difluoride membrane (NEN Life Science Products, Boston, MA, USA). After blocking the membrane with Tris buffered saline with Tween 20 (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.05% Tween 20) containing 1% (w/v) bovine serum albumin, the membrane was incubated with different monoclonal antibodies (R&D SYSTEMS) and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) respectively. For chemiluminescence detection, Western blot membranes were incubated in the dark with ECL (Amersham), which is a luminol-based enhanced chemiluminescence substrate for horseradish peroxidase. The luminescent signal was recorded and quantified with the Syngene G Box (Syngene, UK), which consists of a high-performance CCD video camera with focus stabilized optics in a mini darkroom enclosure. The instrument is linked to a computer that controls the instrument and handles the data. The luminescent signal is detected by the CCD camera and transmitted to the controller unit, and the data are sent to the computer for analysis and documentation.

Statistical analysis

The data were expressed as mean ± SD and analyzed using SPSS10.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Inhibitory effect of U0126 on the growth of HeLa cells

The small molecule MEK inhibitor U0126 specifically inhibits MEK1/2 from phosphorylating and activating ERK without affecting p38 or JNK pathways (8,30,36) . The growth of HeLa cells was measured by MTT assay at varying time points after U0126 treatment. The inhibition of cell growth in high

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concentration was higher than that in low concentration. At 48 hours and 72 hours, there were significant differences between the control group and the 15 µmol/L or 20 µmol/L U0126 treatment groups ($p < 0.05$), suggesting that inhibition occurred in a U0126 dose-dependent manner (Fig. 1A). Because the cell cycle and apoptosis are involved in the regulation of cell growth, we examined these processes using a flow cytometer 24 h after treatment. The cell cycles were arrested slightly at G1/G0 (Fig. 1B) and early apoptosis increased 48 h post-treatment (Fig. 1C).

Antagonistic effect of AG490 on inhibition of HeLa cell growth induced by U0126

Several studies have implicated STAT proteins in both pro-apoptotic (1) and anti-apoptotic(24) signaling. Among them, STAT1 and STAT2 play an important role in promoting apoptotic cell death. To show that STAT is involved in the promotion of apoptotic cell death induced by U0126, we analyzed the antagonistic effecte of AG490 on inhibition of HeLa cell growth induced by U0126. We found that inhibition of cell growth induced by U0126 was decreased partially by AG490 in HeLa cells at 48 hours and 72 hours (Fig. 2A). The arrest of the cell cycle at the G1/G0 stage in the U0126 treatment group was reversed by 5 µmol/L and 10 µmol/L of AG490 (Fig. 2B) compared to the control group. Moreover, the apoptosis induced by U0126 was inhibited partially by AG490 (Fig. 2C), suggesting that the JAK/STAT pathway was involved in inhibition of HeLa cell growth induced by U0126.

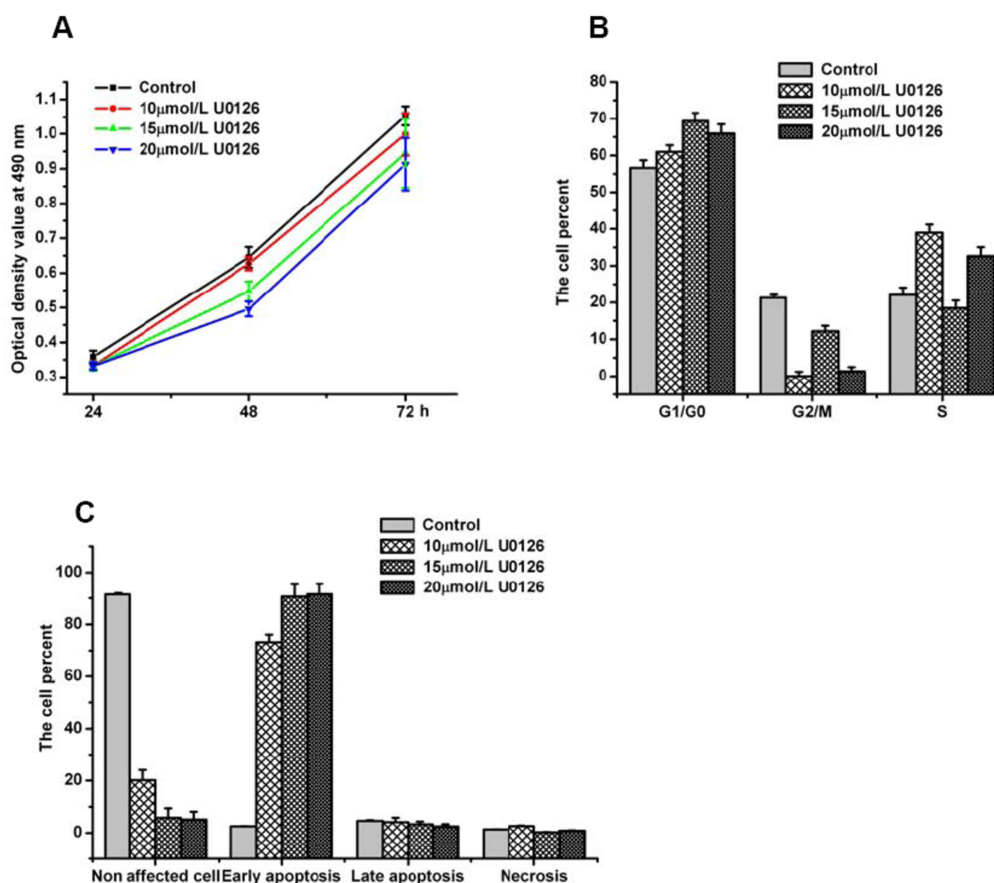

Figure 1. Effects of U0126 on the growth of HeLa cells. A. The inhibition of cell growth was tested using an MTT assay. The results are presented as means ± SD from six independent experiments. B. The results of flow cytometry analysis of the cell cycle in HeLa cells were visualized via PI staining. The data show the percentage of cells in the G1/G0, S, and G2 phases. C. The results of flow cytometry analysis of apoptosis in HeLa cells were visualized using Annexin-V/PI staining. The data show the percentage of non-affected, early apoptotic, late apoptotic, and necrotic HeLa cells. Data are given as mean ± SD from three repeat experiments.

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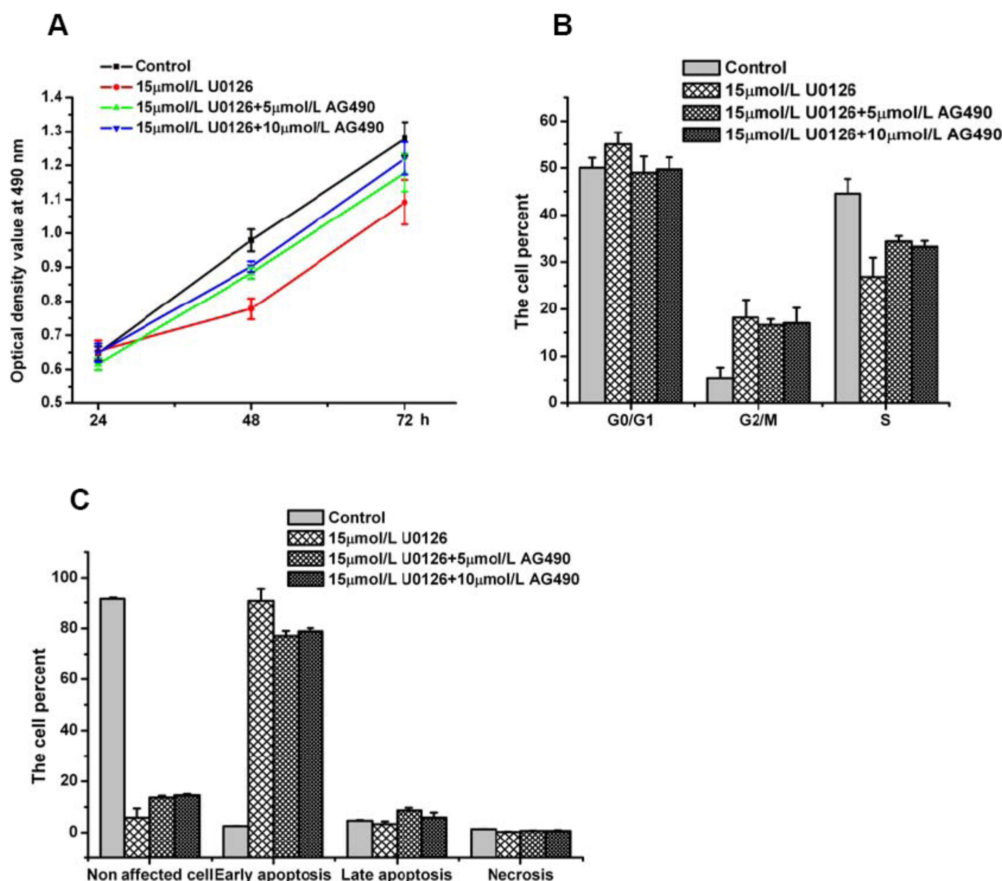

Figure 2. Antagonism of AG490 on inhibition of cell growth induced by U0126 in HeLa cells. A. An MTT assay was used to test the effect of reduction of AG490 on the inhibition of cell growth induced by U0126. The results are presented as means ± SD from six independent experiments. B. Flow cytometry analysis was used to test the effect of reversion of AG490 on the arrest of the cell cycle induced by U0126 in HeLa cells. The data show the percentage of cells in the G1/G0, S, and G2 phases. C. Flow cytometry analysis of apoptosis was used to test the effect of reduction of AG490 on the apoptosis induced by U0126 in HeLa cells. The data show the percentage of non-affected, early apoptotic, late apoptotic, and necrotic HeLa cells. Data are given as mean ± SD from three repeat experiments.

Western blot analysis of the expression and activity on ERK1/2 and STAT1/2

We compared the expression and activity of ERK1/2 and STAT1/2 in HeLa cells treated with U0126 or U0126 plus AG490 at 24 h. U0126 induced the up-regulation of STAT1 and STAT2 expression in HeLa cells in a dose-dependent manner, whereas STAT1 expression was inhibited slightly by AG490. U0126 at 20 μmol/L decreased the expression of ERK1/2 compared to doses of 10 μmol/L and 15 μmol/L. AG490 at 20 μmol/L inhibited the expression of ERK1/2 compared with 15 μmol/L of U0126 (Fig. 3A). When we tested ERK1/2 and STAT1/2 phosphorylation by Western blotting, we found that the activity of STAT1 and STAT2 increased in response to U0126 but that it could be inhibited by AG490 (Fig. 3B). ERK1/2 phosphorylation was inhibited by U0126, but this condition was reversed by AG490. And the activity of ERK1/2 increased in response to AG490 (Fig. 3C).

DISCUSSION

The overexpression and activation of ERK1/2 have been documented in leukemia, renal cell carcinoma, breast cancer, metastatic esophagogastric cancer, and in several ovarian cancer cell lines, suggesting that the ERK MAPKs pathway plays a crucial role in cancer proliferation. Inhibition of MEK/ERK activity with inhibitors of ERK activation is sufficient to induce apoptotic cell death and to attenuate invasiveness of tumours (16,39). U0126 appears to be a potent inhibitor of MEK (13), which

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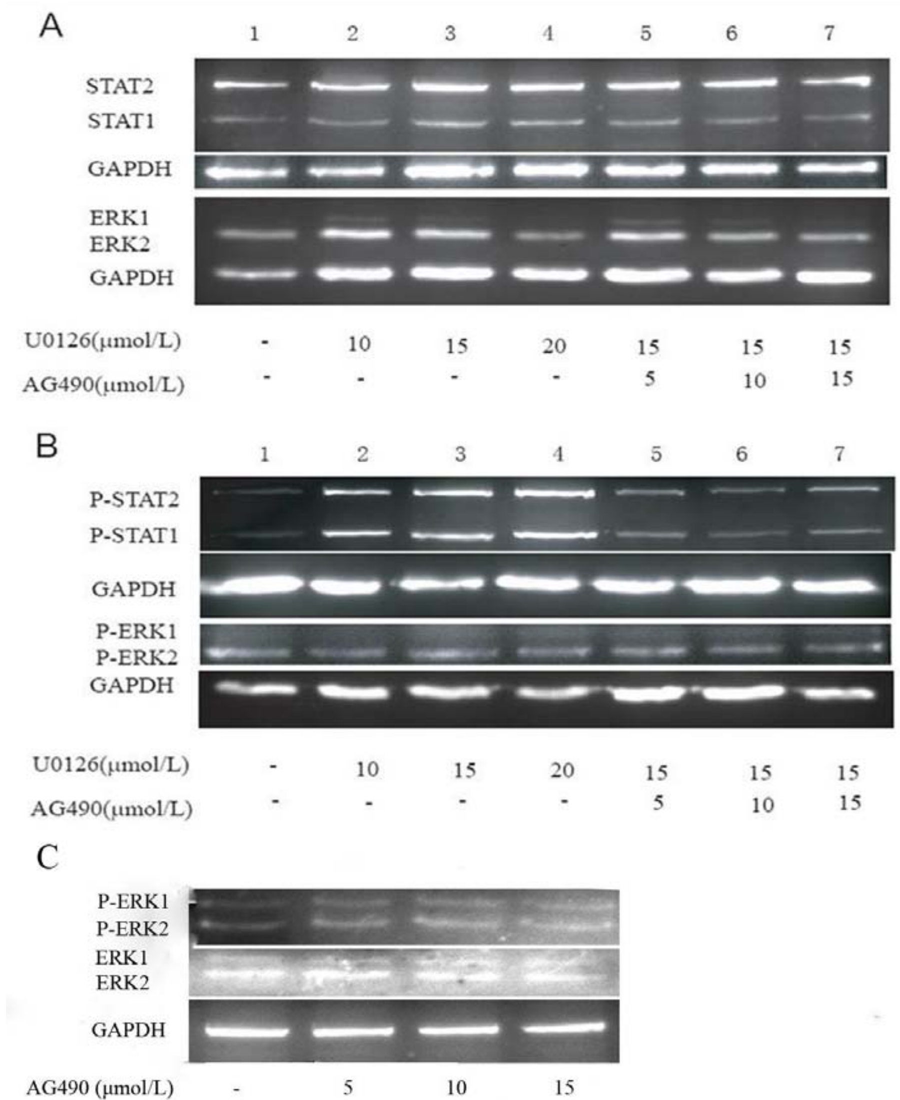

Figure 3. The expression and activity on ERK1/2 and STAT1/2 as shown by Western blot analysis. A. Non-phosphorylated protein. B. Phosphorylated protein.C. ERK1/2 and P- ERK1/2 protein under treated with AG490 only.

binds both MEK isoforms (MEK1 and MEK2) and prevents them from activating their substrates (ERK1 and ERK2) (26). Inhibition of cell growth by U0126 was found to be mediated by cell cycle arrest and apoptosis. The two important arrest points in the cell cycle are at G1/G0 and G2/M (31). The cell cycle phase distributions exist differences in different type of cell lines treated by U0126 (36). In this study, HeLa cells accumulated at the G1/G0 phase in the U0126 treatment groups compared to the control (Fig. 1B), and they also exhibited early apoptosis (Fig. 1C). STAT1 and STAT2 were up-regulated and activated in response to U0126 (Fig. 3 A2–4 and B2–4).

Recent studies have reported that STAT1 is involved in the promotion of apoptosis induced by diverse stimuli, including cytokines, ischemia, heat, DNA damage, and oxysterolalso (18,33,38). In a previous study, we found that siRNAs targeting ERK2 induced the apoptosis of HeLa cells with an increase in STAT1 and STAT2 expression, implying that STAT1 and STAT2 were involved in the apoptosis induced by down-regulation of ERK2 (15). Direct inhibition of MEK is sufficient to trigger JAK-dependent STAT1 expression, suggesting that cross-talk between the MEK/ERK and JAK/STAT pathways plays a key role in regulating neuronal survival (22). AG490, the pharmacological inhibitor of JAK2, inhibits the apoptosis of rat cardiomyocytes by Ang II-mediated

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signaling(23). This is in agreement with our finding that AG490 decreased the growth inhibition, reversed the arrest of the cell cycle at G1/G0, and reduced the apoptosis in groups treated with U0126.

Incubation of HeLa cells with U0126 induced a slight increase of expression and a marked activity of phosphorylation in the transcription factors STAT1 and STAT2. STAT1 and STAT2 are putative tumor suppressor proteins that are proapoptotic in cancer cells (24), and defects in the STAT pathway are observed in some human tumors(19). The JAK-dependent up-regulation of STAT1 following inhibition of Rac GTPase may contribute to the mitochondrial apoptosis of cerebellar granule neurons by modulating the expression of pro-apoptotic Bcl-2 family proteins (22). In our previous study, the up-regulation of STAT1 and STAT2 was mediated by the siRNAs targeting for MAPKs p42 in company with the increase of BAX and the decrease of BCL-2 (15,21,). STAT1 has been shown to cooperate with p53 to regulate the expression of other BH3-only proteins (e.g., Noxa) as well as the multidomain, pro-apoptotic protein Bax in mouse embryonic fibroblasts (32). AG490 was not observed to inhibit the expression of STAT1 and STAT2, but it reduced STAT1/2 phosphorylation activated by U0126 in HeLa cells, suggesting that U0126 partly induced apoptosis in HeLa cells via the STAT1/2 pathways (Fig. 3A5–7 and B5–7).

In conclusion, The MEK/ERK pathway is pro-survival, whereas the JAK/STAT pathway is pro-apoptotic. U0126 inactivates MEK/ERK and stimulates JAK/STAT, ultimately triggering HeLa apoptosis. Most significantly, we suggest that cross-talk occurs between the MEK/ERK pathway and the JAK/STAT signaling pathway in HeLa apoptosis induced by U0126.

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REFERENCES

1. Auernhammer, C.J., Dorn, F., Vlotides, G., Hengge, S., Kopp, F.B., Spoettl, G., Cengic, N., Engelhardt, D. and Weber, M.M., The oncostatin M receptor/gp130 ligand murine oncostatin M induces apoptosis in adrenocortical Y-1 tumor cells. *J. Endocrinol.* 2004, **180**: 479-864.
2. Boulton, T.G., Nye, S.H., Robbins, D.J, Nancy, Y.I, Radzlejewska, E., Sharon, D.M., Ronald, A.D., Nikos, P., Melanie, H.C. and George, D.Y., ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell.* 1991,**65**:663–675.
3. Chmidt, C.M., Wang, Y.and Wiesenauer, C., Novel combination of a COX-2 inhibitor, NS398, and MEK inhibitor, U0126, in human hepatocellular carcinoma provides synergistic increase in apoptosis. *J. Gastrointest Surg*. 2003, **7**:1024-1033.
4. D'Amico, T.A. and Harpole, D.H., Molecular biology of esophageal cancer. *Chest Surg Clin N Am.* 2000, **10**:451-469.
5. deFazio, A., Chiew, Y.E., Sini, R.L., Janes, P.W. and Sutherl, R.L., Expression of c-erbB rec- eptors, heregulin and oestrogen receptor in human breast cell lines. In: *J.Cancer.* 2000,**87**: 487–498.
6. Derijard, B., Hibi, M., Wu, I.H., Barrett, T., Su, B., Deng, T.L., Karin, M. and Davis, R.J., JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell.* 1994, **76**:1025–1037.
7. Elahi, A., Zhang, L.,Yeatman, T.J., Gery, S., Sebti, S. and Shibata, D., HPP1-mediated tumor suppression requires activation of STAT1 pathways. In: *J. Cancer* 2008, **122**:1567-1572.
8. Favata, M.F., Horiuchi, K.Y., Manos, E.J., Daulerio, A.J., Stradley, D.A., Feeser, W.S., Van, Dyk, D.E., Pitts, W.J., Earl, R.A., Hobbs, F., Copeland, R.A., Maqolda, R.L., Scherle, P.A. and Trzaskos, J.M., Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem.* 1998, **273**:18623–18632.
9. Gamero, A.M. and Larner, A.C., Vanadate facilitates interferon alpha-mediated apoptosis that is dependent on the Jak/Stat pathway. *J. Biol. Chem.* 2001,**276**: 13547-13553.
10. Han, J., Lee, J.D., Bibbs, L. and Ulevitch, R.J., A MAP kinase targeted by endotoxin and hy- perosmolarity in mammalian cells. *Sci.* 1994, **265**: 808–811.
11. Haybaeck, J., Obrist, P., Schindler, C.U., Spizzo, G. and Doppler, W., STAT-1 expression in human glioblastoma and peritumoral tissue. *Anticancer Res.* 2007, **27**:3829-3835.
12. Hellstrom,I.,Goodman,G.,Pullman,J.,Ynag,Y.and Hellstrom,K.E., Overexpression of HER-2 in ovarian carcinomas. *Cancer Res*. 2001,**61**:2420–2423.
13. Hotokezaka, H., Sakai, E., Kanaoka, K., Saitor, K., Matsuo, K., Kitaura, H., Yoshida, N. and Nakayama, K., U0126 and PD98059, specific inhibitors of MEK, accelerate differentiation of RAW264.7 cells into osteoclast-like cells. *J. Biol. Chem.* 2002, **277**:47366-47372.
14. Huang, C., Liu, L.Y., Song, T.S., Ni, L., Song, L.P. and Si, L.S., Small interfering RNA- mediated MAPK p42 silencing induces apoptosis of HeLa cells. *Nan Fang Yi Ke Da Xue Xue Bao*. 2006,**26**:11-15.
15. Huang, C., Liu, L., Li, Z., Song, T.S., Ni, L. and Song, L.P., Effects of Small interfering R- NAs targeting MAPK1 on gene expression profile in HeLa cells as revealed by microarray analysis. *Cell. Biol. Int.* 2008, **32**:1081-1090.
16. Huang, D., Ding, Y., Luo, W.M., Bender, S., Qian, C.N., Kort, E., Zhang, Z.F., Kristin, V.B., Duesbery, N.S., James, H.R. and The, B.T., Inhibition of MAPK kinase signaling pathways suppressed renal cell carcinoma growth and angiogenesis in vivo. *Cancer Res.*2008,**68**:81-88.
17. Imaizumi, M., Nishimura, M., Takeuchi, S., Murase, M. and Hamaguchi, M., Role of tyrosine specific phosphorylation of cellular proteins, especially EGF receptor and p125FAK in human lung cancer cells. *Lung Cance.*1997,**17**:69–84.
18. Janjua, S., Stephanou, A. and Latchman, D.S., The C-terminal activation domain of the STA- T-1 transcription factor is necessary and sufficient for stress-induced apoptosis. *Cell. Death.Differ.* 2002, **9**:1140-1146.

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19. Kaplan, D.H., Shankaran, V., Dighe, A.S., Stochert, E., Aguet, M., Old, L.J. and Schreiber, R.D., Demonstration of an interferon gamma-dependent tumor surveillance system in immuno competent mice, *Proc. Natl. Acad. Sci. USA.* 1998,**95**: 7556–7561.
20. Kyriakis, J.M, and Avruch, J., Mammalian mitogen-activated protein kinase signal transducti- on pathways activated by stress and inflammation. *Physiol.Rev.* 2001,**81**: 807–869.
21. Liu, L., Huang, C., Li, Z., Song, T.S. and Ni, L., Related genes for HeLa cell apoptosis induc- ed by siRNA-mediated MAPK p42 silencing. *Acta. Med. Univ. Sci. Technol. Huazhong.* 2008,**37**:129-132.
22. Loucks, F.A., Le, S.S., Zimmermann, A.K., Angela, K.Z., Kristen, R.R., Holger, B., Klaus, A. and Daniel, A.L., Rho family GTPase inhibition reveals opposing effects of mitogen-activated protein kinase kinase/extracellular signal-regulated kinase and Janus kinase/signal transducer and activator of transcription signaling cascades on neuronal survival. *J. Neurochem.* 2006, **97**:957-967.
23. Mascareno, E., Beckles, D.L., Siddiqui, M.A.Q., Janus kinase-2 signaling mediates apoptosis in rat cardiomyocytes. *Vasc Pharmacol* 2005, **43**:327–335.
24. Negoro, S., Kunisada, K., Tone, E., Funamoto, M., Oh, H., Kishimoto, T. and Keiko, Y.T., Activation of JAK/STAT pathway transduces cytoprotective signal in rat acute myocardial infarction. *Cardiovasc. Res.* 2000,**47**:797-805.
25. Rolitsky, C.D., Theil, K.S., McGaughy, V.R., Copeland,L.J. and Niemann,T.H., HER-2/neu amplification and overexpression in endometrial carcinoma. *Int. J. Gynecol. Pathol* .1999, **18**:138-143.
26. Sandberg, E.M., Ma, X., VonDerLinden, D., Godeny, M.D. and Sayeski, P.P., Jak2 tyrosine kinase mediates angiotensin II-dependent inactivation of ERK2 via induction of mitogen- activated protein kinase phosphatase 1. *J. Biol. Chem.* 2004, **279**:1956–1967.
27. Scoccia, B., Lee, Y.M., Niederberger, C. and Ilekis,J.V., Expression of the ErbB family of receptors in ovarian cancer. *J. Soc. Gynecol. Investig.* 1998, **5**: 161-165.
28. Sebolt-Leopold, J.S. and Herrera, R., Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nat. Rev. Cancer.* 2004, **4**:937-947.
29. Seger, R. and Krebs, E.G., The MAPK signaling cascade. *FASEB. J.* 1995, **9**: 726-735.
30. Simon, C., Hicks, M.J., Nemechek, A.J., Mehta, R., O'Malley, B.W. Jr, Goepfert, H., Flaitz, C.M. and Bovd, D., PD098059, an inhibitor of ERK1 activation, attenuates the in vivo invasiveness of head and neck squamous cell carcinoma. *Br. J. Cancer.* 1999, **80**:1412-1419.
31. Steinmetz, R., Wagoner, H.A., Zeng, P., Hammond, J.R., Hannon, T.S., Meyers, J.L. and Pescovtz, O.H., Mechanisms regulating the constitutive activation of the extracellular signal-regulated kinase (ERK) signaling pathway in ovarian cancer and the effect of ribonucleic acid interference for ERK1/2 on cancer cell proliferation. *Mol. Endocrinol.* 2004, **18**:2570-2582.
32. Townsend, P.A., Scarabelli, T.M., Davidson, S.M., Knight, R.A., Latchman, D.S. and Stepha- nou, A., STAT-1 interacts with p53 to enhance DNA damage-induced apoptosis. *J. Biol. Chem.* 2004, **279**:5811-5820.
33. Townsend PA, Cragg MS, Davidson SM, Knight, R.A., Latchman, D.S. and Stephanou, A., STAT-1 facilitates the ATM activated checkpoint pathway following DNA damage. *J. Cell. Sci.* 2005, **118**:1629-1639.
34. Tuziak, T., Olszewski, W.P., Olszewski, W. and Plenkowski, T. Expression of HER2/neu in primary and metastatic breast cancer. *Pol. J. Pathol.* 2001,**52**:21-26.
35. van der Valk, P., Lindeman, J. and Kamphorst, W., Growth factor profiles of human gliomas. Do non-tumour cells contribute to tumour growth in glioma? *Ann. Oncol.* 1997, **8**: 1023-1029.
36. Wiesenauer, C.A., Yip-Schneider, M.T., Wang, Y. and Schmidt, C.M., Multiple Anticancer Effects of Blocking MEK-ERK Signaling in Hepatocellular Carcinoma. *J. Am. Coll. Surg.* 2004, **198**:410-421.
37. Yang, J. and Stark, G.R., Roles of unphosphorylated STATs in signaling. *Cell. Res.* 2008, **18**:443-451.
38. Youlyouz-Marfak, I., Gachard, N., Le Clorennec, C., Najjar, I., Baran-Marszak, F., R eminieras, L., May, E., Bornkamm, G.W., Fagard, R. and Feuillard, J., Identification of a novel p53-dependent activation pathway of STAT1 by antitumour genotoxic agents. *Cell. Death. Differ.* 2008, **15**:376-385.
39. Zhang, S., Li, Z., Wu, X., Huang, Q., Shen, H.M. and Ong, C.N., Methyl-3-indolylacetate inhibits cancer cell invasion by targeting the MEK1/2-ERK1/2 signaling pathway. *Mol. Cancer. Ther.* 2006, **5**:3285-3293.

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