Anti-apoptotic Role of Focal Adhesion Kinase (FAK)

INDUCTION OF INHIBITOR-OF-APOPTOSIS PROTEINS AND APOPTOSIS SUPPRESSION BY THE OVEREXPRESSION OF FAK IN A HUMAN LEUKEMIC CELL LINE, HL-60*

Yoshiko Sonoda‡, Yaeko Matsumoto‡, Megumi Funakoshi‡, Daisuke Yamamoto‡, Steven K. Hanks§, and Tadashi Kasahara‡¶

From the ‡Department of Biochemistry, Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo, 105-8512, Japan and the §Department of Cell Biology, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232-0146

Focal adhesion kinase (FAK) has an anti-apoptotic role in anchorage-dependent cells via an unknown mechanism. To elucidate the role of FAK in anti-apoptosis, we have established several FAK cDNA-transfected HL-60 cell lines and examined whether FAK-transfected cells have resistance to apoptotic stimuli. FAK-transfected HL-60 (HL-60/FAK) cells were highly resistant to apoptosis induced with hydrogen peroxide (1 mM) and etoposide (50 μg/ml) compared with the parental HL-60 cells or the vector-transfected cells, when determined using viability assay, DNA fragmentation, and flow cytometry analysis. Because no proteolytic cleavage of pro-caspase 3 to mature caspase 3 fragment was observed in HL-60/FAK cells, FAK was presumed to inhibit an upstream signal pathway leading to the activation of caspase 3. HL-60/FAK activated the phosphatidylinositide 3'-OH-kinase-Akt survival pathway and exhibited significant activation of NF-κB with marked induction of inhibitor-of-apoptosis proteins (IAPs: cIAP-1, cIAP-2, XIAP), regardless of the hydrogen peroxide-treated or untreated conditions, whereas no significant IAPs were detected in the parental or vector-transfected HL-60 cells. Apoptotic agents induced higher NF-κB activation in HL-60/FAK cells than in HL-60/Vect cells, and it appeared that sustained NF-κB activation is critical to the anti-apoptotic states in HL-60/FAK cells. Mutagenesis of FAK cDNA revealed that Y397 and Y925, which are involved in the tyrosine-phosphorylation sites, were prerequisite for the anti-apoptotic activity as well as induction of IAPs, and that K454, which is involved in the kinase activity, was also required for the full anti-apoptotic activity of FAK. Taken together, we have demonstrated definitively that FAK-transfected HL-60 cells, otherwise sensitive to apoptosis, become resistant to the apoptotic stimuli. We conclude that FAK activates the phosphatidylinositide 3'-OH-kinase-Akt survival pathway with the concomitant activation of NF-κB and induction of IAPs, which ultimately inhibit apoptosis by inhibiting caspase-3 cascade.

Apoptosis (programmed cell death) contributes to the normal development and tissue remodeling of multicellular organisms.

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† To whom correspondence should be addressed: Dept. of Biochemistry, Kyoritsu College of Pharmacy, Shibakoen, Minato-ku, Tokyo 105-8512, Japan. Tel./Fax: 81-3-5400-2697; E-mail: kasahara-td@kyoritsu-ph.ac.jp.

1 The abbreviations used are: CAD, caspase-activated DNase; FAK, focal adhesion kinase; PI3-kinase, phosphatidylinositide 3'-OH-kinase; PDTC, pyrrolidine dithiocarbamate; Ac-YVAD-AMC, N-acetyl-Tyr-Val-Ala-Asp-7-amino-4-methylcoumarin; Ac-DEVAD-AMC, N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin; ICE, interleukin-1β-converting enzyme; FACS, fluorescence-activated cell sorter; IAP, inhibitor-of-apoptosis protein; ROS, reactive oxygen species; TNF, tumor necrosis factor; mAb, monoclonal antibody; RT-PCR, reverse transcription-polymerase chain reaction; PMA, phorbol 12-myristate 13-acetate.
medium containing 5% fetal bovine serum (Nippon Bio-Supply Center, Tokyo, Japan). For oxidative stress experiments, growing cells were subcultured at a density of 2 × 10^6/ml cell in medium containing 1% fetal bovine serum. Monoclonal anti-hemagglutinin epitope peptide (HA) antibody (mAb 12CA5) was purchased from Roche Molecular Biochemicals GmbH (Mannheim, Germany). Rabbit anti-FAK mAb from Transduction Laboratories (Lexington, KY) and rabbit anti-Akt and phospho-Akt antibodies from New England Biolabs Inc. (Boston, MA), anti-phospho-potyrosine mAb (4G10) and rabbit anti-P13-kinase (p85) antibody from Upstate Biotechnology Inc. (Lake Placid, NY), rabbit goat anti-human caspase-3 (CPP32) antibody from Santa Cruz Biotechnology (Santa Cruz, CA), and horseradish peroxidase-conjugated secondary antibody from DAKO (Denmark). Enhanced chemiluminescence reagents were obtained from Amersham Pharmacia Biotech. Substrates for protease activity, N-acetyl-Tyr-Val-Ala-Asp-7-amino-4-methylcoumarin (Ac-YVAD-AMC) (caspase-1) and N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (Ac-DEVAD-AMC) (caspase-3), were obtained from Peptide Institute, Inc. (Osaka, Japan). Pyrrolidine dithiocarbamate (PDTC) and LY294002 were purchased from Sigma. PD98059 was purchased from Alexis Biochemicals (San Diego, CA).

**Transfection of FAK cDNA into HL-60 Cells—**HA-tagged FAK cDNA and mutated FAK cDNA subcloned into the plasmid pRcCMV were prepared as described elsewhere (28). Each 10 μg of pRcCMV/FAK or pRcCMV control vector was transfected into HL-60 cells using electroporation with a Gene Pulser (Bio-Rad) at 0.85 kV. After transfection with 0.5 mg/ml Genetecin (Life Technologies, Inc.), six clones expressing high FAK among several FAK-expressing clones were established.

**Electrophoresis and Immunoblotting—**For the preparation of cell lysate, 1 × 10^8 packed cells were lysed with lysis buffer as described elsewhere (27, 29). In brief, samples were added by Laemmli sample buffer and boiled for 5 min, and equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis. After being transferred to nitrocellulose membranes, they were blocked with 3% bovine serum albumin in phosphate-buffered saline for 1 h and then incubated with primary antibody for 1 h at room temperature. After incubation with the secondary antibody coupled to horseradish peroxidase, detection was achieved using the enhanced chemiluminescence system (Amersham Pharmacia Biotech). Molecular sizes were determined by the relative mobilities of prestained molecular weight markers. PI3-kinase activity in the immunoprecipitates was determined as described previously (27).

**Analysis of DNA Fragmentation and Cell Viability Assay—**A DNA fragmentation assay was performed as described previously (26). In brief, cells were gently lysed for 30 min at 4°C in a buffer containing 5 mM Tris HCl (pH 7.4), 20 mM EDTA, and 0.5% Triton X-100. After centrifugation at 15,000 rpm for 15 min, supernatants containing soluble fragmented DNA were collected and treated with RNase (20 μg/ml, Wako Pure Chemicals, Tokyo, Japan), followed by proteinase K (20 μg/ml) digestion. DNA fragments were precipitated in 99% ethanol. Samples were then electrophoresed on a 2% agarose gel and visualized with 0.1% ethidium bromide. Cell viability was determined using trypan blue exclusion test. The existence of apoptotic cell death was confirmed as well by the appearance of a sub-G0/G1 peak fraction in the cell cycle analysis. For the cell cycle analysis, ethanol-fixed cells were stained with propidium iodide (50 μg/ml) in the presence of RNase A (100 μg/ml), and then analyzed using the fluorescence-activated cell sorter (FACS) Calibur with a CELLQuest program (Becton Dickinson, Mountain View, CA).

**Caspase-1 and Caspase-3 Protease Activity—**Following phosphate-buffered saline washing, cell lysate was prepared as described by Nicholson et al. (2). The cell lysate (50 μg of protein) was incubated at 37°C with 50 μM Ac-DEVD-AMC as a caspase-3 substrate for 30 min or Ac-YVAD-AMC as a caspase-1 (interleukin-1β converting enzyme (ICE)) substrate for 60 min. The amounts of released 7-amino-4-methylcoumarin (AMC) were measured with fluorescence spectrophotometer (Hitachi F-4000, Tokyo, Japan), with excitation at 380 nm and emission at 490 nm. Caspase-1 and -3 activities were expressed as picomoles/min/mg of protein.

**Transfection and Luciferase Assays—**The NF-κB luciferase reporter gene plasmid (pNF-κB4-XP2), containing four tandem copies of the NF-κB binding sequence of the immunoglobulin light chain kappa gene, was obtained from Dr. Hiroya Sato (Chugai Pharmaceutical Co., Japan). Transient cotransfection of NF-κB luciferase (firefly) reporter gene and luciferase (Renilla; sea pansy) expression vector pRL-TK for normalization of transfection efficiency into cells was performed using the DEAE-dextran method as described previously (29). The cells were cultured in RPMI 1640 containing 5% fetal calf serum for 15 h and treated with or without hydrogen peroxide (0.2 mM) or etoposide (10 μg/ml) for 5 h.

**RESULTS**

**Establishment of HL-60 Cell Lines That Overexpress FAK—**To investigate the effect of overexpressed FAK on various stimuli-induced apoptosis, we established six stable transfectants of HL-60 cells that harbor FAK cDNA in the pRcCMV construct and were designated as HL-60/FAK (clones 1–6). Immunoblot analysis revealed significantly higher FAK expression in these HL-60/FAK clones than in the parental HL-60 cells or the control vector transfectants, HL-60/Vect (clones 7–10), in which virtually no or minimal level of FAK was detected (Fig. 1). Endogenous FAK protein in HL-60 could not be detected in unstimulated conditions, but was detected by the stimulation with phorbol 12-myristate 13-acetate (PMA, 10 nM) for 1 day and its expression continued at least till 3 days as shown in Fig. 2. We tested whether the PMA-treated cells were resistant to the hydrogen peroxide-induced apoptosis, i.e. PMA-treated cells for 1–3 days were stimulated with 1 mM hydrogen peroxide, and the apoptotic cells were analyzed by FACS after propidium iodide staining. As shown in Fig. 2, the amount of apoptosis was 10–25% 4 h after treatment with hydrogen peroxide, which was apparently lower than the 50% observed in untreated HL-60 cells. These results showed that intrinsic FAK also has an anti-apoptotic role. These results also suggest that expression of FAK might correlate with the anti-apoptotic capacity in the HL-60 cell line.

**Inhibition of Various Stimuli-induced Apoptosis by FAK Overexpression—**To induce apoptosis, cells were treated with hydrogen peroxide (1 mM) or etoposide (50 μg/ml) for various time periods, and cell viability was assessed by trypan blue exclusion test. All the HL-60/FAK clones showed essentially similar results with marked resistance, and all the HL-60/Vect clones showed the marked apoptosis by the agents during the 4-h incubation period. Therefore, each representative clone (clones 1 and 7) from HL-60/FAK and HL-60/Vect was used for further studies. HL-60/FAK cells (clone 1) showed marked resistance against these stimuli-induced apoptosis compared with both parental HL-60 and HL-60/Vect (clone 7) (Fig. 3). That is, parental HL-60 and HL-60/Vect cells showed 40–60% cell death 4 h after treatment either with hydrogen peroxide or etoposide, whereas HL-60/FAK cells died only less than 3% by

**Induction of IAPs by FAK Activation**
these stimuli. As shown in Fig. 4, the absence of DNA fragmentation by these stimuli in HL-60/FAK cells confirmed the anti-apoptotic activity of FAK. FACS analysis also confirmed the above observation. When cells were treated with these apoptotic stimuli for 4 h, a sub-G2/G1 apoptotic population in parent HL-60 cells or HL-60/Vect cells increased up to 40–50%, whereas the sub-G2/G1 population was only 3–5% in HL-60/FAK cells (data not shown).

Tyrosine Phosphorylation of FAK, Serine Phosphorylation of Akt, and the Association with PI3-kinase in HL-60/FAK Cells—We have previously described that the FAK-PI3-kinaseAkt survival pathway was activated before occurring apoptosis by hydrogen peroxide in a human glioblastoma cell line, T98G (27). To investigate signal transduction of the anti-apoptotic effect of FAK in FAK-transfected cells, we examined tyrosine phosphorylation in these cells. HL-60/FAK and HL-60/Vect cells were incubated with 1 mM hydrogen peroxide for 1 h then lysed and immunoblotted with anti-phosphotyrosine mAb. As shown in Fig. 5A, tyrosine phosphorylation on several protein bands was detected in hydrogen peroxide-treated or non-treated HL-60/FAK cells, showing the activation of these proteins by FAK overexpression regardless of hydrogen peroxide stimulation. The molecular masses of these prominent bands were 120, 52, and 40 kDa, respectively. We identified the band of 120 kDa to be FAK by immunoprecipitation with anti-FAK mAb, followed by blotting with anti-phosphotyrosine mAb (Fig. 5B). From these results, we presumed that the survival pathway (FAK-PI3-kinase-Akt) was activated in HL-60/FAK cells constitutively. Next, we studied the activation of PI3-kinase and Akt, to investigate the link from FAK to PI3-kinase. Cell lysates of HL-60/Vect and HL-60/FAK cells were immunoprecipitated using the anti-FAK mAb, followed by the detection of its associated PI3-kinase, as well as the p85 subunit of PI3-kinase. Without the stimulation of hydrogen peroxide, a significant increase of PI3-kinase activity in HL-60/FAK cells was found in the anti-FAK immunoprecipitates (Fig. 5C). No significant PI3-kinase activity was detected in the HL-60/Vect cells with or without the stimulation of hydrogen peroxide. Immunoblotting of anti-FAK immunoprecipitates with anti-p85 antibody substantially detected the p85 subunit of PI3-kinase (Fig. 5D). These results confirmed that PI3-kinase associates clearly with FAK in HL-60/FAK cells. No significant p85 band was detected in the immunoprecipitates with anti-p85 antibody of HL-60/Vect cells with or without the stimulation of hydrogen peroxide.

In addition, we examined the serine phosphorylation of Akt using anti-phospho-Akt antibody in HL-60/Vect and HL-60/FAK cells. When the cell lysates with or without the stimulation of hydrogen peroxide were subjected to immunoblotting with anti-phospho-Akt or anti-Akt antibodies, the corresponding band of phospho-Akt was consistently detected in HL-60/FAK cells (Fig. 5E). However, no band of phospho-Akt was detected in HL-60/Vect cells with or without the stimulation of hydrogen peroxide. Taken collectively, these findings suggest that FAK-PI3-kinase-Akt survival pathway was constitutively activated in HL-60/FAK cells.

Overexpressed FAK Inhibits the Activation of Caspase-3 Protease—Recent studies have shown that members of the protease caspase family play pivotal roles in apoptosis. To examine the possible involvement of FAK in the regulation of the activity in caspase family proteases, we measured caspase-1 and caspase-3 protease activities using specific peptide substrates in cells treated with hydrogen peroxide or etoposide, with or without FAK overexpression. Although no caspase-1 activity was detected, caspase-3 activity in parent cells and HL-60/Vect cells was markedly elevated with these apoptotic inducers (Fig. 6A). In contrast, no significant caspase-3 activity was detected in HL-60/FAK cells, suggesting that enhanced FAK expression blocks apoptosis by inhibiting the increase of caspase-3 activity when induced by these apoptotic stimuli.
Caspase-3 protease is synthesized as a 32-kDa inactive precursor (pro-caspase-3), which is proteolytically cleaved to produce a mature enzyme composed of 17- and 12-kDa subunits (2). To determine at which stages of caspase-3 protease activation FAK inhibition occurs, we examined the effects on cleavage of the pro-caspase-3 protein in response to these apoptotic inducers. As shown in Fig. 6B, pro-caspase-3 protein disappears concomitantly with apoptosis by hydrogen peroxide treatment in HL-60/Vect cells, indicating that inhibition occurs at the proteolytic cleavage of the pro-caspase-3, which generates active caspase-3 protease fragment. In contrast, pro-caspase-3 protein remains uncleaved in hydrogen peroxide-treated HL-60/FAK cells. Similar results were obtained with etoposide treatment. These results together suggest that FAK suppresses a common upstream step in the pathway leading to the activation of caspase-3 in response to various stimuli rather than directly inhibiting the protease activity.

Overexpressed FAK Induces Sustained NF-κB Activation—It is important to know at which step(s) FAK actually interacts with the apoptosis signal pathway. Therefore, we tested specific inhibitors to locate the inhibition site of the signaling pathway. Addition of 10 μM LY-294002 or 50 μM PDTC, an inhibitor of PI3-kinase or NF-κB, respectively, abrogated the survival effect of FAK partially but not completely (data not shown). The inhibitory effect by LY-294002 and PDTC suggested the involvement of PI3-kinase and NF-κB in the survival pathway used by FAK. Because the importance of PI3-kinase in the anti-apoptotic effect of HL-60/FAK was demonstrated (see Fig. 5), this experiment further confirmed the previous findings.

To further confirm the NF-κB activation in FAK-transfected cells, HL-60/Vect and HL-60/FAK cells were transfected with a luciferase reporter gene that contains putative binding sites for NF-κB (30), followed by the stimulation with hydrogen peroxide or etoposide. A 1.5- and 1.6-fold increase (p < 0.05) in luciferase reporter gene activity was observed in the presence of hydrogen peroxide or etoposide in HL-60/Vect cells compared with unstimulated control cells. In contrast, a 3.2-fold increase (p < 0.005) was observed in HL-60/FAK cells compared with HL-60/Vect cells in unstimulated conditions, indicating that FAK enhances basal NF-κB activity, which was further enhanced with hydrogen peroxide or etoposide treatment (Fig. 7A). The increase of NF-κB activity was inhibited by the addition of 50 μM PDTC or 10 μM LY-294002 down to the unstimulated levels, but still higher than that of HL-60/Vect cells (data not shown), indicating that high basal NF-κB activity in FAK-transfected cells may be related to the anti-apoptotic states of these cells.

Apoptotic Agents and FAK Induce IAP Family Protein Expression—Because NF-κB activation appeared to be critical to the inhibition of hydrogen peroxide- or etoposide-induced apoptosis by FAK, we then explored the expression of survival...
genes, particularly the inhibitory apoptosis protein (IAP) and Bcl-2. The IAP family proteins that block caspase cascade, particularly the proteolytic activation of caspase-3, -7, and -9 (31–33) were reported to be up-regulated followed by the NF-κB activation (34). Therefore, we examined the expression of the IAP family, cIAP-1,2 and XIAP by RT-PCR (Fig. 7B).

Expression of IAPs in HL-60/FAK cells was markedly elevated compared with those in HL-60/Vect cells, and their levels were sustained by hydrogen peroxide or etoposide stimulation. Expression of IAPs in HL-60/Vect cells was only minimally elevated by the above apoptotic stimulation. Expression profiles of the three IAP family proteins were essentially similar. It should be noted that expression of IAPs in HL-60/FAK cells was reduced only partially by the treatment of LY-294002 or PDTC.

As the anti-apoptotic function of the Bcl-2 family proteins involves the inhibition of caspase-3, HL-60/FAK cells may also up-regulate Bcl-2 family proteins. Therefore, we examined the amount of Bcl-2 by immunoblotting, and this indicated that these proteins remained unchanged after hydrogen peroxide or etoposide treatment in HL-60/Vect and HL-60/FAK cells (Fig. 7C).

Anti-apoptotic Effect in Mutated FAK-transfected Cells—To examine further the function of FAK in anti-apoptosis, three mutated FAK cDNA were constructed (35) and tested. One is a kinase-inactive FAK in which the ATP binding site Lys-454 is replaced with Arg (K454R), the second is a mutated form of FAK in which the autophosphorylation site Tyr-397 is replaced with Phe (Y397F), and the third is a mutated FAK of the tyrosine-phosphorylation site Tyr-925 replaced with Phe (Y925F). These FAK mutants were transfected into HL-60 cells, and several clones from each construct were established. The expression level of FAK was confirmed in three lines from the kinase-inactive K454R mutation, six lines with the Y397F mutant, and six lines with the Y925F mutant. The results with the two representative cell lines expressing each of these mutated FAK are shown in Fig. 8A. The anti-apoptotic effect for hydrogen peroxide or etoposide was tested in these cell lines. Because each of the three mutated FAK clones showed similar results, respectively, each representative clone was indicated in Fig. 8B–D.

Anti-apoptotic Effect in Mutated FAK-transfected Cells

Fig. 7. NF-κB activation, IAPs induction, and Bcl-2 expression.

A, HL-60/Vect or HL-60/FAK cells were transfected with luciferase reporter plasmid containing NF-κB binding elements (NF-κBLuc) as described under "Experimental Procedures." After 15-h incubation, cells were stimulated with hydrogen peroxide or etoposide. HL-60/FAK resulted in NF-κB-dependent gene transcription compared with HL-60/Vect cells. The data are presented as fold increases from the control group of HL-60/Vect cells with mean ± S.D. Experiments were repeated at least three times using independent culture conditions. B, HL-60/Vect or HL-60/FAK cells were stimulated with hydrogen peroxide (1 mM) or etoposide (50 μg/ml) in the absence or presence of PDTC (50 μM) for 3 h. Total RNA was isolated at 3 h after stimulation and 1 μg of total RNA was used for cDNA synthesis, amplified by specific primers for IAPs and GAPDH. C, HL-60/Vect or HL-60/FAK cells were stimulated with hydrogen peroxide (1 mM) or etoposide (50 μg/ml) for 3 h. Total cell lysates were prepared and immunoblotted using anti-Bcl-2 mAb.

Fig. 8. Anti-apoptotic effects and induction of NF-κB and IAPs in mutated-FAK-transfected HL-60 cells. A, lysates were prepared from the indicated cell clones and analyzed by immunoblotting using anti-HA mAb. B, the wild-type FAK or mutated FAK-transfected cells were treated with hydrogen peroxide (1 mM) or etoposide (50 μg/ml) for 4 h, and the cell viability was assayed using trypan blue exclusion. Means ± S.D. of triplicate determination are shown. C, mutated FAK-transfected cells were transfected with NF-κB luciferase reporter plasmid, and luciferase activities were assayed as described in Fig. 7A. D, total RNA was isolated from HL-60/Vect or HL-60/FAK or mutated-FAK cells, and 1 μg of total RNA was used for cDNA synthesis, amplified by specific primers for IAPs and GAPDH.

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NF-κB activation and the expression of IAP proteins, cIAP-1,2 and XIAP, were studied. As shown in Fig. 8, C and D, K454R-transfected cells alone showed a marginal increase of the NF-κB activation and IAPs expression, whereas Y397F- or Y925F-transfected cells did not induce the NF-κB activation and IAPs expression at all.

**DISCUSSION**

In this study, we demonstrated that overexpression of FAK endowed HL-60 cells to protect against apoptosis otherwise induced by two representative apoptosis-inducers, an oxidative stress or an anticancer drug, etoposide. In addition to hydrogen peroxide and etoposide, FAK-overexpressed cells were also found to be resistant to C₂-ceramide-induced apoptosis (data not shown).

It has been widely recognized that HL-60 cells do not adhere, but 10 nM PMA-treated cells adhered mildly after 1 day of treatment with PMA and differentiated to macrophage-like cells. HL-60/FAK cells adhered mildly similarly to PMA-treated HL-60 cells (data not shown). It is unknown whether the expression of FAK is prerequisite for or merely concomitant with the differentiation into macrophage form. It should be noted that PMA-treated cells exhibited enhanced FAK expression and acquired the resistance to the hydrogen peroxide-induced apoptosis similar to the HL-60/FAK, strongly suggesting that FAK plays a role in the anti-apoptosis during the differentiation into macrophages. There have been several reports describing the anti-apoptotic roles of FAK in various apoptosis-inducing systems. Hungerford et al. (36) reported on anchorage-dependent cells that became apoptotic when cells were microinjected with anti-FAK antibody, or with a peptide corresponding to the portion of the β₁-integrin cytoplasmic domain presumed to be required for the β₁-integrin-FAK interaction. In another study, Frisch et al. (24) reported that constitutively activated FAK protected MDCK cells from apoptosis consequent to the loss of matrix contact. Furthermore, Xu et al. (37) reported that attenuation of FAK expression leads to apoptosis in some tumor cells. Interestingly, Ilic et al. (38) reported that the extracellular matrix survival signals transduced by FAK suppressed a p53-regulated apoptosis by serum withdrawal in anchorage-dependent cells. Whether FAK has an anti-apoptotic effect on other stress or drug-induced apoptosis has not been explored, and the mechanism of FAK anti-apoptotic role in the apoptosis induced by oxidative stress. Recently, Chan et al. (47) reported that overexpression of FAK in Madin-Darby canine kidney cells suppressed the UV-induced apoptosis. Furthermore, they compared the anti-apoptotic activity of wild type FAK with those of FAK mutants (D395A, Y397F, p712715A, and Y925F). These mutants failed to promote cell survival upon UV irradiation. The interaction of FAK with these proteins might lead to survival signals from FAK, proposing that the binding of PI3-kinase and p130cas with FAK is required for the anti-apoptotic function of FAK. We demonstrated here that FAK protects against oxidative stress-induced apoptosis in HL-60 cells as well as the glioblastoma cell line T98G (27). In addition, Chan et al. (47) indicated that FAK protects against the UV-induced apoptosis in Madin-Darby canine kidney cells, supporting the notion of the general feature of FAK as a role of anti-apoptosis in various cells. Particularly, the involvement of NF-κB and IAPs and the resulting abrogation of caspase-3 activation was evidenced in the suppression of apoptosis in this study. To our knowledge, the data presented here provide the first line of evidence for the regulation of IAPs through NF-κB by FAK.

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