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Mutated focal adhesion kinase induces apoptosis in a human glioma cell line, T98G☆

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Abstract

We have established that focal adhesion kinase (FAK)-transfected HL-60 (HL-60/FAK) cells were highly resistant to hydrogen peroxide and etoposide-induced apoptosis compared to vector-transfected cells. Mutagenesis study revealed that Y397 is required for anti-apoptotic activity in HL-60/FAK, since Y397F-mutated FAK (397FAK) lost anti-apoptotic function. Assuming that 397FAK functions as a dominant negative FAK, we introduced 397FAK cDNA into a human glioma cell line, T98G, using an adenoviral vector. We found that 397FAK induced marked apoptosis with significant FAK degradation. As PI3-kinase–Akt survival pathway was constitutively activated in T98G cells, we hypothesized that this pathway was shut off by 397FAK gene transfection. As expected, activation of PI3-kinase–Akt survival pathway was decreased by the 397FAK gene transfection. 397FAK activated mainly caspase-6 which induced degradation of transfected FAK as well as endogenous FAK. These results indicated that 397FAK induces apoptosis in T98G cells, by interrupting signals of FAK leading to the survival pathway in T98G glioma cells.

Keywords: Glioma; Caspase-6; FAK; PI3-kinase; Akt; Y397F-mutated FAK; Adenovirus; FAK cleavage; IκBdN; Survival pathway

Focal adhesion kinase (FAK) has been implicated in the integration of signals from integrins, oncogenes, and neuropeptides [1]. FAK has also been shown to play an important role in the survival of anchorage-dependent cells [2]. Proteolytic cleavage of FAK by caspase-3 has been reported during growth factor deprivation-induced apoptosis in human umbilical vein endothelial cells [3], which implies an association between FAK and apoptosis. Chan et al. [4] indicated that FAK protects against UV-induced apoptosis in MDCK cells, supporting the notion that FAK has an anti-apoptotic role in various cells and cell lines.

FAK was tyrosine-phosphorylated in response to oxidative stress before apoptosis occurred [5]. Subsequently, PKB/Akt, which has been implicated in the pathway of survival signaling, was serine-phosphorylated following tyrosine-phosphorylation of FAK therefore proposing FAK as an anti-apoptotic role in oxidative stress-induced apoptosis in a human glioma cell line, T98G [6]. We have established stable clones overexpressing FAK in an anchorage-independent cell line, HL-60, and found that these clones were highly resistant to apoptosis induced by oxidative stress, anti-cancer drugs, revealing constitutive activation of the survival pathway [7]. Overexpression and/or deregulation of tyrosine kinases have been reported to lead to constitutive downstream kinase activation, infinite proliferation, and oncogenic transformation [8]. FAK functions in signal transduction from epidermal growth factor receptor (EGFR) amplified in breast and lung carcinomas [9]. FAK is thus assumed to be an oncogene and potential target in anti-cancer therapy.

☆ *Abbreviations:* CAD, caspase-activated DNase; FAK, focal adhesion kinase; 397FAK, Y397F-mutated FAK; PI3-kinase, phosphatidylinositol 3-kinase.

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Human glioma cells are relatively resistant to apoptotic stimuli. We hypothesized that mutated FAK might function as a dominant negative FAK and induce apoptosis in glioma cells. Although Tyr^{397} (Y397) and Tyr^{925} (Y925) were required for the anti-apoptotic activity [7], we focused on Y397 in this study based on the linking of Y397 to the phosphatidylinositol 3 (PI3)-kinase. We thus constructed adenoviral overexpression system of Y397F-mutated FAK (397FAK) and we found that 397FAK induced significant apoptosis in the human glioma cells. Furthermore, we attempted to determine how this mutated FAK induced apoptosis in the glioma cells.

Materials and methods

Cells and reagents. A human glioma cell line, T98G, was obtained from American Type Culture Collection (ATCC, Rockville, MD). T98G cells were maintained in RPMI 1640 containing 5% fetal bovine serum (FBS). For the various stress experiments, growing cells were subcultured at a density of 1×10^5 cells/ml in RPMI 1640 containing 1% FBS. Anti-FAK kinase domain (anti-FAK KD) monoclonal antibody (MAb) and caspase-3 MAb were purchased from Transduction Laboratories (Lexington, KY), rabbit anti-IκB from New England Biolabs (Beverly, MA), anti-phosphotyrosine (PY) MAb (4G10), and rabbit anti-PI3-kinase (p85) antibody (Ab) from Upstate Biotechnology (Lake Placid, NY). Anti-FAK COOH-terminal domain (anti-FAK CD) MAb was purchased from Santa Cruz Biotechnology (San Diego, CA). Anti-caspase-6 MAb was purchased from Oncogene (Boston, MA), anti-human caspase-8 MAb was from Pharmingen (San Diego, CA), and the horseradish peroxidase-conjugated secondary Ab from DAKO (Denmark). Enhanced chemiluminescence reagents were obtained from Amersham-Pharmacia-Biotech (Tokyo, Japan). Substrates for protease activity, Ac-YVAD-7-amino-4-methylcoumarin (AMC) (caspase-1), Ac-DEVD-AMC (caspase-3), Ac-VEID-MCA (caspase-6), Ac-IETD-MCA (caspase-8), and Ac-LEHD-MCA (caspase-9) were obtained from R&S (Minneapolis, MN). LY294002 was obtained from Alexis Biochemicals (San Diego, CA). Human embryonic kidney 293 cells were cultured in DMEM containing 10% FBS.

Generation of recombinant adenoviral vectors. pRcCMV-FAK or -397FAK fused to the HA epitope tag was digested with *Kpn*I/*Xba*I and blunted. The obtained fragment was inserted into the *Swa*I site of the cosmid pAXCAwT. Recombinant adenoviruses, Adv-FAK, and Adv-397FAK, were generated by cotransfection of the cosmids and adenovirus using the method described by Miyake et al. [10]. Large amounts of Adv-FAK or Adv-397FAK were generated by subsequent infection of 293 cells with Adv-FAK or Adv-397FAK for 48 h in DMEM containing 10% FBS. Cells were then harvested, resuspended in PBS, and lysed by freezing and thawing three times. Cell debris was removed by centrifugation, and the supernatant was collected. The number of virions per milliliter was estimated by infection of 293 cells and the virus was stored at −80 °C. Adv-IκB fused to the Flag tag and Adv-LacZ have been described elsewhere [11].

Adenoviral infection. Cells were plated at 1×10^5 ml^{-1} in 6-cm culture plates and infected with Adv-FAK, Adv-397FAK, or Adv-LacZ for various periods at an optimal concentration of virus [10,11]. Optimal concentrations of virus were determined by infection of cells with β-galactosidase-expressing virus (Adv-LacZ) at different doses and subsequent staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). We used titers that gave the expression of β-galactosidase in >90% of the cells without a visible toxic effect and confirmed the expression of each infected Adv as follows. Cells were infected with 3 or 5×10^2 virions per cell (multiplicities of infection, MOI 300 or 500) and detected HA-tagged FAK or 397FAK or IκB–Flag expression was observed within $85 \pm 5\%$ of the cells using an anti-HA MAb or anti-Flag MAb by fluorescence microscopy. For the co-transfection experiments, cells were infected with Adv-397FAK MOI (500) and Adv-IκBdN (MOI 300). The total number of virions was adjusted with Adv-LacZ.

Assays of cell death and DNA fragmentation. Cell death was determined by trypan blue dye exclusion and MTT assay as described elsewhere [7,8]. DNA fragmentation assay was performed as reported previously [6]. Briefly, cells were gently lysed for 30 min at 4 °C in a buffer containing 5 mM Tris buffer (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 Chem, Tokyo, Japan), followed by proteinase K (20 μg/ml). DNA fragments were precipitated in 99% ethanol. Samples were then electrophoresed on a 2% agarose gel, and visualized with 0.1% ethidium bromide.

Electrophoresis and immunoblotting. For the preparation of cell lysate, 1×10^6 packed cells were lysed as described [12]. Briefly, samples were mixed with Laemmli sample buffer, the mixture was boiled for 5 min, and equal amounts of protein were separated by SDS–PAGE. After being transferred to nitrocellulose membranes, blocked with 3% BSA in PBS for 1 h, and then incubated with primary Ab for 1 h at room temperature followed by the secondary Ab coupled to horseradish peroxidase, protein was detected using the enhanced chemiluminescence system (Amersham-Pharmacia-Biotech). Expression of HA-tagged FAK and 397FAK was confirmed by immunoblotting of infected cells using an anti-HA MAb (12CA5, Roche Mol. Biochem). PI3-kinase activity in the immunoprecipitates was determined as described previously [7].

Caspase-1, -3, -6, -8, and -9 activity. Following a wash in PBS, cell lysate was prepared as described by Nicholson et al. [13]. The cell lysate (50 μg protein) was incubated at 37 °C with 50 μM Ac-DEVD-AMC, Ac-VEID-AMC, Ac-IETD-AMC, and Ac-LEHD-AMC as caspase-3, -6, -8, and -9 substrates, respectively, for 30 min or Ac-YVAD-AMC as a caspase-1 substrate for 60 min. The amount of 7-amino-4-methylcoumarin (AMC) released was measured using a fluorescence spectrofluorometer (Hitachi F-4000, Tokyo, Japan), with excitation at 380 nm and emission at 460 nm. Caspase activities were expressed as pmol/min/mg protein.

Results

397FAK induces apoptosis in T98G glioma cells

Transfection of Adv-FAK or Adv-397FAK into T98G cells induced significant expression of each FAK protein in an MOI-dependent manner (Fig. 1A). Here, we detected a HA-tag protein, since the sequence encoding the HA epitope was fused in-frame with the C-terminal coding sequence of FAK. We found that transfection of 397FAK induced high incidence of cell death in T98G cells at MOI 300 and 500. Therefore, we evaluated the effect of Adv-FAK or Adv-397FAK on the cell proliferation quantitatively (Fig. 1B). 397FAK caused an MOI-dependent increase in the number of dead T98G cells, with a marked increase (40%) at MOI 500. However, no cytotoxicity was induced by wild-type FAK transfection. T98G cells 3 days after being infected with 397FAK at MOI 500 were analyzed for the DNA fragmentation as shown in Fig. 2A. Typical DNA fragmentation was observed,

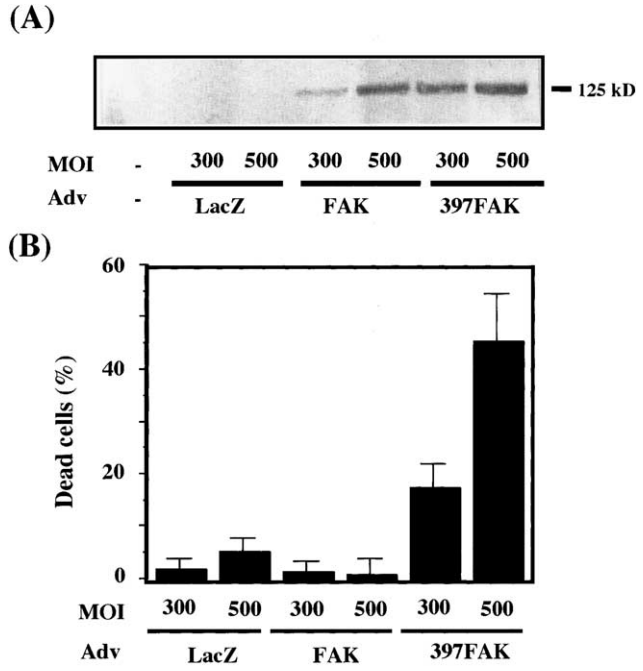

Fig. 1. (A) Immunoblot analysis after infection with Adv-LacZ, Adv-FAK, or Adv-397FAK. (B) Percentage of dead cells in T98G cells after infection with Adv-LacZ, Adv-FAK, or Adv-397FAK T98G glioma cells were infected with Adv-LacZ, Adv-FAK, or Adv-397FAK at various MOIs. Cell lysates were prepared 1 day after infection and immunoblotted as described in Materials and methods. Results are representative of three independent experiments. Dead cells were measured by trypan blue exclusion and MTT assay 3 days after infection. Results were shown as mean ± SD from three independent experiments.

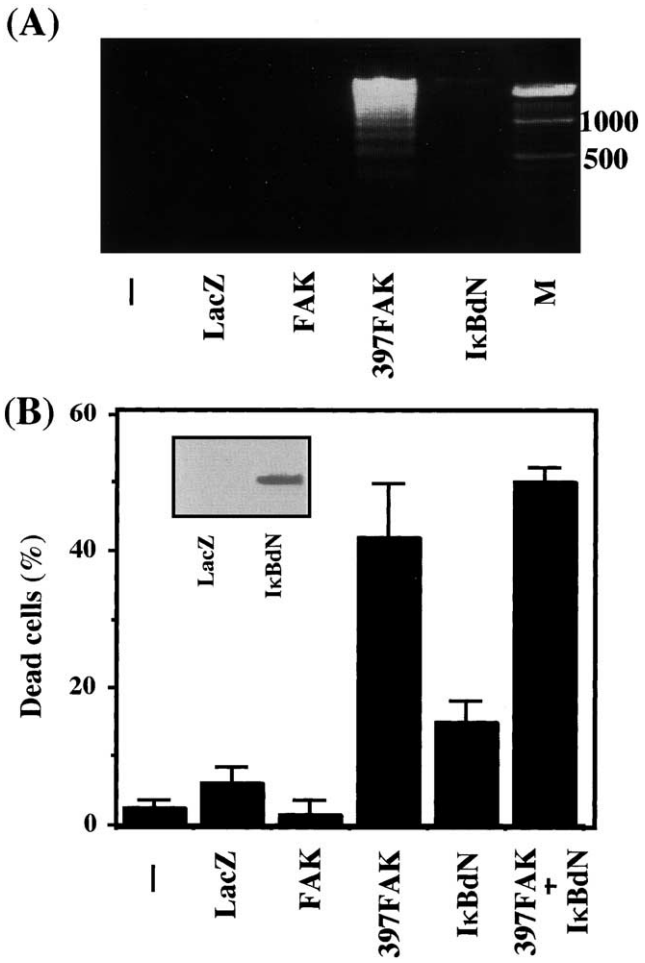

Fig. 2. (A) DNA fragmentation assay. (B) Percentage of dead cells in T98G cells after coinfection with Adv-397FAK, Adv-IκBdN, Adv-397FAK, or Adv-IκBdN. (A) T98G glioma cells were infected with Adv-LacZ, Adv-FAK, Adv-397FAK, or Adv-IκBdN at MOI 500 and DNA fragmentation was analyzed 3 days after infection. Results are representative of three independent experiments. Molecular size markers (M) are indicated in the right lane. (B) T98G glioma cells were infected with Adv-IκB (MOI 300), Adv-397FAK (MOI 500), Adv-397FAK (MOI 500), and Adv-IκBdN (MOI 300) and dead cells were measured by trypan blue exclusion and MTT assay 3 days after infection. Expression of IκBdN with a Flag tag was detected by anti-Flag MAb (B, inset). Results were shown as mean ± SD from three independent experiments. The total MOI was kept constant by supplementing with Adv-lacZ.

confirming that these cells were apoptotic. In a previous report, we found that FAK-transfected HL-60 cells accompanied activation of NF-κB, which plays a key role in the anti-apoptotic mechanism. Since IκB is an inhibitor of NF-κB, which translocates to the nucleus on degradation of IκB under stimulated conditions, we assumed that N-terminal deleted IκB (IκBdN), a supersuppressor of NF-κB, enhances the apoptotic event. When T98G cells were cotransfected with IκBdN (at MOI 300) and 397FAK (at MOI 500), IκBdN only slightly induced cell death and IκBdN enhanced minimally the apoptosis-inducing capacity of 397FAK (Fig. 2B). To test whether other human glioma cell lines were sensitive to Adv-397FAK, U373MG glioma cells were also studied and similar results were obtained (data not shown).

397FAK mainly activates caspase-6 in T98G cells

It should be quite important which caspase family proteases are activated during apoptosis. To examine which caspases are involved in 397FAK-induced apoptosis, the activities of caspase-1, -3, -6, -8, and -9 were measured using specific peptide substrates. We have previously reported that caspase-6 was activated during FTY720-induced apoptosis of T98G glioma cells [14]. As expected, caspase-6 was mostly activated, while caspase-3 and -8 were moderately activated in 397FAK-infected T98G cells and no significant caspase-1 and -9 activities were detected (Fig. 3A). The above caspase activities were the highest at 3 days after the 397FAK transfection. In contrast, neither LacZ nor wild-type FAK transfection induced activation of these caspases at all (data not shown). Since caspase-3, -6, and -8 are synthesized as a 32, 34, and 55 kDa inactive precursors, which are proteolytically cleaved to corresponding mature fragments, respectively, we examined

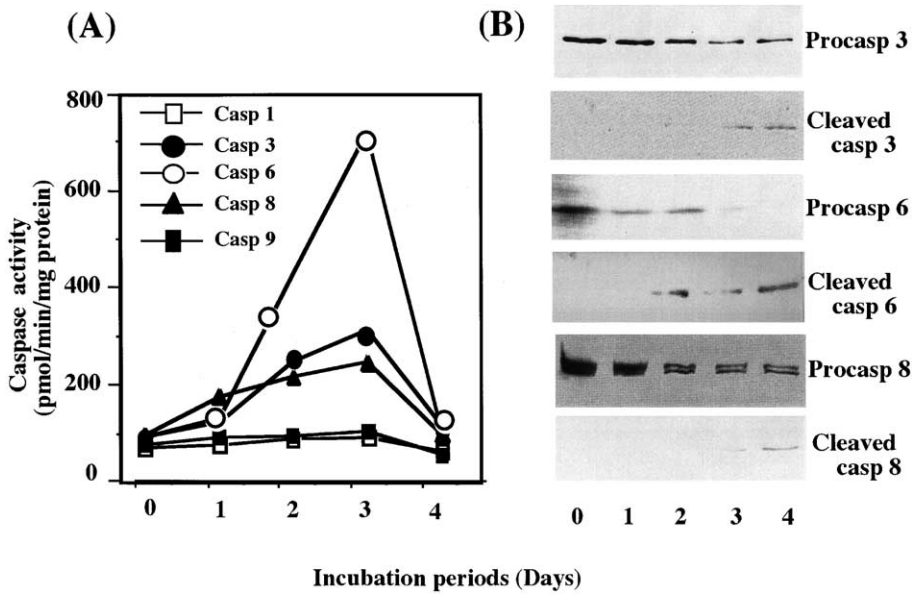

Fig. 3. (A) Activation of caspase-3, -6, and -8 after infection with Adv-397FAK. (B) Detection of procaspase-3, -6, and -8 and cleaved caspase-3, -6, and -8 after infection with Adv-397FAK T98G glioma cells were infected with Adv-397FAK at MOI 500 and caspase 1, 3, 6, 8, and 9 activities were determined for 4 days after infection. Results represent the average of three independent experiments. Immunoblot analysis was performed for 4 days after infection. Results are representative of three independent experiments.

the cleavage of these caspases by specific Abs recognizing cleaved fragments of 17 (caspase-3), 18 (caspase-6), or 10 kDa (caspase-8). As shown in Fig. 3B, 34 kDa procaspase-6 band disappeared and the active fragment (p18) appeared upon the 397FAK-induced apoptosis. Both 32 kDa caspase-3 and 55 kDa caspase-8 bands decreased and the active fragments appeared faintly. These results indicate that 397FAK induces marked activation of caspase-6, and moderately caspase-3, which are presumably initiated by the activation of caspase-8.

397FAK causes cleavage of FAK

Since FAK is a substrate for both caspase-3 and -6, we explored whether FAK was cleaved upon treatment with 397FAK transfection. Four cleavage products of FAK, i.e., 92, 84, 41, and 33 kDa fragments were identified (Fig. 4A-1) by using anti-FAK kinase domain (anti-FAK KD) MAb or anti-FAK carboxy-terminal domain (anti-FAK CD) MAb (Fig. 4A-2). Wen et al. [15] indicated that FAK is cleaved by caspase-3/7 and -6, and suggested that the cleavage sites of caspase-3/7 and caspase-6 in human FAK are presumed to be at DQTD772 and VSWD704 as shown in Fig. 4C. Caspase-3/7 site, DQTD772, is absent in mouse FAK (DQTE772) [16]. As we transfected mouse HA-tagged FAKcDNA or 397FAKcDNA in this study, 92 and 33 kDa bands must therefore be the cleaved fragment of endogenous human FAK. Anti-HA MAb recognizing only transfected FAK with HA-tag in the C-terminus revealed 41 kDa band as well as 125 kDa band in 397FAK-transfected cells (Fig. 4B). In FAK-transfected cells, only 125 kDa band was detected by Western blot with these three antibodies. Thus, it is assumed that 397FAK-transfection caused cleavages of 397FAK and endogenous FAK generating 41 and 33 kDa fragments by activating caspase-6 and -3/7. The cleavage products, 41 and 33 kDa generated as carboxy-terminal fragments, may act like FRNK, a naturally occurring variant of FAK [17]. It should be noted that cells treated with LacZ, FAK, or IκBdN did not generate these bands. Taken collectively, it is assumed that overexpression of 397FAK resulted in the cleavages of endogenous FAK as well as transfected 397FAK, thus leading to the shutting off of the survival pathway.

Interruption of the survival pathway

We described that the FAK–PI3-kinase–Akt survival pathway is activated by hydrogen peroxide in T98G cells before the induction of apoptosis [6]. We therefore assumed that 397FAK-induced apoptosis in glioma cells might interrupt this survival pathway. To test this notion, FAK phosphorylation and the association of FAK with PI3-kinase were studied. As shown in Figs. 5A and B, marked tyrosine phosphorylation of FAK and association of p85 regulatory subunit of PI3-kinase with FAK were observed in the FAK-transfected cells. In contrast, no discernible tyrosine phosphorylation of FAK or association with p85 was observed in 397FAK-transfected cells. Similarly, enhanced serine

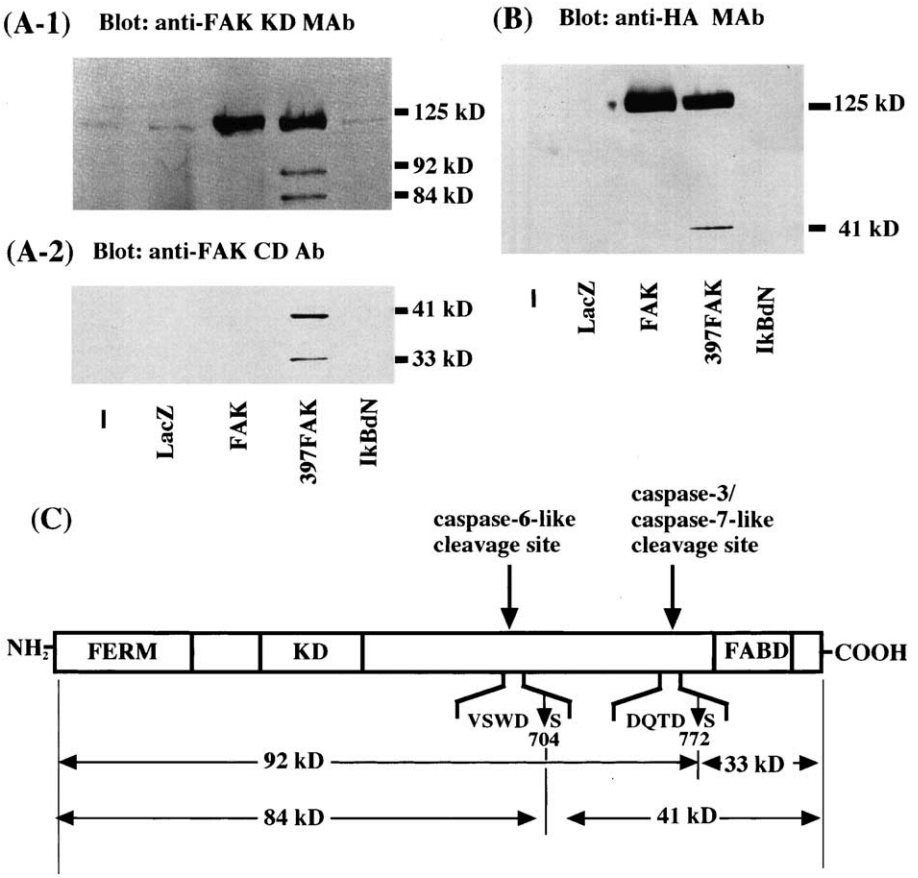

Fig. 4. Cleavage of FAK by 397FAK T98G glioma cells were infected with Adv-LacZ, FAK, 397FAK, IκBdN as described in Materials and methods. After 3 days, cell lysates were separated by SDS–PAGE followed by immunoblotting with anti-FAK KD MAb (A-1), anti-FAK CD MAb (A-2) or anti-HA MAb (B). Results are representative of three independent experiments. (C) Expected FAK fragments cleaved by caspases This figure is cited and modified from Wen et al. [15]. Abbreviations used: FERM, Band 4.1, Ezurin, Radixin, Moesin: Integrin binding domain; KD, kinase domain; FABD, focal adhesion binding domain, binds Talin, Paxillin.

phosphorylation of Akt (Fig. 5C), which was seen in the FAK-transfected cells, was not observed in the 397FAK-transfected cells.

Concomitantly, significant PI3-kinase activity observed in the LacZ-transfected or FAK-transfected cells was not detected in 397FAK-transfected cells at all (Fig. 6D), confirming that PI3-kinase is associated clearly with FAK in FAK-transfected cells, but not in 397FAK-transfected cells. These observations supported the notion that the FAK–PI3-kinase–Akt survival pathway is low but is constitutively activated and markedly enhanced by the FAK-transfection in T98G cells. In contrast, 397FAK-transfection did not induce but suppressed PI3-kinase activity and Akt phosphorylation. Thus, these results confirmed that 397FAK disrupts the FAK–PI3-kinase–Akt pathway in T98G cells.

PI3-kinase inhibitor induces apoptosis

To determine whether apoptosis in 397FAK-transfected cells might be attributable to a decrease of a PI3-kinase–Akt activity, we tested the effect of LY294002 (LY), a PI3-kinase inhibitor (Fig. 6). In the presence of LY, cell death was induced in a dose-dependent manner. Since typical DNA fragmentation and caspase-3 and -6 activation were observed in the LY-induced cell death (data not shown), this type of cell death was presumed to be due to apoptosis. In addition, LY-induced apoptosis was also associated with a marked decrease in phospho-Akt protein (Fig. 6A), resembling the 397FAK-induced apoptosis. We also detected degradation products of FAK, i.e., 92, 85, 41, and 33 kDa fragments (Fig. 6B). This degradation pattern is similar to that by 397FAK-induced apoptosis. Thus, we assumed that 397FAK caused inhibition of the PI3-kinase–Akt survival pathway, which is basically similar to the events induced by a specific PI3-kinase inhibitor.

Discussion

We demonstrated in this paper that (1) Y397F-mutated FAK (397FAK) transfection but not wild-type FAK induced significant apoptosis in T98G glioma

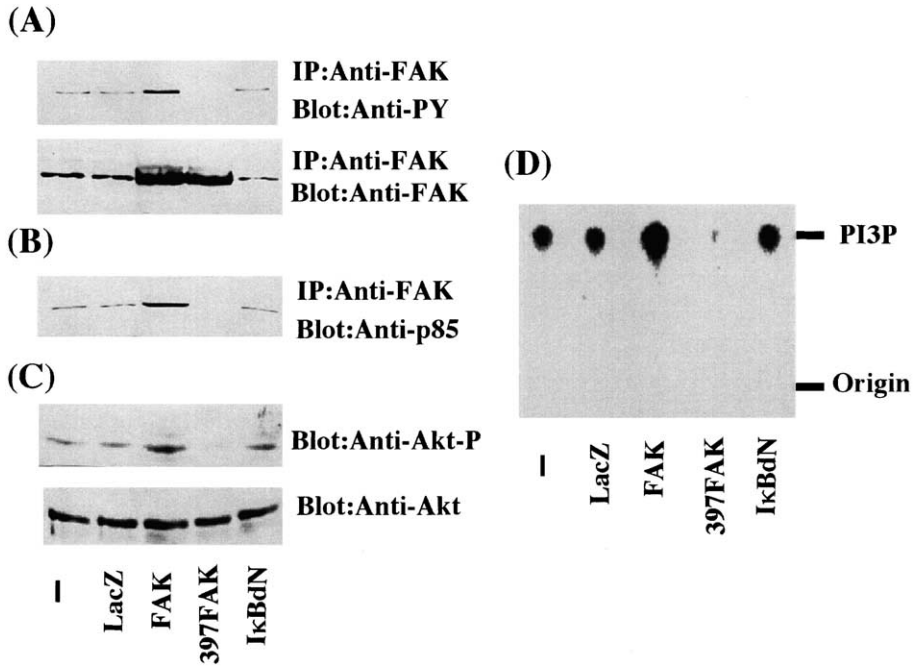

Fig. 5. (A) Detection of tyrosine-phosphorylated proteins in T98G cells. Cells were infected as described in Fig. 3 Tyrosine phosphorylation was analyzed 1 day after infection by immunoprecipitation using anti-FAK MAb followed by immunoblotting with anti-PY or anti-FAK MAb. (B) Anti-FAK immunoprecipitates were also analyzed by immunoblotting with anti-PI3-kinase (p85) Ab. (C) Lysates were prepared and analyzed by immunoblotting using anti-phospho-Akt or anti-Akt Ab. (D) Detection of PI3-kinase activity lysates were prepared and immunoprecipitated by anti-FAK mAb, and the associated PI3-kinase activity was assayed by thin-layer chromatography. Results are representative of three independent experiments. PI3P: Phosphatidylinositol 3-phosphate.

cells; (2) caspase-6 was the main mediator of this apoptosis; (3) 397FAK interrupted the FAK–PI3-kinase–Akt survival pathway, and finally; (4) LY294002, a PI3-kinase inhibitor similarly induced apoptosis by interrupting PI3-kinase–Akt survival pathway, largely mimicking 397FAK-induced apoptosis. Our finding that 397FAK transfection per se induced apoptosis in T98G cells should be of great interest. Tyr-397 of FAK is an autophosphorylation site and a high-affinity binding site for Src homology 2 (SH2) domains of Src family kinases. PI3-kinase and phospholipase Cγ (PLCγ) also interact with this site. Thus, signals through Src family kinase, PI3-kinase and/or PLCγ appear to be essential for survival, particularly emphasizing that PI3-kinase leads to the activation of survival pathway.

Akt inhibits apoptosis in multiple ways, i.e., by the phosphorylation of BAD, caspase-9, Forkhead transcription factors, IKKα, p21, ASK1, and/or cAMP-responsive element binding protein (CREB) [18]. In fact, LY294002, a PI3-kinase inhibitor, induced dose-dependent apoptosis with a marked decrease of Akt activation, also supporting a possible involvement of PI3-kinase–Akt pathway for survival of T98G cells.

FAK cleavage has been reported in other models of apoptosis, but the cleavage by 397FAK has not been documented. Thus, to our knowledge, this is the first report that 397FAK activates caspase-6 and induces cleavage of FAK. Xu et al. [19] reported that the carboxy-terminal domain of FAK (FAK-CD) induced apoptosis in human breast cancer cells accompanied with activation of caspase-3 and -8. It should be noted that in our study of 397FAK-induced apoptosis, caspase-6 was activated mainly as well. Presumably which caspase family is involved is largely dependent of which cell types are used. As reported by Wagenknecht et al. [20], inhibitor of apoptosis proteins (IAP) family proteins are widely expressed by glioma cell lines, and these anti-apoptotic proteins might be related to the striking resistance of gliomas to apoptosis induced by radiotherapy and cancer chemotherapy. IAP proteins which inhibit the process of caspase-3, -7, and -9, were expressed constitutively in T98G cells. Caspase-3, -6, and -7 are known to be executioner caspases. In T98G cells, caspase-6 may be activated instead of caspase-3 and -7 due to the IAP proteins which inhibit the activation of caspase-3 or because of low-level expression of caspase-3 and -7. It appears that cleavage of FAK and other cytoskeletal proteins is required for processing of apoptosis. While the cleaved band in the 397FAK-transfected cells is a minor portion compared with transfected 397FAK, this appears reasonable since the endogenous FAK is much less than transfected 397FAK (approximately 1/50–1/100). As described in the Result section, a 92 kDa band is exclusively derived from the endogenous

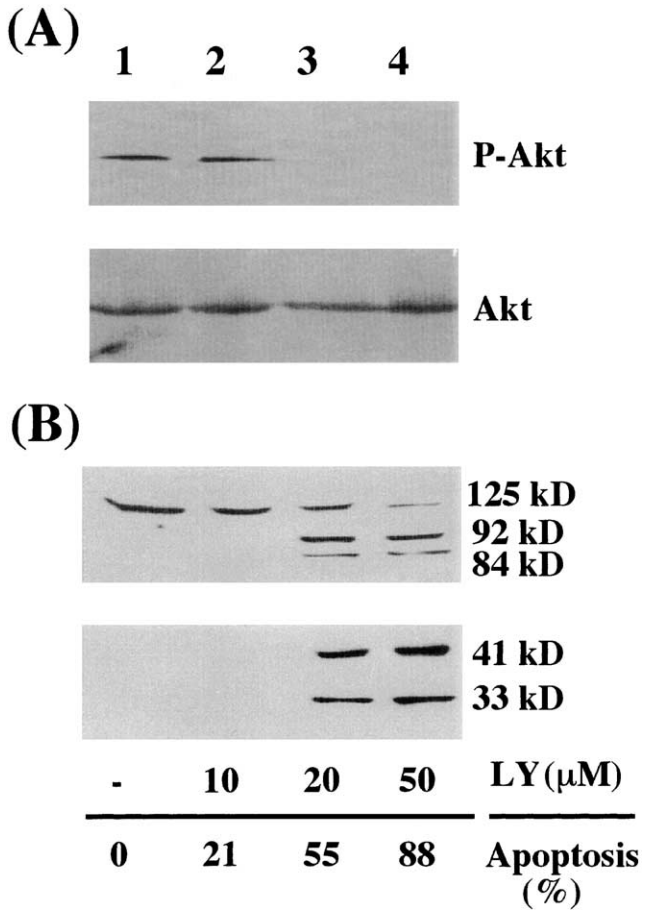

Fig. 6. Dose-dependent inhibition of Akt activation and cleavage of FAK by LY294002 treatment T98G cells were incubated with 10–50 μM LY294002 for 2 days. (A) Immunoblot analysis. The cell lysates were subjected to SDS–PAGE and blotted with phospho-Akt Ab or Akt Ab. (B) Cleavage of FAK. The cell lysates were subjected to SDS–PAGE and blotted with anti-FAK KD MAb (upper panel), anti-FAK CD MAb (lower panel).

FAK which is presumably cleaved by the 397FAK-activated caspase-3. Taken collectively, we assumed that 397FAK binds to the FAK-associated proteins such as Cas and Paxillin and, as a result, endogeneous FAK turns out to be unable to bind these proteins. Liberated FAK without associated proteins, thus, might be unable to be tyrosine-phosphorylated and the survival pathway comes down to be shut off. These associated proteins might be necessary for the activation of the FAK–PI3-kinase–Akt survival pathway.

FAK enhances NF-κB activation through an unknown mechanism that might involve the activation of the Akt–IKK pathway [21]. NF-κB is known to exert its anti-apoptotic effect by inducing the expression of anti-apoptotic genes. In this study, inhibition of NF-κB by Adv-IκBdN was insufficient to suppress massive cell death. Presumably, factors other than NF-κB, various phosphorylated proteins by Akt work well for the survival of T98G cells.

In summary, the present findings demonstrate that 397FAK interrupts the FAK–PI3-kinase–Akt survival pathway, proceeds with the activation of caspase-6 mainly and -3 slightly, subsequent cleavage of FAK, and induces apoptosis. Thus, the inhibition of the function of FAK links to the induction of apoptosis. FAK and caspase-6 might be a target for cancer therapy.

Acknowledgments

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