CD44 signaling through focal adhesion kinase and its anti-apoptotic effect

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Abstract **Adhesion molecules can initiate intracellular signaling. Engagement of CD44 either by its natural ligand hyaluronan or a specific antibody on a cell line induced tyrosine phosphorylation and activation of focal adhesion kinase (FAK), which then associated with phosphatidylinositol 3-kinase (PI3K) and activated mitogen-activated protein kinase at its downstream. However, the introduction of dominant negative Rho into the cells inhibited the CD44-stimulated FAK phosphorylation. Cells expressing CD44 were significantly resistant to etoposide-induced apoptosis. This anti-apoptotic effect was cancelled by the inhibition of either Rho, FAK or PI3K. These results may indicate a signaling pathway from CD44 to mediate the resistance against drug-induced apoptosis in cancer cells.**

Key words: CD44; Signal transduction; Focal adhesion kinase; Phosphatidylinositol 3-kinase; Mitogen-activated protein kinase; Apoptosis

1. Introduction

CD44 is a broadly distributed type I transmembrane glycoprotein receptor for the glycosaminoglycan hyaluronan (HA), a major component of extracellular matrix (ECM) [1–4]. CD44 has been recognized for its structural roles in linking ECM with the cellular actin cytoskeleton to regulate cell shape and cell migration [2–4]. At its N-terminus, CD44 has the HA-binding domain whose primary structure has significant similarity with other HA-binding proteins such as cartilage link protein [2–4]. The 72 amino acid cytoplasmic domain of CD44 has been shown to associate with actin microfilaments mediated by ezrin, radixin, and moesin family members of proteins [4,5]. In the extracellular membrane proximal region, there may be insertion of various amino acid sequences caused by alternative splicing among at least 10 variable exons. Thus, a single CD44 gene would potentially produce over 1000 isoforms. A form of CD44 without any variable exons is referred to as the standard form (CD44s; also called CD44H) and is most widely distributed. CD44 is overexpressed in many cancers including colorectal carcinomas [6], at least partly because it is one of the target genes of the Wnt pathway [6] that is up-regulated in the carcinomas due mainly to the loss of APC gene function [7]. Furthermore, there are numerous reports concerning several CD44 isoforms, especially those containing the sequence of exon v6, that plays an important role in the processes of invasion and metastasis in neoplastic cells [2–4,8–10]. In addition, one of the major CD44 isoforms, which contains the sequence from exons v8–v10, is called the epithelial form (CD44E; also referred to as CD44R1 in the literature), and it is expressed in normal epithelial cells as well as in many cancer cells [2,9].

Focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase (PTK) that has been considered to play a central role in cellular responses to ECM glycoproteins like fibronectin, which signal through integrin receptors [11,12]. However, FAK is also implicated in the responses to a number of other cellular stimuli that generate signals through either G-protein linked receptors (e.g. bombesin and thrombin), transmembrane growth factor receptors, or other, as yet unknown mechanisms [12]. For some of these stimuli like bombesin and thrombin, the events leading to increased FAK tyrosine phosphorylation have been shown to be dependent upon the activity of the small GTP binding protein p21 Rho [12–14]. Rho functions as a molecular switch for many intracellular processes by converting its inactive GDP binding form to its active GTP binding form [15]. FAK is associated with a number of signaling proteins that enable this PTK to couple to a variety of intracellular signaling pathways, extracellular signal-regulated kinase mitogen-activated protein kinase (MAPK) cascade and phosphatidylinositol 3-kinase (PI3K) pathway being but two of them [12,16,17].

In addition to its connection to cytoskeleton, the cytoplasmic domain of CD44 has been shown to associate with Src family PTKs whose activities are up-regulated by ligation of CD44 in hematopoietic cells [18–20]. However, the biological

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Abbreviations: CD44E, epithelial form of CD44; FAK, focal adhesion kinase; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; HA, hyaluronan; ECM, extracellular matrix; CD44s, standard form of CD44; mAb, monoclonal antibody; pTyr, phosphotyrosine; IMEM, Iscove's modified Eagle's medium; FBS, fetal bovine serum

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significance of this phosphorylation has remained unclear. Through our experiments using a non-hematopoietic human CD44-negative cancer cell line, we found that cells expressing CD44E bind more avidly to HA than those expressing CD44s (Nakamura, S. and Harigaya, K., unpublished result). Using these cell lines, we attempted to elucidate the molecular mechanisms of intracellular signaling from CD44.

2. Materials and methods

2.1. Reagents

An anti-CD44 monoclonal antibody (mAb), Hermes 3, was purified from conditioned medium of hybridoma HB-9480 that had been purchased from American Type Culture Collection (ATCC, Rockvill, MD, USA). An anti-CD44 mAb that can block its ligand binding (BRIC 235) [21] was purchased from Biogenesis (New Field, UK). An anti-hemagglutinin (12CA5) and anti-Myc (9E10) monoclonal antibodies were also prepared from hybridomas. Anti-phosphotyrosine (pTyr) antibody (PY20), anti-FAK antibody (sc-558), anti-Tiam1 antibody (sc-872) and anti-PI3K p85 (sc-423) were purchased from Santa Cruz (Santa Cruz, CA, USA). Anti-phosphoMAPK was from New England Biolabs (Beverly, MA, USA). HA preparations of various molecular weights were generously provided by Seikagaku Co. (Tokyo, Japan). Protein contents of these preparations were under the detection limit as judged by UV absorption at 280 nm. Bovine serum albumin (BSA) (Fraction V), LY294002 and PD98059 were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture

A human lung small cell cancer cell line, NCI-H82 (ATCC HTB-175), was cultured in Iscove's modified Eagle's medium (IMEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS). Various transfectants of NCI-H82 were grown in the same medium containing appropriate selection agents to stabilize the transgenes.

2.3. DNA construction and transfection to cells

The NCI-H82 cells were stably transfected with full-length human CD44E cDNA [22] cloned into pCIneo mammalian expression vector (Promega, Madison, WI, USA) by lipofection procedure with DMRIE-C reagent (Gibco BRL). As a control, pCIneo was transfected to the cells. Stable transfectants were selected in the growth medium containing 1.0 mg/ml of G418 (Gibco BRL). The transfected cells were cloned by limiting dilution. The resultant clones were termed 82E and 82pCI, respectively.

The cDNA of mutant FAK is known to have two mutations (Tyr397Phe, the binding site for Src and PI3K p85, and Lys454Arg, the ATP-binding site in the kinase domain) and three hemagglutinin epitopes at its N-terminus [23]. The cDNA of RhoA dominant negative mutant (Thr19Asn) with a Myc-epitope tag was as described [24]. These cDNAs were cloned into pMx retrovirus vector [25]. Recombinant retroviruses were produced by co-transfecting various pMx vectors and amphotropic helper virus DNA [26] into 293T cells. 82E cells and their derivatives were infected with the recombinant retroviruses by culturing in conditioned media in the presence of polybrene (Sigma). For expression of the mutant FAK and the dominant negative mutant of RhoA, retroviral introduction was performed three times to increase the fraction of the cells expressing the proteins. Empty vector control was also introduced three times. The transfectants were designated as 82E-mutFAK, 82E-dnRhoA and 82E-pMX, respectively.

2.4. Cell adhesion assay

Cells (1×10^5 cells/well) were incubated for 30 min at 37°C in a 96-well plate (Sumitomo Bakelite, Tokyo, Japan) which had been coated with HA (32 kDa; 10 mg/ml) together with BSA (100 mg/ml) as an anchor for the immobilization. Wells coated with BSA alone were used as controls. Following the incubation, cells that had not been adhered were washed away with phosphate-buffered saline. Proportion of the adhered cells to the total cells incubated was estimated with the MTT assay as described [27,28].

For assessing the dependency of the adhesion on CD44, cells were preincubated with concentrations of an anti-CD44 mAb (BRIC 235) or an isotype-matched control IgG (IgG2b) (Immunotech, Marseille, France) for 30 min at 4°C before the adhesion assay.

2.5. Cell stimulation

Cells were starved in IMEM containing 0.5% FBS for 24 h before stimulation. The cells (5×10^5 cells/ml) were then cultured in IMEM (serum-free) containing 10 μg/ml of HA or 10 μg/ml of anti-CD44 mAb (Hermes 3) at 37°C for the indicated periods.

2.6. Immunoprecipitation and Western blotting

Cells were lysed by suspension in an ice-cold buffer (20 mM HEPES–NaOH (pH 7.9), 0.5% Nonidet P-40, 15% glycerol, 300 mM NaCl, 1 mM EDTA, 10 mM NaF, 1 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μg/ml aprotinin, 1 mM dithiothreitol and 0.5 mM phenylmethylsulfonyl fluoride) for 30 min [28,29]. The supernatants were saved as total cell lysates following centrifugation. The lysates from each cell were immunoprecipitated by incubation with the anti-FAK or the anti-Tiam1 antibody for 2 h on ice. Immune complexes were collected on protein G-Sepharose (Pharmacia, Uppsala, Sweden), washed five times in the lysis buffer, and proteins were eluted with sample buffer (62.5mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.02% bromophenol blue) for SDS–polyacrylamide gel electrophoresis (PAGE). The proteins were resolved on SDS–PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA). Primary antibodies reacted to the antigens on the membranes were detected using the appropriate horseradish peroxidase-conjugated secondary antibodies (Pharmacia or Santa Cruz) and the Super Signal chemiluminescence detection system (Pierce, Rockford, IL, USA) as suggested by the manufacturer.

2.7. Kinase assay

PTK assay for FAK was performed with a non-radioactive enzyme-linked immunosorbent assay kit (Universal Tyrosine Kinase Assay Kit, Takara Shuzou Co., Shiga, Japan) according to the manufacturer's instructions. Cell lysates equivalent to 250 μg of protein were immunoprecipitated with the anti-FAK antibody. After washing, the immunoprecipitates were incubated with ATP in poly(Glu-Tyr)-coated 96-well plates. After washing, pTyr was detected with peroxidase-conjugated anti-pTyr (PY20) antibody and TMBZ chromogenic substrates.

2.8. DNA fragmentation and cell viability assays

Cells (4×10^5 cells/ml) were preincubated for 1 h with 10 μM LY294002 (Sigma) or 50 μM PD98059 (Sigma) in IMEM with 0.5% FBS on 96-well plates. Then, etoposide (10 μg/ml) was included in the culture for 24 h. Cell viability was determined by the MTT assay [27,28]. For DNA fragmentation assay, cells (1.5×10^6) were lysed in a lysis buffer (10 mM Tris–HCl (pH 7.4), 10 mM EDTA, 0.5% Triton X-100), and then treated sequentially with RNase (20 μg/ml) and proteinase K (20 μg/ml). Lysates were electrophoresed on 2% agarose gel with visualization by 0.1% ethidium bromide.

3. Results

To investigate the functions of CD44, an expression vector for CD44E, or its empty counterpart, was transfected into a CD44-negative cancer cell line to establish stable transformants. We isolated several clones expressing CD44E (82E) (Fig. 1A). 82pCI clones, which had been transfected with the empty vector, expressed no detectable CD44 protein (Fig. 1A). As shown in Fig. 1B, 82E clones exhibited robust adhesion to the culture wells coated with HA, while 82pCI clones showed little adhesion to the wells. Adhesion to BSA-coated wells was essentially invariant among the 82E and 82pCI clones (Fig. 1B). The HA-mediated adhesion of the 82E clone was blocked by pre-incubating the cells with a well-characterized blocking antibody for CD44 (BRIC 235) [21] in a dose-dependent manner (Fig. 1C). This antibody also inhibited the adhesion of other 82E clones to a similar extent with the clone at the concentration of 10 μg/ml (data not shown). An isotype-

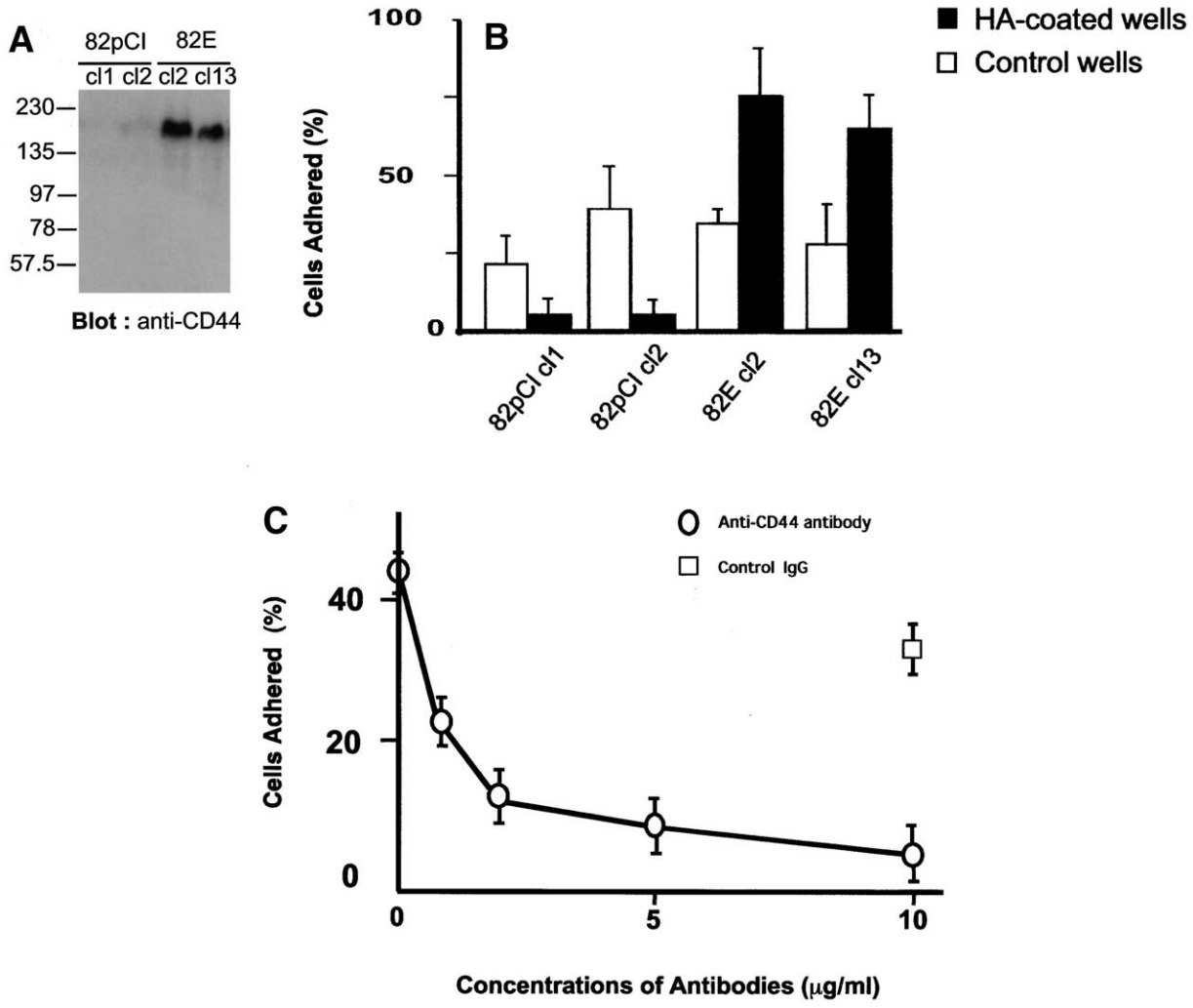

Fig. 1. Establishment of human cancer cell lines with or without CD44 expression. A: Expression of CD44 in NCI-H82 cells. Cell lysates from 82pCI and 82E cells were immunoblotted with the anti-CD44 mAb (Hermes 3). B: HA-mediated adhesion of 82E cells. Vertical axis represents the percentage of adhered cells among the cells seeded. Error bars indicate standard deviations ($n=6$). C: Effect of an anti-CD44 blocking mAb on the HA-mediated adhesion of the 82E cl13 cells. Vertical axis represents percentage of adhered cells. Error bars indicate standard deviations ($n=3$). Open circles represent groups treated with the anti-CD44 mAb and an open square indicates a group treated with isotype-matched control IgG2b. The results given by other clones were essentially invariable (data not shown).

matched antibody did not inhibit the adhesion of the clones at this concentration (Fig. 1C; data not shown). The adhesion was also significantly inhibited by adding soluble HA in the medium (250 μg/ml; data not shown). These results indicate that CD44 expressed in the cell line can reconstitute HA-mediated cell adhesion, one of the major functions of CD44 molecule [2–4]. Thus, 82E cells would be a valid model system to assess physiological functions of CD44.

Upon stimulation by HA, we observed tyrosine phosphorylation of several cellular proteins in 82E clones but not in 82pCI clones (Fig. 2A). The phosphorylated proteins included those with approximate molecular weights of 130, 125, 100, 80 and 65 kDa (Fig. 2A). For further studies, we employed one from each group (clone1 from 82pCI and clone13 from 82E) as representatives.

HA exists as a high molecular weight polymer (usually over 1000 kDa) in its native state [30–32]. However, under conditions such as tissue injury, low molecular weight HA accumulates [31–37]. As shown in Fig. 2B, although HA of all molecular weights (including that with high molecular weight) induced tyrosine phosphorylation, HA with a low molecular weight was the strongest inducer of the response. For subsequent studies, we utilized the low molecular weight HA (32 kDa) that evoked the strongest reaction.

CD44 has no recognizable tyrosine kinase domain in its intracellular domain [4]. To identify the kinase(s) involved in the tyrosine phosphorylation of 82E, the lysates of the cells stimulated with the low molecular weight HA were immunoprecipitated with an anti-FAK antibody and immunoblotted with an anti-pTyr mAb. As shown in Fig. 2C, FAK with a molecular weight of 125 kDa was tyrosine-phosphorylated in 82E cells but not in 82pCI cells at their non-stimulated state. Ligand stimulation strongly increased the phosphorylation of FAK in 82E, but not in 82pCI. Furthermore, ligation of CD44 with a mAb directed against the extracellular domain of CD44 (Hermes 3) also increased the tyrosine phosphorylation of FAK (Fig. 2C). To examine whether this phosphorylation reflects the activation of FAK, the kinase activity of the anti-FAK immunoprecipitates was assayed with poly(Glu-Tyr) as a substrate [38]. Fig. 2D shows that the CD44 ligation

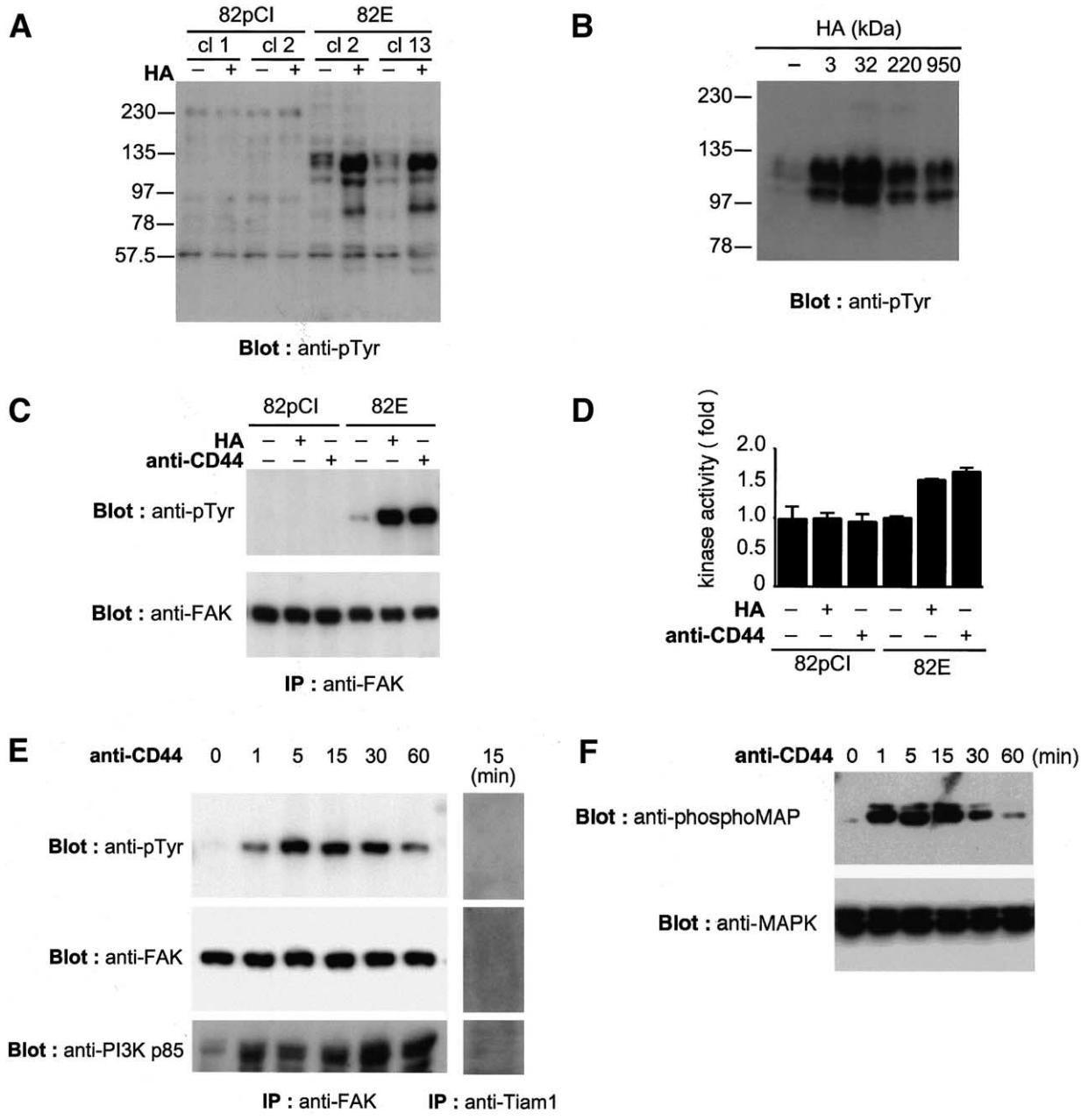

Fig. 2. CD44 ligation-induced activation of FAK and its downstream molecules. A: CD44-dependent tyrosine phosphorylation of cellular proteins activated by HA. 82pCI and 82E cells were stimulated with HA (molecular weight = 32 kDa) for 5 min or left untreated. Lysates were immunoblotted with the anti-pTyr mAb (PY20). B: Strength of tyrosine phosphorylation induced by HA is dependent on its molecular weight. HA of various molecular weights was used as stimulant in the same assay as A. C: CD44E ligation-induced tyrosine phosphorylation of FAK. 82pCI or 82E cells were stimulated with HA (molecular weight = 32 kDa) or the anti-CD44 mAb for 5 min. Lysates were immunoprecipitated with anti-FAK antibody followed by immunoblotting with anti-pTyr. The filter membrane was re-blotted with anti-FAK to demonstrate equal protein loading. D: CD44 ligation-induced activation of FAK. The anti-FAK immunoprecipitates were subjected to a PTK assay. Results are shown as mean ± S.D. ($n = 3$). E: Kinetics of tyrosine phosphorylation of FAK and its association with PI3K p85 after ligation of CD44. 82E was stimulated with anti-CD44 mAb for various periods. Lysates were immunoprecipitated with anti-FAK and blotted sequentially with anti-pTyr, anti-PI3K p85 and anti-FAK. As a control for co-immunoprecipitation, the lysate from the cells stimulated for 15 min was immunoprecipitated with the anti-Tiam1 antibody. F: CD44E ligation-induced activation of MAPK. Lysates analyzed in E were also immunoblotted with anti-phosphoMAPK antibody, followed by anti-MAPK.

by either HA or the antibody up-regulated the FAK-associated PTK activity by about 1.5- to 1.7-fold in 82E as compared with the non-stimulated cells or 82pCI control cells.

After the ligation of CD44 by the antibody, tyrosine phosphorylation of FAK could be observed as early as 1 min later, peaking at 5 min and subsequently decreasing (Fig. 2E). The time-course of tyrosine phosphorylation after the stimulation with HA was similar to that with the antibody (data not shown). Tyrosine kinases may initiate downstream signaling reactions including kinase cascades [39]. A major autophosphorylation site of FAK, Tyr-397 [40], has been shown to be the binding site for the p85 subunit of PI3K [23]. As shown in

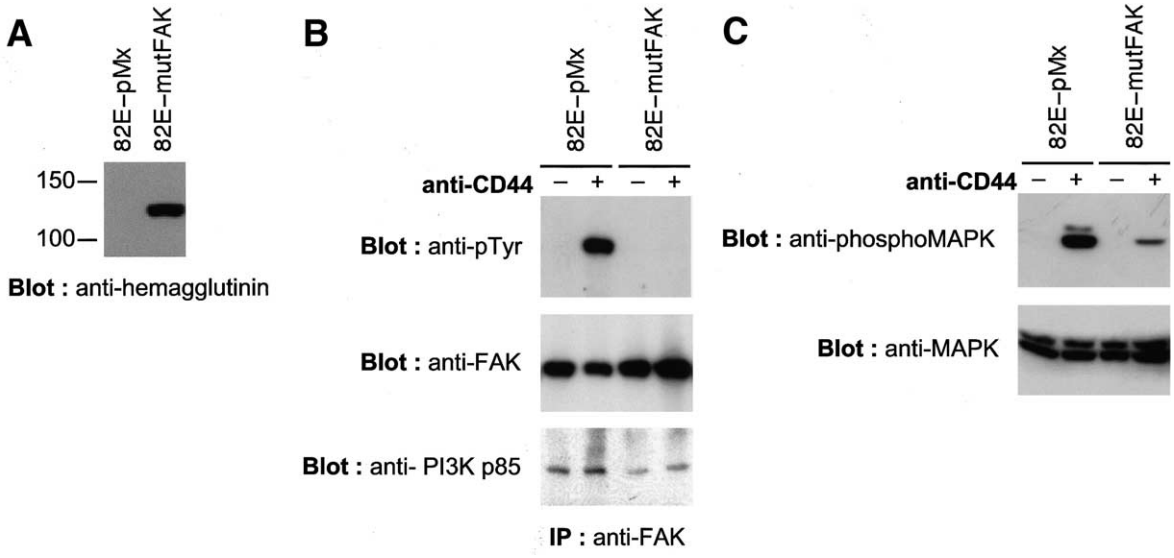

Fig. 3. Expression of mutant FAK inhibited CD44 ligation-induced FAK–PI3K association and MAPK activation. A: Expression of mutant FAK in 82E cells. Cell lysates from 82E-pMx and 82E-mutFAK cells were immunoblotted with anti-hemagglutinin-tag mAb. B: Expression of mutant FAK inhibited CD44 ligation-induced tyrosine phosphorylation of FAK and its association with PI3K p85. The cells were stimulated with anti-CD44 mAb for 5 min. Anti-FAK immunoprecipitates were immunoblotted with anti-pTyr, anti-PI3K p85 and anti-FAK. C: Expression of mutant FAK inhibited CD44 ligation-induced activation of MAPK. Lysates analyzed in B were immunoblotted with anti-phospho-MAPK or anti-MAPK.

Fig. 2E, the immunoprecipitates of the anti-FAK antibody from the antibody-stimulated 82E cells contained PI3K p85. Fig. 2E also shows that an unrelated antibody (anti-Tiam1 antibody) does not co-precipitate PI3K p85, indicating the association of PI3K complex with the tyrosine-phosphorylated FAK. This association began 1 min after the ligation and thereafter was sustained for at least 60 min. Another well-documented downstream target molecule of FAK is MAPK [16,28]. As shown in Fig. 2F, both Erk-1 and Erk-2 were activated by the CD44 ligation. This activation also began 1 min after the ligation, remained at a high level for 30 min, and then decreased by 60 min (Fig. 2F).

In order to investigate whether these biochemical reactions resulted from the activation of FAK, we introduced a mutant FAK into 82E cells by a retrovirus-mediated gene transfer system (Fig. 3A). In these cells (82E-mutFAK), CD44 ligation-induced tyrosine phosphorylation of FAK was almost completely inhibited (Fig. 3B). As shown in Fig. 3B,C, the augmentation of FAK–PI3K association and MAPK activation by CD44 ligation were strongly inhibited in the cells expressing the mutant FAK, while these effects were still evident in mock-infected control cells. These results indicate that FAK mediates outside–in CD44 signaling to activate PI3K and MAPK.

Clarifying the functional link between CD44 and FAK is of major interest. In repeated experiments, however, we could not detect any physical association of FAK with CD44. There is evidence that FAK is one of the targets of Rho [12–14]. Furthermore, we obtained results to indicate that CD44 ligation induces activation of Rho (Kumagai, S., Kitagawa, M., and Harigaya, K., unpublished result). When 82E cells were treated with C3 exotoxin, a specific inhibitor of Rho, CD44 ligation-induced FAK activation was inhibited (data not shown), indicating the involvement of Rho in this process. Retrovirus-mediated transfection of the dominant negative form of RhoA into 82E cells (82E-dnRhoA) (Fig. 4A) almost completely inhibited the tyrosine phosphorylation of FAK in response to CD44 ligation (Fig. 4B). These results indicate that Rho mediates the CD44 ligation-induced FAK activation in this system.

There are some indications that CD44 ligation renders cells resistant to apoptosis [41,42]. Furthermore, targeted deletions of exon 7, or exons 6 and 7, of the CD44 gene in mice results in increased apoptosis of infiltrating mononuclear cells in models of enterocolitis [43]. Consistent with these reports, 82E cells showed a marked resistance against induction of cell death by the anti-cancer drug etoposide (VP16) compared

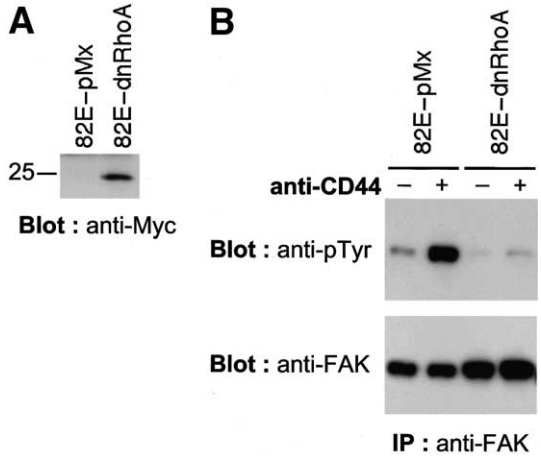

Fig. 4. Expression of a dominant negative form of Rho inhibited CD44 ligation-induced tyrosine phosphorylation of FAK. A: Expression of dominant negative RhoA mutant. Cell lysates of 82E-pMx and 82E-dnRhoA cells were immunoblotted with anti-Myc-tag antibody. B: Expression of dominant negative RhoA mutant inhibited CD44 ligation-induced tyrosine phosphorylation of FAK. The cells were stimulated with anti-CD44 mAb for 5 min. Anti-FAK immunoprecipitates were immunoblotted with anti-pTyr and anti-FAK.

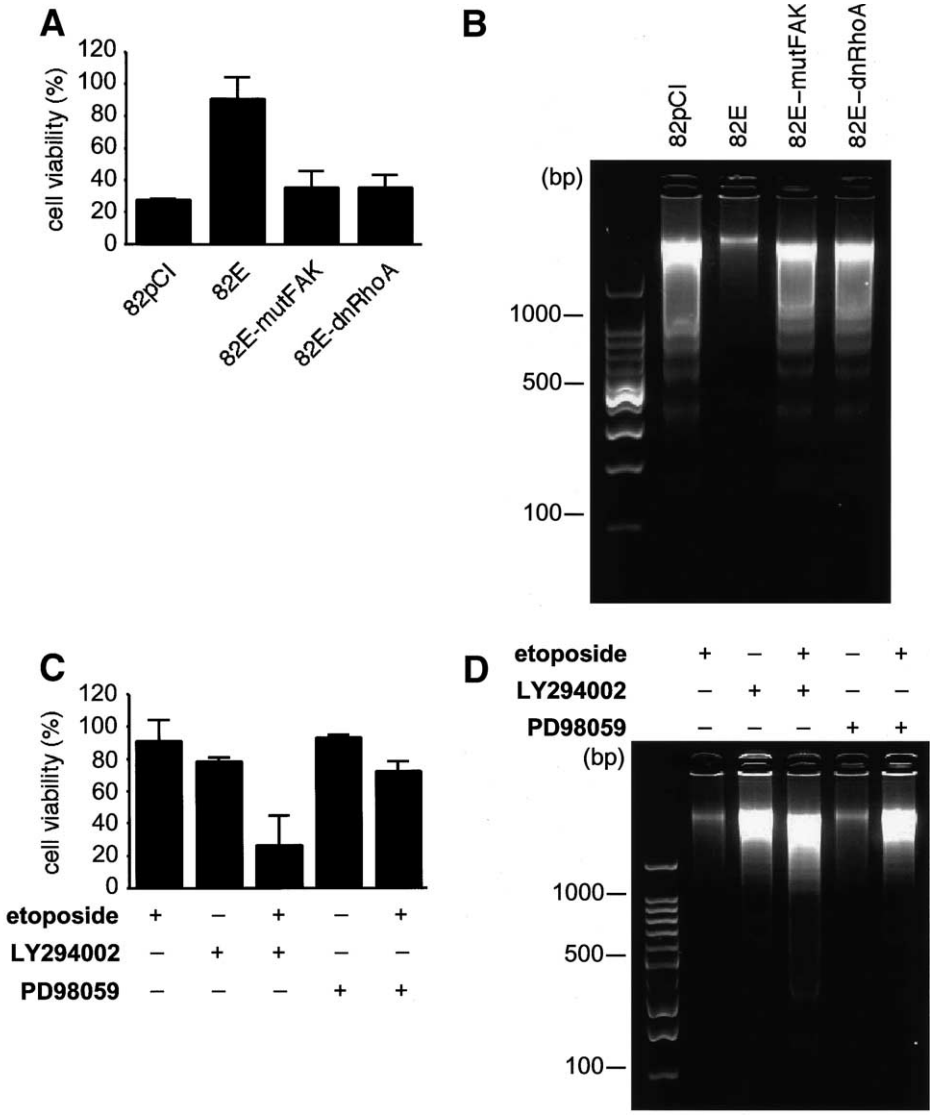

Fig. 5. Resistance to apoptosis mediated by CD44/Rho/FAK pathway. A: Resistance to etoposide-induced cell death caused by CD44 expression and its inhibition by FAK or Rho mutants. 82pCI, 82E, 82E-mutFAK and 82E-DNRhoA cells were treated with etoposide and cell viability was determined by MTT assay. Results are shown as means ± S.D. ($n = 3$). B: Resistance to etoposide-induced DNA fragmentation caused by CD44 expression and its inhibition by FAK or Rho mutants. Cells analyzed in A were also analyzed for DNA fragmentation. C: A specific inhibitor of PI3K but not an inhibitor of MAPK inhibited CD44-induced resistance to apoptosis. 82E cells were treated with etoposide after LY294002 or PD98059 pretreatment. Cell viability was measured by MTT assay. Results are shown as means ± S.D. ($n = 3$). D: A specific inhibitor of PI3K but not an inhibitor of MAPK inhibited CD44-induced resistance to DNA fragmentation. Cells analyzed in C were also analyzed for DNA fragmentation.

to 82pCI cells (Fig. 5A). 90% of 82E cells were viable after treatment with etoposide for 24 h, whereas 82pCI showed no more than 30% viability (Fig. 5A). Notably, the viability of both 82E-mutFAK and 82E-dnRhoA cells was 35% or less, a similar level to that of 82pCI (Fig. 5A). In 82pCI, 82E-dnRhoA and 82E-mutFAK cells, the etoposide treatment induced DNA fragmentation, an indication of apoptosis (Fig. 5B). These results suggest that the CD44/Rho/FAK pathway transduces an anti-apoptotic signal(s).

Both of the downstream kinases of FAK in this system, PI3K and MAPK, were reported to have anti-apoptotic effect [44]. However, the inhibition of cell viability loss and DNA fragmentation by etoposide was markedly reversed by treatment with a specific PI3K inhibitor, LY294002, but not with a specific MEK inhibitor, PD98059 (Fig. 5C,D). Additionally, LY294002 treatment also reduced the survival of NCI-H82 cells expressing CD44s to the levels of the control 82pCI cells in etoposide-containing cultures (data not shown). These results indicate that PI3K, but not MAPK, is essential for the anti-apoptotic signal downstream of FAK. In this study, the CD44-dependent anti-apoptotic phenotype was conferred to the cells without the addition of HA (Fig. 5). This suggests that the HA present in the serum-containing culture medium [30,45] and/or produced by the cells themselves would be sufficient for the weak but still significant FAK phosphorylation (Fig. 2C) and the anti-apoptotic effect.

4. Discussion

There have been a number of reports concerning CD44 ligation-induced cellular responses. They include enhancement of cell motility [46,47], expression of adhesion molecules

[48,49] and a growth factor receptor [49], cytokine and chemokine secretion [31,50–52], and protection from apoptosis [41–43]. At the biochemical level, CD44 ligation has been shown to activate Rac1 [53–55] and nuclear factor-κB [36]. It has also been reported that ligation of CD44 activates Src family PTKs associated with the cytoplasmic domain of CD44 [18–20], although none of the cellular responses has been linked to the activation of PTKs. We have demonstrated a novel functional association between FAK and CD44, which leads to a CD44-mediated anti-apoptotic effect. In 10 other non-transfected CD44-positive cell lines examined, FAK was heavily tyrosine-phosphorylated without any stimulation and the blocking antibodies against CD44 did not reduce the level of the phosphorylation (data not shown). These results suggest that the activation of FAK by the ligand stimulation of CD44 may take place under the limited situation of the cells. It could be cell lineage specific or an alteration concomitant with neoplastic transformation.

Stimulation of another HA receptor, RHAMM, has also been demonstrated to activate FAK and MAPK [56,57]. However, the activation of FAK and MAPKs shown in this report is clearly dependent on CD44, as CD44-negative control cells did not respond to HA stimulation, and the specific antibody against CD44 mimicked the natural ligand in its effect.

Activation of FAK by ligation of an adhesion molecule is reminiscent of integrin signaling [11,12]. As opposed to integrin signaling [12], however, the CD44-induced FAK activation is dependent on Rho. Thus, it is possible that integrins are not involved in this signaling from CD44 to FAK.

In addition to providing structural tethering points for cells, some adhesion molecules transduce signals that result in a broad range of cellular processes. We presented a molecular mechanism for CD44 signaling that directs cell survival against exposure to a cytotoxic agent. This study indicates that FAK would be a mediator of outside–in CD44 signaling under a limited cellular environment.

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