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Cell adhesion aside from integrin system can abrogate anoikis in rat liver cells by down-regulation of FasL expression, not by activation of PI-3K/Akt and ERK signaling pathway☆

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Abstract

Epithelial cells require contact with extracellular matrix (ECM) to inhibit detachment-induced apoptosis (anoikis). The ERK and PI-3K/Akt signaling pathways have been identified to inhibit anoikis. We present here a different story. An adult rat liver cell line, ARLJ301-3, underwent apoptosis within 4 h under suspension conditions even with active forms of Akt and ERK1/2. Once ARLJ301-3 cells are plated on tissue culture plates coated with synthetic polymer, such as poly-(*N*-*p*-vinyl benzyl-*O*-β-D-galacto-pyranosyl-D-gluconamide) (PVLA), poly-L-lysine or polystyrene, instead of functional ECM such as fibronectin, they could survive and proliferate without activation of Akt and ERK1/2. The expression of Fas receptor ligand (FasL) are specifically detected in cells under suspension conditions or treated with cytochalasin-D. We present here the first report that FasL expression is up-regulated by the cytoskeletal disruption directed by cytochalasin-D treatment or cell detachment from ECM.

Keywords: Anoikis; Apoptosis; ERK; PI-3K/Akt; Synthetic polymer; FasL; Fas

Anchorage-independent growth is a hallmark of malignant transformation [1,2]. Epithelial cells and fibroblasts undergo apoptosis (anoikis) and G1 cell cycle arrest, respectively, when deprived of contact with matrix (ECM) [3–5]. It has been reported that extracellular factors such as insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) or genetically mutated ras can rescue epithelial cells from anoikis via the PI-3K/Akt signaling pathway [6–8,15–18]. Under adherent conditions, cell-to-matrix contact itself mediates survival signals by the binding of cell surface integrin receptor with fibronectin or laminin, which has been confirmed to activate the PI3-K/Akt signaling pathway by phosphorylation of focal adhesion kinase (FAK) [9–15]. The specific integrin type -α5β1- suppressed anoikis by upregulation of bcl-2 via fibronectin binding and other types of integrin receptors such as -αvβ3- and -α1β1-might give survival signals through the phosphorylation of the tyrosine residue of the adaptor protein Shc followed by activation of the mitogen-activated protein kinase (MAPK) [14,19]. Taken together, cell matrix contact via integrin/fibronectin binding is apparently a prerequisite factor for epithelial cell survival, leading to activation of PI-3K/Akt and ERK pathways.

However, we think these results were obtained from only limited cell types and present here a different story in that an adult rat liver cell line, ARLJ301-3, underwent anoikis even though the Akt and ERK pathways were fully activated. Furthermore, we identified that FasL was specifically expressed in cells under anchorage-deprived

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conditions or treated with cytochalasin-D which induced apoptosis in ARLJ301-3 as well. The cells could survive when in contact with plates coated with different kinds of synthetic polymers, PVLA, poly-L-lysine and polystyrene, on which no integrin/fibronectin signaling pathway was activated. These cells grown on synthetic polymer showed no difference in Akt and ERK activation profile compared with the functional cell/ECM systems. From these observations, we propose here that any type of cell-to-matrix contact can protect cells from anoikis not by PI-3K/Akt or ERK activation but down-regulation of FasL expression and in addition this FasL expression is stimulated by cytoskeletal disruption.

Materials and methods

Cell culture. The rat liver cell line, ARLJ301-3 [20], was obtained from the Riken Cell Bank (Tsukuba, Japan) and maintained in William's medium E containing 10% FCS on regular tissue culture plates (Corning, NY, USA). We think this cell line is an immortalized cholangiocyte, but not hepatocyte, because we observed little expression level of asialo-glycoprotein-receptor (ASGPR) and α-fetoprotein (data not shown) and positive staining with anti-cytokeratin antibody in ARLJ301-3. To generate an adherent condition, cells were cultured on polystyrene plates or plates coated with fibronectin (Becton–Dickinson Labware, Franklin Lakes, NJ, USA), collagen type I (Funakoshi, Tokyo, Japan) or type IV (Becton–Dickinson Labware), poly-(*N*-*p*-vinyl benzyl-*O*-β-D-galactopyranosyl-D-gluconamide) (PVLA) or poly-L-lysine (Becton–Dickinson Labware). PVLA-coated plates were prepared as previously described [20–22]. Briefly, PVLA was dissolved in PBS at a concentration of 100 mg/ml at room temperature, sonicated for 8 min, and then used to coat the plates. To obtain a culture condition for suspension, cells were allowed to stand without rocking and grown on 10 mg/ml poly-HEMA (Sigma Aldrich, St. Louis, MO, USA) coated polystyrene plates (Becton–Dickinson Labware) or 1% BSA-coated plates. Poly-HEMA was dissolved in 100% ethanol at 10 mg/ml and added onto polystyrene plates to cover the surface of the plate. After casting away the ethanol, the surface was rinsed with PBS three times and the plate was used for the experiments [23]. On polystyrene plates, half of the cells attached onto the plate surface and others did not attach and continuously floated. We collected cells separately as an adherent fraction (polystyrene (adh) in Fig. 2) and a floating fraction (polystyrene (sus) in Fig. 2). When cells were treated with PD98059 or LY294002, they were washed with PBS, incubated in serum free medium containing 50 μM of PD98059 (New England Biolabs, Beverly, MA, USA) or LY294002 (Sigma Aldrich) for 30 min at 4 °C with rocking, and then transferred to plates that provided either adherent or suspension conditions supplemented with 10% FCS at 37 °C. Trypan blue exclusion assay was used to count the number of dying or surviving cells.

Apoptosis assay. For DNA laddering, total genomic DNA was extracted from cells attached to plates or cultivated under suspension conditions using the Quick apoptotic DNA ladder detection kit (MBL, Nagoya, Japan) according to the manufacturer's instructions and analyzed on 2% agarose gel electrophoresis. DNA content was analyzed by flow cytometric analyzer (FACScan) after nuclear staining with propidium iodide (PI) [24]. The sub-G1 population was counted as apoptotic cells.

Immunoblot analysis. Cells were lysed on ice for 30 min with ELB solution (250 mM NaCl, 0.5% NP40, 1 mM DTT, 20 mM Hepes, pH 7.4, containing 1 mg/ml aprotinin (Sigma Aldrich), 1 mg/ml leupeptin (Sigma Aldrich), 50 mg/ml PMSF (Sigma Aldrich), 0.1 mM $NaVO_4$, 0.5 mM NaP_2O_7, and 5.0 mM NaF) and cell lysates were collected as a supernatant after centrifugation at 15 krpm for 15 min and separated on SDS–PAGE. After being transferred to a nitrocellulose membrane, proteins were reacted with antibodies and detected with ECL (Amersham Biosciences, Piscataway, NJ, USA). The specific antibodies used for this experiment were: anti-caspase-9 (MBL 54-3), anti-phospho-Akt (Cell Signaling Technology, Beverly, MA, USA #9274), anti-Akt (Cell Signaling Technology #9272), anti-phospho-ERK (Cell Signaling Technology, #9101), anti-ERK (Santa Cruz Biotechnology, SC94-6), and anti-FasL (Transduction Laboratories, Lexington, KY, USA).

FasL expression by RT-PCR. Total RNA was extracted from cells using Trizol solution according to the manufacturer's instruction (Invitrogen). One μg total RNA was used as a first-strand reaction that included oligo(dT) primer and reverse transcriptase (Invitrogen). These reverse-transcribed products were amplified with the following primers, corresponding to the rat FasL cDNA, 5′-TGCCTCCACTAAGC CCTCTA-3′ and 5′-CCTCCATTAGCACCAGATCC-3′ by Ex Taq polymerase (Takara Biomedicals, Otsu, Japan). The PCR products were analyzed on 2% gel electrophoresis.

Results

Rat liver cell line underwent anoikis under suspension conditions

The rat liver cell line, ARLJ301-3, proliferated under adhesion conditions on regular plates, however, decreased after incubation under suspension conditions even with supplementation of 10% FCS over poly-HEMA-coated plates (Fig. 1A). On the other hand, the human hepatocellular carcinoma cell line, HuH7, survived and increased at the same doubling time when kept in suspension as when grown under adherent conditions on regular plates (Fig. 1A). We confirmed that death of ARLJ301-3 cells was due to an apoptotic process by showing a DNA ladder formation on gel (Fig. 1B) and an increase of a sub-G1 population of cells by FACScan after staining nucleus with PI at different incubation times (Fig. 1C).

Synthetic polymer rescued cells from anoikis

The fact that the rat liver cell line ARLJ301-3 undergoes anoikis soon after being kept under suspension conditions prompted us to determine what type of cell surface-to-matrix contacts can efficiently abrogate anoikis in rat liver cells. We adopted plates coated with collagen type I, collagen type IV or fibronectin as physiological adhesion model systems, which mimicked regular plates, and plates coated with PVLA, poly-L-lysine or polystyrene as synthetic adhesion substrates. Tissue culture plates coated with synthetic polymer, PVLA, efficiently adhere primary hepatocytes through binding to asialo-glycoprotein-receptor (ASGPR) on the cell surface and this adhesion stimulates [^{3}H]thymidine incorporation into the cell [21]. More than 70% of ARLJ301-3 cells could bind with PVLA-coated plates and survive. We analyzed the sub-G1 population of

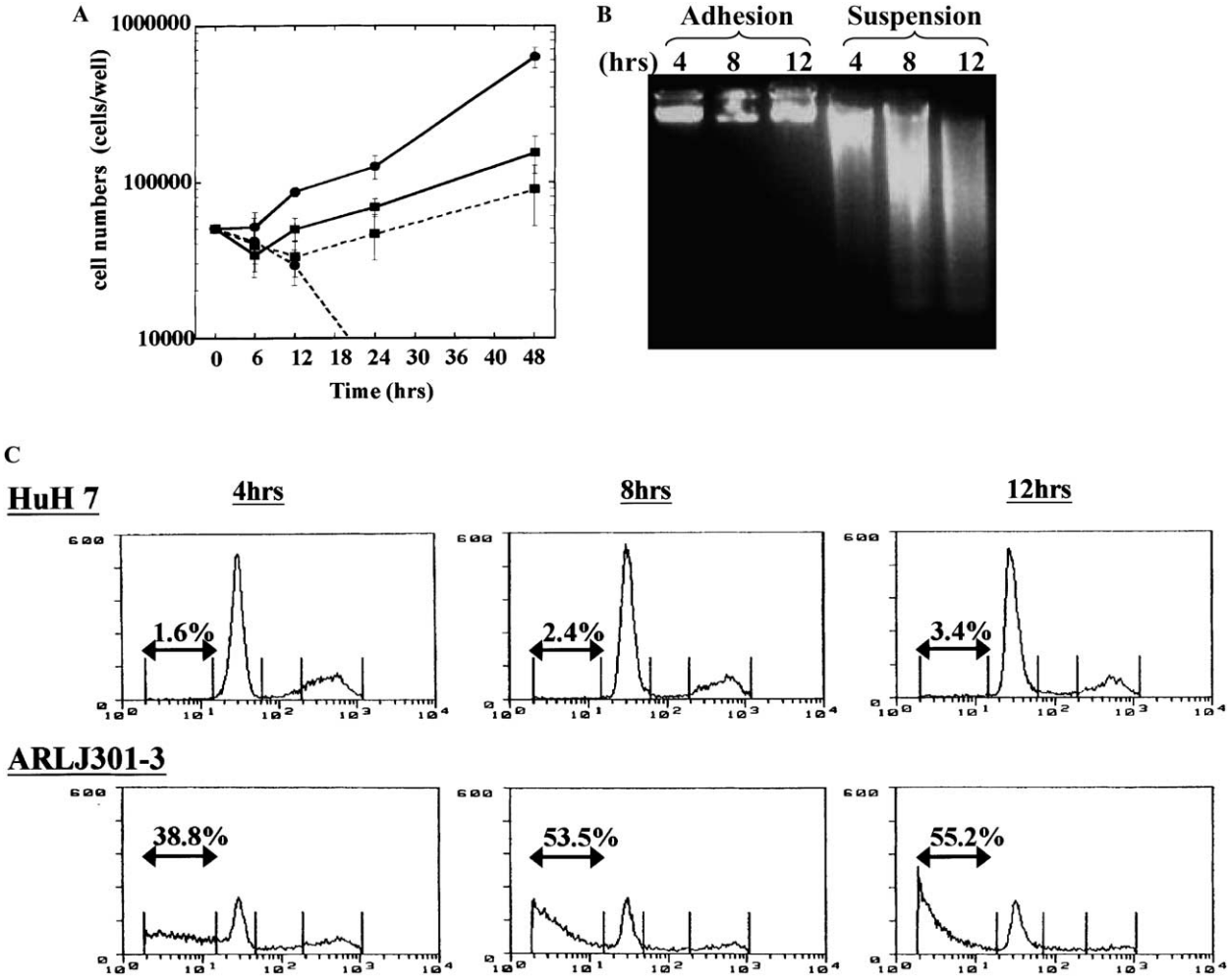

Fig. 1. ARLJ301-3 underwent anoikis under an anchorage-deprived condition. (A) Number of ARLJ301-3 cells and HuH7 cells on regular plates (ARLJ301-3 ●——●, HuH7 ■——■) and poly-HEMA-coated plate (ARLJ301-3 ●------●, Huh7 ■------■) at indicated time. Cells were counted with the trypan blue exclusion assay. (B) DNA ladder formation of ARLJ301-3 on regular plate (adhesion) and poly-HEMA-coated plate (suspension). (C) FACS profile of HuH7 and ARLJ301-3 in suspension. DNA content was analyzed by FACScan after cell nuclei were stained with propidium iodide.

cellular DNA after an 8-h incubation under various adhesion or suspension conditions (Fig. 2A). As expected, the sub-G1 populations of ARLJ301-3 cells grown on collagen I, collagen IV or fibronectin were 2–6%, as low as control cells on regular plates. Surprisingly, poly-L-lysine-coated plates, on which cells presumably attached to the surface by some electrical charge, could grow cells with a sub-G1 population as low as collagen IV. ARLJ301-1 cells attached to polystyrene plates and PVLA-coated plates had a relatively low sub-G1 population compared with cells floating on polystyrene plates (Fig. 2 polystyrene (sus)) or poly-HEMA-coated plates.

Recently accumulated data have shown that different types of cysteine protease were activated in anoikis [26,27]. By immunoblotting, we detected an active form of caspase-9 in anchorage-deprived cell lysates, such as on poly-HEMA- or 1% BSA-coated plates and the floating fraction on polystyrene plates (Fig. 2B). On collagen I-coated plates, some fraction of cells did not attach onto the plates surface, floated, and underwent apoptosis (Fig. 2A). These apoptotic cells were collected together with adherent cells to make a cell lysate. That is a reason why the cell lysate from collagen I-coated plates provided a relatively high level of the active form of caspase-9 (Fig. 2B). Adherent cells gave no active form of caspase-9. Caspase-3 was processed and activated in cells under suspension conditions in which caspase-9 was activated as well (data not shown).

PI-3K/Akt and ERK activation could not rescue ARLJ301-1 cells from anoikis

Signaling pathways for PI-3K/Akt and ERK have been identified to play a central role in protecting cells from apoptosis, including anoikis. While PI-3K/Akt

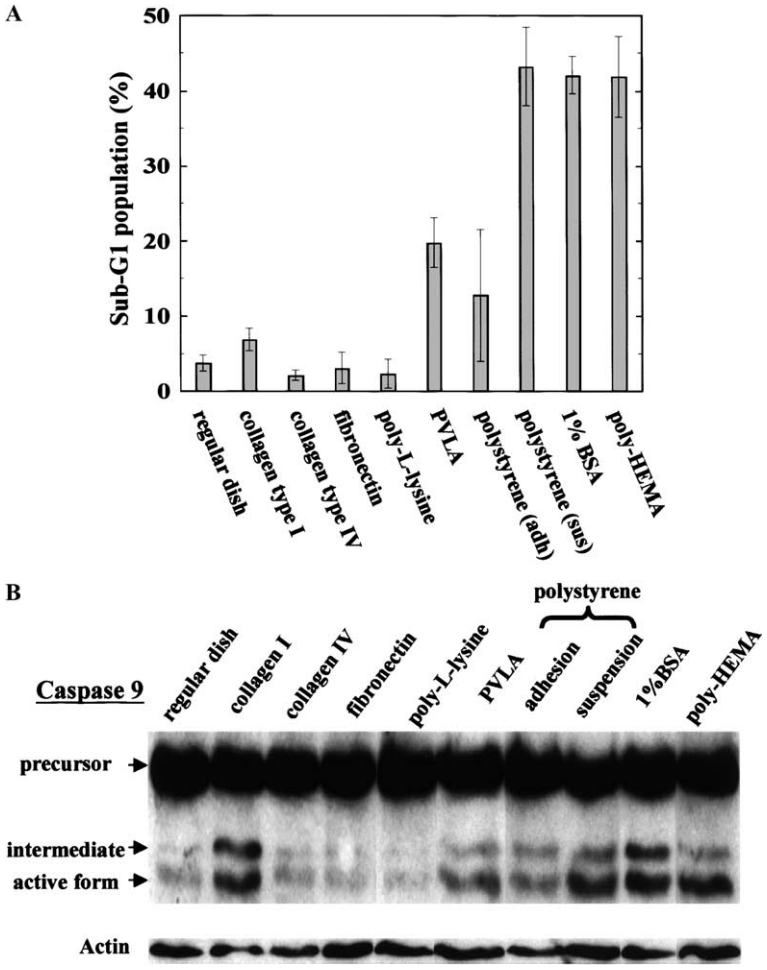

Fig. 2. Different types of adherent conditions reduced the population of apoptosis in ARLJ301-3 and suppressed caspase-9 activity. (A) Cells were plated onto the indicated plates and sub-G1 populations were determined by FACS analysis at 8 h of incubation. (B) Intermediate and active forms of caspase-9 were detected in floating, apoptotic cells at 8 h of incubation. Adherent conditions are: regular dish, collagen type I, collagen type IV, fibronectin, poly-L-lysine, PVLA, and adherent fraction of polystyrene (polystyrene (adh)). Anchorage-deprived culture conditions are: suspended fraction of polystyrene (polystyrene (sus)), 1% BSA and poly-HEMA. Note, on polystyrene plates, half of the cells were attached to plate surface and grew, however, another set of cells did not attach to the plates, floated during experiments, and underwent apoptosis. We termed the former as polystyrene (adh) and the latter as polystyrene (sus).

acts as a protective molecule against anoikis in different cell types [7,28,29], it is controversial whether ERK functions as an anti-apoptotic or a pro-apoptotic molecule [16–18,30–32]. We studied ERK activation following the loss of adherence by detection of the phosphorylated form of ERK by immunoblotting. Surprisingly, phosphorylated ERK was detected only on non-adherent cells after an 8-h incubation in suspension (Fig. 3A). In sharp contrast, no active form of ERK was detected on adherent cells. As these results indicated that ERK activation might promote an apoptotic process in ARLJ301-1 cells, we attempted to confirm whether the pretreatment of PD098059, a specific inhibitor of the ERK cascade, rescued cells from anoikis. This pretreatment to suppress the ERK cascade increased the apoptotic population of cells under both adherent and suspension conditions (Fig. 3B). With the thought that transient activation of ERK at an early stages in the anchorage-deprived condition was of importance in protecting cells from anoikis [33], we analyzed the early expression profile of the active form of ERK. Notably, the phosphorylated form of ERK was detected constantly for 8 h in anchorage-deprived cells. However, in adherent cells, active ERK was expressed as early as 4 h and gradually declined, leading to absence at 8 h after plating (Fig. 3C). These results imply that in our system, activated ERK did not act as an executioner for anoikis but acted as a survival factor for ARLJ301-1 cells in suspension even with little effects.

In contrast, phosphorylated Akt was easily detected in cells under both adherent and anchorage-deprived conditions, and there was no difference in the expression level among functional and non-functional adhe-

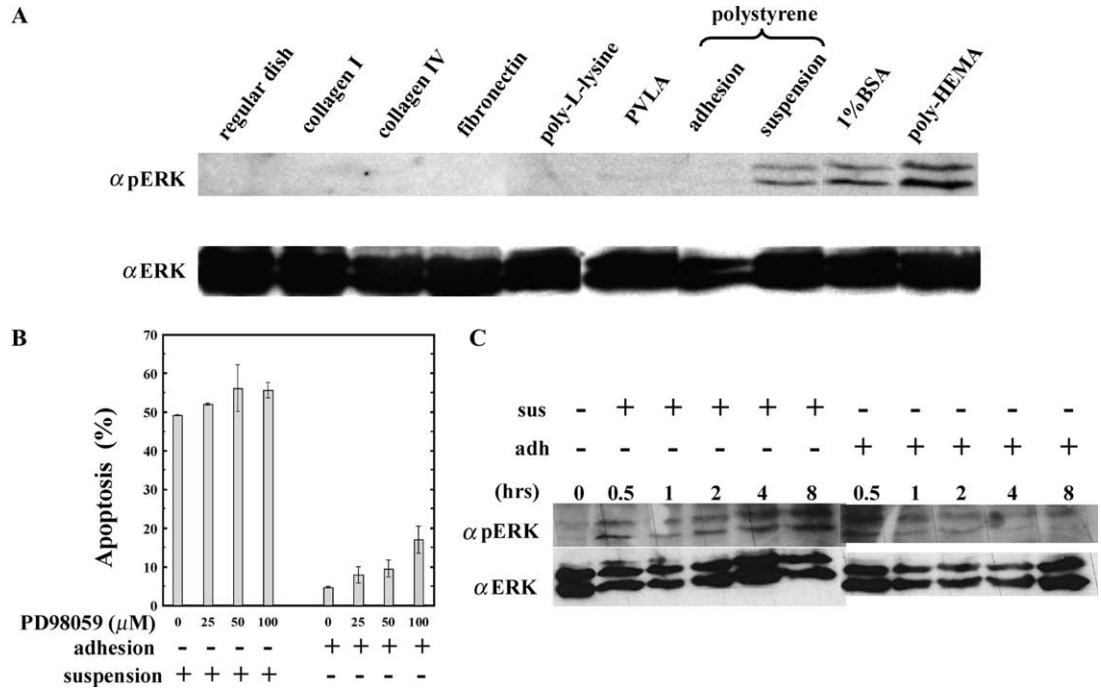

Fig. 3. The active form of ERK was detected only in cells in suspension. (A) Phosphorylated ERK (upper panel) was detected in the suspended fraction of cells on polystyrene, 1% BSA and poly-HEMA-coated plates by immunoblotting. Lower panel shows total ERK protein. (B) Pretreatment with PD098059 enhanced the apoptotic cell population to some extent. Sub-G1 population was analyzed with FACScan. (C) Phosphorylated ERK (upper panel) was determined at the indicated time after cells were transferred to adherent or anchorage-deprived conditions.

sion substrates (Fig. 4A). Furthermore, the time course experiment revealed a slight decrease in the active form of PI-3K/Akt at 8 h of culture under both adherent and anchorage-deprived conditions (Fig. 4C). We wished to confirm that this detectable, phosphorylated form of PI-3K/Akt still worked as a protector of ARLJ301-3

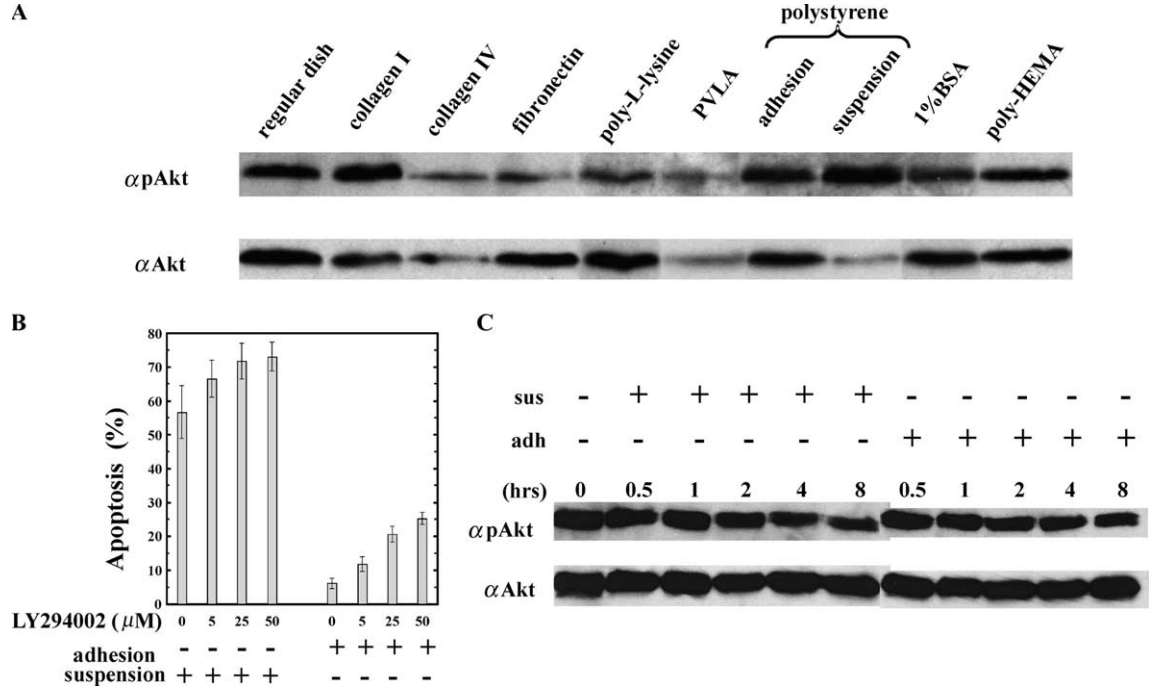

Fig. 4. Active form of Akt constitutively expressed in ARLJ301-3. (A) Phosphorylated Akt (upper panel) was readily detected under adherent and suspension conditions. Lower panel shows total Akt protein. (B) Pretreatment with LY294002 enhanced the apoptotic cell population to some extent. Sub-G1 population was analyzed with FACScan. (C) Phosphorylated Akt was determined at the indicated time after cells were transferred to adherent or anchorage-deprived conditions.

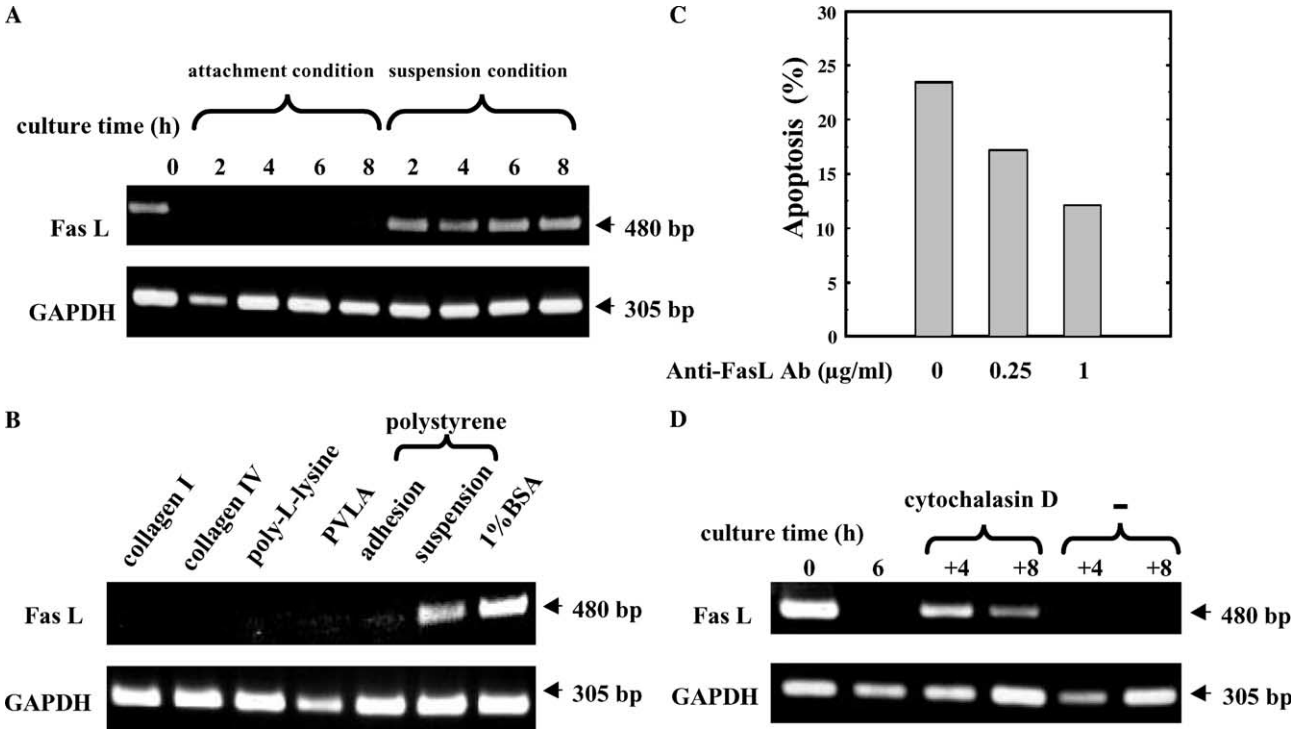

Fig. 5. The FasL expression was determined by RT-PCR. (A) The FasL was already expressed in cells after trypsinization (most left side lane, 0 h) and its expression continued for 8 h. The ARLJ301-3 cells attached to plate showed no FasL expression. GAPDH expression was determined as an internal positive control for RT-PCR. (B) FasL was specifically expressed in cells not under adherent but anchorage-deprived conditions. (C) Anti-FasL antibody was added into medium at the same time with plating cells on poly-HEMA plates and sub-G1 populations were analyzed by FACS. (D) Cells were incubated for 6 h after plating on regular plates and then treated with cytochalasin-D with further incubation for 4 or 8 h. Note, this treatment did not induce cell detachment.

cells from anoikis. To do this, we pretreated cells with LY294002, a specific inhibitor of PI3-K/Akt, at 4 °C for 30 min and then transferred them to suspended or adherent conditions. This pretreatment induced apoptosis in adherent cells and further enhanced the proportion of apoptotic cells deprived of anchorage (Fig. 4B).

The up-regulation of FasL expression in cells under cytoskeletal disruption

Thus, we could not find out any survival function in PI-3K/Akt and ERK signaling pathways and considered that our system of anoikis could be a fast type of anoikis because it took place within 2–4 h after ARLJ301-1 cells were transferred to the anchorage-deprived conditions. That is the reason why we investigated the involvement of death receptor signaling pathways. The FasL expression determined by RT-PCR revealed that its up-regulation was specifically observed in cells under anchorage-deprived conditions and not in adherent cells (Figs. 5A and B). Interestingly, ARLJ301-1 cells that expressed FasL soon after detached from regular plates by treatment with trypsin–EDTA (Fig. 5A, 0 h). This is the first report about Fas/FasL signaling pathway implicated in anoikis aside from that about the involvement of FAS-associated death domain protein (FADD) [34]. As Fas receptor was constitutively expressed in cells under either adherent or anchorage-deprived conditions (data not shown), adherent conditions including synthetic polymer presumably function to maintain the down-regulation of FasL expression to protect cells from anoikis. In the next step, we confirmed the fact that this up-regulated FasL could induce apoptosis in cells under suspension conditions. Anti-FasL antibody was added to the medium at the same time on plating cells on poly-HEMA-coated plates. As shown in Fig. 5C, apoptotic population of ARLJ301-1 cells decreased in a dose-dependent manner with anti-FasL antibody. Thus, the detachment-induced FasL stimulated anoiki in the cells.

ARLJ301-3 cells turned out to be of a round shape from spindle shape when transferred to an anchorage-deprived condition and in addition this morphological change was observed when cells are treated with cytochalasin-D as well, which disrupt filamentous actin. On next step, we confirmed that cytochalasin-D treatment induced the FasL expression and apoptosis in ARLJ301-3 cells (Fig. 5D), however, this treatment did not induce cell detachment. These results suggested that the morphological change such as round shape triggered by cytoskeletal disruption plays an important role in anoikis through the up-regulation of FasL expression.

Discussion

The regular process of adhesion of epithelial cells to ECM is initiated with the contact of cell surface β1-integrin receptors to fibronectin, an ECM component, leading to recruitment of a wide variety of proteins, including FAK, and the formation of focal adhesions which mediate survival signals to downstream targets [10–14]. However, we wished to address the question as to whether the integrin/fibronectin cell adhesion system is necessary and sufficient to protect ARLJ301-3 cells from anoikis or what substrate is able to maintain survival signals much higher than integrin/fibronectin system. ARLJ301-3 cells could efficiently attach to synthetic polymer-coated plates and survive, as did cells to collagen- or fibronectin-coated and regular plates (Fig. 2). To exclude the possibility that components in serum, including fibronectin, might conceal the dish surface and allow cells to adhere to the plate during a long cultivation and de novo synthesis of matrix components from ARLJ301-3 could aid cells in contacting the plate surface, we examined the efficiency of cell adhesion at 4 °C. ARLJ301-3 cells could not attach to fibronectin-coated plates (adherent: 31.5%, floating: 68.5%) whereas cells efficiently adhered to poly-L-lysine-coated plates (adherent: 94.1%, floating: 5.9%) at 8 h. Thus, cell adhesion with synthetic polymer-coated plates proved not to be mediated by fibronectin in serum or produced by de novo synthesis.

To identify the anti-apoptotic signaling pathway, we analyzed the ERK and PI-3K/Akt cascades by immunoblotting. Surprisingly, ERK was extensively phosphorylated throughout the 8-h incubation under anchorage-deprived conditions, whereas under an adherent condition ERK was activated only at an early phase after plating (Fig. 3). In sharp contrast to ERK, PI-3K/Akt was extensively and equally phosphorylated under all adherent or anchorage-deprived conditions (Fig. 4). These results suggest that PI-3K/Akt and ERK activation could not inhibit anoikis under a physiological condition.

Recently, it was shown that cell shape was another influential factor in protecting cells from anoikis [25,35], where the cell surface area for attachment to plates was crucial. If the area would be small, cells were rounded and were susceptible to apoptosis. On the other hand, if cells were forced to stretch and maintain a wide cell-to-ECM contact area, spindle-shaped cells could survive and proliferate [25,35]. Microscopic examination revealed that the morphological phenotype of cells on synthetic polymers was very similar to that under functional ECMs (data not shown). These previous reports led us to investigate the involvement of death receptor signaling pathways in cells in anoikis or under cytoskeletal disruption. The FasL expression was specifically up-regulated in cells in anoikis and treated with cytochalasin-D. Cytochalasin-D treatment changed the cell morphology from spindle to round shape and stimulated apoptotic process, consistent with anoikis. The evidence obtained by anti-FasL antibody which could inhibit anoikis in a dose-dependent manner supports the fact that the up-regulated FasL actually induced anoikis in ARLJ301-3 cells. In contrast, Fas was expressed at the same level between adherent and anchorage-deprived ARLJ301-3 cells (data not shown) consistent with constitutive expression in hepatocytes.

This is a first report that FasL expression is regulated by cytoskeletal disruption and related to anoikis. We need further investigation on how so fast FasL is inducible soon after being detached from ECMs and this may give us the new insight into anchorage-independent growth in cancer cells.

References

[1] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[2] L.A. Liotta, E.C. Kohn, The microenvironment of the tumor–host interface, Nature 411 (2001) 375–379.
[3] J.E. Meredith Jr., B. Frazeli, M.A. Schwartz, The extracellular matrix as a cell survival factor, Mol. Biol. Cell 4 (1993) 953–961.
[4] X. Zhu, M. Ohtsubo, R.M. Böhmer, J.M. Roberts, R.K. Assoian, Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E:cdk2 and phosphorylation of the retinoblastomaprotein, J. Cell Biol. 133 (1996) 391–403.
[5] F. Re, M. Zanetti, N. Sironi, L. Dolentarutti, E. Lanfrancone, E. Dejana, F. Collotta, Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells, J. Cell Biol. 127 (1994) 537–546.
[6] B. Valentinis, A. Morrione, F. Peruzzi, M. Prisco, K. Reiss, R. Baserga, Anti-apoptotic signaling of the IGF-I receptor in fibroblast following loss of matrix adhesion, Oncogene 18 (1999) 1827–1836.
[7] A. Khwaja, P. Rodriguez-Viciana, S. Wennstrom, P. Warne, P. Downward, Matrix adhesion and ras transformation both activate a phosphoinositoide 3-OH kinase and protein kinase B/Akt cellular survival pathway, EMBO J. 16 (1997) 2783–2794.
[8] S. Kennedy, A. Wagner, S. Conzen, J. Jordan, A. Bellucosa, P. Tscichlis, N. Hay, The PI3-kinase/Akt signaling pathway deliver an anti-apoptotic signal, Genes Dev. 11 (1997) 703–713.
[9] S.M. Frish, K. Vuori, E. Ruoslahti, P.Y. Chan-Hui, Control of adhesion dependent cell survival by focal adhesion kinase, J. Cell Biol. 134 (1996) 793–799.
[10] A. Aplin, A. Howe, R. Juliano, Cell adhesion molecules, signal transduction and cell growth, Curr. Opin. Cell Biol. 11 (1999) 737–744.
[11] D. Illic, C.H. Damsky, T. Yamamoto, Focal adhesion kinase, J. Cell Sci. 110 (1997) 401–407.
[12] D.D. Schlaepfer, C.R. Hauck, D.J. Sieg, Signaling through focal adhesion kinase, Prog. Biophys. Mol. Biol. 71 (1999) 435–478.
[13] T. Seufferiein, E. Rozengurt, LPA stimulates tyrosine phosphorylation of focal adhesion kinase, paxillin and p130. Signaling pathways and cross-talk with PDGF, J. Biol. Chem. 269 (1994) 9345–9351.
[14] D.D. Schlaepfer, S.K. Hanks, T. Hunter, P. Van der Geer, Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase, Nature 372 (1994) 786–791.

[15] A. Kaufmann-Zeh, P. Rodriguez-Viciana, E. Ulrich, C. Gilbert, P. Coffer, J. Downward, G. Evan, Suppression of c-Myc-induced apoptosis by Ras signaling through PI(3)K and PKB, Nature 385 (1997) 544–548.
[16] J.M. Shirakawa, R. Elisei, J.A. Knauf, T. Hara, J. Wang, H.I. Saaveara, J.A. Fagin, Conditional apoptosis induced by oncogenic Ras in thyroid cells, Mol. Endocrinol. 14 (2000) 1725–1738.
[17] A. Danilkovitch, S. Donley, A. Skeel, E.J. Leonard, Two independent signaling pathways mediate the antiapoptotic action of macrophage-stimulating protein on epithelial cells, Mol. Cell. Biol. 20 (2000) 2218–2227.
[18] C.R.L. Webster, M.S. Anwer, Phosphoinositoide 3-kinase, but not mitogen-activated protein kinase, pathway is involved in hepatocyte growth factor-mediated protection against bile-acid-induced apoptosis in cultured rat hepatocytes, Hepatology 33 (1998) 608–615.
[19] K. Wary, F. Mainiero, S. Isakoff, E. Marcantonio, F. Giancotti, The adaptor protein Shc couples a class of integrins to the control of cell cycle progression, Cell 87 (1996) 733–743.
[20] K. Furukawa, T. Yokoi, T. Kodama, Y. Mochizuki, Properties of a newly established adult rat liver epithelial cell strain and its application to transformation assay to detect epigenetic carcinogen, Tumor Res. 22 (1987) 87–102.
[21] A. Kobayashi, M. Goto, K. Kabayashi, T. Akaike, Receptor-mediated regulation of differentiation and proliferation of hepatocytes by synthetic polymer model of asialoglycoprotein, J. Biomater Sci. Ed. 6 (1994) 325–342.
[22] A. Kobayashi, K. Kobayashi, T. Akaike, Control of adhesion and detachment of parenchymal liver cells using lactose-carrying polystyrene as substratum, J. Biomater Sci. Polym. Ed. 3 (1992) 499–508.
[23] S.M. Frish, H. Francis, Disruption of epithelial cell–matrix interactions induces apoptosis, J. Cell. Biol. 124 (1994) 619–626.
[24] H. Nagahara, S.A. Ezhevsky, A.M. Vocero-Akbani, P. Kaldis, M.J. Solomon, S.F. Dowdy, Transforming growth factor-β targeted inactivation of cyclin E: cyclin-dependent kinase 2 (Cdk2) complexes by inhibition of Cdk2 activating kinase activity, Proc. Natl. Acad. Sci. USA 96 (1999) 14961–14966.
[25] C.S. Chen, M. Mrkisch, S. Huang, G.M. Whitesides, D. Ingber, Geometric control of cell life and death, Science 276 (1997) 1425–1428.
[26] M.H. Cardone, G. Salvesen, C. Widman, G. Johnson, S.M. Frish, The regulation of anoikis: MEKK-1 activation requires cleavage by caspases, Cell 90 (1997) 315–323.
[27] M. Rytomaa, K. Lehmann, D. Julian, Matrix detachment induces caspase-dependent cytochrome C release from mitochondria: inhibition by PKB/Akt but not Raf signaling, Oncogene 19 (2000) 4461–4468.
[28] D.A. Flusberg, Y. Numaguchi, D.E. Ingber, Cooperative control of Akt phosphorylation, bcl-2 expression and apoptosis by cytoskeletal microfilaments and microtubules in capillary endothelial cells, Mol. Biol. Cell 12 (2001) 3087–3094.
[29] A.K. Howe, R.L. Juliano, Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase, Nat. Cell Biol. 2 (2000) 593–600.
[30] O. Zugasti, W. Rul, P. Roux, C. Peyssonnaux, A. Eychene, T. Franke, P. Fort, U. Hibner, Raf-MEK-ERK cascade in anoikis is controlled by Rac1 and Cdc42 via Akt, Mol. Cell. Biol. 21 (2001) 6706–6717.
[31] M.L. Gall, J.-C. Chambard, J.-P. Brettmayer, D. Grall, J. Obberghen-Schilling, The p42/p44 MAP kinase pathway prevents apoptosis induced by anchorage and serum removal, Mol. Biol. Cell 11 (2000) 1103–1112.
[32] Z. Xia, M. Dickens, J. Raingeaud, R.J. Davis, M.E. Greenberg, Opposing effects of ERK and JNK-p38 MAP kinase on apoptosis, Science 270 (1995) 1326–1331.
[33] M. Jost, T.M. Huggett, C. Kari, U. Rodeck, Matrix-independent survival of human keratinocytes through an EGF receptor/MAPK-kinase-dependent pathway, Mol. Biol. Cell 12 (2001) 1519–1527.
[34] M. Rytömaa, L.M. Martins, J. Downward, Involvement of FADD and caspase-8 signalling in detachment-induced apoptosis, Curr. Biol. 9 (1999) 1043–1046.
[35] D.A. Flusberg, Y. Numaguchi, D.E. Ingber, Cooperative control of Akt phosphorylation, bcl-2 expression, and apoptosis by cytoskeletal microfilaments and microtubules in capillary endothelial cells, Mol. Biol. Cell 12 (2001) 3087–3094.