Ligand density and integrin repertoire regulate cellular response to LPA

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Abstract

Engagement of integrin receptors by the extracellular matrix (ECM) protein fibronectin (FN) activates intracellular signaling, cytoskeletal reorganization and cellular tension. The soluble factor lysophosphatidic acid (LPA) acts through Rho GTPase and its effector Rho kinase (ROCK) to enhance α5β1 integrin-mediated cell spreading on the Arg-Gly-Asp (RGD) cell-binding domain of FN. A second cell-binding site for α4 integrins resides in the CS1 segment of the alternatively spliced V region of FN. We show here that LPA treatment of α4β1-expressing CHOα4 cells on FN induced a significant decrease in spread cell area. LPA also decreased apoptosis induced by serum-deprivation in CHOα4 and human A375 melanoma cells in an α4β1-dependent manner. Improvement in cell viability and changes in cell morphology were dependent on ROCK and on the number of substrate binding sites for α4β1. LPA signaling combined with α4β1-mediated adhesion appears to sustain cell viability in situations where FN matrix is limiting. Such cooperation may impact dynamic cellular events such as wound healing, fibrosis, and metastasis.

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1. Introduction

The extracellular matrix (ECM) elicits mechanical and biochemical signals primarily through integrin receptors that link the ECM to the actin cytoskeleton. Integrins are heterodimeric transmembrane receptors consisting of α and β subunits (Hynes, 2002). Engagement of integrins with ECM ligands leads to receptor clustering at the plasma membrane, recruitment of structural and signaling molecules to adhesion sites, reorganization of the actin cytoskeleton and induction of signal transduction cascades (Giancotti and Tarone, 2003; Schoenwaelder and Burridge, 1999; Schwartz et al., 1995). The specific cellular outcome of adhesion depends on multiple factors including receptor levels, ECM ligand and the action of soluble factors that enhance adhesion-mediated events. Combined signals from the ECM and growth factors can have synergistic effects on cell proliferation, migration, and differentiation.

Fibronectin (FN) is a major ECM component that is present in both tissue and blood plasma, and affects multiple cellular functions (Hynes, 1990). FN exists as a dimer containing multiple repeating motifs including two cell-binding sites. The central cell-binding domain contains the Arg-Gly-Asp (RGD) and synergy sites for binding of α5β1 integrin (Nagai et al., 1991; Pierschbacher and Ruoslahti, 1984). Alternative splicing of the variable (V) region of the FN transcript introduces the CS1 cell-binding site for α4 integrins (Guan and Hynes, 1990; Wayner et al., 1989). While all FN subunits contain the RGD cell binding site, available CS1 binding sites for α4β1 comprise only about 40% of human plasma FN (Paul et al., 1986). However, during certain dynamic processes such as fetal development and wound repair, changes in splicing of the V region yield FN enriched in α4β1 binding sites (ffrench-Constant et al., 1989; Hynes, 1990).

Lysophosphatidic acid (LPA) is a soluble lipid that binds to cell-surface edg-2, edg-4 and edg-7 receptors (Moolenaar, 2000; Moolenaar et al., 1997), members of the heptahelical superfamily of G protein-coupled receptors. LPA is present in normal serum and is released locally by activated platelets during the early stages of wound repair and by certain cancer cells such as ovarian carcinoma (Moolenaar, 2000, 2002). Much evidence links LPA with FN and cytoskeletal changes. For example, both LPA and FN activate RhoA (Barry et al., 1997; Jalink et al., 1994; Kumagai et al., 1993), a small GTPase involved in
cytoskeletal reorganization and cell shape changes (Ridley, 1996). Rho activates a number of downstream effector molecules including Rho kinase (ROCK) (Riento and Ridley, 2003). ROCK activation induces myosin II contractility (Kimura et al., 1996), and the resulting internal cellular force promotes actin reorganization into stress fibers and enhances focal adhesion formation in fibroblasts (Chrzanowska-Wodnicka and Burridge, 1996; Hotchin and Hall, 1995) and rounding of neural cells (Jalink and Moolenaar, 1992; Jalink et al., 1994).

FN has both RGD-dependent and RGD-independent integrin binding sites, so we tested the possibility that cells respond differently to LPA depending upon the FN receptor that is engaged. We took advantage of cells that ectopically express α4β1 (CHOα4) or α5β1 (CHOα5) integrins, which bind FN at different sites. In contrast to CHOα5 cells, CHOα4 cells on substrates coated with a low concentration of FN had reduced survival that could be rescued by stimulation with LPA. Our results indicate that the cell surface integrin repertoire along with the concentration of integrin ligand dictate how a cell responds to LPA.

2. Experimental procedures

2.1. Cell culture

Chinese Hamster Ovary B2 (CHO(B2)) cells, which are α5 integrin deficient (Schreiner et al., 1989), were cultured in DMEM (Cellgro) containing 10% Fetal calf serum (HyClone Laboratories), 1% Non-Essential Amino Acids (GIBCO BRL), and 2 mM l-glutamine (GIBCO BRL). CHO(B2) stable transfected populations expressing human α4 integrin (CHOα4) or α5 integrin cDNAs (CHOα5) (provided by Siobhan Corbett, Robert Wood Johnson Medical School) were cultured in the above medium plus 1 mg/ml G418 (GIBCO BRL). These cell lines expressed similar levels of α4β1 and α5β1 integrins as measured by FACS analysis and by immunoprecipitation of the appropriate integrin from cells metabolically labeled with 35S-methionine (NEN). A375 human melanoma cells (ATCC, Rockville, MD) were cultured in DMEM containing 10% fetal bovine serum (Hyclone Laboratories) and 4 mM l-glutamine.

Cells expressing chimeric integrins were generated using CHO(B2) cells and LipofectAMINE 2000 (Invitrogen) in accordance with the manufacturer’s guidelines. Cells were transfected with DNA constructs containing the extracellular and transmembrane domain of α4 integrin connected to the cytoplasmic domain of α5 integrin (X4C5) or no cytoplasmic domain (X4C0) (gift of Siobhan Corbett, Robert Wood Johnson Medical School) in LipofectAMINE 2000 reagent and OptiMem medium. Individual colonies were chosen and, after selection with G418 at 1 mg/ml, colonies were expanded and subjected to fluorescence activated cell sorting (FACS) based on cell surface α4 integrin expression. Briefly, cells were trypsinized, washed in FACS buffer (PBS plus 2% FN-depleted fetal bovine serum), and incubated for 30 min on ice with a monoclonal antibody against α4 integrin (Chemicon International). After washing, cells were incubated with goat-anti-mouse FITC labeled secondary antibody for 30 min on ice in the dark. Cells were washed twice with FACS buffer, filtered to separate clumps and resuspended in PBS. Samples were analyzed and sorted using a FACS Vantage SE w/DiVa flow cytometer (BD Biosciences) with excitation at 488 nm and a 530/30 bandpass filter for FITC detection. CHO cells expressing equivalent cell surface levels of α4 and chimeric integrins were isolated by FACS and used in these studies.

2.2. Preparation of fibronectin and cell plating

Human plasma fibronectin (FN) was purified by gelatin–Sepharose affinity chromatography (Pharmacia Biotech) from spent human plasma (Princeton Medical Center, Princeton, New Jersey) as previously described (Wilson and Schwarzbauer, 1992). Recombinant FNs rFN(V+) and rFN(V+RGD−) are identical to recFNαM(RGD+R) and recFNαM(RGD−) (Corbett and Schwarzbauer, 1999).

Surfaces were coated overnight with varying concentrations of FN or purified recombinant FN at 4 °C. To determine the amount of FN adsorbed to glass coverslips, unlabeled FN was spiked with 125I-labeled FN and surfaces were coated with solutions of 10 μg/ml (low FN) or 100 μg/ml (high FN) as above. Coverslips were washed and exposed to a phosphorimager screen. The amount of adsorbed FN was calculated using a standard curve of known concentrations of 125I-labeled FN. Coverslips coated with a 100 μg/ml solution had 10-fold more adsorbed FN, on average, than surfaces coated with a 10 μg/ml solution.

FN-coated surfaces were blocked with 1% fatty-acid free BSA (ICN) in PBS for 1 h at 37 °C. Cells were lifted with TPCK-treated trypsin (Sigma) and washed with soybean trypsin inhibitor in PBS. Seeding densities were 5 × 10^4 cells/24-well dish for immunofluorescence staining, 2.5 × 10^4 cells/96-well dish for viability assays, and 5 × 10^5 cells/35 mm dish for FACS analysis and for Rho assays. Cells were allowed to attach for 1 h at 37 °C in DMEM without serum. Medium was then replaced with DMEM containing LPA (Sigma) at the indicated concentrations for the indicated periods of time. In some experiments, cells were pretreated for 10 min with 10 μM Y27632 (Biomol) or 1 mM Mn2+ before addition of LPA.

2.3. Immunofluorescence and cell viability

Cells were washed, fixed and permeabilized for staining with rhodamine-conjugated phalloidin (Molecular Probes Inc.) used at 1:1000 dilution (Midwood et al., 2002). Coverslips were mounted with Fluoroguard (BioRad) and cells were visualized with a Nikon TE2000U microscope equipped with a Cooke SensiCamQE High Performance camera. Images were captured and normalized using IPLab software. For figures, images were captured with a Nikon Plan Fluor ELWD 40× objective with 0.60 aperture. For cell areas, six random fields of cells per each condition were captured with a Nikon Plan Fluor ELWD 20× objective with 0.45 aperture and the cell areas were calculated using IPLab software. On average, approximately 100 total cells were counted for each condition in each experiment.
Cell viability was measured after 48 h of treatment with LPA and/or Y27632. Cells were trypsinized and stained in suspension with 0.08% trypan blue (Sigma). Percent viable cells are the number of trypan blue-negative cells divided by the total number of cells. For apoptosis assays, cells were processed using an In Situ Cell Death Detection kit (Roche Applied Sciences) following manufacturer’s instructions. TUNEL-positive fluorescent nuclei were counted and data are expressed as percent apoptotic cells. For both assays, 50–150 total cells were counted for each condition and experiments were done in duplicate.

2.4. Rho activation assays

Cells were lysed with RIPA buffer and active Rho was isolated from the lysate using a pull-down assay (Cytoskeleton Inc.) according to the manufacturer’s instructions. Total Rho protein in the lysates and isolated active Rho were separated in a 15% polyacrylamide-SDS gel and visualized using an anti-RhoA monoclonal antibody (Cytoskeleton, Inc.) and Super Signal ECL reagents (Pierce). Films were scanned using an Epson Perfection 4870 Photo scanner and adjusted in Adobe Photoshop 7.0. Since CHOα4 cells expressed much less active Rho than CHOα5 cells, bands from CHOα4 cell lysates were adjusted separately in Adobe Photoshop than bands from CHOα5 cells lysates in order to visualize weaker bands.

2.5. Statistics

Statistics were performed and p values were calculated using a standard t test. P values less than 0.05 were considered significant.

3. Results

3.1. Fibronectin-dependent changes in cell shape are induced by LPA

In order to directly compare the effects of LPA on α4β1-FN versus α5β1-FN interactions, the behaviors of CHOα4 and CHOα5 cells on different concentrations of FN were compared. Both cell types attached and spread within 1 h on surfaces coated with 10 and 100 μg/ml solutions of FN (Fig. 1A). For convenience, we refer to 10 μg/ml as “low FN” and 100 μg/ml as “high FN”. Addition of 10 μM LPA to CHOα4 cells on low FN resulted in rapid cell rounding with loss of stress fibers (Fig. 1A) and average cell areas decreased by approximately 35% as compared to areas of untreated cells on the same substrate (Fig. 1B). LPA-induced rounding in CHOα4 cells on low FN occurred with as little as 0.1 μM LPA and was observed even after CHOα4 cells had been adherent on low FN for 8 h (data not shown). LPA did not have these effects in CHOα5 cells plated on high FN (Fig. 1A–B). Furthermore, the change in CHOα4 cell areas varied with the coating concentration of FN. LPA-treated cells on surfaces coated with 20 or 50 μg/ml FN solutions had intermediate cell areas and these cells did not spread in 1 h on tissue culture plastic or on glass coverslips without FN (data not shown). Together, these results indicate that the amount of substrate FN regulates cell responses to LPA.

The effect of LPA on CHOα5 cells was the opposite, as treatment of these cells enhanced spreading and formation of stress fibers (Fig. 1A) and increased cell area (Fig. 1B) on both concentrations of FN. In fact, CHOα5 cells never showed reduced cell areas in response to LPA treatment, even on FN concentrations as low as 0.1 μg/ml. Increasing the concentration
of FN is essentially increasing the number of integrin binding sites in the ECM. Therefore, these results suggest that the number of available α4β1 binding sites in the FN ECM regulates cell rounding versus spreading in response to LPA, while LPA-induced spreading is independent of the number of α5β1 binding sites.

α4β1 binds to the alternatively spliced V region of FN (Guan and Hynes, 1990; Wayner et al., 1989). To mimic an increase in α4β1 binding sites by alternative splicing, CHOα4 cells were plated on coverslips coated with a purified recombinant FN (rFN(V+)) in the absence or presence of LPA. In rFN(V+), each FN subunit includes a binding site for α4β1 within the V region (Fig. 2A), and thus contains a 1:1 molar ratio of α4β1 to α5β1 binding sites. This is in contrast to rat plasma FN in which only 40% of subunits contain the V region (Paul et al., 1986). To compensate for the increased V region levels, rFN(V+) solutions of 1 and 10 μg/ml were considered low and high, respectively.

CHOα4 cells spread on both low and high rFN(V+)-coated surfaces (Fig. 2B). Addition of LPA to CHOα4 cells on the high rFN(V+) concentration increased cell area by as much as 20% (Fig. 2B–C). In contrast, CHOα4 cells on low rFN(V+) showed a more rounded morphology with LPA (Fig. 2B–C). A similar change in cell area was observed with rFN(V+RGD−) which lacks the binding site for α5β1 integrin (data not shown). Thus, increasing the number of α4β1 binding sites, either by increasing FN concentration or by alternative splicing of the V region binding site, regulates α4-mediated cell responses to LPA.

3.2. LPA effects require ROCK activity

LPA induces shape changes and cytoskeletal rearrangement in a variety of cell types, including fibroblasts and neuronal cells, via the Rho/ROCK pathway (Moolenaar et al., 1997). CHOα4 and CHOα5 cells express the EDG-2 receptor for LPA (not shown) suggesting that this pathway might also be active in these cell lines. Indeed, LPA increased the proportion of activated Rho in both CHOα4 and CHOα5 cells on FN (Fig. 3A) although the overall levels of active Rho were higher in CHOα5 cells. Cell shape changes were dependent on Rho activation of ROCK, since LPA-induced rounding of CHOα4 cells was attenuated in the presence of Y27632, a specific biochemical inhibitor of ROCK (Fig. 3B–C). Y27632 also prevented LPA-induced spreading and the increase in CHOα5 cell areas (Fig. 3C). These results indicate that LPA activates the Rho/ROCK pathway in both CHOα4 and CHOα5 cells and that this pathway is required for LPA-induced shape changes, both spreading and rounding.

3.3. LPA effects depend on the integrin ectodomain but not the α integrin cytoplasmic tail

Functional analyses using chimeric integrins with different α subunit cytoplasmic domains has been used to show the contributions of α4 and α5 cytoplasmic tails to cell spreading, migration, and fibronectin matrix assembly (Kassner et al., 1995; Na et al., 2003). We used a similar approach to determine if the intracellular domain of α4 determines cellular response to LPA. CHO cells stably expressing an integrin composed of the extracellular and transmembrane domains of α4, but the cytoplasmic domain of α5 (X4C5 cells) spread and formed stress fibers on low and high concentrations of FN (Fig. 4A). Addition of LPA to cells on low FN, however, promoted cell shape changes and loss of stress fibers, a response that is identical to CHOα4 cells. CHO cells expressing an α4 subunit with no cytoplasmic tail (X4C0 cells) did not spread significantly on either concentration of FN and LPA did not affect this morphology (Fig. 4B). Thus, an α integrin cytoplasmic domain is required to promote cell spreading on FN, but α4 and α5 domains are equally active.

Integrins α4β1 and α5β1 differ in the strength of binding to FN as illustrated by differential abilities to assemble FN into
fibrils. However, α4β1 can be stimulated to assemble FN matrix by incubation with the integrin activator Mn²⁺ (Sechler et al., 2000). Treatment of CHOα4 cells on low FN with Mn²⁺ reversed the effect of LPA on cell area. Cells showed an increase in size compared to untreated, in sharp contrast to the decrease with LPA alone (Fig. 4C). These results link the spreading changes induced by LPA with differences in integrin ectodomains and the FN binding activities of these two integrins.

### 3.4. LPA promotes survival of CHOα4 and A375 human melanoma cells on low FN

Our results define a novel mechanism in which cells differentially change their shapes in response to LPA in an integrin- and ligand-dependent manner. To understand the biological consequences of this mechanism, we analyzed cell survival and apoptosis since FN and LPA independently affect these processes (Stupack and Cheresh, 2002; Ye et al., 2002). Cells were serum-starved in medium with or without LPA and then assayed
for cell viability or apoptosis. The number of viable CHOα4 cells significantly increased by almost 2-fold in the presence of LPA on low FN, but this effect was not seen on high FN (Fig. 5A). CHOα5 cell viability was the same under all conditions and was similar to CHOα4 cells with LPA on low FN (data not shown). X4C5 cells behaved in a similar manner to CHOα4 cells, as the number of viable X4C5 cells increased with LPA treatment on low FN, but no effect was seen on high FN (Fig. 5B). X4C0 cells were less viable on both FN substrates, and LPA did not rescue this defect (Fig. 5B).

Increased viability corresponded with reduced apoptosis. LPA treatment dramatically decreased TUNEL-positive CHOα4 cells on low FN to levels observed on high FN (Fig. 6A). Reduced cell death and increased viability were partially reversed by treatment with Y27632 (Figs. 5A and 6A), indicating a role for ROCK. Other inhibitors such as pertussis toxin, PD98059 and LY294992 did not detectably affect cell viability, suggesting that Rho and ROCK are the primary mediators of the LPA effects. In addition, LPA had a similar protective effect on CHOα4 cells plated on low rFN(V+) (data not shown). The effects were FN-specific, as LPA did not improve CHOα4 or CHOα5 cell viability on tissue culture plastic (data not shown). These results show that low levels of α4β1-FN binding are not sufficient to fully support cell survival in the absence of serum. However, concomitant stimulation with LPA circumvents the requirement for high levels of α4β1 engagement and promotes survival.

The human melanoma cell line A375 naturally expresses both α4β1 and α5β1 integrins (Mould et al., 1990). In order to determine if LPA could similarly induce effects in these cells in
an α4β1-dependent manner, apoptosis was measured in A375 cells on different concentrations of rFN(V+) lacking RGD (rFN (V+RGD−)), in order to prevent α5β1-mediated adhesion. LPA dramatically reduced the proportion of TUNEL-positive A375 melanoma cells on low but not on high rFN(V+RGD−) (Fig. 6B) and similarly enhanced viability as measured by trypan blue exclusion (data not shown). These results indicate that in environments with low levels of FN, these cancer cells behave similarly to CHOα4 cells and enhance their survival by utilizing LPA signals to supplement α4β1–FN interactions.

4. Discussion

Our data provide evidence that the integrin expression profile and density of integrin binding sites in the ECM are important parameters in regulating cell shape and survival responses to soluble factors. We found that, in cells using α4β1 to adhere to FN, modulation of cell shape by LPA signaling is dependent on the FN concentration in the substrate. Cell rounding was induced by LPA treatment on low FN substrates, but this effect was counteracted by stimulation of α4β1–FN interactions with Mn2+. These modulatory effects required ROCK activity downstream of Rho but did not require signals specific to the α4 cytoplasmic tail. In addition to changes in cell shape, LPA signaling also rescued the survival defect that occurred in α4β1-expressing cells on low FN substrates. Therefore, cell viability, when compromised by an environment with limiting amounts of adhesive ligands, can be enhanced by concomitant stimulation with LPA.

Adherent cells require attachment to the ECM in order to survive — removal of ECM proteins promotes cell death (Frisch and Francis, 1994; Meredith et al., 1993; Re et al., 1994; Ruoslahti and Reed, 1994). In comparing cell behaviors on different amounts of FN, shape changes and survival mediated by α4β1 and α5β1 were equivalent on high FN substrates but α5β1–FN interactions were much more effective than α4β1–FN on low FN. However, activation of LPA signaling in serum-starved CHOα4 cells and A375 melanoma cells was able to compensate for reduced FN. Cells were protected from apoptosis and cell survival on low FN was maintained at levels equivalent to survival of these cells on high FN. LPA also elicited a FN concentration-dependent reduction in spreading with α4β1 cells, while cells using α5β1 increased in size regardless of the amount of FN. Other cell types have shown similar responses to either LPA or FN signals. Interactions between α4β1 and the CS1 region of FN have previously been shown to prevent apoptosis of serum-starved B cells (Garcia-Gila et al., 2002). LPA treatment of neural cells induced neurite retraction (Jalink and Moolenaar, 1992), a response similar to the cell rounding observed with CHOα4 cells. Our results add to these findings by demonstrating that LPA differentially affects cells depending on the amount of ECM ligand and the particular integrin receptor that is engaged.

Differences between α4β1 and α5β1 ligation have been observed in other systems and probably result from differential integrin signaling. Rabbit synovial fibroblasts expressing both α4β1 and α5β1 increased matrix metalloprotease levels in an α5β1-FN dependent manner, while inhibiting matrix metalloprotease transcription in an α4β1-FN dependent manner (Huhtala et al., 1995). Erythroid progenitor cells responded to stem cell factor-1 differentially depending upon which integrin was ligated to FN; α5β1–FN interactions promoted survival, while α4β1–interactions promoted cell death in response to stem cell factor-1 (Kapur et al., 2001). PKCα activation was induced in melanoma cells by α5β1 ligation, but not α4β1 ligation (Mostafavi-Pour et al., 2003). “Cytoplasmic swap” experiments using chimeric integrins have demonstrated that different α integrin cytoplasmic domains support different cell functions (Hemler et al., 1992). When attached to the α4 integrin extracellular and transmembrane domains, the cytoplasmic tails of α2 and α5 supported focal adhesion formation and the α4 cytoplasmic domain promoted cell migration by CHO cells on FN (Kassner et al., 1995). In amphibian embryos, a similar α4 extracellular–α5 cytoplasmic chimera was more effective than wild type α4 integrin at assembling FN into fibrils (Na et al., 2003). Based on these experiments, α5β1 integrin has been associated with firm attachment and α4β1 integrin with motility. This difference can be explained in part by differences in cytoplasmic domain interactions. α4 integrin directly binds the cytoplasmic protein paxillin and this association inhibits cell spreading and actin stress fiber formation, thus promoting a migratory phenotype (Liu and Ginsberg, 2000; Liu et al., 1999).

In our system, the effects of LPA on cell spreading and survival were not dependent on signals initiated by the α4 cytoplasmic tail, since cells expressing the chimeric X4C5 integrin responded identically to CHOα4 cells. This result suggested that the integrin extracellular domain modulates CHO cell responses to LPA which most likely occurs through differences in affinity/avidity for FN between α4β1 and α5β1. This idea is supported by reversal of LPA effects when α4β1 binding to FN was stimulated by Mn2+.

LPA exposure induced changes in cell spreading and actin cytoskeletal organization. Concomitantly, the activities of Rho/ROCK and Erk (unpublished observations) were enhanced. Active Erk is known to contribute to cell survival and proliferation (Stupack and Cheresh, 2002). Although the Rho/ROCK pathway is typically associated with control of the actin cytoskeleton, its contribution to cell survival has been documented in lung and ovarian carcinoma cells (Baudhuin et al., 2002; Igishi et al., 2003; Radeff-Huang et al., 2004). Furthermore, ROCK activation promotes FAK phosphorylation (Sinnett-Smith et al., 2001), which can affect cell survival (Schlaepfer et al., 1999). Our findings suggest that simultaneous activation of these pathways supplements and enhances integrin signaling which then allows cells to overcome deficiencies caused by circumstances of stress and suboptimal levels of α4β1 binding sites. Thus, various combinations of signaling pathways can lead to the same cellular output. In our case, LPA can rescue α4β1 cell defects caused by insufficient levels of FN–integrin interactions.

Our results suggest that cells using α4β1 for adhesion are more sensitive to changes in FN levels than are α5β1 cells. This is due in part to alterations in protein expression profiles of CHOα4 cells and A375 melanoma cells. Levels of expression of
the cell survival protein Bcl-2 and the cell cycle regulator p21 were significantly diminished in serum-starved CHO cells, and A375 cells expressed virtually no p21 whether or not serum was present (unpublished observations). Combined with conditions of inadequate ECM binding sites, this sensitized cell phenotype requires an additional stimulus in order to overcome insufficient integrin survival signaling. Under these conditions, LPA prolongs survival partially via ROCK-mediated reorganization of the actin cytoskeleton, but Erk and other pro-survival signals are probably also important. Activation of these pathways by LPA may supplement adhesion ligand signaling thus overcoming a threshold level needed to allow survival. This type of activation supplementation could have significant benefits to cells in vivo. For example, α4β1-positive cells within sites of tissue injury are exposed to variable amounts of ECM ligand, which might compromise survival (Clark, 1996). As our results show, stimulation with LPA, which is released in injuries, would improve viability and allow continued participation in the healing process.

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