Interactions of cells with basement membranes and the extracellular matrices are crucial for various biological processes, including the maintenance of tissue integrity, embryogenesis, wound healing, and the metastasis of tumor cells. These processes involve cell adhesion and migration. Adhesive and migratory events require certain biochemical entities, formation of multiprotein complex between these entities, and their functional communication one another. Cell adhesion is mediated through cell adhesion receptors or transmembrane glycoproteins binding to extracellular matrix (ECM) or counter-receptors on neighbor cells. Cell adhesion receptor families include cadherins, selectins, syndecans, and the immunoglobulin superfamily of cell adhesion molecules (IgCAMs). In this review, integrin-mediated cellular processes such as cell proliferation and apoptosis and their molecular basis will be discussed based on recent observations, although other cell adhesion receptors can play important roles or be involved in the processes and recent outstanding reviews are also available (1, 2).

1. INTEGRINS

Integrins comprise a large family of transmembrane glycoprotein or integrins are heterodimeric molecules composed of an α and a β subunit, with long extracellular domains binding to ECM and short cytoplasmic domains associating with the actin cytoskeleton and affiliated proteins (3). The integrin receptor family in mammals includes at least 18 distinct α subunits and 8 or more β subunits which can potentially generate 24 distinct αβ heterodimeric receptors (4). Major ECMs include fibronectin, laminin, and collagen. By communicating with both ECM and intracellular proteins simultaneously, cells are able to respond to extracellular cues through actions of intracellular signaling components as well as to transduce intracellular signal toward outside of a cell. Further, evidences are being accumulated over the past several years to show that integrins are

OVERVIEW

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important component not only for the structure and architecture of tissues but also for signal transduction leading to regulation in many biological functions in a cell. The signaling through integrin receptors can be classified to two categories; direct signaling by engagement itself of cells via integrins on the surface of a cell and co-signaling upon cooperation with growth factor stimulation of receptor tyrosine kinases (RTKs) of an anchored cell (Figure 1). In next sections, both signaling will be discussed one by one.
focal contacts (7). In a focal adhesion, integrins are linked to actin filaments by virtue of a number of signaling and structural molecules including FAK, c-Src, PI-3-Kinase, RhoGAP, paxillin, talin, PI30Cas, integrin-linked kinase (ILK), and phosphorylated caveolin-1 (8). The protein complex resulting from these interactions can serve as a structural and functional link between integrins and the actin-containing cytoskeleton.

FAK has a unique structure as a member of the family of protein tyrosine kinases. FAK shares little sequence homology with other tyrosine kinases outside its kinase domain, is not a transmembrane protein, contains no lipid modification or acylation sites, and lacks SH2 and SH3 domains. It contains three domains: a central kinase domain, an N-terminal domain binding to β1 integrin cytoplasmic domain peptides (9), and a COOH-terminal domain with two proline-rich sequence motifs and a region required for focal adhesion targeting called the “FAT” sequence (9). FAK is phosphorylated in many tyrosine residues and activated upon integrin-mediated adhesion, and dephosphorylated when cells are detached (10). Although how these happen is not clearly revealed yet, the consequent signaling following activation of FAK have been actively studied by many groups worldwide.

FAK is autophosphorylated in response to integrin activation, predominantly on Tyr397, which conforms to the consensus binding for SH2 domains for c-Src (11), p85 regulatory subunit of PI-3-Kinase and Grb7 (12) (Figure 2). Src interaction with FAK leads to phosphorylation of FAK at several sites including Tyr407, Tyr576, Tyr577, Tyr861, and Tyr925, which then results in the adaptor protein Grb2 binding to FAK at residue Tyr925 (13). Grb2 binding to FAK has been proposed as a possible link between FAK tyrosine phosphorylation and the integrin-mediated activation of MAPK (14). It has become apparent that FAK, Src, PI30Cas and paxillin form a signaling complex at cell adhesion sites, whose assembly is normally initiated by autophosphorylation of FAK (15).

Cell adhesion and spreading has been indexed by FAK activation in numerous studies (16). FAK also appear to control cell spreading and migration (17). FAK is also an important mediator of cell survival in some models (18). Previous studies indicated that a constitutively active form of FAK can facilitate anchorage-independent growth and protection from...
suspension-mediated apoptosis (anoikis) in MDCK epithelial cells (18). Inactivation of FAK by microinjection of a peptide representing the FAK-binding site of the beta 1 tail coupled to a carrier protein into fibroblasts caused rapid apoptosis (19). The mechanisms by which FAK protects cells from apoptosis (and anoikis) are not clear, but FAK association with Src and/or PI-3-K at Tyr397 is predicted to be necessary (18). Recently the FAK-P130Cas complex was shown to activate JNK via a Ras/Rac1/Pak1-MAPK kinase 4 (MKK4) pathway, resulting in survival of fibroblasts on fibronectin when serum was withdrawn (20). In addition, a recent report implicated phospholipase A2 (PLA2), PKC, and p53 in FAK-mediated cell survival (21). However, FAK is not always involved in cell survival. In a study where α5 integrin subunit mediated cell survival of CHO transfectants stressed by serum deprivation, FAK phosphorylation was not correlated with the anti-apoptotic effects (22).

2.2 Direct signaling for activation of MAP kinase cascade.

In addition to FAK, MAP kinases including of Erk1/2 and c-Jun kinase (JNKs), and p38 MAPK are well known to be activated by integrin-mediated cell adhesion. Activation of MAPK (or Erk1/2) pathway by cell adhesion provides a common route leading to transcriptional regulation of certain genes. The products of these genes are critical for cell growth and differentiation (23). However, the intriguing and somewhat controversial aspects of their activation mechanisms have not yet clearly explained.

The exact mechanism of integrin-mediated activation of Erk1/2 is not yet known. However, there are several plausible mechanisms for the integrin-mediated activation of Erk1-2. One mechanism involves Src mediated phosphorylation of FAK Tyr925, as explained above. FAK autophosphorylates Tyr97, creating a docking site for the Src homology 2 (SH2) domain of Src or Fyn (24). Src phosphorylates FAK at Tyr925, creating a binding site for the signaling complex including adaptor protein Grb2 and Ras GTP-exchange factor mSOS (24). These interactions between signaling or structural molecules link FAK to signaling pathways that modify the organization of cytoskeleton and Erk1/2 cascade (25).

It has also been proposed that Shc may be responsible for the initial high level activation of Erk1/2 through a complex formation with Shc/Fyn/Cav-1 upon cell adhesion, and FAK, which is activated more slowly, may sustain the Erk1/2 activation (26). The pathway involving Shc is quite distinct from the other proposed pathways involving FAK, which involve p subunit cytoplasmic domains. Certain integrin subunits bind to the membrane protein caveolin-1, through their external and transmembrane domains (26). Upon interaction with Fyn, Shc is phosphorylated at Tyr117 and recruits Grb2, leading to Ras/Erk1/2 cascade activation. Recently it was reported that the Shc and FAK pathways are activated independently and function in a parallel fashion, and that FAK might enhance and prolong integrin-mediated activation of Erk1/2 through p130Cas, CrkII, and Rap1 in cells expressing B-Raf (27). This suggests that, depending on the Raf isoforms (Raf-1 vs. B-Raf), integrin signaling to Erk1/2 is either through Shc (when Raf-1 is high) or FAK (when B-Raf is high). This suggestion is consistent with another observation that the C3G-CrkII complex (upstream of B-Raf) plays a negative role in anchorage-dependent regulation of Erk1/2 activation, but hat this was reversed by over-expression of B-Raf (28). Therefore, these indicate that the ratio of Raf-1 vs B-Raf depending on cell contexts determine the precise details of integrin signaling to Erk1/2. Another possible mechanism for integrin-stimulated Erk1/2 activation involves PI-3-K, which may activate Raf-1 through activation of PKC isoforms.
These studies do not indicate a direct role for FAK in the activation of Erk1/2. In one study, overexpression of a constitutively activated form of FAK failed to activate Erk1/2, and in a separate study overexpression of a dominant-negative form of FAK blocked FAK activation, but had no effect on Erk1/2 activation (31). The models described above include the activation of Ras in the integrin-mediated activation of Erk-1/2, supported by studies using a Ras dominant-negative (Ras N17) to block the adhesion activation of Erk1/2 (32). Other studies, however, indicate a Ras-independent activation of Erk1/2. When the N-terminal domain of Raf was used as a specific dominant-negative inhibitor of Ras signaling, the activation of Erk1/2 was not affected (33). Further, a recent study shows that Raf can be activated via integrin-mediated adhesion when it contains mutations in the Ras-binding domain of Raf and thereby cannot bind to Ras, also supporting a Ras-independent activation of Erk1/2 (34). In addition to Erk1/2, JNKs and p38 MAPK are also known to be activated by integrin signaling. The integrin-mediated JNKs activation is dependent on FAK and involved in cell survival (20), cell cycle progression (35), or cell spreading (36). On the other hand, p38 is activated by integrins for regulation of collagen gene expression (37), cell motility (38), or functional expression of integrin αv subunit and urokinase plasminogen activator (uPA) (39). The physiological significance of integrin-mediated activation of MAP kinases is not clear, since the activity of Erk1/2 mediated by cell adhesion alone is not sufficient for the cell cycle traverse. That is, additional signals provided by growth factors though integrin co-signaling with receptor tyrosine kinases (RTKs) are still needed. In addition, morphological rearrangements modulated by integrin-mediated activation of small GTPase family members and/or actin cytoskeletal integrity is also shown to be importantly involved in G1/S cell cycle transition (25,40-42).

2.3. Integrin-mediated regulation of RhoA GTPases.

Beyond the trend that many researchers have indicated a link between integrin-ECM interaction and thereby signaling and activation of Ras/Raf-1/MEK/MAPK(Erk) pathway, a pathway downstream of the prototype member of the Ras superfamily of small GTPase, recently much research has also indicated that integrin-mediated signaling also activates other members of the Ras superfamily, notably the Rho family members of small GTPase including RhoA, Rac1, and CDC42 (Figure 3). It is well known that integrin-mediated activation of RhoA, Rac1, or CDC42 rearranges the actin cytoskeletal network, leading to morphological features of stress fiber formation/local adhesion turnover, lamellipodia, or filopodia formation, respectively (43). By virtue of that these small GTPases are important to regulate actin cytoskeleton, they influence diverse key cellular processes (44). The mechanisms how the GTPases regulate actin cytoskeleton are being actively studied and revealed some downstream effectors. Both RhoA-stimulated kinase ROCK and Rac-1 stimulated kinase p21activated kinase (PAK1) can phosphorylate and thereby activate LIM kinase, which in turn phosphorylates and inactivates cofilin (an actin depolymerizing protein), thus leading to promotion of actin filament assembly (45). On the other hand, activation of Rac1 and CDC42 can be linked to actin polymerization, though mediation by WASP, WAVE adaptor proteins, and the Arp2/3 complex in forming actin filaments (45,46). During branching of actin filaments via the Arp2/3 complex, c-Src-mediated activation of cortactin is required for the bridge of the Arp2/3 complex to actin filaments (47). It is now clear that activation or deactivation of RhoA GTPase family members when cells adhere to ECMs is a key event for localized control of actin filament...
assembly, contractility, and cell motility. In addition to their roles in actin filament reorganization, RhoA GTPases have been shown to be important for cell cycle progression (25). Interestingly, activated GTPases are shown to regulate cell cycle progression, by modulating the levels of the G1 phase cyclin, cyclinD, transcriptionally or translationally, or the induction timing of the cyclin D during the G1 phase (25, 40, 41, 48). It was shown that Rac1 activation leads to an enhanced transcription of cyclin D (49), via NF-κB transcription activity in fibroblast cells (50). Rac1 is also shown to regulate cyclin D level at the translational level of endothelial cells following activation of Shc and FAK, through mediation of SOS and PI-3-K (41). In addition, it was found that Rho is involved in the accumulation of cyclin D protein by fibronectin and the same time reduction of p21Cip/Waf was observed (51). Interestingly, Welsh et al. (48) reported that Rho inhibited an alternative Rac/Cdc42-dependent pathway, which resulted in a strikingly early G1-phase expression of cyclin D1, and that cyclin D1 was induced in mid-G1 phase because a Rho switch couples its expression to sustained ERK activity rather than Rac-1 and Cdc42, allowing a RhoA switch crucial for maintaining the correct timing of cyclin D1 expression in G1 phase. Being consistent with the implication by the small GTPase studies to cell proliferation, evidences were reported that intact actin cytoskeletal network integrity is also critical for G1/S transition; demolishing of the network by a pharmacological reagent inhibited
G1/S transition (42). Interestingly, Rac1 activation in hepatocarcinoma cells resulted in preferential G1/S transition, depending on ECM on which cells were newly replated, indicating, although cyclin D levels were similar one another in an ECM-independent manner but cyclin A levels were differentially regulated (78), indicating another step of cell proliferation presumably regulated by small GTPases. In the cases that Rho GTPases are involved in cell cycle progression through regulation of cyclin levels, not only integrin-mediated activation of GTPases but also mitogenic stimulation of signal activation (e.g., Erk1/2) will be required, because integrin-mediated activation of signaling alone is not sufficient for cell cycle traverse. Therefore, in the observations explained above for the roles of the GTPases in cell cycle regulation would be performed in the conditions where cells were treated with growth factors.

3. INDIRECT SIGNALING OF INTEGRINS COLLABORATIVE WITH GROWTH FACTOR RECEPTORS

By virtues of collaborative signaling by integrins with other cell surface receptors such as receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs), integrin-mediated cell anchorage has been observed to have profound effects, in terms of amplitude, duration, and/or spatial aspects on signaling processes. There are several known intracellular signaling molecules that are activated synergistically by both integrins and growth factor receptors. They include the Ras/Raf-1/-MEK/Erk1/2 pathway, Rho family GTPase, PI-3-K, ribosomal S6 kinase (RSK), Jun amino-terminal kinase (JNK), FAK and paxillin, and p130cas (reviewed in (52,53). Since the G1 to S phase transition or S phase entry has been shown to be regulated by mitogenic reagents, cell adhesion, and intact cytoskeletal network integrity (as explained earlier), this collaboration allows cells to integrate positional information as to cell-cell or cell-ECM contacts, with information of rendering cell growth.

3.1. Integrin co-signaling with RTKs.

Over the last few years, there have been profound evidences that integrin-mediated signaling can influence the RTK-initiated signaling. The collaboration has been reported that integrin engagement and the presence of growth factors are required to promote efficient activation (and autophosphorylation) of RTK, such as EGF, PDGF, and FGF receptors (1,52). On the other hand, there were at least two examples that integrin mediated regulation of RTK activation occurs in the absence of growth factors one with PDGF-β receptor (54) and the other with EGFR (55). In these systems, enhanced RTK phosphorylation was correlated with cell proliferation and/or survival. The first demonstration of a growthfactor-dependent physical association between an RTK and an integrin molecule involved PDGFRβ and αvβ3 in human foreskin fibroblasts (56). This study showed that fibroblasts on vitronectin, a ligand for the αvβ3 integrin, display enhanced growth and MAPK activation in response to PDGFRβ compared to cells cultured on non-αvβ3 ligands. Further, an association between a highly phosphorylated fraction of the PDGFRβ and αvβ3 integrin was promoted by PDGF, indicating a direct effect of ECM on the RTK signaling pathway. In contrast, normal human bronchial epithelial cells on collagen type I showed enhanced retinoid-induced differentiation, by restriction of the amount of EGFR-dependent Erk1/2 activation; integration between the ECM- and EGFR-derived signaling pathways lies downstream of EGFR autophosphorylation and at, or upstream of, Raf-1 activation (57). Another recent study has shown that integrins can induce EGFR tyrosine phosphorylation in the absence of EGFR ligands, leading to Shc tyrosine phosphorylation and Erk1/2 acti-
vation (55). In this study, physical interaction between β1 integrin subunit and EGFR has been shown. Further, it was concluded that integrin-mediated tyrosine phosphorylation of the EGFR requires activation of receptor tyrosine kinase activity, based on an EGFR kinase inhibitor study. Importantly, Moro et al. (55) showed that adhesion-induced EGFR activation does not induce cell proliferation, but was shown to protect EGFR-transfected NIH3T3 cells from serum deprivation-mediated apoptosis. Therefore, it is possible that the intensity and duration of EGFR transactivation and subsequent MAPK (Erk1/2) (or other downstream effectors) activation represent a decision point between cell proliferation and alternative pathways such as cell survival.

3.2. Regulation of Ras/Raf-1/MEK/Erks cascade by integrin co-signaling with RTKs.

Integrin regulation of RTK-emanated signaling to the Ras/Raf-1/MEK/Erks cascade has been interesting due to the dependency of cell cycle progression on the presence of growth factors and at the same time cell adhesion. The levels where integrins can modulate RTKs/Ras/Raf-1/MEK/Erks have been shown the RTK (55), activation of Raf-1 (58), activation of MEK (59), and nuclear translocation of activated Erk1/2 (60) (Figure 1). As for the RTK level regulation by integrins, it appears obvious that formation of direct or indirect complexes between the RTKs and the integrins could lead to enhanced opportunities for RTK dimerization and cross-phosphorylation. This author have reported that integrin α5β1, but not α2β1, expression in normal rat intestinal epithelial cells allows preferential survival of cells from serum deprivation through differential activation of PKB/Akt (61), and that this effect was possible via the differential activation of EGFR signaling to PI-3-K/PKB survival pathway, but equal activation to Erk1/2, through differential formation of signaling complexes including activated EGFR, ErbB3, p85 regulatory domain of PI3-K, and integrin α5 subunit (62). Presumably indirect interaction between EGFR or PDGF and integrins via a complex including activated FAK (N-terminus binding to RTKs), paxillin (binding to α4 integrin subunit), and talin (binding to integrin β1 subunit) has also been reported leading to activation of Erk1/2 (63). In cases that integrin signaling regulates the coupling between upstream and downstream components in the Ras/Raf-1/MEK/Erks cascade, the regulation steps were diverse, such as from Ras to Raf-1 (58), from Raf-1 to MEK (59), upstream of Raf-1 (64), association of RasGAP to RTK (i.e., upstream of Ras) (65). These differences may be due to differences in cell types and signaling contexts. These anchorage-dependent regulations of Ras/Raf-1/MEK/Erks cascade appear clearly dependent on actin-cytoskeleton, since disruption of the focal adhesions and stress fibers by cytochalasin D treatment did not affect Erk activation by growth factors (66). However, in the same study CDC42-dependent promotion of cortical actin assembly was shown important for the Erk activation, indicating that integrin-mediated cell adhesion and subsequent actin cytoskeleton organization can regulate upstream to downstream coupling of Ras/Raf-1/MEK/Erks intracellular signaling cascade. Another point of integrin-mediated regulation for RTK signaling is interesting. Recently it was reported that cell adhesion can regulate trafficking of activated Erk1/2 from cytosol to nucleus (60). Thus, in suspension cells, or in cells treated with cytochalasin D, the normal nucleus translocation of activated Erk is impossible because of disrupted cytoskeleton presumably acting as a traffic guide. Therefore, even enforced activation of Erk by using of active Raf-1 or MEK could not allow translocation of active Erk1/2 to nucleus and phosphorylation of its target, Elk-1 (60).

3.3. Integrin co-signaling with
TGF-β pathway

Integrin engagement has been shown to influence expression of cytokines and their receptors, especially TGF-β1 and its receptors. Transforming growth factor-β (TGF-β1) is a multifunctional cytokine to inhibit epithelial cell growth. It triggers intracellular signal transduction involving SMAD proteins to regulate numerous developmental and homeostatic processes via regulations in genes induction (67). Recently, TGF-β1-mediated, but SMAD-independent signaling pathways have also been evidenced in many model systems (68). For example, TGF-β1-mediated activation of p38 MAPK and JNKs resulted in increases in ECMs such as collagen and fibronectin levels, respectively (69,70). Similarly, in diverse model systems, expression profiles of integrins are susceptible to regulation by TGF-β1 (71), and integrin-mediated signaling also regulates the expression level of TGF-β1 (72). Overexpression of integrin α5β1 leads to upregulation of TGF-β1 receptor type II and growth inhibition (73), and conversely TGF-β1 treatment of NRK fibroblasts leads to upregulation of integrin α5β1 expression and a loss of anchorage-dependent growth (74). Similar to growth factor receptors or GPCRs, TGF-β1 receptor mediated signal pathway is also cooperative with integrin signaling. Recently, it was reported that TGF-β1 treatment to mammary epithelial cells resulted in epithelial-mesenchymal transdifferentiation (EMT) and this effects were dependent on both functional integrin β1 and activity of p38 MAPK (75). In addition, this author and colleagues found that TGF-β1 treatment to epithelial cells results in c-Src activity dependent cell adhesion and Erk activity (77), and cause morphological changes leading to activation of c-Src and small GTPases such as Racl and CDC42, resulting in preferential S-phase entry in a GTPase activity-dependent manner when cells were seeded on fibronectin (78).

4. CONCLUDING REMARKS.

The interaction of cells via cell adhesion receptors, integrins, to extracellular matrix proteins has profound effects on diverse cellular processes. These roles of integrins seem to be driven by the not only structural bridge between extracellular matrix proteins and intracellular actin cytoskeleton, but also signal transduction by integrin alone and/or collaboration with other receptors such as RTKs. During these roles, cytoskeleton and other signaling molecules such as small GTPases function very closely and most importantly. Since the cellular processes regulated by integrin and their signaling are involved diversely in cellular function, studies on integrin and their signaling may have critical impacts on understanding or development of reagent to cure human diseases such as cancers. So, as expected in other scientifically (more directly, in terms of cell signaling) developed countries, coming years can be fruitful for our domestic researches.

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