Integrin regulation of epidermal growth factor (EGF) receptor and of EGF-dependent responses

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Abstract

Integrin signalling co-ordinates with signalling originating from growth factor receptors in the co-operative control of cell proliferation, survival and migration. Increasing evidence suggests that integrins form physical complexes at the cell membrane with growth factor receptors, giving rise to signalling platforms at the adhesive sites. It is probable that at these sites integrins regulate adhesion and at the same time physically constrain and direct the response to soluble growth factors towards proliferation or survival stimuli. These co-operative effects might depend on integrin ability to activate growth factor receptors. In the present paper, we summarize our recent study showing that integrin-dependent adhesion triggers ligand-independent EGFR (epidermal growth factor receptor) activation to transduce downstream signalling. In addition, we also show that integrin-induced signalling pathways are necessary for EGF-dependent transcriptional response, demonstrating the requirement of the co-operation between cell-matrix adhesion and EGFR to achieve full biological responses.

Introduction

Integrins are adhesive receptors formed by α and β subunits, which anchor extracellular matrix proteins to the actin cytoskeleton. Integrins also trigger multiple signalling pathways, which, on the basis of differential expression and specific localization of the receptors, are involved in cell migration, proliferation, differentiation and survival from apoptosis [1]. Integrin signalling includes Ca2+ influx, cytoplasmic alkalinization, activation of potassium channels, tyrosine phosphorylation of cytoplasmic proteins and activation of the MAPKs (mitogen-activated protein kinases) [2–6].

To promote intracellular signalling, integrins interact with transducing molecules such as the Fak (focal adhesion kinase) and SRC kinase family. The N-terminal domain of Fak interacts with β1 and β3 integrins [7,8], whereas its C-terminal part binds SH2 (Src homology 2) and SH3 domains of several proteins involved in focal adhesion assembly and downstream signalling [9]. After integrin activation, Fak is phosphorylated on Tyr-925, which becomes an high-affinity binding site for the SH2 domain of c-Src. The Src kinase then phosphorylates focal adhesion components, such as the cytoskeletal adaptors talin, paxillin, p130Cas, and the Fak itself on the Tyr-925, leading to signalling functions [10,11].

Key words: adhesion, epidermal growth factor (EGF), extracellular matrix, growth factor receptor, integrin, signalling.

Abbreviations used: EGF(β), epidermal growth factor (receptor); Egr-1, early growth response gene product 1; ERK, extracellular-signal-regulated kinase; Fak, focal adhesion kinase; Grb2, growth-factor-receptor-bound protein 2; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; RPTK, receptor protein tyrosine kinase; SH, Src homology; VEGFR, vascular endothelial growth factor receptor.

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Phosphorylated Fak interacts with the adaptor molecule Grb2 (growth-factor-receptor-bound protein 2), leading to MAPK activation [12], through a B-Raf-dependent pathway [13]. In addition to Fak, some β1 and αv integrins activate the Src family member Fyn and the adaptor Shc (Src-homology collagen). The assembly of this transduction complex involves caveolin, a transmembrane protein that co-operates with integrins to activate signalling pathways. After cell-matrix adhesion, integrin–caveolin–Fyn complexes associate with phosphorylated Shc, which in turn, interacts with the Grb2–Sos complex leading to the activation of the Ras-MAPK cascade [14]. Integrins can also associate with proteins belonging to the tetraspan family (CD9, CD63 and CD81) to modulate intracellular signalling [15].

In addition to the pathways described above, growth factor receptors are integrin partners in assembling a transduction machinery. Increasing evidence indicates that integrins can co-operate with RPTKs (receptor protein tyrosine kinases) in transducing proliferative signals and in regulating survival and migration [4,5]. The molecular mechanisms involved in such events include integrin–dependent activation of RPTKs [16], enhancement of growth factor signals [17,18], recruitment of crucial transducing proteins to membrane cytoskeletal complexes [19] and enhancement of nuclear translocation of transcriptional regulators [20,21]. In the present paper, we will discuss the ability of integrins to induce EGFR (epidermal growth factor receptor) activation and to regulate the responses of EGFR to its ligand EGF.

Integrin cross-talk with RPTKs

Integrin–growth factor receptor co-operation has been extensively demonstrated [6], showing that integrins regulate
Integrin-dependent activation of RPTKs: the EGFR

Direct phosphorylation of growth factor receptors by integrin signalling represents a potential mechanism by which integrins can enhance signalling pathways emanating from growth factor receptors. It has been shown, in fact, that integrins stimulate phosphorylation, and at least partial activation, of several RPTKs, including the EGFR [16], HGF-R [27,28], PDGFR-R [29], Ron kinase [30] and VEGFR (vascular endothelial growth factor receptor) [31], in the absence of any growth factor ligand. The number of RPTKs involved suggests that this activation scheme can be a broadly used mechanism in adhesion-mediated signalling. In other words, cell-matrix adhesion represents a priming event, and a limiting factor in addition to soluble ligand for RPTKs activation.

Recently, we found that integrins induce EGFR tyrosine phosphorylation in the absence of EGFR ligands [16] (Figure 1A). Molecular mechanisms regulating integrin-dependent EGFR phosphorylation are distinct from the classical activation induced by mitogenic concentrations of soluble EGF. In the early phases of cell adhesion integrins associate with EGFR on the cell membrane in a macromolecular complex (Figure 1B), suggesting that a close proximity between integrins and RPTK could locally increase receptor densities and lead to oligomerization and transactivation. This simple mechanistic explanation is made complex by the fact that integrin-dependent EGFR phosphorylation requires Src kinase activity (Figure 1B), and the assembly of Src and the adaptor protein p130Cas in a multimeric complex with integrins and EGFR [32]. The requirement of Src kinase is in line with the mechanism proposed for the integrin-induced activation of Ron [30]. In this model, in fact, integrins activate Src tyrosine kinase, which in turn mediates Ron activation. A second feature of integrin-dependent EGFR activation is that it leads to phosphorylation of EGFR on a specific subset of tyrosine residues, only partially overlapping with those phosphorylated by EGF. After adhesion, EGFR is phosphorylated on Tyr-845, Tyr-1068, Tyr-1086 and Tyr-1173, but not on Tyr-1148, a major site of phosphorylation in response to EGF [32]. Interestingly, integrin-dependent phosphorylation of Tyr-845, which is located in the activation loop of the RPTK, could occur through Src kinase activity as a priming event for subsequent activation of the EGFR kinase activity, which in turn is required for phosphorylation of the other tyrosine residues.

As an alternative mechanism, integrins can cause the recruitment of tyrosine phosphatases such as SHP-2 at the plasma membrane in close proximity to the PDGF-R.
SHP-2 de-phosphorylates PDGF-R on specific tyrosine residues involved in Ras–GAP binding, thus decreasing Ras-GAP activation and potentiating Ras signalling [33], resulting in PDGF-R-downstream events, without involving the activation of the receptor tyrosine kinase.

By treatment with the specific EGFR inhibitor tyrphostin AG1478 and by expression of a dominant-negative EGFR mutant, lacking the intracellular C-terminal domain (EGFR/ΔC), we have shown that integrin-dependent EGFR activation leads to adhesion-dependent ERK1/ERK2 (extracellular-signal-regulated kinase) MAPK (Figure 1C, lower panels and [16]) and AKT activation (Figure 1C, upper panels). These results indicate that integrins can utilize EGFR as a transducing element in the matrix-induced signalling pathways. To strengthen further the relevance of integrin-induced EGFR activation, Marcoux and Vuori [34] have shown recently that it is instrumental for integrin-dependent Rac activation, through PI3K (phosphoinositide 3-kinase) activation and GTP loading on Vav2, a known exchange factor for Rac.

A third feature of integrin-dependent EGFR activation is a lower apparent stoichiometry, which leads to a lower level of tyrosine phosphorylation compared with that observed with mitogenic doses of EGF [16]. Phosphorylation of EGFR on a specific subset of tyrosine residues [32] could account for this event. According to this characteristic, integrin-induced EGFR activation is not sufficient for G1–S cell-cycle progression and proliferation [16], confirming that in untransformed cells anchorage-induced signalling is a key control step that co-operates with pathways activated by growth factors to induce cell proliferation.

On the other hand, integrin-dependent EGFR activation is sufficient for adhesion-dependent cell survival, by an EGFR kinase-dependent mechanism [16]. It is well defined that cells plated on the matrix proteins activates signals that protect cells from anoikis [35]. In cells expressing EGFR, the ability of cells to survive on fibronectin is blocked by the specific EGFR kinase inhibitor AG1478 or by the expression of the dominant-negative form EGFR/ΔC [16]. EGFR-mediated cell survival is blocked by the PI3K inhibitor wortmannin [16], suggesting that PI3K and AKT, well-defined players in adhesion-dependent survival to anoikis [35], are involved (see also Figure 1C). Integrin-EGFR signalling had also been recently implicated in the regulation of expression of the pro-apoptotic protein Bim (Bcl-2-interacting mediator of cell death), a critical mediator of anoikis in epithelial cells. Bim is strongly induced after cell detachment by concomitant lack of β1 integrin engagement, down-regulation of EGFR expression and inhibition of MAPK signalling [36]. The use of EGFR inhibitor AG1478 and of EGFR/ΔC also demonstrated that integrin-dependent EGFR activation plays a crucial role in lamellipodia formation, cell spreading and migration [34]. Therefore, taken together, these results show that after adhesion, EGFR transactivation accounts for a specific repertoire of mechanisms, namely cell survival and actin cytoskeleton organization involved in cell migration (Figure 2).

**Integrin requirement for propagation of EGFR-dependent signalling**

Integrins have been shown to potentiate signalling pathways in response to different growth factors, such as insulin, PDGF, EGF, FGF (fibroblast growth factor) and VEGF [17,37–45]. Many integrins form complexes with RPTKs and some appear to have preferred partners. For example, the αvβ3 integrin associates and synergizes with the insulin receptor, the PDGF-R and the VEGFR [38,41,45,46]. β1 integrins associate with the EGFR [17,47,48] and α6β4 combines with the EGFR [25], Erb-B2 [49] and the HGF-R [26]. Biochemical analysis revealed that αvβ3 integrin occupancy by its matrix ligand is required to get full tyrosine phosphorylation of insulin and PDGFβ receptors and their binding to signalling molecules, such as IRS-1 (insulin receptor substrate), PLCγ, Ras-GAP, p85-PI3K and the tyrosine phosphatases SHP-1 and SHP-2 [38,41,50–52]. In endothelial cells, moreover, αvβ3 integrin potentiates the activation of VEGFR and of p85-PI3K by its ligand [45].

Integrin-dependent adhesion is also required for EGF-dependent activation of downstream signalling [17,18]. To dissect integrin-dependent pathways in EGF responses, we treated epithelial ECV304 cells with EGF in two distinct conditions, either attached to matrix proteins or kept in suspension, and we analysed the pattern and the extent of downstream signalling pathways. As shown in Figure 3(A), EGF treatment induces phosphorylation of the EGFR both in cells kept in suspension and in cells attached to the matrix, indicating that the receptor is activated by its ligand in suspension or in adherent conditions. When the immunoprecipitates were analysed for Shc and Grb2 binding.
Integrins are required for EGF-dependent transcriptional regulation

ECV304 cells were kept in suspension (−) or allowed to adhere on fibronectin-coated dishes (+) with or without 50 ng/ml EGF for 30 min. (A) Cell extracts were immunoprecipitated with EGFR antibodies and the immunoprecipitates were separated on an SDS/6% polyacrylamide gel. Western blots were analysed with phosphotyrosine mAb PY20 and re-blotted with EGFR antibodies. (B) Cell extracts were separated on an SDS/10% polyacrylamide gel, and analysed with phospho ERK1/ERK2 antibodies (upper panel) and Egr-1 antibodies (lower panel).

Conclusions

In conclusion, integrins exert prominent signalling functions through their co-operations with RPTKs. In different cell types, their association with RPTKs provides positional cues to control RPTK function, allowing the recruitment of signalling molecules in the proximity of the receptors. They induce RPTK activation in the absence of growth factors, thus priming the RPTKs towards distinct adhesion-dependent biological processes, such as cell survival and migration. On the other hand, integrin signalling is essential for RPTK response to their specific ligands leading to control of transcriptional events and cell-cycle progression. Future studies will investigate the involvement of integrin/RPTKs co-operation in development and diseases, and will determine whether dysregulation of integrin/RPTKs cross-talk can contribute to the onset of pathological events.

References


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