Integrating with Integrins

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There has been great interest in the recent finding that integrins, the major family of proteins that mediate cell adhesion to extracellular matrix (ECM), also function as regulatory receptors that can activate intracellular chemical signaling pathways (reviewed in Damsky and Werb, 1992; Schwartz, 1992). In addition, integrins also physically associate with cytoskeletal proteins (reviewed in Burridge *et al.*, 1988), thereby providing a continuous path for mechanical force transfer between cells and their extracellular environment. These findings are exciting because they indicate that, by linking adhesion, mechanical forces, and intracellular chemical signaling pathways, integrins may provide a mechanism to integrate these different types of events. The purpose of this essay is to explore the implications of these concepts for development, physiology, and disease.

SIGNAL INTEGRATION

Cells in multicellular organisms make virtually all decisions regarding growth, gene expression, migration, etc. on the basis of input from their environment. Environmental stimuli include soluble hormones, transmitters or growth factors, direct cell-cell or cell-ECM contacts, and mechanical stresses resulting from local tension, compression, twisting, or fluid flow. In complex organisms, most cellular behaviors represent an efficient response to multiple simultaneous inputs. Cells must therefore have the ability to integrate these different types of signals.

Cell growth in vitro, for example, requires appropriate nutrients, mitogens, and contact with ECM molecules (Alberts *et al.*, 1989). In addition, most cells must physically extend themselves and spread on a rigid surface that resists cell tension to enter S phase (reviewed in Ingber, 1991). For many cells, certain soluble factors, ECM components, and mechanical stress conditions increase growth rates whereas others are inhibitory. For example, basic fibroblast growth factor (Ingber *et al.*, 1990), fibronectin (Ingber *et al.*, 1990), and turbulent fluid shear stress (Levesque *et al.*, 1990) stimulate endothelial cell growth, whereas transforming growth factor- β (Heimark *et al.*, 1986), thrombospondin (Tolsma *et al.*, 1993), and laminar fluid shear stress (Levesque *et al.*, 1990) inhibit. In vivo, growth factors can drive tissue development and remodeling, but both ECM molecules and the mechanical context in which they

are presented (i.e., the ability to resist cell-generated tension) dictate whether individual cells will grow, differentiate, or involute in response to these signals (Emerman and Pitelka, 1977; Ingber and Folkman, 1988, 1989; Opas, 1989; Mooney *et al.*, 1992; Massague, 1990; Talhouk *et al.*, 1992).

Development of specialized tissue forms in the embryo and subsequent maintenance of three dimensional tissue architecture similarly requires exquisite integration between hormonal, adhesive, and mechanical events. In fact, development of complex organisms depends upon relatively simple cellular behaviors, primarily adhesion, movement, cell shape changes, and gene expression. These events, however, must be tightly coordinated in space and time to generate complex structures (Alberts *et al.*, 1989). Clearly the ability of adhesion and mechanical forces to generate feedback that regulates gene expression is an essential element of these processes.

INTEGRATION OF CHEMICAL SIGNALS FROM SOLUBLE FACTORS AND ECM

Insight into the mechanism of ECM-dependent growth control has come from analyses of the chemical basis of integrin signaling. Recent studies suggest that chemical signals from integrins and growth factor receptors are rapidly integrated after cell surface receptor binding. For example, stimulation of inositol lipid turnover and subsequent signaling events in fibroblasts requires both platelet-derived growth factor (PDGF) and adhesion to ECM (Schwartz and Lechene, 1991; McNamee *et al.*, 1992). In suspended cells, PDGF binds its receptors and activates phospholipase C (PLC), yet there is minimal inositol lipid breakdown or release of IP_3 . Instead, cellular levels of 4,5 phosphatidylinositol bisphosphate (PIP_2), the substrate for PLC, decline when cells are placed in suspension and recover following reattachment to fibronectin (FN) (McNamee *et al.*, 1992). Thus, binding to integrins stimulates synthesis of a critical regulatory substrate (PIP_2), whereas growth factor receptors activate the enzyme that is responsible for its breakdown. These findings provide one possible explanation for why both soluble mitogens and insoluble matrix components are required for growth.

Synergism between adhesion- and soluble agonist-induced signals also has been observed in myeloid cells.

Adhesion of both neutrophils (Fuortes *et al.*, 1993) and mast cells (Hamawy *et al.*, 1993) to FN greatly enhanced stimulation of protein tyrosine phosphorylation by soluble agonists (tumor necrosis factor and IgG, respectively). Adhesion to FN alone or addition of soluble agonists to suspended cells induced only modest effects on protein tyrosine phosphorylation. However, addition of the same agonists to adherent cells resulted in large increases in phosphorylation. In neutrophils, addition of a tyrosine kinase inhibitor blocked the respiratory burst, suggesting that this increased phosphorylation may be responsible for the enhanced functional responsiveness of adherent cells (Fuortes *et al.*, 1993).

A third instance where the mechanism is less well understood concerns activation of the Na^+/H^+ antiporter and growth. In endothelial cells, both basic fibroblast growth factor (FGF) and adhesion to FN trigger elevation of intracellular pH (pHi) (Ingber *et al.*, 1990). Unlike the signal integration in fibroblasts, the effects of FGF and FN on pHi were found to be independent and additive. Nevertheless, when different doses and combinations of FGF and FN were tested, pHi (a measure of antiporter activation) always correlated with DNA synthesis. Thus, the results argue that these different stimuli are integrated early in the growth signaling cascade.

MECHANICAL SIGNALING

Soluble mitogens and ECM cues play key roles in morphogenesis, but mechanical stresses are also critical for determining tissue pattern. Examples include the effects of gravity on bone (Carter *et al.*, 1989; Duke *et al.*, 1990), tension on muscle (Vandeburgh *et al.*, 1991), and hemodynamic forces on blood vessels (Davies and Tripathi, 1993). These observations suggest that individual cells are able to sense and respond to mechanical stresses in their local microenvironment. How can cells integrate these different types of signals so that morphogenesis can proceed?

Cell spreading, stretching of the substratum, or pulling cells against their basal ECM adhesions (e.g., by apical shear stress) activates many of the same chemical signaling pathways as ligation of integrins and growth factor receptors (Ingber, 1991). This conversion of mechanical force into a chemical signal requires a transducer that fulfills two basic criteria: 1) it must physically interconnect with the cytoskeleton to sense stresses and strains, and 2) it must regulate biochemical signaling events. Integrins fulfill both these criteria. They connect the ECM and the cytoskeleton and thereby transmit contractile forces, for example, when cells spread on a substratum or contract a collagen gel (Schirotto *et al.*, 1991; Schmidt *et al.*, 1993). Integrins also can transfer external forces inward across the plasma membrane, as when application of mechanical stresses to integrins produce cytoskeletal stiffening (Wang *et al.*, 1993). As integrins

also regulate signaling pathways, they are therefore potential mechanotransducers. We therefore propose that mechanical forces could directly change the conformation or clustering of integrins, resulting in altered associations with cytoplasmic signaling molecules and subsequent activation or inhibition of chemical signaling pathways.

Alternatively (or additionally), integrins also influence organization of the cytoskeleton, which serves as a scaffold for many signaling proteins (Ingber, 1993). By altering the organization of the cytoskeleton, integrins could alter the behavior of cytoskeleton-associated signaling molecules, for example, by changing the proximity of immobilized enzymes and substrates. If these molecules also respond to adhesion and growth factors, signals would be integrated almost immediately at that level.

CANCER

The origin and behavior of cancer cells illustrates several kinds of connections between adhesion, chemical signals, and mechanical forces. Our present view of cancer centers on oncogenes and tumor suppressors, which are believed to be key steps in the regulatory pathways that are controlled by soluble growth factors and inhibitors (reviewed in Bishop, 1991). Activation of dominant oncogenes or inactivation of recessive tumor suppressors leads to irreversible activation of growth regulatory pathways and uncontrolled growth. Yet, in the clinic, cancer is identified not by changes in growth rates but by invasion and loss of cellular and tissue architecture (Robbins *et al.*, 1984; Ingber *et al.*, 1991). Growth of cancer cells is not only independent of serum or growth factors; it is also independent of adhesion to ECM (Tucker *et al.*, 1981) and apparently insensitive to the mechanical determinants that normally limit cell movement (Danowski and Harris, 1988), growth (Wittelsberger *et al.*, 1981), and tissue expansion (Ingber *et al.*, 1991).

Conversely, tumor cells are frequently less adhesive, with lower expression of at least one integrin ($\alpha_5\beta_1$) (Plantefaber and Hynes, 1989) and a less well-organized cytoskeleton (Burrage, 1986). Overexpression of integrin $\alpha_5\beta_1$ or of cytoskeletal proteins involved in cell-ECM adhesion enhances adhesion, restores cytoskeletal organization, and reduces tumorigenicity (Giancotti and Ruoslahti, 1990; Symington, 1990; Fernandez *et al.*, 1992; Gluck *et al.*, 1993). Several genes defined as tumor suppressors by virtue of their loss during tumor progression code for proteins that involve ECM (reviewed in Hedrick *et al.*, 1993). Thus, hypotheses based solely on oncogenes and growth factors clearly are insufficient to explain carcinogenesis.

Though much remains to be learned about the specific pathways and mechanisms of integrin-mediated signal transduction, the general concept that integrins trans-

duce signals required for cell growth allows us to propose a broader hypothesis. We suggest, first, that oncogenes and (classical) tumor suppressor genes irreversibly activate pathways controlled by integrins as well as by growth factors. Where growth factor- and integrin-regulated pathways intersect, a single oncogene could render a cell independent of both. Thus, growth independent of both anchorage and soluble mitogens can be induced by many transforming agents (Bishop, 1991). The *v-ras*, *src*, and polyoma middle T oncogene appear to activate integrin-mediated pathways independent of adhesion, in support of this view (Schwartz *et al.*, 1990; Guan and Shalloway, 1992). This hypothesis is also supported by the demonstration that synthesis of cyclin A during the G1 phase of the cell cycle depends upon adhesion and that overexpression of cyclin A allows cells to grow independent of anchorage (Guadagno *et al.*, 1993).

Second, we observe that loss of adhesion and disorganization of cell and tissue architecture are not immediate consequences of growth deregulation because of oncogene activation or tumor suppressor loss. Rapidly growing but benign tumors are well known. We propose instead that genetic changes that lead to irreversible activation of growth regulatory pathways are permissive for subsequent mutations in genes that are involved in cell-ECM adhesion, cytoskeletal structure, or possibly mechanical signaling. Once growth becomes independent of adhesion, selection for any one of a range of different phenotypes becomes possible, much as occurs when cell lines are cultured continuously and spontaneously transform in vitro. In the absence of the initial mutations in growth regulatory pathways, however, loss of normal adhesion and structure would result in a loss of cell viability and would therefore be selected against. Indeed, recent studies confirm that detachment of anchorage-dependent cells in vitro from their ECM induces apoptosis, and that inappropriate protein tyrosine phosphorylation induced by orthovanadate prevents cell death (Meredith *et al.*, 1993).

By contrast, once cells can survive and proliferate free of adhesion to ECM, selective pressures associated with changes in adhesion to ECM may accelerate disorganization of cell and tissue architecture. Loss of ECM contacts and normal cytoskeletal structure can stimulate growth of cancer cells, whereas restoration of ECM adhesions reverses these effects (Ingber *et al.*, 1981; Watanabe *et al.*, 1984; Giancotti and Ruoslahti, 1990; Symington, 1990; Gluck *et al.*, 1993). Similarly, expression of matrix-degrading proteases in normal tissues induces regression and apoptosis (Talhouk, 1992) but in cancer cells enhances invasion and metastasis (Dano *et al.*, 1985). Altered adhesive and motile properties that promote invasion or metastasis will enable the cells to escape the nutrient-depleted tumor cell mass, and colonize other, more favorable environments. This hypothesis is consistent with genetic analyses of tumors

that indicate that mutations in growth-regulatory genes can be detected in early precancerous lesions, whereas changes in putative adhesion molecules occur at later stages (Fearon and Vogelstein, 1990). Thus, the concept that integrins transduce growth regulatory signals allows us to begin to understand features of carcinogenesis that cannot be explained on the basis of growth factors, oncogenes, and tumor suppressors alone.

These ideas represent at most a framework that might help guide future experimentation. Understanding in detail how integrins signal, how these pathways control cell functions, and how different integrins mediate different cellular behaviors will be essential for understanding tumor progression. For example, tumor cells do not show a complete loss of adhesion but rather an alteration in integrin repertoire (Plantefaber and Hynes, 1989). Certain integrins are sometimes expressed at high levels in tumor cells and are thought to contribute to increased invasion or metastasis (Albeda *et al.*, 1990; Chan *et al.*, 1991; Felding-Habermann, 1992). These integrins may transduce distinct signals that give rise to distinct cellular behaviors. There is, in fact, direct evidence that $\alpha_5\beta_1$ and $\alpha_v\beta_3$ activate different signaling pathways (Schwartz and Denninghoff, 1994).

The role of cell-cell adhesion molecules in these processes is less clear. Cadherins are clearly important for tissue organization and have been suggested to function as a type of tumor suppressor, as their decreased expression or functional inactivation have been observed in tumors, and their overexpression leads to decreased tumorigenicity (reviewed in Takeichi, 1993). Cadherins also connect to the cytoskeleton and mediate mechanical force transfer between cells. There is no evidence, however, that cadherins directly regulate intracellular growth control pathways, or that cell-cell adhesion contributes to cell growth in a manner similar to cell-ECM adhesion. Cadherins are therefore likely to play different sorts of roles in tumor progression.

SUMMARY

Our central claim is that signaling by integrins provides a mechanism by which signals generated in response to adhesion, soluble hormones, and mechanical forces can interact. Such interactions permit cells to integrate these different classes of external stimuli and hence to orchestrate an efficient response. This integrating function of integrins is likely to be essential for much of development and physiology, as well as complex pathologies such as cancer. Understanding in detail how these signals are transduced and processed is likely to be an important area of research in the near future.

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