The winding road from adhesive receptors to the nucleus

Conference on molecular biology of cellular interactions: adhesion receptor signalling and regulation of gene expression

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EURESCO conferences on adhesive interactions were established in 1993 in the Life Sciences program of the European Science Foundation. Every 2 years, scientists involved in the different fields of adhesive interactions come for a 3-day meeting and exchange their latest results in a conference format of about 40 invited speakers and a poster session. This format promotes discussion and a fruitful exchange of results and ideas and is considered by 'old' and 'new' investigators in the field to be one of the most attractive European meetings on cell adhesion.

survival and development were the topics of the 2001 European Science Foundation Conference, organized by G. Tarone (Turin, Italy) and J.-L. Duband (Paris, France).

Adhesive receptors, which include integrins, cadherins, selectins and the immunoglobulin superfamily, have no catalytic function, and their interactions with other transducing molecules are crucial for signalling. Many examples of interactions of adhesive receptors with cellular effectors were presented, and the data generated from *in vitro* cell cultures were often corroborated in *in vivo* systems, designed to determine the contribution of each pathway to a given function. In this report, we highlight some of the salient themes that emerged.

Pathways leading to gene expression: lessons from global expression analyses

In the late 1980s, the pioneering work of S. Haskill (Eierman *et al.*, 1989) indicated that cell adhesion to the extracellular matrix (ECM) controls the expression of specific genes. It is now known that integrins are the main receptors through which cells respond to the ECM, and these are glycoprotein heterodimers consisting of one of 16 α -subunits and one of eight β -subunits. At this meeting, V. Kotliansky (Boston, MA) presented the results of a global expression analysis of the consequences of ECM–integrin interactions in the monocytic cell line THP-1. Integrin-dependent adhesion was shown to regulate a large number of genes, representing 2.7–4.4% of the expressed genome in THP-1 cells. Most of these are involved in the inflammatory and immune responses, confirming and extending the importance of ECM–integrin interactions in multiple aspects of monocyte biology. Adhesion to the ECM protein fibronectin primarily up-regulates genes under the control of the NF- κ B pathway, whereas the combination of fibronectin and growth factors also regulates

Introduction

Adhesive receptors are responsible for general tissue architecture and cell behaviour. They modulate mechanical adhesive interactions and regulate a cascade of intracellular signalling pathways (for reviews, see Giancotti and Ruoslahti, 1999; Gumbiner, 2000). In most cases, the end-point of the receptor-induced signalling cascade is the regulation of gene expression and corresponding modifications in cell metabolism, differentiation or proliferation. Adhesion receptor signalling, regulation of gene expression and their relevance to cell growth, differentiation,

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genes of the JAK/STAT transcriptional pathway. In general, adhesion to specific ECM proteins has been found to regulate distinct downstream genes, indicating that the degree of integrin-specific gene expression is considerable.

However, adhesion-dependent gene regulation is reliant not only on these two transcriptional pathways, but also on pathways such as the MAP kinases (MAPKs) and the β -catenin/LEF-TCF signal transduction systems (Figure 1), both of which were discussed extensively at the meeting.

Epithelial cells are unable to grow or survive when they lose contact with appropriate ECM proteins. However, transformation by the Ras oncoprotein bypasses the need for signals from adhesion receptors and protects them from undergoing apoptosis. Ras-dependent survival in other systems is known to have two effectors, PI 3-K and Raf, the latter of which functions by activating the ERK (a MAPK) pathway. To address the contribution of ERK, J. Downward (London, UK) used an inducible active form of Raf to monitor early changes in gene transcription that impart protection from detachment-induced apoptosis. Among the genes that are strongly up-regulated by activated Raf are the autocrine factors HB-EGF, amphiregulin and TGF α . All of these are members of the epithelial growth factor (EGF) family, indicating that protection by Raf is dependent on the function of an autocrine loop involving transcriptional induction of cell survival EGF-like factors. Interestingly, Raf-dependent survival is independent of its cell proliferation effects, even though MAPKs are known to regulate the cell cycle as well as cell death.

Like the contacts between cells and their substrates, intercellular adhesions also provide survival signals that are under the control of MAPKs. D. Ramarli (Verona, Italy) demonstrated that β 1 and β 4 integrin-mediated thymic epithelial cell adhesion to thymocytes induces expression of the survival factor IL-6. By using selective inhibitors, p38 and ERKs were shown to play crucial roles in β 4-induced activation of transcription factors (NF- κ B, NF-IL-6), necessary for IL-6 production.

To further define the contribution of the β 1 integrin cytoplasmic domain in cell survival, L. Barberis (Turin, Italy) used mouse embryonic fibroblasts (MEFs) expressing a knock-in gene for the β 1 subunit carrying a mutated cytoplasmic variable region. Homozygous embryos die 10.5 days after fertilization, and cells derived from these embryos show strongly impaired growth and increased apoptosis. Activation of signalling molecules such as AKT, p38, JNK and Rac is defective, and nuclear translocation of

ERK is inhibited, although it is phosphorylated. Overexpression of an activated form of Rac rescues cell proliferation and survival and promotes ERK nuclear translocation, though the mechanism whereby this occurs remains unknown. A. Aplin (Albany, NY) showed that translocation of ERK, but not of JNK, to the nucleus is sensitive to cell adhesion and disruption of the actin cytoskeleton. Here again, activated Rac could substitute for adhesion, whereas dominant active forms of Raf and MEK, the kinase which phosphorylates ERK, mimicked growth factor stimulation. As a result, active ERK is able to propagate signalling to the nucleus in non-adherent cells and to efficiently phosphorylate the transcription factor Elk-1.

Integrins can also directly bind to modulators of transcription. R. Pardi (Milan, Italy) reported that Jun activation domain binding protein 1 (JAB1), a coactivator of the c-Jun transcription factor, binds to the cytoplasmic domain of the β 2 integrin subunit. Integrin engagement leads to an increase in the nuclear pool of JAB1, enhanced binding of c-Jun-containing AP-1 complexes to DNA and increased transactivation of an AP-1-dependent promoter. JAB1 can be tyrosine phosphorylated by Src, and this event allows JAB1 translocation to the nucleus, where it can stabilize c-Jun and increase AP-1 activity.

A classical pathway of transcriptional regulation controlled by adhesive events relies on the ability of cell-cell interactions to modulate β -catenin/LEF-TCF signalling. β -catenin binds to the cytoplasmic domain of cadherins and transduces signals by its translocation to the nucleus and subsequent interaction with the LEF/TCF class of transcription factors. The versatility of β -catenin was highlighted by the discovery of its dual role in stem cells. W. Birchmeier and colleagues (Berlin, Germany) targeted β -catenin deficiency to the skin using the Cre/lox system, revealing that it first specifies hair follicle formation and subsequently is required for its maintenance. The phenotype obtained in the mutant mice, i.e. absence or loss of hair follicles without apparent epidermal cell adhesion defects, is quite distinct from that observed in mice in which α -catenin, another component of adherens junctions, is deleted in the skin. Targeted inactivation of β -catenin in endothelial cells, however, causes embryonic lethality, as does the deletion of VE-cadherin. In addition, A. Cattellino (Milan, Italy) reported that β -catenin inactivation does not affect the early events of vasculogenesis, but rather the later events of maturation. Remarkably, the reported differences of the more severe phenotype induced by targeted inactivation of VE-cadherin suggests that the effects of this adhesion molecule do not require signalling through β -catenin for the early stages of vascular development.

A role for β -catenin in cancer transformation has also been postulated. The abnormal accumulation of this protein is a characteristic of various types of cancer and may be due to defects in the adenomatous polyposis coli (APC) protein, in the β -catenin molecule itself, or in the p53 tumour suppressor (at later stages in tumour progression). A. Ben-Ze'ev (Rehovot, Israel) showed that deregulated β -catenin induces the accumulation of p53 through the induction of the tumour suppressor p19ARF, which binds to and blocks the Mdm2 protein (which is involved in p53 degradation by the proteasome system). The increase in p53 levels was also shown to be followed by the down-regulation of β -catenin by a mechanism requiring GSK3 β and involving β -TrCP for ubiquitylation and degradation. Thus, reciprocal regulation of β -catenin and p53 can be broken by selective pressure occurring in cancer cells where, in the absence of p19ARF or wild-type p53, the oncogenic potential of β -catenin is revealed (Figure 2).

Fig. 1. Pathways involved in adhesion-dependent gene regulation. A schematic representation of nuclear transcriptional pathways that control adhesion-dependent gene expression, leading to cell survival and control of cell proliferation.

Fig. 2. Multiple roles of the β -catenin/LEF-TCF system. A summary of the system's main activities that are reported in the text.

Alternative co-receptors and transducers

In addition to feeding into classical pathways for downstream signalling, this conference highlighted that adhesive receptors associate with transmembrane molecules and growth factor receptors to reciprocally modulate activities and downstream pathways (Figure 3).

Fibronectin is an ECM protein that binds $\alpha 5 \beta 1$ and $\alpha 4 \beta 1$ integrins. However, it also binds to the transmembrane proteoglycan syndecan-4, and it does so through its 13th type III repeat. G. Orend (Basel, Switzerland) showed that this fibronectin repeat also binds tenascin C, an adhesive ECM molecule highly expressed in the surrounding stroma of solid tumours. Tenascin-C binding to this repeat counteracts fibronectin adhesive and signalling functions by displacing it from syndecan-4. This results in enhanced tumour cell proliferation that can be neutralized by addition of the recombinant 13th type III repeat.

An alternative way of modulating cell adhesion is represented by tissue transglutaminase (tTG), a member of the transglutaminase family that connects fibronectin to $\beta 1$ and $\beta 3$ integrins. A. Belkin (Rockville, MD) showed that cell surface tTG is degraded by the matrix metalloprotease MT1-MMP at the leading edge of invasive glioma and fibrosarcoma cells. Fibronectin, presented either in soluble form or attached to a substrate, protected tTG

Fig. 3. Cross-talk between adhesive and growth factor receptors. Network of interactions among CD44, the growth factor receptors EGF-R and MET, integrins and tetraspanins described in the text. Each of these molecules can act by itself or in concert with the others. FN, fibronectin; tTG, tissue transglutaminase; TSP, tetraspanins.

from proteolysis, thereby supporting cell adhesion and locomotion. In contrast, collagen does not protect tTG from proteolysis and stimulates migration on collagen matrices, suggesting that the localized composition of the surrounding ECM may also control proteolysis and migration. tTG may, therefore, modulate integrin function in cancer cell adhesion and locomotion regulated by membrane-anchored MMPs.

Tetraspanins are a large family of transmembrane proteins that associate with other transmembrane proteins, such as integrins, to create a tetraspanin web. F. Berditchevski (Birmingham, UK) demonstrated that the minimal region required for the association of tetraspanin CD151 and $\alpha 3 \beta 1$ integrin maps within the large extracellular loop (LECL) of CD151. Recombinant soluble LECL of two distinct tetraspanins, CD151 and CD82, does not modify cell adhesion but affects specific integrin-dependent signalling, decreasing the levels of phosphorylation of the effectors p125Fak and AKT and suggesting that integrin-tetraspanin association can control integrin-dependent signalling.

Integrins are able to activate growth factor receptors in the absence of soluble ligands. P. Defilippi (Turin, Italy) showed that integrin-dependent adhesion leads to the assembly of a macromolecular complex containing integrins and the EGF receptor. The $\beta 1$ integrin cytoplasmic domain, the adaptor protein p130Cas and the c-Src kinase are required to trigger phosphorylation of specific tyrosine residues on the EGF receptor that are distinct from those whose phosphorylation is induced by EGF binding. Integrin-dependent EGF receptor activation sustains ERK and AKT activation and leads to cell survival, suggesting that, in cells expressing the EGF receptor, its activation represents a primary transducing pathway in integrin signalling.

Growth factor receptors can also cooperate with integrins to achieve full response to their ligand. L. Trusolino (Turin, Italy) reported that $\alpha 6 \beta 4$ integrin co-immunoprecipitates with the transmembrane tyrosine kinase MET (the HGF receptor) in a constitutive fashion and is required for MET-dependent responses. It is interesting to note that $\alpha 6 \beta 4$ integrin cooperates with MET independently of its adhesive function. MET activation results in phosphorylation of the $\beta 4$ subunit, which can recruit Shc and other effectors. Deletion of the Shc binding site on the $\beta 4$ subunit leads to a loss of HGF-dependent responses, suggesting that phosphorylation of this integrin subunit creates a scaffold for SH2 domains of signalling effectors.

V. Orion-Rousseau (Karlsruhe, Germany) reported that CD44 can also bind and control MET activity. CD44 isoforms containing the exon v6 can be co-immunoprecipitated with HGF and MET, and antibodies to CD44 block HGF-induced MET phosphorylation as well as scattering and invasion, suggesting that CD44-MET interactions could play a role in HGF-induced responses. This mode of action could account for the tumour-promoting activity of CD44 proteins.

Role of Src kinase in adhesive events

Src family members are key players in cell adhesion: following adhesion-dependent activation, Src phosphorylates important substrates involved in integrin signalling, such as the p125Fak kinase and the adaptor proteins paxillin and p130Cas. Its intracellular targeting has been defined by the work of M. Frame (Glasgow, UK), who showed that inactive Src has a peripheral distribution

but translocates to focal adhesions and associates with actin after integrin-dependent activation. The focal adhesion targeting of Src does not require kinase activity, but rather a functional SH3 domain that associates with the p85 subunit of phosphatidylinositol kinase. v-Src activation in integrin-dependent adhesion controls targeting of active ERK to focal adhesions, supporting a role for this MAPK in the regulation of the cytoskeletal/adhesion network and cell migration. In colon carcinoma cells, in which Src is up-regulated, p125Fak is highly phosphorylated on specific tyrosines by a Src-dependent mechanism, which is required for the combined role of these associated kinases during tumour development.

Organization of adhesive structures and signalling complexes

In addition to focal adhesions and focal contacts, other specialized cell membrane domains are likely to be involved in adhesion-dependent redistribution of integrin heterodimers and signal transducing molecules. F. Giancotti (New York, NY) reported that, whereas α v and β 1 integrin heterodimers are preferentially localized in a Triton X-100-soluble fraction containing focal complexes, caveolin, c-Src kinase and cytoskeletal components, α 6 β 4 integrin is enriched in a lipid raft fraction, in association with cholesterol and GPI-anchored molecules. Association with lipid rafts also coincides with the coupling of β 4 subunit with c-Fyn and c-Yes kinases. The cytoplasmic domain of the β 4 integrin subunit is palmitoylated, and β 4 palmitoylation-defective mutants fail to associate with lipid rafts and with c-Fyn and c-Yes, suggesting an important role for post-transcriptional processing and microdomains in integrin signalling.

Other specialized cell membrane structures in which β 4 integrin is functionally important are hemidesmosomes, which form major cell surface attachment sites for cell-substrate contacts. A. Sonnenberg (Amsterdam, The Netherlands) showed that, within these structures, α 6 β 4 integrin interacts with plectin, which directly links intermediate filaments to the cytoplasmic domain of the β 4 integrin subunit. The presence of proline residues in the β 4 cytoplasmic domain is required for mediating plectin binding through a putative SH3 domain localized on the plectin molecule.

Cell migration

Several protein complexes have been implicated in the control of actin filament dynamics. Green fluorescent protein (GFP) is a powerful tool with which to look more closely at the intracellular trafficking of vesicles to the leading edge of migrating cells. J.V. Small (Salzburg, Austria) and his collaborators expressed GFP-VASP (vasodilator-stimulated phosphoprotein), a member of the Ena/VASP protein family, and localized this protein at the leading edges in living cells. Localization was in direct proportion to the speed of protruding lamellipodia, suggesting a role in promoting actin assembly and, thus, protrusion. Similarly, Scar1/WAVE is specifically recruited to the tips of lamellipodia and not filopodia, whereas the Abl-interacting protein Abi-1 is localized to the tips of both extensions. I. de Curtis (Milan, Italy) described that one key molecule in this event is p95APP1, a multi-domain ArfGAP protein. The functionally split p95-N region containing GAP activity accumulates with defective

Fig. 4. Pathways involved in cell migration. Mechanisms regulating adhesion-dependent cell motility delineated at the meeting.

Arf6 in endocytosis vesicles. Similarly, the p95-C2 truncated protein, consisting of the ankyrin repeats and the paxillin binding domain, will also accumulate in endocytosis vesicles with the adapter protein paxillin. Overexpression of a dominant active Rac relocates p95-C2 to the leading edge of the cell, indicating that Rac functionally interacts with p95APP1.

Paxillin tyrosine phosphorylation is another way of regulating cell migration on collagen by providing binding sites for the SH2 domain of the Crk adapter protein. A.M. Vallés (Orsay, France) showed that the SH3 domain of Crk, in turn, forms a complex with DOCK180, which functionally interacts with Rac. Thus, one common theme that comes from studying dynamic processes like cell migration is the formation of multicomplexes that continually recycle from the plasma membrane and that these complexes consist of structural as well as regulatory proteins (Figure 4).

In addition to the described role in cell migration, paxillin participates in the redistribution of cellular components. As described by D. Critchley (Leicester, UK), paxillin binds with high affinity to poly(A)-binding protein 1 (PABP-1), a 70-kDa protein known to bind to polyadenylated mRNAs. PABP-1 shuttles from the nucleus to the cytoplasm, where it co-localizes with paxillin in the endoplasmic reticulum and at the leading edge of migrating cells. Interestingly, leptomycin B, which inhibits nuclear export of PABP-1, leads to the accumulation of paxillin in the nucleus. This suggests a new mechanism whereby a paxillin/PABP-1 complex facilitates transport of mRNA from the nucleus to sites of protein synthesis at the endoplasmic reticulum and the leading lamella.

Rho family GTPases in epithelial cell adhesion

The Rho family of GTPases is known to regulate cadherin-mediated cell-cell adhesion. In particular, a balance between Rac and Rho controls the epithelial mesenchymal transition, a crucial event in tissue organization and tumour cell dissemination. Ezrin is a member of the ERM family of proteins that link the plasma membrane to the actin cytoskeleton. D. Louvard (Paris, France) showed that, in epithelial cells, the expression of the T567D ezrin mutant, which mimics a phosphorylated and active conformation, impaired intercellular junctions. This effect was accompanied by the activation of the small GTPase Rac and by

a decrease in the E-cadherin pool at the plasma membrane, suggesting that ezrin may regulate assembly/disassembly of adherens junctions through activation of Rac.

J. Collard (Amsterdam, The Netherlands) presented data on the Rac-specific exchange factor Tiam-1, a key molecule in the regulation of the epithelial state. Epithelial cells transformed by oncogenic RasV12 suppress Tiam-1 expression and Rac activity, up-regulating Rho and leading to an invasive and mesenchymal-like phenotype. Skin carcinogenesis on mice deficient for Tiam-1 leads to a lower number of benign papillomas than in wild-type mice. In contrast, progression to invasive spindle carcinoma was more elevated in mutant mice, supporting a model in which Tiam-1 and Rac are required for tumour initiation and promotion, and their inactivation favours progression to a more invasive phenotype.

Cell adhesion and the control of morphogenesis

Inductive signals also regulate adhesive events during the migration of neural crest cells (NCCs). This population of cells exists in the neuroectoderm, where they are specified to migrate along defined pathways after stimulation by inductive signals. J.-L. Duband (Paris, France) presented *in vitro* data showing that Sonic hedgehog (Shh), a member of the hedgehog family of signalling molecules expressed by the notochord and the floor plate, negatively regulates avian NCC adhesion and migration on fibronectin. In accordance, ectopic injection of Shh into the chick neural tube causes accumulation of NCCs, due to inhibition of cell migration. Interestingly, Shh does not affect the identity nor the proliferation capacity of NCC, indicating that the novel function of Shh on cell adhesion is independent of its inductive and mitogenic roles.

The mammary gland is a favourable model system to study how cell–cell and cell–ECM interactions influence morphogenesis and differentiation processes. C. Streuli (Manchester, UK) reported that desmosomal intercellular adhesions are as important as E-cadherins for mammary epithelial cell morphogenesis. In an *in vitro* assay of morphogenesis, the matrix-dependent formation of spherical alveolar structures of luminal cells is prevented by the addition of CAR peptides corresponding to the binding sites of the luminal desmosomal cadherins Dsc2 and Dsg2. Moreover, the normal cell sorting of these two populations is perturbed by CAR inhibitory peptides, indicating that, besides their known role in maintenance of epithelia, desmosomes participate in the organization and polarity of the multilayer tissue.

Conclusions

In this report, we have underlined how much the adhesion field has moved towards exploring the details of signalling pathways

that lie downstream of specific adhesion receptors. A decade ago, the idea that adhesion molecules might also have a signalling function was almost heretical. Now, the data, both *in vivo* and *in vitro*, indicate that every adhesion molecule is involved in signalling, and it is likely that this concept will be extended and clarified in the near future. As shown by N. Brown (Cambridge, UK), the completion of the sequencing of the *Drosophila* genome now allows the rapid identification of genes encoding *Drosophila* orthologues of vertebrate integrins and integrin-related signalling molecules and the application of a reverse genetic approach to the study of their biological role. This type of genetic analysis will eventually provide a complete description of the chain of molecules required to connect the ECM, the cytoskeleton and the signalling pathways towards the nucleus.

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Paola Defilippi & Ana M. Vallés

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