CELL MATRIX INTERACTIONS

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Cellular adhesion to other cells or to extracellular matrix (ECM) components is a key event in a number of biological processes such as: (1) cell growth & proliferation; (2) differentiation; (3) morphogenesis; (4) cellular immunity; (5) inflammation; (6) invasion and metastasis.

Despite this abundance, the number of known molecules participating in such processes is at the present time, relatively small. Thus, by employing a few selected elements, interacting each in a small range of different ways, nature has been able to achieve enormous phenotype and behavioral diversity. That is to say, the intrinsic nature of biological phenomena is simple, even though they might appear complex.

Essentially, cellular interactions can be divided into homophilic and heterophilic in which the chemical nature of the interacting molecules is identical or not, respectively; obviously homophilic interactions can occur only between cells, which display at their surfaces the same ligands. These are more commonly known as CAMS or cadherins and again can be divided into $Ca^2 + -$ dependent or $Ca^2 + -$ independent CAMS (Edelman, 1987).

Heterophilic interactions may take place both between cells or with ECM components. The cell receptors involved in such interactions are members of an ever increasing family of molecules called the *integrins*.

Integrins are heterodimers composed of an α chain which always contains a $Ca^2 + -$ binding domain since such interactions are always $Ca^2 + -$ dependent, a transmembrane domain and a cytoplasmic domain. The β chain also contains a transmembrane domain and a cytoplasmic domain (Ruoslahti, 1988). Though the latter displays a tyrosine kinase domain, at least for the β_1 integrin chain, it is not clear that docking of the ligand triggers alterations of its phosphorylated state. Studies suggestive of this (Hirst et al., 1986) have not been confirmed. Rather independent studies have indicated that empty receptors have a serine phosphorylated instead and that it is dephosphorylated by docking of fibronectin (FN) (Duband et al., 1988).

The retinoic acid-induced differentiation of F9 teratocarcinoma cells is accompanied by the accumulation of fibrillar fibronectin deposits, the appearance of a highly structured actin cytoskeleton and the localization of integrin at sites of substrate contact. Integrin is phosphorylated at a cytoplasmic domain serine residue very close to a previously describe site and its level of phosphorylation is drastically reduced at a time coinciding with the above changes (Dahl & Grabel, 1989).

Integrins may be subclassified by the different β chains but it is known by now that not only different α chain interact with the same β chain but also that the same α chain can interact with different β chains (Ruoslahti & Giancotti, 1989).

Figure 1 depicts some properties of the main ECM components as well as their respective integrin receptors.

It is possible that the specificity towards given ligands is determined by different assembly patterns between α and β integrin chains, but on the other hand, cross linking experiments (Santoro & Lawing, 1987) as well as photo affinity labeling (Parise & Phillips, 1986) have indicated that the site for fibronectin binding lies on the β_1 chain.

Employing the complementary hydropathy concept (Brentani et al., 1988) we have been able to show that a peptide which inhibits cell adhesion to fibronectin and GPIIbIIIa binding to the latter's 120 kd fragment which contains the RGD cell binding peptide, mimics a peptide present both in the β_1 and β_3 chains. Antibodies raised against this peptide were found to be able to bind in Western blots to integrin β_1 and β_3 chains (Pasqualini et al., 1989).

If however one looks at the integrins listed in figure 1, one sees that both laminin and fibronectin receptors are β_1 integrins. Thus, apparently ligand specificity is conferred by association of β_1 chains to different α chains. If, as mentioned above, the ligand binding site resides in the β_1 chain then it follows that such an association to differ-

	EGF	Ca²⁺	FN	RGD	Integrin Receptors
Fibronectin (FN)	—	+	+	+	α3β1,α4β1, α5β1 αIIbβ3, αVβ5
Laminin (LN)	+(B₁)	+(B₂)	—	+(A)	α1β1, α2β1, α3β1 α6β1, α6β4
Tenascin (TN)	+	?	+	+	α/β heterodimer *
Thrombospondin (TS)	+	+	+	+	αVβ3

*under characterization

Major extracellular matrix macromolecular components and their respective cell-surface integrin receptors.

ent α chains must induce different conformations of the same β_1 chain. Indeed, chromatography of B16F10 melanoma cell extracts on laminin sepharose columns leads to isolation of a fibronectin binding integrin which contains a β_1 chain characterized as such by its reactivity towards an anti- β_1 antibody. In the eluate, the LN-binding integrin, likewise contains a β_1 integrin.

I shall now describe the participation of the molecules that were described above in the biological processes that were outlined at the beginning of this review.

CELL GROWTH AND PROLIFERATION

It has been known for a number of years that normal cell proliferation is contingent upon adhesion to solid surfaces, a property which has been called "anchorage dependence". Growing normally adherent 3T3 cells in suspension causes them to enter a G_0 state of growth arrest characterized by inhibition of DNA synthesis and suppression of c-myc and histone mRNA expression. Even in the absence of serum, that is of growth factors, reattachment to fibronectin-coated surfaces leads to cell

spreading and synthesis of c-myc, c-fos and actin (Dike & Former, 1988). Otsuka & Moskowitz (cited in Cell 44: 489, 1986), on the other hand, report arrest in G_1. FN was able to act as a growth factor for fibroblasts grown on collagen gels (Bitterman et al., 1983).

The growth-promoting effect of laminin has also been detected in a number of cellular models (Panayotou et al., 1989). Its effect has been pinpointed to the regions of the component peptides which harbor the EGF-like domains. Likewise, thrombospondin (TS) has been shown to display a cell proliferation effect on smooth muscle cell growth (Majack et al., 1986; 1988). The rapid induction of TS by PDGF is suggestive of its participation in the "competence" gene family. TS promotes facilitation of the EGF effect on such cells. Heparin, on the other hand, is a known inhibitor of smooth cell growth and apparently it may act by reducing the incorporation of TS into the ECM (Majack et al., 1986).

Tenascin (TN) has also been shown to be the most effective ECM component in promoting growth of mammary tumor cells (Chiquet-Ehrismann, 1986).

It is worth noticing that, like laminin, as discussed above, TS and TN also display numerous EGF repeats (Fig. 1) and it is tempting to speculate that likewise these are the active domains in the context of growth promoting activity. It has been shown that TGF-α, a peptide related to EGF is cleaved from a conserved integral membrane glycoprotein. Such a cleavage may resemble the processing of EGF and the related vaccinia virus growth factor.

It has further been shown that the uncleaved TGF-α precursor even in the absence of processing can bind to the EGF receptor on other cells leading to signal transduction (Wong et al., 1989). One could then imagine a novel mechanism for cell-cell adhesion. On the other hand, one could see, in this way, the consequences of cell adhesion to the above mentioned EGF-like containing ECM molecules. Another independent role of ECM components modulating cell proliferation could be as a deposit of growth factors. It has been shown, for example, that bFGF synthesized by endothelial cells is deposited in the subendothelial ECM (Vlodovsky et al., 1987).

DIFFERENTIATION

Stable, prolonged hepatocyte proliferation *in vitro* is dependent on the use of a serum-free, hormonally defined medium and extracellular matrix-derived substrate such as type I collagen gels (Enat et al., 1984). On the other hand, expression of genes characterizing an hepatic phenotype such as albumin, α_1-inhibitor III and α_1 antitrypsin is dependent upon a higher cell density, leading to increased cell-cell contacts or preferably, to the presence of an underlying gel matrix derived from the EHS tumor, which makes basement membranes (Ben-Ze'ev et al., 1988).

Likewise, rat granulosa cells from preovulatory follicles, when growing upon an endothelial cell-derived ECM displayed an epithelial shape, formed multilayered aggregates and established numerous gap junctions between neighboring cells. Cytoskeletal proteins such as vinculin, α-actinin and actin were greatly decreased and production of progesterone greatly increased in comparison with cells grown on plastic (Ben-Ze'ev & Amsterdam, 1986). Initiation of the terminal stages of myogenic differentiation in chicken embryos has been shown to be dependent on the interaction between a β_1 integrin and the ECM (Menuko & Boettiger, 1987).

The degree of polysialic acid has been proven

to be critical since functional N-CAM has to be undersialylated (Acheson & Rutishauer, 1988).

Differentiation is strongly affected by a family of polypeptide factors of which TGF-β is the prototype (reviewed in Massagué, 1987). All members of the family, while presenting hardly any growth-promoting activity, heavily altered the expression of specific phenotypes by cells with differentiating potential.

While the exact molecular mechanisms of such activity are still unknown, it is clear that TGF-β strongly induces synthesis of tenascin/citotactin (Pearson et al., 1988), integrins that share the β_1 subunit (Reino et al., 1989), as well as members of the β_2 (LFA-1) and β_3 (fibronectin receptors) subfamilies (Ignotz et al., 1989); and the ECM components collagen, fibronectin (Ignotz & Massagué, 1986) and thrombospondin (Penttinen et al., 1988). A nuclear factor 1 binding site mediates the transcriptional activation of the collagens type I and III promoters by TGF-β (Rossi et al., 1988).

One must finally comment that steady state levels of mRNAS coding for type IV collagens and laminin chains exibit tissue specific variations.

Such a mechanism, coupled to differential splicing like that of the fibronectin gene (Kornblith et al., 1985) may lead to cell-or tissue specific ECMs which would lead in turn to specific interactions typical of a given differentiated state.

MORPHOGENESIS

As pointed out by Edelman (1984) morphogenesis poses the riddle of how to convert a one-dimensional genetic code into the three-dimensional shape of living creatures. To approach this, one must identify the signals that lead to morphogenetic motion which will approximate the appropriate cells leading them to interact through the activity of specific molecules. Some of the involved molecules have been discussed above, as to their chemical nature and to further our understanding we can localize them in so-called "fate maps" which specify in two dimensions, which cells will lead, at a later point in development, to particular organs and structures.

N-CAM is predominantly found in the neural plate, notochord, somites and some lateral plate mesodermal derivatives as heart and smooth muscle.

It is worth noticing that the heart and smooth muscle N-CAMS differ from those in nervous tissues by a short insert specified by 4 exons present in the gene but which are spliced out in the latter N-CAMS (Predigen et al., 1988). There is a cephalo-caudal decreasing N-CAM gradient; furthermore, whereas the cephalic region will express N-CAM throughout the whole lifetime, in the notochord first there is no N-CAM, then it is expressed and then it disappears after neurulation. Simultaneously with the loss of N-CAM these cells migrate through fibronectin-rich regions.

L-CAM on the other hand, is contained by ectodermal and endodermal derivatives. Kidney development is a fine example of coordinate expression of CAMs. L-CAM first appears in the inductor tissue or Wolffian duct; N-CAM appears in mesonephric mesenchyne as it organizes into tubules and is replaced by L-CAM as such tubules extend (Edelman, 1984).

Epithelial-to-mesenchymal transitions seem therefore correlated with CAM expression variations. Cytotactin/tenascin seems to be involved in such interactions and is expressed at specific locations in the developing embryo (Crossin et al., 1986) mediating neuron-glia interactions (Grumet et al., 1985) and epithelial-mesenchyme interactions during gut development (Aufderheide et al., 1988).

During kidney development the epithelization of non-polarized mesenchymal stem cells is dependent upon laminin, in particular of its A chain (Klein et al., 1988).

Although the exact nature of the signals that trigger the expression of adhesion molecules which will in turn govern morphogenetic movements still eludes us, one should point out that with the aid of the polymerase chain reaction, PDGF, TGF-α and TGF-β were found to be expressed already in the 16-cell pre-implantation embryo (Rappolee et al., 1988).

The role of sugar in morphogenesis — N-CAM is a glycoprotein characterized by the presence of an unusual carbohydrate, α polysialic acid which is abundant in the embryonic form (E) but decreased in the adult N-CAM (A). Homophilic N-CAM binding is inhibited by this sugar and consequently A to A binding is much stronger (Edelman, 1987).

· One of the ligands of cytotactin is a proteoglycan which is initially diffused throughout the sclero-

tome but subsequently restricted to the caudal half after the appearance of cytotactin and invasion of neural crest cells in the rostral half. Both molecules are effective in altering migration on fibronectin (Tan et al., 1987).

CELLULAR IMMUNITY

In order to mount an effective immune response, lymphocytes must interact with antigen specific surface receptors. Since the repertoire of antigens is very large, cells displaying receptors specific for any antigen are rare. To promote such interactions, vertebrates have evolved lymphoid organs, in which antigens are received, processed by accessory cells and presented to lymphocytes in order to promote the immune response. B lymphocytes, regardless of their origin home preferentially to Peyer patches, through the interaction with their high endothelial venules (HEV), whereas T lymphocytes home preferentially to peripheral lymph node (Gallatin et al., 1986). The latter's receptor for T cells, called the MEL-14 antigen, is an ubiquitinated glycoprotein (St. John et al., 1986). In contrast, it has been shown that the Peyer patch receptor is an integrin comprised by the same α 4 chain present in VLA-4 and a specific β (p) chain which must be related to the β_1 chain since the chain α of the receptor is also able to interact with β (Holzmann et al., 1989). The respective ligands at the surface of lymphocytes are still unknown.

Within the immune context, cell-cell interactions occur between helper and killer T cell subsets and their respective targets. Such an interaction is always mediated by LFA-1, a member of the integrin family, expressed at the surface of the effector cells and ICAM-1, expressed on target cells (Springer et al., 1987). Expression of ICAM-1 can be stimulated several-fold by either IL-1 or interferon (Dustin et al., 1986). Antibodies that block this interaction, through reactions with either LFA-1 or ICAM-1 can inhibit widely differing activities such as T-cell-mediated B-cell activation, cytotoxic cell function and immunoglobulin production (Boyd et al., 1988).

In auto immune diseases, a common complication is renal involvement. This was thought to be in part the consequence of deposition of immune complexes mostly involving anti DNA auto antibodies. We have recently shown however (Sabbaga et al., 1989) that a mouse monoclonal anti double stranded DNA auto antibody isolated from lup-

ic mice cross reacts specifically with mouse laminin. Thus, it appears that the renal picture could be due, at least in part, to specific reactions between antibodies and renal basement membrane laminin. We have since shown that in lupic mice monoclonal antibodies can be obtained which react only with laminin. Furthermore, fusing spleens from mice of ages varying between newborn and six months an increasing proportion of exclusively anti laminin antibodies was found (Sabbàga et al., unpublished data).

The role of sugars in cellular immunity — Sequencing of the MEL-14 antigen has provided evidence that it is a lectin. In fact, there is a domain which displays considerable homology with a number of animal lectins which share a consensus motif for carbohydrate binding such as rat mannose binding proteins A and C, human mannose binding protein, canine pulmonary surfactant, rat asyaloglycoprotein receptor and the human F_c epsilon receptor (Siegelman et al., 1989). Furthermore, competition experiments have pointed to the importance of sialylation of HEV cells (Rosen et al., 1985). Other studies have indicated that the MEL-14 lectin antigen recognizes a ligand composed at least in part by mannose-6-phosphate derivatives (Gallatin et al., 1986).

INFLAMMATION

Answering chemotactic signals which are produced and liberated by endothelial cells from target areas, white blood cells proceed to such areas and their initial access, for exit from the circulation relies on cell-cell adhesion between these cells and the endothelial layer. Such an interaction is mediated, on the endothelial side, by the expression of a surface glycoprotein called ELAM-1 (endothelial-leukocyte adhesion molecule) wchich is inducible by cytokines such as IL-1, TNF and endotoxin like ICAM-1, but its induction is more rapid and transient than that of the latter. The primary sequence of cloned ELAM-1 shows an aminoterminal lectin-like domain, an EGF-like domain and six repeats of a motif also found in complement regulatory proteins like complement receptors CR1 and CR2, decay accelerating factor (DAF) and membrane cofactor protein (MCP) (Bevilacqua et al., 1989). The great degree of structural homology between this protein and the MEL-14 antigen suggests the existence of a new family of adhesion molecules (Marx, 1989).

A disease called leukocyte adhesion defficien-

cy has been described and is characterized by recurrent life-threatening bacterial and fungal infections, progressive periodontitis, lack of pus formation and leukocytosis. It has been shown that severity of the clinical complication correlates with the degree of LFA-1 defficiency. Such a defficiency, as well as those of Mac-1 and p150,95 seem due to a defect in the common β_2 subunit. Apparently normal levels of α chains are synthesized but little is processed or transported to the cell surface. Mouse x human hybrids show surface expression of hybrid complexes (Springer et al., 1987).

Since antibodies against LFA-1, Mac-1 and p150,95 inhibit partially the adhesion of polymorphonuclear to endothelial cells (Pohlman et al., 1986) it is possible that such ligands interact directly with ELAM-1 but formal proof of this association is still lacking.

INVASION AND METASTASIS

Metastatic dissemination occurs through a multistep pathway which has been recently reviewed (Brentani et al., 1988; Brentani, 1989).

Arrest can take place through at least two different ways: cell to ECM and cell to cell adhesion. It seems quite clear that in the first case, the interaction between laminin and specific cell receptors (LNR) is important. We have been able to show that interaction with the LNP_1 domain correlates with metastatic potential whereas the E8-mediated interaction seems of little importance. Thus, EJ rastrasfected 3T3 cells, which are metastatic display increased numbers of P1 receptors. Furthermore, the anti S aureus LNR Mab, which cross-reacts with the 68kD mammalian receptor (Mota et al., 1988) inhibits their metastatic capacity in a dose-dependent fashion (Brentani et al., 1989), through a mechanism unrelated to complement-mediated cytotoxicity. A direct association has been observed between Mab positivity in a series of 238 human breast cancer specimens and both survival and disease-free interval (Marques et al., 1990). This can be explained by assuming that receptor occupancy correlates with metastatic potential. Since our Mab recognizes the binding site (Brentani et al., 1987) positivity reflects the presence of empty sites; then the good prognosis. It is worth mentioning that stromal tenascin expression has been considered as a marker for epithelial malignancy (Marckie et al., 1987) and that in Cystosarcoma phyllodes, a benign stromal tumor, recurrence has been associated to CEA expression by the other-

wise normal mammary epithelium (Alberti et al., 1986).

These markers are indicative of mesenchyme-epithelial traffic during mammary gland carcinogenesis.

Acquisition of metastatic potential by otherwise "benign" cancer cells has been attributed in some cases to spontaneous cell fusion to host macrophages (Larizza et al., 1984). This could be mediated by the interaction of LFA-1 on the leukocytes and ICAM-1 on human melanomas, since it has been shown that expression of ICAM-1 on the latter cells correlates with an increased risk of metastasis (Johnson, 1989). Similarily, polysialylated N-CAM (ICAM-1) is re-expressed in Wilm's tumor (Roth et al., 1988). In this context one must point out that both sialylation (Collard et al., 1986; Ishikawa et al., 1988; Passaniti & Hart, 1988) and expression of asparagine-linked β 1-6 branched oligosaccharides (Denis et al., 1987) are directly correlated with metastatic potential. Fucosylation of surface elements, on the other hand (Finne et al., 1989) seems inversely correlated. Another step worthy of consideration is the enzymatic dissolution of barriers to host tissue invasion by tumor cells. Several enzymes have been shown to play pivotal roles in this context. Among them is plasminogen activator, an enzyme induced by estrogen and thyroxine. We have shown that there is in fact a direct correlation between levels of tPA and ER in human breast cancer (Pacheco et al., 1988). The enzyme can also be induced by TGF-β (Keski-Oja et al., 1988). Interestingly, cells grown on a LN-coated substratum express increased levels of t-PA (Pourreau-Schneider et al., 1989).

Other enzymes also involved in metastatic dissemination are cathepsin L, the main inducible protein by the *ras* mutation (Joseph et al., 1987) and collagenases (Tryggvason et al., 1987). It is interesting to note that several tissues normally express a gene coding for a tissue inhibitor of metalloproteinases (TIMP) which has been cloned and shown to display also an erythroid-potentiating activity. Recombinant TIMP has been shown to inhibit *in vitro* human amnion invasion and lung colonization of B16-F10 (Schultz et al., 1988). Transfection of Swiss 3T3 cells with a plasmid coding for antisense RNA leads to a reduction in TIMP levels and to the acquisition of oncogenicity (Khokha et al., 1989). We are currently applying the principle of complementary hydropathy to the determination of the enzyme's active site, in the hope of developing useful antibodies and inhibitors.

The molecular basis of the homing mechanism — While abundant clinical and experimental evidence have lent strong support for the "seed and soil" hypothesis, we still know very little about the molecular mechanisms involved. The knowledge obtained concerning lymphocyte homing which was discussed above of course is important and might be extrapolated to cancer cells. Auerbach (Auerbach et al., 1987) has provided evidence in support of interactions between specific tumor cells and regional endothelial cells but no information is currently available concerning the molecular events involved.

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