Surface molecular recognition

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The spatial display of cellular ligands and receptors is important for cell adhesion and communication. Two approaches that emphasize developing selective methods to dissect, modify, and control receptor–ligand interactions at the cellular interface are discussed.

Cells display on their surface a complex mixture of receptors and ligands that mediates cell adhesion and cell communication. Receptors and ligands are protein molecules that clasp one another, much like a hook and an eye. Receptors are typically integral to the cell membrane: part of the molecule is confined to the lipid bilayer, the remainder extends beyond the membrane inside and outside of the cell. Ligands may be integral to the membrane of another cell, part of the matrix outside the cell, or freely diffusing in the medium outside the cell. Moreover, these receptor and ligand proteins are frequently glycosylated; that is, sugar polymers are covalently linked to the proteins. Depending on the receptor and the ligand, the protein amino acids, sugars, or both, may be the functional moieties involved in cell adhesion and communication.

The milieu outside a cell is quite complex. It consists of insoluble proteins and glycosaminoglycans that form a matrix, a variety of cells of different types, and small water-soluble molecules. The cell surface–extracellular milieu interactions are analogous to complex cellular Velcro. Cells attach to the matrix and to each other to form the structure of a tissue. The ligands presented by the milieu can provide an address code for a cell to adhere or to migrate, or a signal for a cell to grow, undergo alterations, or die. The signals from the ligated receptor may be transmitted to the nucleus to activate transcription. Alternatively, a receptor ligated to the extracellular matrix may provide a direct link to the cytoskeleton. The cell skeleton of the cell, much like the skeleton of an organism, provides structural integrity and dictates the shape of the cell. For example, the $\alpha_5\beta_1$ integrin receptor connects the extracellular matrix protein fibronectin to the intracellular molecules talin and vinculin that, in turn, latch onto actin. In a third scenario, ligands presented by a cell recruit a second cell type into the tissue. This type of recruitment is the basis for the immune system to function. For example, lymphocytes are recruited from the circulatory system to initiate wound repair. Moreover, cellular receptors may provide the means for alien invasion by bacteria, viruses, or metastatic spread of cancerous tumors. Many different receptors are present on the surface of a single cell. Likewise, a large variety of ligands are displayed in the cellular milieu. In fact, one individual matrix molecule may present different ligands to the cell surface.

The question addressed was how to study these elaborate, heterogeneous systems at the molecular level. The complexity of ligand presentation and the diversity of receptors on the cell surface make it difficult to dissect the individual roles of each receptor–ligand pair. In addition, it would be advantageous to target specific unique receptors or ligands in a single cell type. Crossover between classical areas of research has led to the development of new methods to study and/or modify the role of a receptor–ligand pair by displaying a two-dimensional array of the receptor or ligand, i.e., mimic the cell-surface display. This spatial display is important for function because generally more than one pair is required for adhesion or communication, much like the hooks and eyes of Velcro.

Discussed below are two complementary approaches to study these cellular systems that interface chemistry with engineering and biology. The first describes a microfabrication method to model the extracellular matrix that allows selective presentation of extracellular matrix ligands in well defined densities and arrays. The second uses metabolic delivery methods to create a new set of nonnaturally occurring sugar ligands on the cell surface that may be used to target the cells with unique receptors. The two methodologies described are just two of many approaches being developed to address the molecular complexity of cellular systems.

Tailored Substrates as Extracellular Matrix Models

The matrix that serves as the scaffold for localization of mammalian cells comprises an insoluble aggregate of large glycoproteins, which include fibronectin, laminin, and the collagens. Studies of ligand influence on cell behavior have traditionally relied on nonspecific adsorption of matrix proteins to plastic surfaces that form complex and heterogeneous structures. These ambiguous structures have motivated the development of model matrix-substrates that control the presentation of individual ligands.

Self-assembled monolayers are easily formed by immersing gold-coated glass slides into solutions of long-chain alkanethiols. The alkanethiolates can be derivatized with the appropriate peptide or carbohydrate ligand that mimics the extracellular matrix ligand of interest, or with a short oligomer of ethylene glycol to prevent the nonspecific adsorption of proteins (1). These thin films have further advantages in that they are optically transparent and electrically conductive, allowing the use of spectroscopy and electrochemistry to monitor and alter the ligand activities. Microcontact printing can be used to produce patterns of different alkanethiol ligands on the surface that can subsequently control the positions, sizes, and even shapes of attached cells (2).

This monolayer strategy has been useful for investigating whether the peptide G-R-G-D-S, present in fibronectin, is responsible for adhesion, signaling to the cytoskeleton, or both, by means of its integrin receptor. In earlier work, it was difficult to distinguish between the effects of binding to immobilized fibronectin and nonspecific adsorption to protein. Monolayers were prepared that presented this peptide at the terminus of an alkanethiol (Fig. 1 *A–C*). The peptide-containing alkanethiols were mixed with oligo-(ethylene glycol) alkanethiols to vary the density of peptide in the monolayer (3, 4). With densities between 0.1–1.0%, the cells attached to the monolayers efficiently. Furthermore, the adherent cells had signaled assembly of focal adhesion complexes and actin stress filaments.

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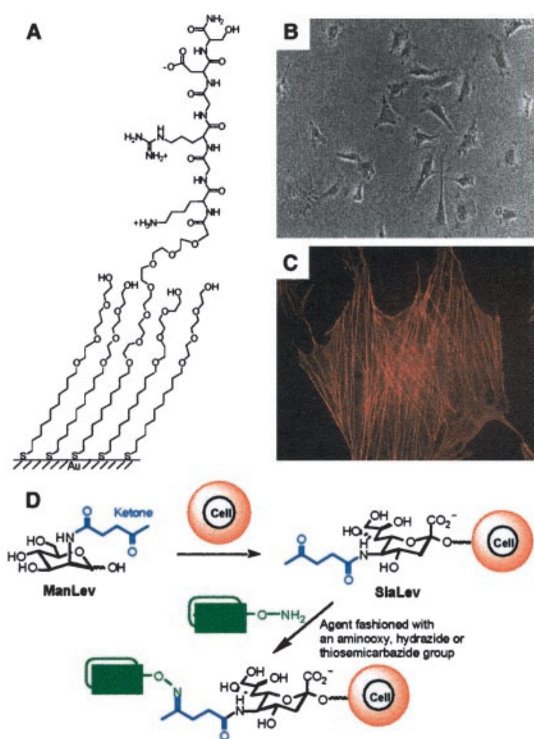

Fig. 1. (*A*) Self-assembled monolayer that presents the peptide G-R-G-D-S and tri(ethylene glycol) groups. (*B*) Optical micrograph of fibroblast cells attached to the monolayer in *A*. (*C*) Fluorescent micrograph of a cell that was labeled to visualize the actin cytoskeleton of the cell. (*D*) Metabolic delivery of ketones to sialic acid residues and cell-surface engineering.

An exciting direction of this work is the development of dynamic matrix-substrates that can alter, in real time, the activities of immobilized ligands with which cells interact. These matrix-substrates are based on the design of ligands that are electrically active and that can be switched on and off by application of electrical potentials to the substrate. In one strategy, a monolayer presenting a hydroquinone group was prepared. After application of an electrical potential to the gold film, the hydroquinone undergoes oxidation to produce a benzoquinone, which subsequently can undergo a Diels–Alder reaction with a cyclopentadiene. When the diene is conjugated to a ligand of interest, the Diels–Alder reaction results in the immobilization of the ligand (5). This active matrix-substrate provides new opportunities for studying the migration of adherent cells and for patterning the attachment of two distinct cell types to generate patterned cocultures (6).

A related strategy has been used to create matrix-substrates that release a ligand after application of an electrical potential (7). These examples illustrate new strategies for designing and preparing tailored matrix-substrates for mechanistic studies of the interactions of cells with extracellular matrix. These resulting matrix-substrates provide new tools for investigating the responses of cells to changes in their environments and are particularly relevant for studies of differentiation, wound regeneration, and metastasis.

Altering the Landscape of the Cell Surface

Glycoconjugates (glycoproteins and glycolipids) have defied traditional genetic approaches to biological study as a result of the heterogeneous and template-independent nature of their biosynthesis. In this work, the metabolic machinery of the cell has been harnessed to remodel cell surfaces with reactive organic functional groups. The foundation of this approach is the observation that many of the enzymes involved in oligosaccharide biosynthesis are fairly permissive with respect to substrate specificity and will convert unnatural monosaccharide precursors into cell surface-associated oligosaccharides. These pathways have been exploited as vehicles for the delivery of unnatural sugars bearing uniquely reactive electrophilic functional groups, such as ketones (8) and azides (9), to cell surfaces. For example, an unnatural analog of *N*-acetylmannosamine bearing a ketone group, *N*-levulinoylmannosamine (ManLev), is metabolized by human cells to *N*-levulinoyl sialosides on the cell surface, resulting in the cell-surface display of ketone groups. The cell surface can then be selectively decorated with rationally designed organic structures bearing a complementary nucleophile, such as an aminooxy group, that reacts to form a stable covalent adduct (Fig. 1*D*). Synthetic oligosaccharides functionalized with aminooxy, hydrazide, and thiosemicarbazide groups can be attached to endogenous cell-surface glycans, affording remodeled cells with novel receptor-binding activities. Other applications being pursued include targeted gene delivery (10) and antitumor diagnostics (11). This latter application was prompted by reports from the last decade that correlate sialic acid expression on the cell surface with a malignant phenotype.

Paramagnetic contrast reagents for use in MRI that specifically target the tumor cell surface have the potential to enhance the image of the tumor tissue. Some progress has been achieved by using contrast reagents conjugated to mAbs that bind to tumor cell-surface antigens (12, 13). However, Ab targeting has been hampered by several factors, such as structural heterogeneity of the epitopes, low density of antigenic determinants ($<10^5$ per cell), and Ab crossreactivity *in vivo*. It was hypothesized that ketone expression levels on cells treated with *N*-levulinoylmannosamine (ManLev) would mirror intrinsic sialic acid expression levels, enabling preferential targeting of highly sialylated cells with an aminooxy-functionalized contrast reagent.

An aminooxy-functionalized analog of the clinically used contrast reagent Magnevist [gadolinium-diethylenetriamine pentaacetic acid (DTPA)] was synthesized. Cultured Jurkat cells, a heavily sialylated human T-lymphoma cell line, were grown in the presence or absence of *N*-levulinoylmannosamine (ManLev) and then treated with the contrast reagent at clinically relevant concentrations. By using a fluorescence assay, the contrast reagent was found to be highly localized on the ketone-coated cells compared with control cells devoid of ketones. When preincubated with ManLev and treated with contrast reagent, heavily sialylated normal Jurkat cells accumulated twice the amount of contrast reagent than mutant Jurkat cells with lower sialic acid (Sia$\alpha 2 \rightarrow 3$Gal) expression levels. Thus, subtle differences in cell metabolism caused by malignancy may be amplified into a detectable difference in cell-surface display of ligands for MRI contrast reagents.

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