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Rewiring Cell Adhesion

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Cell adhesion to the extracellular matrix (ECM) is integral to the survival and function of most cells.[1,2] Cells use a wide range of receptors, which function by binding ligands in the substratum and transducing signals through the intracellular domain, to regulate functions as diverse as tissue maintenance, immune response, and development. Studies that address the roles for individual receptors are important for understanding these physiological processes but are often complicated by overlapping ligand specificities between receptors.[3] Here we report a strategy for "rewiring" the interactions between a cell and surface to create unique ligand−receptor bonds between a cell and ECM, but at the same time preserving the biological role of the receptor. We demonstrate this strategy by rewiring a cell-surface integrin receptor for adhesion to a synthetic ligand. The integrins are transmembrane $\alpha\beta$ heterodimeric receptors that are found on all cell surfaces and that mediate cell adhesion.[4−6] Of the 25 known integrins, approximately one-third bind to the ligand, Arg-Gly-Asp, which is frequently used for in vitro studies of cell−ECM adhesion.[4] Strategies that give specific activation of integrin receptors would prove valuable for studies of signaling and would also enable many applications.

Our approach is based on a complementary engineering of the cell and model substrate using genetic and surface chemistry approaches, respectively, to install uniquely specific ligand−receptor interactions (Figure 1a). We constructed a chimeric receptor that contained the intracellular and transmembrane domains of β_1 integrin, an extracellular stalk domain from fractalkine, and a carbonic anhydrase IV (CAIV) domain at the terminus. The CAIV protein selectively binds to benzenesulfonamide (BzS) ligands and provides a new specificity for the binding of receptor to ECM. With a dissociation constant of 6 μM, this interaction is similar to that of $\alpha_5\beta_1$ integrin for its natural ligand, fibronectin.[7,8] The fractalkine stalk domain serves to extend the ligand binding domain of the receptor approximately 26 nm from the cell surface, which is somewhat longer than the integrin stalk.[9,10] Upon binding, the chimeric receptor participates intracellularly as a β_1 integrin in focal adhesion formation and signaling. Because previous reports have used chimeric receptors that act as dominant negatives to establish the sufficiency of the intracellular domain of β_1 integrin for signaling and receptor localization to focal adhesions, we reasoned that the β_1 subunit should allow adhesion function, provided that a monomeric ligand binding domain is introduced.[11−13]

In parallel we prepared a model surface that presents a ligand for the chimeric receptor (Figure 1b). We used self-assembled monolayers (SAMs) of alkanethiolates on gold because these surfaces have been developed as models of ECM.[14,15] Monolayers were prepared from two alkanethiols terminated in a benzenesulfonamide and a tri(ethylene glycol) group.[16] The latter resists nonspecific protein adsorption and ensures that cell adhesion is mediated only by specific interactions between the ligand and cell-surface chimeric receptors.[17,18]

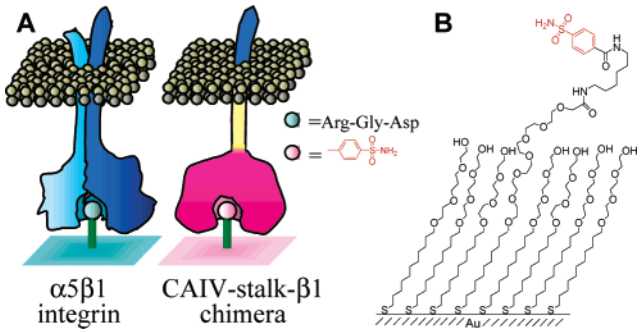

Figure 1. Schematic diagram of the complementary engineering of receptor and ligand. (A) The native $\alpha_5\beta_1$ integrin (left) binds a tripeptide ligand, Arg-Gly-Asp. A chimeric receptor (right) was engineered to retain the intracellular and transmembrane domain of the β_1 integrin (blue), but on the extracellular side, to comprise a fractalkine stalk domain (yellow) and the enzymatic domain of carbonic anhydrase IV (CAIV) (pink). A surface presenting a small-molecule inhibitor of CAIV, benzenesulfonamide, specifically targets the chimeric receptor. (B) A self-assembled monolayer of alkanethiolates on gold present benzenesulfonamide at a 1% density with an inert background of tri(ethylene glycol).

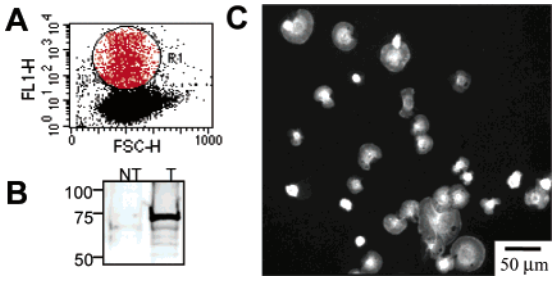

Figure 2. Cells expressing the chimeric receptor adhere to a surface presenting benzenesulfonamide. (A) FACS plot of fluorescence vs forward scatter shows a transfected population of 11% (red dots) that express the chimeric receptor and a nontransfected population (black dots). (B) Western blot analysis with an anti-CAIV antibody shows the chimeric receptor to be ~75 kD. (C) Transfected cells attach and spread on a surface presenting benzenesulfonamide. Cells were stained with phalloidin-TexasRed for better visualization of the lammelipodia.

We transfected Chinese hamster ovary (CHO) cells to express the chimeric receptor. Fluorescence activated cell sorting (FACS) analysis using an antibody against CAIV showed that the chimeric receptor was expressed and presented on the membrane surface of CHO cells (Figure 2a). Western blot analysis of cell lysates using the same antibody confirmed that the full-length chimeric receptor was expressed in cells. The apparent molecular weight of the chimeric receptor is slightly higher than the calculated mass of 63 kD based on the amino acid sequence and is due to the posttranslational glycosylation of the mucin-like fractalkine stalk (Figure 2b).[19]

Cells expressing the chimeric receptor adhered to and spread well on SAMs presenting the benzenesulfonamide ligand and were

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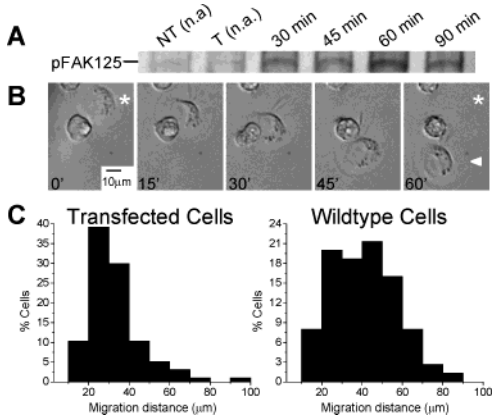

Figure 3. Chimeric receptor supports signaling and cell migration. (A) Phosphorylation of FAK at Y397 correlates to cell attachment and spreading. Nonadherent cells (n.a.), both nontransfected (NT) and transfected (T), have minimal phosphorylation. A marked increase in phosphorylation is observed approximately 1 h after plating in transfected cells. (B) A transfected cell migrates on a surface presenting benzenesulfonamide. (C) The distribution of migration distances of transfected cells on SAMs presenting 1% benzenesulfonamide (left) and nontransfected cells on SAMs presenting 1% cyclic Arg-Gly-Asp (right) over 1 h.

characterized by wide lammelipodia (Figure 2c). The adhesion of cells to the model substrate was entirely specific. Cell adhesion was inhibited by soluble benzenesulfonamide (1 mM), and nontransfected cells showed no adhesion to the substrates. Because cell spreading requires proper signaling from the intracellular domain of the β integrin to promote reorganization of the actin cytoskeleton,[20,21] our observation of efficient cell spreading suggests that the chimeric receptor not only binds ligand but *functions* intracellularly as a β_1 receptor.

Signal transduction analysis showed that the cytoplasmic β_1 subunit of the chimeric receptor is able to activate signaling through focal adhesion kinase (FAK). FAK becomes autophosphorylated at Tyr397 in response to cell spreading and the clustering of β_1 receptors.[22,23] A western blot analysis using anti-phosphoFAK antibody showed that FAK signaling is absent in nonadherent cells but becomes activated as transfected cells attach to and spread on surfaces presenting benzenesulfonamide (Figure 3a). This pattern of signaling is similar to that observed in nontransfected cells adhering to fibronectin, a common ligand for integrin receptors, including $\alpha_5\beta_1$.

Finally, we found that cells transfected with the chimeric receptor were functional in migration assays. Transfected CHO cells, using a gliding motion of the fan-shaped lammelipodia, migrated on SAMs presenting benzenesulfonamide at a rate similar to that of wild-type cells on SAMs presenting Arg-Gly-Asp at the same density (Figure 3b,c). Migration, which requires the coordinated action of attachment at the leading edge and detachment at the trailing end through differential integrin signaling, is a clear indicator that the chimeric receptors have been successfully integrated into the circuitry of the cell.[24]

This work demonstrates a strategy for rewiring the receptor−ligand interactions between a cell and ECM. In this example, we genetically modified the β_1 integrin receptor to promote adhesion to a synthetic surface and found that cell adhesion, signaling, and migration were similar to that observed with wild-type cells on natural ECM proteins. The modularity of this approach allows the β_1 domain of the chimeric receptor to be interchanged with either other integrin β subunits or other transmembrane receptors, while retaining the extracellular interaction between CAIV and benzenesulfonamide ligand. We believe that this strategy is applicable to a wide range of cell-surface receptors that participate in adhesion and therefore offers a new opportunity for understanding fundamental biological processes—ranging from mechanotransduction to stem cell differentiation—and for directing the adhesion of many cell types to artificial scaffolds in tissue engineering applications.[25−28] The broad strategy of rewiring cellular pathways to either enhance or redirect a biological function has proven an important tool in biology—including the yeast two-hybrid screens[29] and synthetic receptors for targeted endocytosis[30]—and is now available for the study and engineering of cell−ECM interactions.

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Supporting Information Available: Additional figures and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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