VIEWPOINT

Reflections on Integrins—Past, Present, and Future
The Albert Lasker Basic Medical Research Award

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Supplemental content

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The 2022 Albert Lasker Basic Medical Research Award has been presented to Richard O. Hynes, PhD; Erkki Ruoslahti, MD, PhD; and Timothy A. Springer, PhD, for discoveries concerning the integrins—key mediators of cell-matrix and cell-cell adhesion in physiology and disease.

Integrins are receptors on the surfaces of most human cells that mediate interactions of cells with their neighbors and their surroundings. These interactions have vital roles in multiple aspects of embryonic development, normal physiology, and disease processes. The integrin family of receptors was recognized in the 1980s in the course of several different lines of investigation, leading to convergence of multiple previously independent areas of research. In the ensuing years, the manifold functions of integrins have been elucidated and these discoveries have directly influenced many areas of biology and medicine.

Three distinct areas of research led to the discovery of integrins. Studies of the cell biology of cancer in the early 1970s led to the discovery of fibronectin as a protein lost from oncogenically transformed cells in vitro and as a component of the extracellular matrix (ECM). It was subsequently shown that fibronectin promoted cell adhesion and organization of the actin cytoskeleton and that the intracellular actin cytoskeleton and the extracellular fibronectin-rich ECM are mutually interdependent and closely coaligned physically.[1] These results suggested a transmembrane link between them and sparked an active search for receptors that bound fibronectin to the cell surface. Analyses of the active sites in fibronectin that mediate binding to cells identified the small peptide sequence arginylglycylaspartic acid (RGD) as being key for promotion of cell adhesion to fibronectin as well as to other adhesive proteins.[2,3] This short peptide motif allowed isolation by affinity chromatography of several adhesion receptors for fibronectin and several other ECM proteins. Monoclonal antibodies blocking cell adhesion and chemical crosslinking provided other avenues leading to identification of ECM receptors.

Meanwhile, 2 other lines of research converged with this ongoing research on ECM-cytoskeleton connections. One came from studies on the aggregation of blood platelets through binding of fibrinogen. As techniques for identification of cell surface proteins were applied to platelets, a heterodimeric complex, glycoprotein IIb/IIIa (GpIIb/IIIa), was defined. This complex was shown to be lost in a genetic disease, Glanzmann thrombasthenia, a bleeding disorder in which blood platelets fail to aggregate. Glanzmann thrombasthenia platelets fail to bind fibrinogen. Platelets also bind to fibronectin and several other adhesive ECM proteins through GpIIb/IIIa via binding to RGD or closely related motifs, and it appeared likely that GpIIb/IIIa was related to the receptors discussed above.

Also in the early 1980s, a separate, independent line of research used monoclonal antibodies to identify cell surface proteins of macrophages and lymphocytes. Lymphocyte function–associated antigen 1 (LFA-1) was identified by antibodies inhibiting antigen-specific killing by T cells requiring direct cell-cell contact. Macrophage antigen 1 and p150,95, both receptors for complement component iC3b, which links to antibody-tagged cells and pathogens, were found to be related to LFA-1. They were shown to be noncovalently associated heterodimers with identical β subunits and distinct α subunits that were homologous in amino acid sequence, providing evidence for a family of related receptors. These receptors were deficient in a genetic disease, known as *leukocyte adhesion deficiency*, due to loss of the common β subunit.[4] Inability of leukocyte adhesion deficiency–patient neutrophils to leave the circulation further showed that these integrins were important in interactions with endothelium and trafficking of leukocytes in the body. Intercellular adhesion molecule 1 was identified as the counter-receptor for LFA-1 and inducible by inflammatory cytokines on binding partners for T cells in immune responses and on endothelium. LFA-1 and macrophage antigen 1 binding to intercellular adhesion molecule 1 on endothelium is important in leukocyte trafficking, with important implications for autoimmune therapeutics.

Sequence data from cloned cDNAs made clear that all 3 lines of research converged on related sets of receptors: heterodimeric transmembrane integral membrane proteins linking the inside and outside of cells. The name *integrins* reflected their integral membrane structures and their roles in integration of internal and external functions of cells.

This convergence of fields including oncology, immunology, hemostasis, and development was a tremendous stimulus to further research because each of the fields contributed different concepts that informed the others. Many laboratories participated in defining and sequencing the spectrum of integrins.[5] It was found that mammals had 8 β subunits and 18 different α subunits in several different subfamilies that could be assembled into 24 different integrins (eFigure, A in the Supplement).

Genome sequences determined since 2000 showed that all metazoa possess integrins and thus indicate that integrins were associated with the evolution of multicellularity approximately half a billion years ago. Invertebrate taxa have fewer integrins, with laminin-binding and RGD-recognizing integrins evolving early. Later-evolving integrins include those with αI domains (eFigure, A in the Supplement) and $\alpha_V\beta_6$ and $\alpha_V\beta_8$, which activate transforming growth factor β.

It is clear that integrins are crucial for the organization of all multicellular animals; they are essential for proper associations among cells in embryonic development and in many aspects of physiology. Engineered variants in genes encoding integrin subunits all cause altered phenotypes, in some cases including embryonic lethality, although the majority have nonlethal phenotypes revealing their functions, such as the genetic defects in platelet and leukocyte adhesion discussed earlier. All human cells except mature erythrocytes have 1 or more integrins and there is significant overlap in their ligand specificity. The spectrum of integrins expressed by a given cell type is under regulation, allowing the adhesive properties of cells to be altered in the course of terminal maturation, differentiation, and oncogenesis.

Importantly, ligand binding by integrins is actively regulated, as well exemplified by the platelet and leukocyte integrins. These integrins are typically in an inactive state in resting; circulating blood cells and tight control of adhesion through integrins is important to avoid adhesion that would cause thrombosis, inflammation, or autoimmunity. More subtle forms of adhesion regulation of integrins play roles in cell migration and processes, such as neuronal outgrowth and many other intercellular connections with neighboring cells or with ECM.

Another aspect of integrin function is their ability to transmit signals both "outside in" and "inside out."[6-8] These signals are transmitted by large conformational changes in the integrins. The 22 integrins with β subunits that bind talin through their cytoplasmic domains (white circles in eFigure, A in the Supplement) have 3 distinct conformational states (eFigure, B in the Supplement). The extended-open conformation binds ligand with approximately 1000-fold higher affinity than the 2 closed conformations. This high-affinity state is stabilized by binding both through an adaptor to the cytoskeleton inside the cell and to ligands embedded in the extracellular environment outside the cell (on adjacent cells or in the ECM). Integrin signal transduction is at least as complex as that mediated by more familiar receptors such as G-protein–coupled receptors or tyrosine kinase receptors, and integrins cooperate with these and other types of receptors. The physical connections with both cytoskeleton inside and ECM outside mediate mechanotransduction as well as biochemical signaling.

Integrins are also needed for ECM assembly around cells. The lack of fibronectin at the surface noted in the early studies is explained by impaired integrin function in cancer cells. Normal cells require ECM attachment for survival and undergo apoptosis in its absence, a phenomenon known as *anchorage dependence*. The apparent reason is that ECM adhesion through integrins enhances the expression of antiapoptotic genes such as BCL-2. Malignant cells are independent of anchorage owing to aberrant expression of antiapoptotic genes. The low adhesion to ECM and ability to survive without adhesion make it possible for cancer cells to metastasize to distant sites in the body.[9]

The genetic defects central in defining the platelet and leukocyte integrins immediately suggested the possibility of modulating integrin function for therapeutic interventions, and that has been an active area of research. Therapies based on monoclonal antibodies against integrins, peptides, and peptidomimetics, often based on the RGD integrin-recognition motif, have been prominent in these efforts, and many more are ongoing. Effective therapies have been developed for thrombosis and for several autoimmune inflammatory diseases, including multiple sclerosis and inflammatory bowel diseases, and many more are under active development and improvement. A long-term hope has been to develop integrin-based drugs targeting cancer. Those hopes have not yet been realized, but many avenues are under investigation[10] and further advances are anticipated.

The discovery of integrins and their pervasive roles in important biological processes has been a fascinating and exciting area of biology and will continue to be so. The rapid advances in molecular cell biological approaches over the past 50 years and the active involvement of many laboratories worldwide has yielded rich insights into the functions of these versatile receptors and their mechanisms of activation and has opened many avenues for future advances, including direct applications for therapeutic and regenerative medicine.

ARTICLE INFORMATION

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Conflict of Interest Disclosures: Dr Hynes reported serving on scientific advisory boards for Genentech, Amgen, and Blade Therapeutics unrelated to this work and having patents in progress of review that address the area of extracellular matrix but not integrins. Dr Ruoslahti reported receiving grants from Sanford Burnaham Prebys Medical Discovery Institute for work mentioned in the article and for unrelated work; serving as consultant, founder, shareholder, chairman of the board for Cend Therapeutics; founder, shareholder, chairman of the board for AivoCode; and founder, shareholder, chairman of the board for Precis Therapeutics outside the submitted work; and having a patent for integrin-binding peptides licensed to Cend Therapeutics Inc; a patent for peptides homing to diseased brain licensed to AivoCode Inc; and a patent for peptides homing to bacteria pending (not directly relevant to the article). Dr Springer reported being a stockholder and board member of Morphic Therapeutic outside the submitted work.

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REFERENCES

1. Hynes RO, Yamada KM. Fibronectins: multifunctional modular glycoproteins. *J Cell Biol*. 1982;95(2 pt 1):369-377. doi:10.1083/jcb.95.2.369

2. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science*. 1987;238(4826):491-497. doi:10.1126/science.2821619

3. Ruoslahti E. RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol*. 1996;12:697-715. doi:10.1146/annurev.cellbio.12.1.697

4. Anderson DC, Springer TA. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med*. 1987;38:175-194. doi:10.1146/annurev.me.38.020187.001135

5. Hynes RO. Integrins: a family of cell surface receptors. *Cell*. 1987;48(4):549-554. doi:10.1016/0092-8674(87)90233-9

6. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002;110(6):673-687. doi:10.1016/S0092-8674(02)00971-6

7. Li J, Yan J, Springer TA. Low-affinity integrin states have faster ligand-binding kinetics than the high-affinity state. *Elife*. 2021;10:e73359. doi:10.7554/eLife.73359

8. Springer TA, Dustin ML. Integrin inside-out signaling and the immunological synapse. *Curr Opin Cell Biol*. 2012;24(1):107-115. doi:10.1016/j.ceb.2011.10.004

9. Ruoslahti E, Reed JC. Anchorage dependence, integrins, and apoptosis. *Cell*. 1994;77(4):477-478. doi:10.1016/0092-8674(94)90209-7

10. Alday-Parejo B, Stupp R, Rüegg C. Are integrins still practicable targets for anti-cancer therapy? *Cancers (Basel)*. 2019;11(7):978. doi:10.3390/cancers11070978

Supplemental Online Content

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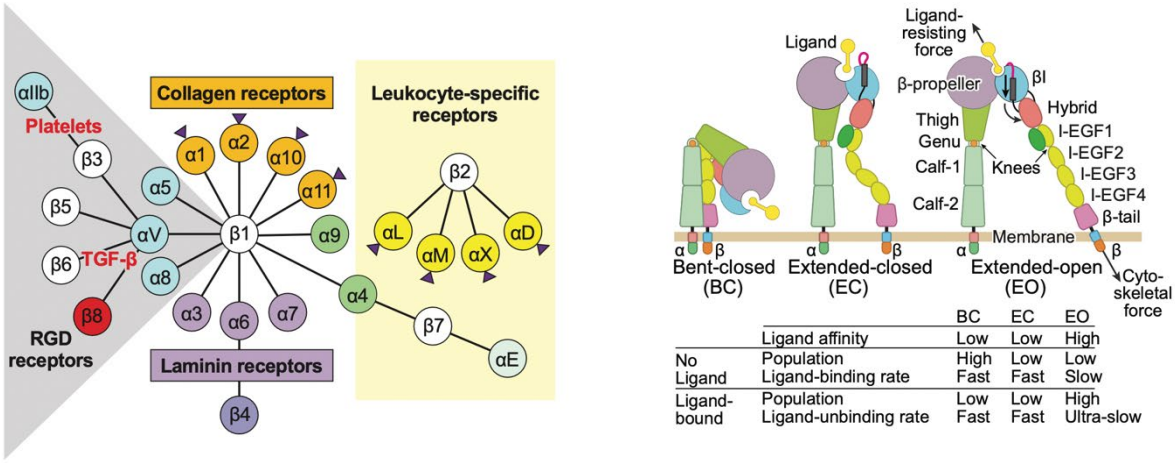
eFigure

This supplemental material has been provided by the authors to give readers additional information about their work.



eFigure, A.The Integrin Receptor Family
Different subfamilies of related integrins are highlighted. The RGD-binding and laminin-binding integrins are evolutionarily the most ancient and have diverged into separate clades in the vertebrate lineage. The α4/α9 clade is vertebrate-specific and two subfamilies of chordate α subunits (collagen-binding and leukocyte-specific) have inserted αI domains (purple arrowheads) that act as ligand-binding domains. Figure adapted with permission from Elsevier.[6]

eFigure ,B. Structure and Conformational States
Integrins extend at their knees. Conformational change at the ligand binding site is linked to integrin opening and to changes in the legs and cytoplasmic domains and they can be triggered either from the outside (ligand) or the inside (cytoskeleton). Integrins appear to bind to ligand in their closed states and then rapidly shift to their high-affinity extended-open state.
Figure modified from *eLife*.[7]