Integrin Profiling of Stem Cells and Differentiated Progeny

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Abstract

To identify integrin subunits that are specifically and/or differentially expressed during proliferation and differentiation, adult rat neural stem cells and their differentiated progeny, neurons and astrocytes, were profiled using Millipore's arrayed panel of α and β integrin antibodies. Relative quantification of the cells captured by specific immobilized integrin antibodies revealed that αvβ5 integrin is highly expressed in both adult rat neural stem cells and differentiated astrocytes that are cultured on laminin. Additionally, αvβ5 integrin has been implicated in the VEGF-induced angiogenesis pathway, providing a possible mechanistic link between angiogenesis and neurogenesis in the central nervous system. Further investigations into the role of integrins in neural stem cell biology are merited, and the differential expression of integrins on different types of stem cells will shed light on the selection of appropriate ECM for the cell culture plate coating and cell adhesion studies in a more efficient manner.

Introduction

Cell adhesion plays a major role in cellular communication and regulation, and is of fundamental importance in the development and maintenance of tissues. A family of cell surface glycoproteins, the integrins, mediate cell adhesion to proteins of the extracellular matrix (ECM). Integrins act as receptors for ECM proteins (collagen, laminin, vitronectin, or fibronectin) or for membrane-bound counter-receptors on other cells. They play an important role in regulating gene expression, cell cycle, programmed cell death, cell migration and proliferation. Integrins exist as heterodimers containing an α and β subunit. Each subunit has a large extracellular domain, a single membrane-spanning region and a short cytoplasmic domain (Figure 1). There are 16 distinct α subunits and 8 or more β subunits, which form more than 20 distinct integrins. The α/β pairings specify the ligand-binding abilities. The extracellular domains contribute to formation of the binding site. Activated

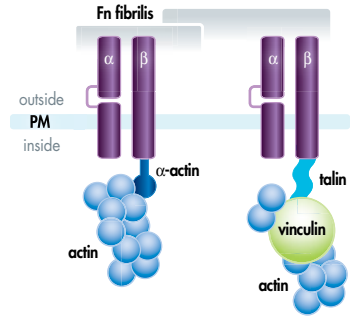

Figure 1. Structure of Ð/Ð integrin dimers. Cytoplasmic domains are key nexus of interaction between the extracellular environment and intracellular structures and signaling cascades. Contribute to cytoskeletal organization, cell motility, signal transduction and modulation of integrin affinity for ligands.

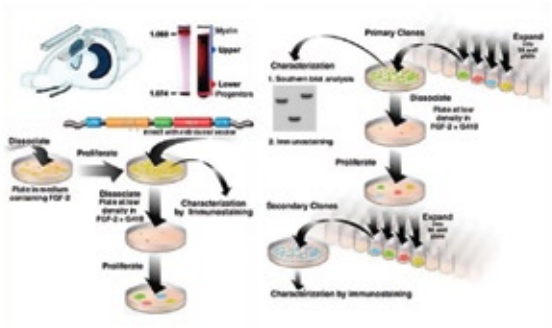

Figure 2. Schematic for isolating and propagating adult NSCs

integrins often form multimers, which have higher affinities to cell adhesion molecules.

Despite their versatility and importance, little is known about the expression pattern and regulation of integrins in stem cells and their differentiated progeny. Furthermore, the intracellular signaling cascades initiated by integrins in response to the binding of laminin, an ECM molecule commonly used to culture neural stem cells, remain poorly understood.

Historically, individual antibodies have been used to determine the integrin profiles of cells by immunoprecipitation, immunofluorescence, immunoblotting, and flow cytometry. These methods are time-consuming, laborious or require sophisticated equipment. Millipore's α/β integrin-mediated cell adhesion array kit is designed as a cost effective, efficient method for identification of cell surface integrins.

Methods, Results and Discussion

Millipore offers a variety of stem cells and characterization kits as research reagents, including embryonic and adult stem cells from different tissues and species. Neural stem cells (NSC) and their differentiated progenies were used in this integrin profiling study, although they require ECM proteins such as laminin and fibronectin for culture, the mechanism and signaling cascades initiated by the integrins in response to the binding of the ECM remain poorly understood. NSCs are self-renewing cells with the capacity to differentiate into neurons, astrocytes and oligodendrocytes.

In this study, we isolated rat adult NSC (Cat. No. SCR022) from discrete regions of the hippocampus and the subventricular zone (Figure 2). Approximately 20 adult rats were sacrificed and their hippocampi were surgically removed. The tissue was then enzymatically digested to dissociate the cells. The cell mixture was subjected to a Percoll density gradient column and the presumptive NSCs were extracted after high speed ultracentrifugation. The extracted cells were grown in media containing FGF-2 and allowed to proliferate.

Using a series of assays, the NSC population was immunostained for stem cell markers and assessed for their ability to differentiate towards specific cell lineages. The Neural Stem Cell Marker Characterization Kit (Cat. No. SCR019) was used to further characterize and QC the purity of the cells. Each lot of primary adult rat hippocampal neural stem cells was validated for a high level of expression of Nestin and Sox 2 and for their self-renewal and multi-lineage differentiation capacities. The NSCs were

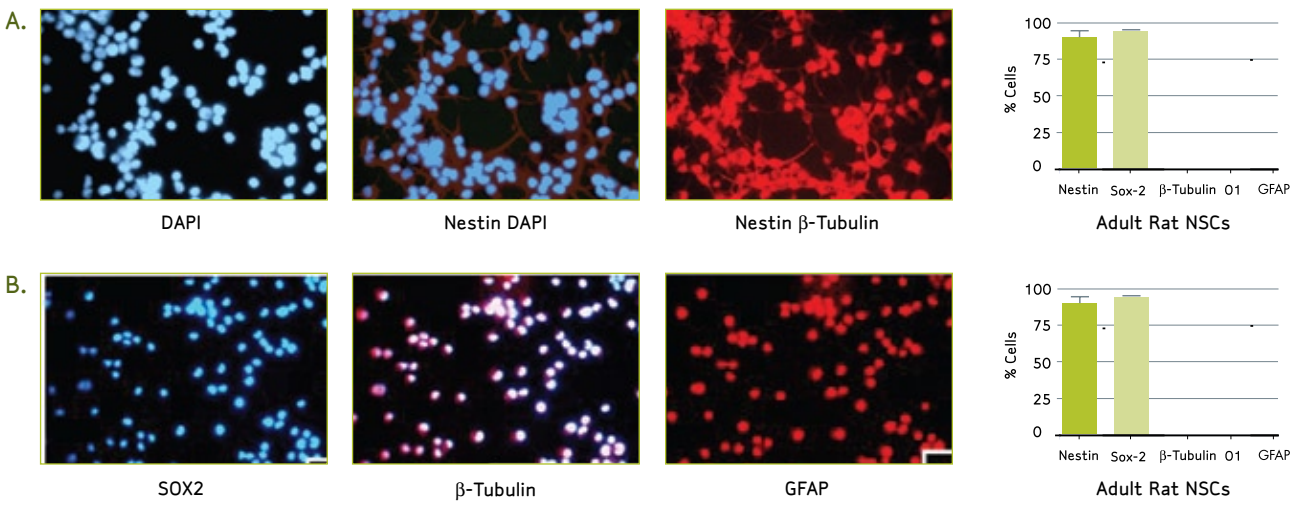

Figure 3. (A) Adult Rat NSCs are Nestin+, βIII Tubulin-, O1- and GFAP-. (B) Adult Rat NSCs are Sox2+, βIII Tubulin-, O1- and GFAP-.

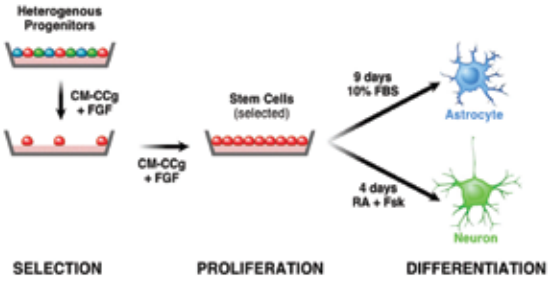

Figure 4. Differentiation of NSCs into neurons and astrocytes.

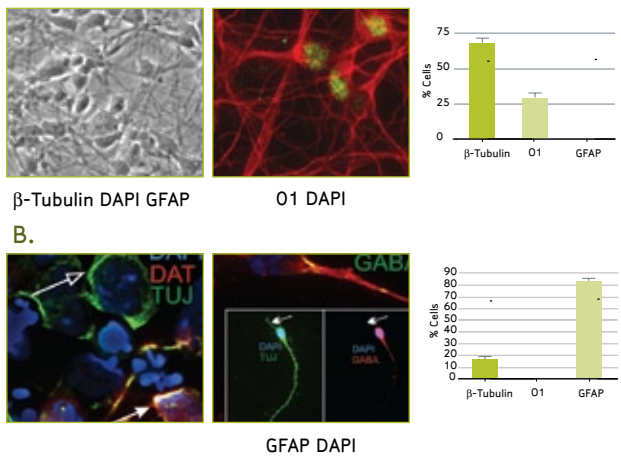

Figure 5. (A) Differentiated neurons and oligodendrocyte express β-Tubulin and O1. (B) Differentiated astrocytes express GFAP.

negative for differentiated markers (β-Tubulin, Map2ab, GFAP, and O1). Cells also displayed normal karyotypes as assessed by chromosome spread and tested negative for mycoplasma (Figure 3). While pluripotent embryonic stem (ES) cells are capable of producing most tissues of an organism and over 200 cell types, adult stem cells possess a limited potential to differentiate. In most cases, adult stem cells can only give rise to specialized cells of the organ system from which they were derived. The isolated adult rat NSCs were then differentiated into neurons, astrocytes and oligodendrocytes (Figure 4). The differentiated lineages were characterized with selective antibodies (Figure 5).

Since the culture of NSCs requires ECM proteins, various integrins must be expressed on the cell surface. Millipore's α/β integrin-mediated cell adhesion arrays were used in order to determine which types of integrins are expressed or activated on both adult rat NSCs and differentiated cells that are cultured on ECMs.

The layout of Millipore's integrin-mediated cell adhesion arrays is shown in Figure 7. The integrin adhesion arrays are a cost effective, efficient method for the identification of cell surface integrins. The kits format of 12 x 8-well removable strips in a plate frame allows for convenience and flexibility in designing assays. Integrin profiling using Millipore's α/β integrin-mediated cell adhesion arrays can be completed within 1–2 hours, providing a rapid, sensitive, and quantitative assays for the characterization of α and β integrin surface expression.

	1	2	3	4	5	6	7	8	9	10	11	12
A	β1	β1	β1	β1	β1	β1	β1	β1	β1	β1	β1	β1
B	β2	β2	β2	β2	β2	β2	β2	β2	β2	β2	β2	β2
C	β3	β3	β3	β3	β3	β3	β3	β3	β3	β3	β3	β3
D	β4	β4	β4	β4	β4	β4	β4	β4	β4	β4	β4	β4
E	β6	β6	β6	β6	β6	β6	β6	β6	β6	β6	β6	β6
F	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5	αVβ5
G	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1	α5β1
H	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Figure7. (A) Differentiated neurons and oligodendrocyte express β-Tubulin and O1. (B) Differentiated astrocytes express GFAP.

The profiling results indicate that both adult rat NSCs and differentiated cells express αVβ5 integrins, but not others, which is consistent with immunocytochemical staining result using anti-αVβ5 integrin antibody (Figure 8). αVβ5 integrin is highly expressed in both adult rat neural stem cells and differentiated astrocytes that are cultured on laminin. αVβ5 integrin has been implicated in the VEGF-induced angiogenesis pathway, providing a possible mechanistic link between angiogenesis and neurogenesis in the central nervous system. Integrin profiling of stem cells will provide a new method for stem cell isolation through Fluorescence Activated Cell Sorting (FACS) using lineage specific anti-integrin antibodies and help identify potentially new ECM coating reagents for the culturing of different types of stem cells.

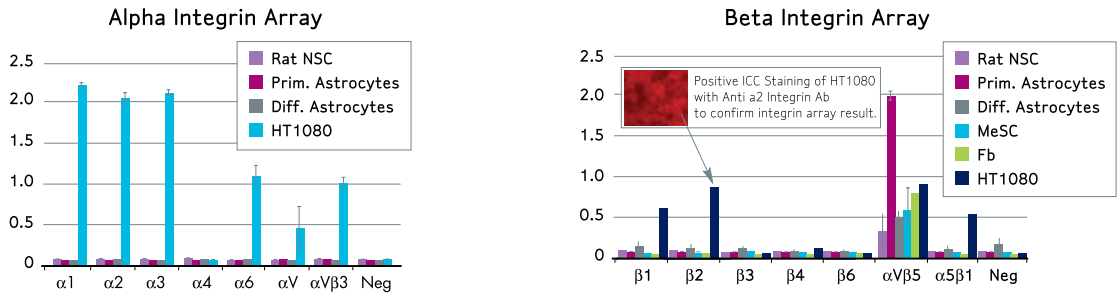

Figure 8. Both integrin (β and α) array profiling and ICC results suggest that adult rat NSCs and differentiated cells express αVβ5 integrins, but not others.

Description	Format	Quantity	Cat. No.
α Integrin-mediated Cell Adhesion Array Kit	Colorimetric	96 wells	ECM530
α Integrin-Mediated Cell Adhesion Array Kit	Fluorimetric	96 wells	ECM533
β Integrin-mediated Cell Adhesion Array Kit	Colorimetric	96 wells	ECM531
β Integrin-Mediated Cell Adhesion Array Kit	Fluorimetric	96 wells	ECM534
α/β Integrin-mediated Cell Adhesion Array Combination Kit: 1 each ECM530 / ECM531	Colorimetric	2 x 96 wells	ECM532
α/β Integrin-Mediated Cell Adhesion Array Combination Kit: 1 each ECM533 / ECM534	Fluorimetric	2 x 96 wells	ECM535
ECM Cell Adhesion Array Kit	Colorimetric	96 wells	ECM540
ECM Cell Adhesion Array Kit	Fluorimetric	96 wells	ECM545
Adult Rat Hippocampal Neural Stem Cells		1 x 10^6 cells	SCR022
Neural Stem Cell Marker Characterization Kit		1 kit	SCR019

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