The Role of Integrin $\alpha 6$ (CD49f) in Stem Cells: More than a Conserved Biomarker

Paul H. Krebsbach¹ and Luis G. Villa-Diaz²

Stem cells have the capacity for self-renewal and differentiation into specialized cells that form and repopulated all tissues and organs, from conception to adult life. Depending on their capacity for differentiation, stem cells are classified as totipotent (ie, zygote), pluripotent (ie, embryonic stem cells), multipotent (ie, neuronal stem cells, hematopoietic stem cells, epithelial stem cells, etc.), and unipotent (ie, spermatogonial stem cells). Adult or tissue-specific stem cells reside in specific niches located in, or nearby, their organ or tissue of origin. There, they have microenvironmental support to remain quiescent, to proliferate as undifferentiated cells (self-renewal), and to differentiate into progenitors or terminally differentiated cells that migrate from the niche to perform specialized functions. The presence of proteins at the cell surface is often used to identify, classify, and isolate stem cells. Among the diverse groups of cell surface proteins used for these purposes, integrin $\alpha 6$, also known as CD49f, may be the only biomarker commonly found in more than 30 different populations of stem cells, including some cancer stem cells. This broad expression among stem cell populations indicates that integrin $\alpha 6$ may play an important and conserved role in stem cell biology, which is reaffirmed by recent demonstrations of its role maintaining self-renewal of pluripotent stem cells and breast and glioblastoma cancer stem cells. Therefore, this review intends to highlight and synthesize new findings on the importance of integrin $\alpha 6$ in stem cell biology.

Keywords: integrin $\alpha 6$, CD49f, stem cells, niche, self-renewal, differentiation

Introduction

DUE TO THEIR CAPACITY for self-renewal and differentiation, stem cells play key roles in the development and homeostasis of tissues and organs throughout the life of most living organisms. Soon after fertilization, the zygote divides into individual blastomeres that have identical genetic information and are totipotent in nature. At the morula stage of embryonic development, the first cell lineage differentiation occurs. The blastomeres on the outside of the embryo acquire a trophoblast cell lineage, while the inner blastomeres give rise to the inner cell mass (ICM) of the forming blastocyst. The cells that constitute the ICM give rise to the primitive endoderm and the pluripotent epiblast cells. Isolation and culture in vitro of cells from the ICM result in the establishment of embryonic stem cells (ESCs), which like their counterpart in vivo are pluripotent. The following three primary germ layers: ectoderm, mesoderm, and endoderm, form from the origins of the ICM and together with the germ line originate at the gastrulation stage. From this developmental stage, specific cell lineage differentiation occurs in a coordinated sequence of events that appears to be controlled both temporally and spatially, resulting in the formation of distinct

tissues and organs. Diverse and specialized stem cell populations emerge during the development of the fetus, and some are maintained during adult life, repopulating and replenishing cells of each tissue and organ. While the capability of cell lineage differentiation is restricted and unique for each adult stem cell population, all maintain the property of self-renewal. Some stem cells remain active, while others become quiescent, and are “awakened” when needed, to prevent depletion of the stem cell pool and to repopulate their specific tissue cell types.

Several biomarkers have been used to characterize, isolate, expand, and study stem cells. The majority of these biomarkers are proteins located at the cell membrane and have the capability of binding or adhering to other signaling molecules and cells, respectively. Each cell type produces a combination of protein receptors on their surface, creating unique profiles of biomarkers that distinguish them from other cell types. The cell surface markers CD34, CD44, CD90, and CD177 (c-kit) are among the many surface proteins found on stem cells. It is important to note, however, that these proteins are also translated in other somatic cells that are not considered stem cells. For example, CD34 is regarded as a marker of hematopoietic stem cells (HSCs)

¹School of Dentistry, University of California, Los Angeles, California.

²Department of Biological Sciences, Oakland University, Rochester, Michigan.

and hematopoietic progenitor cells [1]. CD34 is also found in other populations of stem cells and progenitor cells like mesenchymal stem cells (MSCs) [2], muscle satellite cells [3], and epithelial progenitors [4], in addition to nonstem cell populations like fibroblasts [5,6].

Integrin $\alpha 6$, also known as CD49f, is among the proteins that have been identified in stem cell populations (Table 1) and somatic cells like keratinocytes [7], platelets [8], epithelial cells, and basal cells of the cornea [9]. This subunit of the α family of integrins was first identified as a stem cell marker in keratinocyte stem cells in 1998, when it was reported that a subpopulation ($\sim 10\%$) of cells in neonatal human foreskin with the phenotype $\alpha 6^{\text{bri}}10G7^{\text{dim}}$ exhibit highest regenerative capacity compared to other basal cells and were quiescent at the time of isolation [10]. Since then, the expression of integrin $\alpha 6$ has been found in more than 30 stem cell populations, including pluripotent and multipotent stem cells, and cancer stem cells (CSCs) (Table 1). Importantly, the expression of integrin $\alpha 6$ in some of these

populations is conserved among several mammalian species. Furthermore, integrin $\alpha 6$ has been identified as the only common gene expressed in stem cell signatures from ESCs, embryonic neuronal stem cells (NSCs), and HSCs [11]. Taken together, these studies point to an important role for this transmembrane receptor in stem cell biology.

The Binding Partners and Function of Integrin $\alpha 6$

Integrins are a family of type I transmembrane glycoproteins composed of α and β subfamilies. In mammals, the α subfamily has 18 subunits, while 8 subunits comprise the β subfamily. Together, these subunits form at least 24 different heterodimer combinations that have unique affinities for extracellular matrix (ECM) components, including laminins, collagens, and fibronectin, and have specific and nonredundant functions [12]. Integrins play a key role in cell adhesion to ECM ligands and adjacent cells and serve

TABLE 1. STEM CELL POPULATIONS EXPRESSING INTEGRIN $\alpha 6$ (CD49F)

Cardiac stem cells	CD117 ⁽⁺⁾ /Laminin1 ⁽⁺⁾ /CD49f ⁽⁺⁾	[77]
Cord blood stem cells	CD34 ⁽⁺⁾ /CD90 ⁽⁺⁾ /CD49f ⁽⁺⁾ /CD117 ⁽⁺⁾	[78]
Corneal epithelial stem cells	CD71 ^(dim) /CD49f ^(bri)	[79]
Dental stem cells	Sca-1 ⁽⁺⁾ /CD49f ^(bri) /CD44 ⁽⁺⁾	[80]
Embryonic stem cells	SSEA4 ⁽⁺⁾ /SSEA3 ⁽⁺⁾ /TRA-1-60 ⁽⁺⁾ /TRA-1-81 ⁽⁺⁾ /CD49f ⁽⁺⁾	[29]
Epithelial odontogenic stem cells	CD49f ⁽⁺⁾	[81]
Epithelial intestine stem cells	PHLDA1 ⁽⁺⁾ /CD49f ⁽⁺⁾	[82]
Esophageal epithelial stem cells	CD71 ^(dim) CD49f ^(bri)	[83]
Gallbladder stem cells	EpCAM ⁽⁺⁾ /CD49f ^(hi)	[84]
Hair follicle stem cells	CD34 ⁽⁺⁾ /CD49f ^(bri)	[85]
Hematopoietic stem cells	Thy1 ⁽⁺⁾ /Rho ^(low) /CD49f ⁽⁺⁾	[32]
Hepatic stem cells	Adult: CD49f ⁽⁺⁾ /CD29 ⁽⁺⁾ /c-Kit ⁽⁻⁾ /Thy1.1 ⁽⁻⁾	[86,87]
	Fetal: c-Met ⁽⁺⁾ /CD49f ^(+low) /c-kit ⁽⁻⁾ /CD45 ⁽⁻⁾ /TER199 ⁽⁻⁾	
Keratinocyte stem cells	CD49f ^(bri) /10G7 ^(dim) , CD71 ^(dim) CD49f ^(bri)	[10,88–90]
Lung epithelial stem cells	EpCAM ^(hi) /CD49f ⁽⁺⁾ /CD104 ⁽⁺⁾ /CD24 ^(low)	[91,92]
Mammary epithelial stem cells	CD24 ⁽⁺⁾ /CD49f ^(hi) ; Sca ^(hi) ; CD45 ⁽⁻⁾ /Ter119 ⁽⁻⁾ /CD31 ⁽⁻⁾ /Sca-1 ^(low) /CD24 ^(med) /CD49f ^(hi)	[33,93,94]
Mesenchymal stem cells	PODXL ^(hi) /CD49f ^(hi)	[44,95]
Merkel cell stem cells	Sca1 ⁽⁺⁾ /CD200 ⁽⁺⁾ /CD49f ⁽⁺⁾	[96]
Myometrial stem cells	CD49f ⁽⁺⁾ /CD34 ⁽⁺⁾	[97]
Neural stem cells	CD49f ^(hi) /CD29 ^(hi) /CD133 ⁽⁺⁾ ; GFAPv/LeX ⁽⁺⁾ /CD49f ⁽⁺⁾ /CD29 ⁽⁺⁾	[47,48]
Oral epithelial stem cells	Ki-67 ⁽⁺⁾ /CD29 ⁽⁺⁾ /CD49f ^(hi) /keratin 13 ^(low)	[98]
Primordial germ cells	SSEA1 ⁽⁺⁾ /SSEA3 ⁽⁺⁾ /SSEA4 ⁽⁺⁾ /EMA-1 ⁽⁺⁾ /CD29 ⁽⁺⁾ , CD49f ⁽⁺⁾	[99]
Prostate stem cells	CD45 ⁽⁻⁾ /CD31 ⁽⁻⁾ /Ter119 ⁽⁻⁾ /Sca-1 ⁽⁺⁾ /CD49f ⁽⁺⁾ ; Trop2 ^(hi) /CD49f ^{(hi)*}	[100–102]
Salivary gland stem cells	CD49f ⁽⁺⁾ /Thy-1 ⁽⁺⁾ /Laminin ⁽⁺⁾	[103]
Sweat gland myoepithelial stem cells	CD29 ^(hi) /CD49f ^(hi)	[104]
Spermatogonial stem cells	CD29 ⁽⁺⁾ /CD49f ⁽⁺⁾	[23]
Thymus stem cells	Sca1 ⁽⁺⁾ /PDGFRa ⁽⁺⁾ /PDGFRb ⁽⁺⁾ /CD29 ⁽⁺⁾ /CD44 ⁽⁺⁾ /CD49f ⁽⁺⁾ /CD90 ⁽⁺⁾	[105]
Tracheal epithelial stem cells	CD49f ^(bri) /Sca1 ⁽⁺⁾ /ALDH ⁽⁺⁾	[106]
Breast cancer stem cells	CD44 ⁽⁺⁾ /CD49f ⁽⁺⁾ ; CD44 ⁽⁺⁾ /CD24 ⁽⁻⁾ /CD49f ⁽⁺⁾ /NRP2 ⁽⁺⁾	[57,107]
Cervical cancer stem cells	CD34 ⁽⁺⁾ /CD49f ⁽⁺⁾ /CD133 ⁽⁺⁾ ; CK-17 ⁽⁺⁾ /p63 ⁽⁺⁾ /All ⁽⁺⁾ /CD49f ⁽⁺⁾ /ALDH ^(hi)	[50,51]
Esophageal adenocarcinoma stem cells	CD71 ^(dim) /CD49f ^(bri)	[83]
Glioblastoma cancer stem cells	CD133 ⁽⁺⁾ /CD49f ⁽⁺⁾	[49]
Hepatocellular carcinoma stem cells	CD133 ⁽⁺⁾ /CD49f ⁽⁺⁾	[108]
Prostate cancer stem cells	CD49f ⁽⁺⁾ /CD44 ⁽⁺⁾ /CD133 ⁽⁺⁾ ; Lin ⁽⁻⁾ /Sca-1 ⁽⁺⁾ /CD49f ⁽⁺⁾	[109,110]
Ovarian endometrioma stem cells	Sal-like 4 ⁽⁺⁾ /CD133 ⁽⁺⁾ /Musashi-1 ⁽⁺⁾ /CD49f ⁽⁺⁾	[111]
Squamous cell carcinoma stem cells	CD34 ⁽⁺⁾ /CD49f ^(hi) /CD29 ^(hi)	[112]

The *bold* text highlights the positive and common expression of CD49f among all the stem cell types listed in the table.

as a link between extracellular contacts and the intracellular cytoskeleton. In addition, integrins work together with receptor tyrosine kinases to communicate bidirectional signals between cells and the ECM [13]. The activation of integrins upon ligand binding triggers signal transduction mechanisms involved in cell differentiation, gene expression, motility, polarity, proliferation, shape, and survival/apoptosis [12,14].

By forming heterodimers with either integrin $\beta 1$ (CD29) or integrin $\beta 4$ (CD104), integrin $\alpha 6$ functions as a receptor for the following laminins: LAMA2, LAMA3, LAMB1, LAMB2, LAMB3, LAMC1, and LAMC2. In addition, integrin $\alpha 6$ has two isoforms with distinct cytoplasmic variants, $\alpha 6A$ and $\alpha 6B$, which are generated by alternative mRNA splicing [15,16]. Thus, the signaling directed from integrin $\alpha 6$ varies depending on the dominant isoform expressed, by which β chain is partnered to form a heterodimer and by the ligand that is binding [17,18]. As discussed below in detail, the expression of integrin $\alpha 6$ is found in early stages of development and throughout adult life. Embryonic deletion of integrin $\alpha 6$ in mice leads to neonatal death with a phenotype of cerebral malformations and severe skin blistering [19]. The latter resembles skin lesions observed in epidermolysis bullosa, which is observed in mouse deficient in LAMA3 [20], LAMB3 [21], and LAMC2 [22]. All this information indicates the important role of integrin $\alpha 6$ in development and formation of organs.

Expression of integrin $\alpha 6$ in stem cells: from germ cells to somatic stem cells

The expression of integrin $\alpha 6$ has been identified in somatic stem cells and germ cells from early developmental stages through adulthood. The heterodimer of integrin $\alpha 6\beta 1$ is found in spermatogonial stem cells [23] and female primordial germ cells [24]. Spermatogenesis has been reconstituted in infertile male mice after colonization assays in recipient testes using testis cells expressing integrin $\alpha 6$ and integrin $\beta 1$. Remarkably, the c-kit⁺ populations isolated from testicular cells are not able to repopulate recipient testes, while the success rate in reconstitution of spermatogenesis doubles using integrin $\alpha 6^+$ cells compared to $\beta 1^+$ cells. These findings suggest that integrin $\alpha 6$ selects a pure population of spermatogonial stem cells; while because integrin $\beta 1$ forms heterodimers with many other α subunits, its selected population may be contaminated with nonstem cells [23]. In female primordial germ cells isolated from mouse embryos at 10.5 embryonic days, which give rise to oocytes, both integrin $\alpha 6$ and $\beta 1$ are expressed, and their expression is continuous during all stages of oocyte development [24]. It has been demonstrated that the presence of integrin $\alpha 6\beta 1$ in ovulated oocytes is required for fertilization by facilitating the binding and fusion between sperm and egg plasma membranes [25]. In the developing embryo, the expression of integrin $\alpha 6\beta 1$ has been detected at the mRNA level throughout preimplantation development. At the protein level, $\alpha 6$ is detected from two-cell embryos to the late morula/early blastocyst stage. At the early and late blastocyst stage, $\alpha 6\beta 1$ is present in the ICM and all the rest of the cells except in the external surface of the trophectoderm. In vitro culture of outgrowths from blastocyst embryos reveals that only cells from the ICM express integrin $\alpha 6$, while

trophoblast outgrowing cells are $\alpha 6^-$ [26]. In the appropriate in vitro conditions, the ICM outgrowths (integrin $\alpha 6^+$ cells) become what are known as ESCs [27,28], which have integrin $\alpha 6\beta 1$ in their undifferentiated and pluripotent state [29,30].

As shown in Table 1, integrin $\alpha 6$ is present in many somatic stem cells, and some studies clearly show its importance in identifying true stem cell populations. Below we describe selected studies that demonstrate the importance of integrin $\alpha 6$ in the most well-studied stem cell populations.

Hematopoietic Stem Cells

Mature blood cell lineages are generated from a network of hierarchically distinct progenitors that arise from self-renewing HSCs. Integrin $\alpha 6$ is expressed in nearly 99% of mouse primitive hematopoietic cells (Lin⁻/Sca-1⁺/c-kit⁺ [LSK]) and in about 90% of committed myeloid progenitors (Lin⁻/Sca-1⁻/c-Kit⁺). Interestingly, the blockage of integrin $\alpha 6$ significantly inhibits the homing of mouse HSCs to the bone marrow (BM) and impairs long-term multilineage reconstitution after competitive repopulation assays. Integrin $\alpha 6$ is also found in human primitive HSCs, suggesting a conserved interspecies functional role during hematopoiesis [31]. More recently, further evidence that integrin $\alpha 6$ (CD49f) plays a key role in human HSC function was demonstrated by showing that single Lin⁻/CD34⁺/Cd38⁻/CD45RA⁻/Thy⁺/Rho^{low}/CD49f⁺ cells engrafted in femur BM give rise to long-term multilineage systemic grafts. In addition, the loss of expression of integrin $\alpha 6$ in human hematopoietic cells distinguishes HSCs from multilineage progenitor populations [32].

Mammary Stem Cells

Integrin $\alpha 6$ has also been identified as a biomarker of mammary stem cells (MaSCs), which are cells that display self-renewal properties and are able to regenerate new mammary tissue in vivo. Transplantation of single cells extracted from adult mouse mammary tissue with the phenotype of CD45⁻/Ter119⁻/CD31⁻/Sca-1^{low}/CD24^{med}/CD49f^{high} is able to regenerate mammary glands in the fat pads of virgin host mice [33].

The self-renewal properties of CD45⁻/Ter119⁻/CD31⁻/Sca-1^{low}/CD24^{med}/CD49f^{high} cells have also been confirmed after secondary limiting dilution assays prepared from primary outgrowths after transplantation [33]. Interestingly, the expression levels of integrin $\alpha 6$ from high to low are able to differentiate MaSCs from progenitor cells, respectively, in combination with the expression of CD24. CD24^{med}/CD49f^{high} cells in Matrigel cultures produce solid colonies with irregular shape, branched ductal appearance, and an irregular-shaped lumen, while CD24^{high}/CD49f^{low} cells generate uniformly spherical acinar structures mainly made off cuboidal epithelium [33].

Mesenchymal Stem Cells

MSCs, which have immunomodulatory and engraftment promoting properties [34], are the subject of several promising clinical trials (ClinicalTrials.gov). First identified from mononuclear cells of the BM [35,36], MSCs are also detected and isolated from other tissues like adipose tissue

[37], dental pulp [38], endometrial stroma [39], periodontal ligament [34], placenta [40], and umbilical cord blood (UCB) [41]. The International Society for Cellular Therapy has established that minimum criteria to define a MSC population require: (1) adherence to tissue culture plastic under standard culture conditions, (2) an immunophenotype that includes CD105, CD73, and CD90, while lacking the expression of hematopoietic markers (CD45, CD34, CD14 or CD11b, CD79a, CD19, or HLA-DR), and (3) ability to differentiate into osteoblast, adipocytes, and chondrocytes. Beside these biological markers, other cell surface proteins, including integrin $\alpha 6$ -CD49f- [42], have been used to characterize MSCs [43]. CD49f has been found in MSCs derived from BM [44,45] and UCB [46]. Interestingly, it was reported that the level of CD49f expression in BM-MSC declines with increasing age, while similarly higher levels of expression are found among younger donors of both BM-MSC and UCB-MSCs. This higher expression of CD49f in UCB in relation to adult BM-MSC correlates with a higher lung clearance rate after systemic infusion [46]. The high expression of CD49f and PODXL in BM-MSCs has been also related to significantly better efficiency in generating single cell-derived colonies and in differentiation into mineralizing cells and adipocytes in vitro. Furthermore, after intravenous infusion in mice, the PODXL^{hi}/CD49f^{hi} is less likely to result in lethal pulmonary emboli and these cells survive longer in the lung compared to PODXL^{low}/CD49f^{low} cells [44].

Neuronal Stem Cells

Neural stem cells (NSC) give rise to all major cell types of the central nervous system and are localized in the ventricular and subventricular zones (SVZ) of the brain, where NSC niches have been identified surrounding vascular endothelial cells [47]. NSCs are identified by the intracellular expression of Sox1, Sox2, Sox3, Nestin, and Musashi and extracellular expression of integrin $\alpha 6\beta 1$; and selection of $\alpha 6^{\text{high}}$ or $\beta 1^{\text{high}}$ cells leads to enrichment of NSCs from neurospheres [48]. The expression of $\beta 1$ in these cells correlates with the expression of prominin-1 (CD133), one of the most effective markers of NSCs. In the same population of cells, 20% are characterized as integrin $\alpha 6^{\text{high}}$ cells. Reports show that $\beta 1^{\text{high}}$ cells have upregulated expression of Sox2, Sox3, Musashi1, and Bmi1 compared to $\beta 1^{\text{low}}$ cells and are able to differentiate into neurons and astrocytes [48]. A similar pattern of $\alpha 6^{\text{high}}/\beta 1^{\text{high}}$ expression has been reported in mouse adult NSCs, and this expression is lost as NSCs differentiate and migrate away from their vascular niche in the SVZ of the brain. Interestingly, in vivo treatment with integrin $\alpha 6$ antibodies in the lateral ventricle of adult mice brains results in migration of NSCs from their niche and proliferation of SVZ lineage cells [47], suggesting that modulation in the expression of integrin $\alpha 6$ induces differentiation of NSC and migration out of the stem cell niche.

Cancer Stem Cells

A distinct population of cells within most tumors have the potential for self-renewal and differentiation into cells that populate new tumors and, therefore, are referred to as tumor-initiating cells or CSCs. In glioblastoma cancer stem

cells (GSCs), integrin $\alpha 6$ has been proposed to be an important regulator of self-renewal, proliferation, and tumor formation capacity [49]. An enriched population of GSCs can be obtained from bulk tumors, based on selection of high expression of integrin $\alpha 6$ alone or in combination with CD133 [49]. These cells are able to form tumorspheres in vitro, which confirm their self-renewal capacity. In vivo limiting dilution transplantation assays of integrin $\alpha 6^{\text{high}}$ cells demonstrates a significant increase in tumor formation with lower numbers of transplanted cells and in a shorter time compared to integrin $\alpha 6^{\text{low}}$ cells. Knockdown of *integrin $\alpha 6$* by shRNA on these cells abrogates the formation of tumorspheres and significantly reduces tumor formation in immune-compromised mice [49], suggesting that this stem cell population is depleted.

Finally, integrin $\alpha 6$ has been used to enrich CSC populations from four different cervical uterine cancer cell lines: HeLa, SiHa, Ca Ski, and C-4 I. The integrin $\alpha 6^+$ CSCs obtained from spheroids show self-renewal properties, enhanced tumorigenic capabilities, and increased resistance to ionizing radiation compared to their parental cell lines cultured in monolayer [50,51].

The Role of Integrin $\alpha 6$ in the Mechanisms Used to Maintain Self-Renewal in Stem Cells

Despite the common expression of integrin $\alpha 6$ in stem cells (Table 1), little is known about the molecular mechanisms by which this transmembrane receptor is regulated. It is known that Oct4 and Sox2 bind promoters of integrin $\alpha 6$ and support its transcription [45]. It has also been shown that KLF9 represses the transcription of integrin $\alpha 6$ by binding its promoter region [52]. Interestingly, this repressive action of KLF9 on integrin $\alpha 6$ inhibits both glioblastoma cell stemness and tumorigenicity [52].

Our current understanding of the molecular mechanisms by which integrin $\alpha 6$ regulates self-renewal of stem cells comes largely from studies in ESCs and breast CSCs. We recently reported a critical role of integrin $\alpha 6$ in the self-renewal of human ESCs and identified the molecular mechanisms involved in this process [30]. The heterodimer of integrin $\alpha 6\beta 1$ is dominant in undifferentiated human ESCs, regardless of the substrate on which they are cultured (vitronectin-coated plates (CP), fibronectin-CP, laminin 511-CP, Matrigel-CP, or in supporting polymer coatings). The expression of integrin $\alpha 6$ highly correlates with the undifferentiated state of human ESCs and, therefore, with the expression of Oct4 and Sox2, both pluripotent-related transcription factors [30]. Interestingly, human ESCs express the A and B isoforms of integrin $\alpha 6$, and it has been reported that the A isoform is able to prevent the activation of integrin $\beta 1$ [53]. Indeed, it was observed that $\beta 1$ signaling in hESCs is inactive, since focal adhesion kinase (FAK) is not phosphorylated in undifferentiated cells. Activation of integrin $\beta 1$ signaling by activating antibodies induces the phosphorylation of FAK and the differentiation of human ESCs. Furthermore, during differentiation integrin $\alpha 6$ expression decreases, while FAK becomes phosphorylated. FAK is present in the nucleus of undifferentiated hESCs where it co-localizes and interacts with both Oct4 and Sox2, and the overexpression of these transcription factors induces the nuclear localization of FAK.

Similarly, during the reprogramming of fibroblasts into induced pluripotent stem cells, integrin $\alpha 6$ is upregulated and FAK is dephosphorylated [30]. Finally, it has been reported that human ESCs synthesize and deposit a laminin 511-rich substrate [54], and the knockdown of laminin in these stem cells induces reduction in integrin $\alpha 6$ expression,

phosphorylation of FAK, and degradation of Oct4 and Sox2 [30]. Taken together, these findings suggest a model in which human pluripotent stem cells remodel the microenvironment to sustain integrin $\alpha 6$, prevent integrin $\beta 1$ /FAK activation, and maintain the expression of transcription factors involved in self-renewal and pluripotency (Fig. 1) [30,54].

FIG. 1. Integrin $\alpha 6$ (CD49f) is a conserved biomarker of stem cells involved in their self-renewal. **(A)** Illustration showing the presence of integrin $\alpha 6$ in diverse stem cell populations. From pluripotent stem cells, the inner cell mass of the blastocyst embryo and ESC to adult stem cells (for a complete list of stem cell types refer to Table 1). **(B)** Representative micrographs showing coexpression of integrin $\alpha 6$ with the pluripotent transcription factor Oct4 in undifferentiated (*top panel*) and differentiating (*bottom panel*) human ESC colonies. The *dotted outer line* in the micrographs indicates the margins of colonies, while the *inner dimmed* and *bright dotted lines* delineate colony areas with positive expression of Oct4 and integrin $\alpha 6$, respectively. Notice tightly coexpression of Oct4 and integrin $\alpha 6$ in both undifferentiated and differentiating colonies. A schematic model illustrating a mechanism by which integrin $\alpha 6$ might regulate self-renewal of stem cells, based on data obtained in human ESCs [30]. The depicted drawing on the *left* indicates an undifferentiated ESC expressing pluripotent related transcription factors Oct4, Sox2, and Nanog and integrin $\alpha 6A\beta 1B$. This protein configuration maintains the FAK inactive and localized in the cytoplasm and the nuclei of undifferentiated cells. Change in translation of integrin $\alpha 6\beta 1$ to $\alpha 6B\beta 1A$ in the stem cell (drawing on the *right*) results in recruitment to the plasma membrane and subsequent phosphorylation of FAK, followed by activation of signaling pathways and change in gene expression that include the downregulation of the pluripotent transcription factors and cell differentiation. ESC, embryonic stem cell; FAK, focal adhesion kinase.

The expression of integrin $\alpha 6$ is consistently found in breast CSCs [55–57] and recently it has been shown that the B isoform, rather than the A isoform of $\alpha 6$, defines more precisely the breast CSC population [17]. Phenotypically the integrin $\alpha 6$ B-dominant population has a mesenchymal cell morphology compared to the epithelial cell morphology of the $\alpha 6$ A-dominant population, and they form heterodimers differently: the $\alpha 6$ B heterodimer with integrin $\beta 1$, while $\alpha 6$ A population expresses integrin $\beta 4$. The expression of ALDH1 and BMI1, well-established stem cell markers, as well as augmented retention of PKH, a marker for slow-cycling cells, is increased in the $\alpha 6$ B population. Furthermore, $\alpha 6$ B cells have a greater capacity to form mammospheres and to initiate tumors.

The expression of A and B isoforms of integrin $\alpha 6$ is regulated by alternative splicing mechanisms [58], and the ESRP1-splicing factor has been identified as a key regulator of $\alpha 6$ A while it also functions to repress $\alpha 6$ B [17]. Interestingly, VEGF signaling also promotes the initiation of triple negative breast cancer tumors through suppression of ESRP1 and, consequently, by the induction of $\alpha 6$ B expression [17,57]. Supporting the concept that integrin $\alpha 6$ B is a determinant in breast CSCs, laminin511 is found to be expressed in the niche that supports self-renewal of breast CSCs by engaging with integrin $\alpha 6$ B [59]. The interaction between laminin511 and integrin $\alpha 6$ B activates TAZ [59], a transducer of the Hippo pathway that has been associated in the self-renewal and tumor-initiation capacities of breast CSCs [60].

Integrin $\alpha 6$ Links Stem Cells to Laminins Present in Their Niche

Stem cells reside in specialized microenvironments termed the stem cell niche. The niche provides cues in the form of cell-ECM, cell-cell contact, and secreted factors that regulate the fate of the residing stem cell population. Specific characteristics among niches vary [61–63]. However, a common finding among stem cell niches is the presence of the laminins. These ECM proteins, which are the ligands for integrin $\alpha 6$, have been found in the niches of several somatic stem cells such as corneal [64], colonic [65], epithelial [66], hair follicle [67], hematopoietic [68,69], hepatic [70], spermatogonia [23], neuronal [47,71], as well as in the blastocyst embryo [72], where the ICM is formed, and in the niche of glioblastoma [49] and breast CSCs [59]. The deposition of laminins in stem cell niches may originate from nonstem cells that are part of the niche. For example, vascular endothelial cells [73], which are key elements in several stem cell niches [47,74–76]. However, recent evidence suggests that stem cells secrete laminins as well and may therefore contribute to the formation and connection to the stem cell niche, and with their self-renewal mechanisms. In vitro human ESCs deposit laminin511 [30,54], and knockdown of laminin in these stem cells demonstrates connection to protein levels of integrin $\alpha 6$ and Oct4 [30]. Breast CSCs produce laminin511, which functions as a ligand for $\alpha 6$ B $\beta 1$ expressed in these cells, promoting self-renewal and tumor initiation as well [59]. The deposition of laminins by stem cells in the niche may be regulated by integrin signaling through integrin-linked kinase (ILK), as demonstrated recently in the niche of CD34⁺/CD49f^{high} hair

follicle stem cells (HFSCs) [67]. Deletion of ILK leads to changes in the laminin isoforms present in the stem cell niche, which results in activation of HFSCs and in their exhaustion [67]. This suggests a critical role between laminins in the niche and integrins in stem cells regulating their activities, and the fact that integrin $\alpha 6$ is present in more than 30 stem cell populations (Table 1) gives merit to further investigation of this specific integrin subunit in the maintenance and function of stem cells.

Conclusion

Stem cells play key roles in homeostasis by replenishing tissues with differentiated cells that are lost because of natural causes or during injury. Stem cells share the properties of self-renewal and differentiation, as well as the need for a specialized niche in which to reside. In this literature review, we shed light onto another common characteristic, the expression and functional role of integrin $\alpha 6$ (CD49f). We synthesized data from several lines of evidence in a broad range of stem cells to support the case that integrin $\alpha 6$ can be considered as an authentic and reliable stem cell marker because of its common expression and function in homing and connecting stem cells to their niches and for its role in regulating self-renewal mechanisms in stem cells.

Acknowledgments

The National Institutes of Health grant R01DE016530-08 supported this work. The authors thank AlphaMed Press for permission to use a modified version of a figure published in Stem Cells 2016;34:1753-64 (DOI:10.1002/stem.2349) by the authors.

Author Disclosure Statement

No competing financial interests exist.

References

1. Civin CI, LC Strauss, C Brovall, MJ Fackler, JF Schwartz and JH Shaper. (1984). Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol* 133:157–165.
2. Simmons PJ and B Torok-Storb. (1991). CD34 expression by stromal precursors in normal human adult bone marrow. *Blood* 78:2848–2853.
3. Dupas T, T Rouaud, K Rouger, B Lieubeau, C Cario-Toumaniantz, J Fontaine-Perus, MF Gardahaut and G Auda-Boucher. (2011). Fetal muscle contains different CD34⁺ cell subsets that distinctly differentiate into adipogenic, angiogenic and myogenic lineages. *Stem Cell Res* 7:230–243.
4. Tumber T, G Guasch, V Greco, C Blanpain, WE Lowry, M Rendl and E Fuchs. (2004). Defining the epithelial stem cell niche in skin. *Science* 303:359–363.
5. Brown J, MF Greaves and HV Molgaard. (1991). The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts. *Int Immunol* 3: 175–184.
6. Sidney LE, MJ Branch, SE Dunphy, HS Dua and A Hopkinson. (2014). Concise review: evidence for CD34 as

- a common marker for diverse progenitors. *Stem Cells* 32: 1380–1389.
7. Kligys KR, Y Wu, SB Hopkinson, S Kaur, LC Platanias and JC Jones. (2012). Alpha6beta4 integrin, a master regulator of expression of integrins in human keratinocytes. *J Biol Chem* 287:17975–17984.
 8. Neuber K, RA Hilger and W Konig. (1992). Differential increase in 12-HETE release and CD29/CD49f expression of platelets from normal donors and from patients with atopic dermatitis by *Staphylococcus aureus*. *Int Arch Allergy Immunol* 98:339–342.
 9. Schlotzer-Schrehardt U and FE Kruse. (2005). Identification and characterization of limbal stem cells. *Exp Eye Res* 81:247–264.
 10. Li A, PJ Simmons and P Kaur. (1998). Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci U S A* 95:3902–3907.
 11. Fortunel NO, HH Otu, HH Ng, J Chen, X Mu, T Chevassut, X Li, M Joseph, C Bailey, et al. (2003). Comment on “‘Stemness’: transcriptional profiling of embryonic and adult stem cells” and “a stem cell molecular signature”. *Science* 302:393; author reply 393.
 12. Hynes RO. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* 110:673–687.
 13. Gilcrease MZ. (2007). Integrin signaling in epithelial cells. *Cancer Lett* 247:1–25.
 14. Watt FM. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J* 21: 3919–3926.
 15. Hogervorst F, I Kuikman, AG van Kessel and A Sonnenberg. (1991). Molecular cloning of the human alpha 6 integrin subunit. Alternative splicing of alpha 6 mRNA and chromosomal localization of the alpha 6 and beta 4 genes. *Eur J Biochem* 199:425–433.
 16. Tamura RN, HM Cooper, G Collo and V Quaranta. (1991). Cell type-specific integrin variants with alternative alpha chain cytoplasmic domains. *Proc Natl Acad Sci U S A* 88:10183–10187.
 17. Goel HL, T Gritsko, B Pursell, C Chang, LD Shultz, DL Greiner, JH Norum, R Toftgard, LM Shaw and AM Mercurio. (2014). Regulated splicing of the alpha6 integrin cytoplasmic domain determines the fate of breast cancer stem cells. *Cell Rep* 7:747–761.
 18. Dogic D, P Rousselle and M Aumailley. (1998). Cell adhesion to laminin 1 or 5 induces isoform-specific clustering of integrins and other focal adhesion components. *J Cell Sci* 111 (Pt 6):793–802.
 19. Georges-Labouesse E, N Messaddeq, G Yehia, L Cadalbert, A Dierich and M Le Meur. (1996). Absence of integrin alpha 6 leads to epidermolysis bullosa and neonatal death in mice. *Nat Genet* 13:370–373.
 20. McGrath JA, S Kivirikko, S Ciatti, C Moss, GS Dunnill, RA Eady, CH Rodeck, AM Christiano and J Uitto. (1995). A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in Herlitz junctional epidermolysis bullosa: prenatal exclusion in a fetus at risk. *Genomics* 29:282–284.
 21. Kuster JE, MH Guarnieri, JG Ault, L Flaherty and PJ Swiatek. (1997). IAP insertion in the murine Lamb3 gene results in junctional epidermolysis bullosa. *Mamm Genome* 8:673–681.
 22. Pulkkinen L, AM Christiano, T Airene, H Haakana, K Tryggvason and J Uitto. (1994). Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa. *Nat Genet* 6:293–297.
 23. Shinohara T, MR Avarbock and RL Brinster. (1999). beta1- and alpha6-integrin are surface markers on mouse spermatogonial stem cells. *Proc Natl Acad Sci U S A* 96:5504–5509.
 24. Zuccotti M, P Giorgi Rossi, E Fiorillo, S Garagna, A Forabosco and CA Redi. (1998). Timing of gene expression and oolemma localization of mouse alpha6 and beta1 integrin subunits during oogenesis. *Dev Biol* 200:27–34.
 25. Almeida EA, AP Huovila, AE Sutherland, LE Stephens, PG Calarco, LM Shaw, AM Mercurio, A Sonnenberg, P Primakoff, DG Myles and JM White. (1995). Mouse egg integrin alpha 6 beta 1 functions as a sperm receptor. *Cell* 81:1095–1104.
 26. Sutherland AE, PG Calarco and CH Damsky. (1993). Developmental regulation of integrin expression at the time of implantation in the mouse embryo. *Development* 119:1175–1186.
 27. Evans MJ and MH Kaufman. (1981). Establishment in culture of pluripotent cells from mouse embryos. *Nature* 292:154–156.
 28. Thomson JA, J Itskovitz-Eldor, SS Shapiro, MA Waknitz, JJ Swiergiel, VS Marshall and JM Jones. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–1147.
 29. Xu C, MS Inokuma, J Denham, K Golds, P Kundu, JD Gold and MK Carpenter. (2001). Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol* 19:971–974.
 30. Villa-Diaz LG, JK Kim, A Laperle, SP Palecek and PH Krebsbach. (2016). Inhibition of focal adhesion kinase signaling by integrin alpha6beta1 supports human pluripotent stem cell self-renewal. *Stem Cells* 34:1753–1764.
 31. Qian H, K Tryggvason, SE Jacobsen and M Ekblom. (2006). Contribution of alpha6 integrins to hematopoietic stem and progenitor cell homing to bone marrow and collaboration with alpha4 integrins. *Blood* 107:3503–3510.
 32. Notta F, S Doulatov, E Laurenti, A Poepl, I Jurisica and JE Dick. (2011). Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* 333:218–221.
 33. Stingl J, P Eirew, I Ricketson, M Shackleton, F Vaillant, D Choi, HI Li and CJ Eaves. (2006). Purification and unique properties of mammary epithelial stem cells. *Nature* 439:993–997.
 34. Ozawa K, K Sato, I Oh, K Ozaki, R Uchibori, Y Obara, Y Kikuchi, T Ito, T Okada, et al. (2008). Cell and gene therapy using mesenchymal stem cells (MSCs). *J Autoimmun* 30:121–127.
 35. Pittenger MF, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147.
 36. Krebsbach PH, SA Kuznetsov, P Bianco and PG Robey. (1999). Bone marrow stromal cells: characterization and clinical application. *Crit Rev Oral Biol Med* 10:165–181.
 37. Katz AJ, A Tholpady, SS Tholpady, H Shang and RC Ogle. (2005). Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. *Stem Cells* 23:412–423.

38. Shi S and S Gronthos. (2003). Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 18:696–704.
39. Schwab KE and CE Gargett. (2007). Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. *Hum Reprod* 22:2903–2911.
40. Igura K, X Zhang, K Takahashi, A Mitsuru, S Yamaguchi and TA Takashi. (2004). Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. *Cytotherapy* 6:543–553.
41. Romanov YA, VA Svintsitskaya and VN Smirnov. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* 21:105–110.
42. Majumdar MK, M Keane-Moore, D Buyaner, WB Hardy, MA Moorman, KR McIntosh and JD Mosca. (2003). Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 10:228–241.
43. Lv FJ, RS Tuan, KM Cheung and VY Leung. (2014). Concise review: the surface markers and identity of human mesenchymal stem cells. *Stem Cells* 32:1408–1419.
44. Lee RH, MJ Seo, AA Pulin, CA Gregory, J Ylostalo and DJ Prockop. (2009). The CD34-like protein PODXL and alpha6-integrin (CD49f) identify early progenitor MSCs with increased clonogenicity and migration to infarcted heart in mice. *Blood* 113:816–826.
45. Yu KR, SR Yang, JW Jung, H Kim, K Ko, DW Han, SB Park, SW Choi, SK Kang, H Scholer and KS Kang. (2012). CD49f enhances multipotency and maintains stemness through the direct regulation of OCT4 and SOX2. *Stem Cells* 30:876–887.
46. Nystedt J, H Anderson, J Tikkanen, M Pietila, T Hirvonen, R Takalo, A Heiskanen, T Satomaa, S Natunen, et al. (2013). Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. *Stem Cells* 31:317–326.
47. Shen Q, Y Wang, E Kokovay, G Lin, SM Chuang, SK Goderie, B Roysam and S Temple. (2008). Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* 3:289–300.
48. Hall PE, JD Lathia, NG Miller, MA Caldwell and C Ffrench-Constant. (2006). Integrins are markers of human neural stem cells. *Stem Cells* 24:2078–2084.
49. Lathia JD, J Gallagher, JM Heddleston, J Wang, CE Eyler, J Macswords, Q Wu, A Vasanji, RE McLendon, AB Hjelmeland and JN Rich. (2010). Integrin alpha 6 regulates glioblastoma stem cells. *Cell Stem Cell* 6:421–432.
50. Lopez J, A Poitevin, V Mendoza-Martinez, C Perez-Plasencia and A Garcia-Carranca. (2012). Cancer-initiating cells derived from established cervical cell lines exhibit stem-cell markers and increased radioresistance. *BMC Cancer* 12:48.
51. Ortiz-Sanchez E, L Santiago-Lopez, VB Cruz-Dominguez, ME Toledo-Guzman, D Hernandez-Cueto, S Muniz-Hernandez, E Garrido, D Cantu-De Leon and A Garcia-Carranca. (2016). Characterization of cervical cancer stem cell-like cells: phenotyping, stemness, and Human Papilloma Virus co-receptor expression. *Oncotarget* 7:31943–31954.
52. Ying M, J Tilghman, Y Wei, H Guerrero-Cazares, A Quinones-Hinojosa, H Ji and J Littera. (2014). Kruppel-like factor-9 (KLF9) inhibits glioblastoma stemness through global transcription repression and integrin alpha6 inhibition. *J Biol Chem* 289:32742–32756.
53. Sastry SK, M Lakonishok, S Wu, TQ Truong, A Huttenlocher, CE Turner and AF Horwitz. (1999). Quantitative changes in integrin and focal adhesion signaling regulate myoblast cell cycle withdrawal. *J Cell Biol* 144:1295–1309.
54. Laperle A, C Hsiao, M Lampe, J Mortier, K Saha, SP Palecek and KS Masters. (2015). Alpha-5 laminin synthesized by human pluripotent stem cells promotes self-renewal. *Stem Cell Reports* 11:195–206.
55. Vieira AF, S Ricardo, MP Ablett, MR Dionisio, N Mendes, A Albergaria, G Farnie, R Gerhard, JF Cameselle-Teijeiro, et al. (2012). P-cadherin is coexpressed with CD44 and CD49f and mediates stem cell properties in basal-like breast cancer. *Stem Cells* 30:854–864.
56. Meyer MJ, JM Fleming, AF Lin, SA Hussnain, E Ginsburg and BK Vonderhaar. (2010). CD44posCD49f-hiCD133/2hi defines xenograft-initiating cells in estrogen receptor-negative breast cancer. *Cancer Res* 70:4624–4633.
57. Goel HL, B Pursell, C Chang, LM Shaw, J Mao, K Simin, P Kumar, CW Vander Kooi, LD Shultz, et al. (2013). GLI1 regulates a novel neuropilin-2/alpha6beta1 integrin based autocrine pathway that contributes to breast cancer initiation. *EMBO Mol Med* 5:488–508.
58. Warzecha CC, P Jiang, K Amirikyan, KA Dittmar, H Lu, S Shen, W Guo, Y Xing and RP Carstens. (2010). An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. *EMBO J* 29:3286–3300.
59. Chang C, HL Goel, H Gao, B Pursell, LD Shultz, DL Greiner, S Ingerpuu, M Patarroyo, S Cao, et al. (2015). A laminin 511 matrix is regulated by TAZ and functions as the ligand for the alpha6Bbeta1 integrin to sustain breast cancer stem cells. *Genes Dev* 29:1–6.
60. Cordenonsi M, F Zanconato, L Azzolin, M Forcato, A Rosato, C Frasson, M Inui, M Montagner, AR Parenti, et al. (2011). The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* 147:759–772.
61. Spradling A, D Drummond-Barbosa and T Kai. (2001). Stem cells find their niche. *Nature* 414:98–104.
62. Fuchs E, T Tumber and G Guasch. (2004). Socializing with the neighbors: stem cells and their niche. *Cell* 116:769–778.
63. Watt FM and BL Hogan. (2000). Out of Eden: stem cells and their niches. *Science* 287:1427–1430.
64. Chen Z, CS de Paiva, L Luo, FL Kretzer, SC Pflugfelder and DQ Li. (2004). Characterization of putative stem cell phenotype in human limbal epithelia. *Stem Cells* 22:355–366.
65. Fujimoto K, RD Beauchamp and RH Whitehead. (2002). Identification and isolation of candidate human colonic clonogenic cells based on cell surface integrin expression. *Gastroenterology* 123:1941–1948.
66. Jones PH and FM Watt. (1993). Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* 73:713–724.
67. Morgner J, S Ghatak, T Jakobi, C Dieterich, M Aumailley and SA Wickstrom. (2015). Integrin-linked kinase regulates the niche of quiescent epidermal stem cells. *Nat Commun* 6:8198.

68. Siler U, P Rousselle, CA Muller and G Klein. (2002). Laminin gamma2 chain as a stromal cell marker of the human bone marrow microenvironment. *Br J Haematol* 119:212–220.
69. Gu Y, L Sorokin, M Durbeek, T Hjalt, JI Jonsson and M Ekblom. (1999). Characterization of bone marrow laminins and identification of alpha5-containing laminins as adhesive proteins for multipotent hematopoietic FDCP-Mix cells. *Blood* 93:2533–2542.
70. Suzuki A, Y Zheng, R Kondo, M Kusakabe, Y Takada, K Fukao, H Nakauchi and H Taniguchi. (2000). Flow-cytometric separation and enrichment of hepatic progenitor cells in the developing mouse liver. *Hepatology* 32:1230–1239.
71. Lathia JD, B Patton, DM Eckley, T Magnus, MR Mughal, T Sasaki, MA Caldwell, MS Rao, MP Mattson and C Ffrench-Constant. (2007). Patterns of laminins and integrins in the embryonic ventricular zone of the CNS. *J Comp Neurol* 505:630–643.
72. Leivo I, A Vaheri, R Timpl and J Wartiovaara. (1980). Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev Biol* 76:100–114.
73. Vartanian KB, SJ Kirkpatrick, OJ McCarty, TQ Vu, SR Hanson and MT Hinds. (2009). Distinct extracellular matrix microenvironments of progenitor and carotid endothelial cells. *J Biomed Mater Res A* 91:528–539.
74. Palmer TD, AR Willhoite and FH Gage. (2000). Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425:479–494.
75. Louissaint A, Jr., S Rao, C Leventhal and SA Goldman. (2002). Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron* 34:945–960.
76. Kiel MJ, OH Yilmaz, T Iwashita, OH Yilmaz, C Terhorst and SJ Morrison. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 121:1109–1121.
77. Castaldo C, F Di Meglio, D Nurzynska, G Romano, C Maiello, C Bancone, P Muller, M Bohm, M Cotrufo and S Montagnani. (2008). CD117-positive cells in adult human heart are localized in the subepicardium, and their activation is associated with laminin-1 and alpha6 integrin expression. *Stem Cells* 26:1723–1731.
78. Chaurasia P, DC Gajzer, C Schaniel, S D'Souza and R Hoffman. (2014). Epigenetic reprogramming induces the expansion of cord blood stem cells. *J Clin Invest* 124:2378–2395.
79. Hayashi R, M Yamato, T Saito, T Oshima, T Okano, Y Tano and K Nishida. (2008). Enrichment of corneal epithelial stem/progenitor cells using cell surface markers, integrin alpha6 and CD71. *Biochem Biophys Res Commun* 367:256–263.
80. Chang JY, C Wang, C Jin, C Yang, Y Huang, J Liu, WL McKeenan, RN D'Souza and F Wang. (2013). Self-renewal and multilineage differentiation of mouse dental epithelial stem cells. *Stem Cell Res* 11:990–1002.
81. Xiong J, K Mrozik, S Gronthos and PM Bartold. (2012). Epithelial cell rests of Malassez contain unique stem cell populations capable of undergoing epithelial-mesenchymal transition. *Stem Cells Dev* 21:2012–2025.
82. Sakthianandeswaren A, M Christie, C D'Andreti, C Tsui, RN Jorissen, S Li, NI Fleming, P Gibbs, L Lipton, et al. (2011). PHLDA1 expression marks the putative epithelial stem cells and contributes to intestinal tumorigenesis. *Cancer Res* 71:3709–3719.
83. Zhao R, L Quaroni and AG Casson. (2012). Identification and characterization of stemlike cells in human esophageal adenocarcinoma and normal epithelial cell lines. *J Thorac Cardiovasc Surg* 144:1192–1199.
84. Manohar R, J Komori, L Guzik, DB Stolz, UR Chandran, WA LaFramboise and E Lagasse. (2011). Identification and expansion of a unique stem cell population from adult mouse gallbladder. *Hepatology* 54:1830–1841.
85. Trempus CS, RJ Morris, CD Bortner, G Cotsarelis, RS Faircloth, JM Reece and RW Tennant. (2003). Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J Invest Dermatol* 120:501–511.
86. Fujikawa T, T Hirose, H Fujii, S Oe, K Yasuchika, H Azuma and Y Yamaoka. (2003). Purification of adult hepatic progenitor cells using green fluorescent protein (GFP)-transgenic mice and fluorescence-activated cell sorting. *J Hepatol* 39:162–170.
87. Suzuki A, YW Zheng, S Kaneko, M Onodera, K Fukao, H Nakauchi and H Taniguchi. (2002). Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver. *J Cell Biol* 156:173–184.
88. Webb A, A Li and P Kaur. (2004). Location and phenotype of human adult keratinocyte stem cells of the skin. *Differentiation* 72:387–395.
89. Terunuma A, V Kapoor, C Yee, WG Telford, MC Udey and JC Vogel. (2007). Stem cell activity of human side population and alpha6 integrin-bright keratinocytes defined by a quantitative in vivo assay. *Stem Cells* 25:664–669.
90. Tani H, RJ Morris and P Kaur. (2000). Enrichment for murine keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci U S A* 97:10960–10965.
91. Chernaya O, V Shinin, Y Liu and RD Minshall. (2014). Behavioral heterogeneity of adult mouse lung epithelial progenitor cells. *Stem Cells Dev* 23:2744–2757.
92. McQualter JL, K Yuen, B Williams and I Bertoncello. (2010). Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proc Natl Acad Sci U S A* 107:1414–1419.
93. Matulka LA, AA Triplett and KU Wagner. (2007). Parity-induced mammary epithelial cells are multipotent and express cell surface markers associated with stem cells. *Dev Biol* 303:29–44.
94. Deugnier MA, MM Faraldo, J Teuliere, JP Thiery, D Medina and MA Glukhova. (2006). Isolation of mouse mammary epithelial progenitor cells with basal characteristics from the Comma-Delta cell line. *Dev Biol* 293:414–425.
95. Yang Z, P Dong, X Fu, Q Li, S Ma, D Wu, N Kang, X Liu, L Yan and R Xiao. (2015). CD49f acts as an inflammation sensor to regulate differentiation, adhesion, and migration of human mesenchymal stem cells. *Stem Cells* 33:2798–2810.
96. Woo SH, M Stumpfova, UB Jensen, EA Lumpkin and DM Owens. (2010). Identification of epidermal progenitors for the Merkel cell lineage. *Development* 137:3965–3971.
97. Ono M, T Kajitani, H Uchida, T Arase, H Oda, S Uchida, K Ota, T Nagashima, H Masuda, et al. (2015). CD34 and CD49f double-positive and lineage marker-negative cells isolated from human myometrium exhibit stem cell-like properties involved in pregnancy-induced uterine remodeling. *Biol Reprod* 93:37.

98. Igarashi T, S Shimmura, S Yoshida, M Tonogi, N Shinozaki and GY Yamane. (2008). Isolation of oral epithelial progenitors using collagen IV. *Oral Dis* 14:413–418.
99. Jung JG, DK Kim, TS Park, SD Lee, JM Lim and JY Han. (2005). Development of novel markers for the characterization of chicken primordial germ cells. *Stem Cells* 23: 689–698.
100. Lawson DA, L Xin, RU Lukacs, D Cheng and ON Witte. (2007). Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci U S A* 104: 181–186.
101. Barclay WW, LS Axanova, W Chen, L Romero, SL Maund, S Soker, CJ Lees and SD Cramer. (2008). Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. *Stem Cells* 26:600–610.
102. Goldstein AS, DA Lawson, D Cheng, W Sun, IP Garraway and ON Witte. (2008). Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc Natl Acad Sci U S A* 105:20882–20887.
103. Sato A, K Okumura, S Matsumoto, K Hattori, S Hattori, M Shinohara and F Endo. (2007). Isolation, tissue localization, and cellular characterization of progenitors derived from adult human salivary glands. *Cloning Stem Cells* 9:191–205.
104. Kurata R, S Futaki, I Nakano, A Tanemura, H Murota, I Katayama and K Sekiguchi. (2014). Isolation and characterization of sweat gland myoepithelial cells from human skin. *Cell Struct Funct* 39:101–112.
105. Osada M, VJ Singh, K Wu, DB Sant'Angelo and M Pezzano. (2013). Label retention identifies a multipotent mesenchymal stem cell-like population in the postnatal thymus. *PLoS One* 8:e83024.
106. Ghosh M, KM Helm, RW Smith, MS Giordanengo, B Li, H Shen and SD Reynolds. (2011). A single cell functions as a tissue-specific stem cell and the in vitro niche-forming cell. *Am J Respir Cell Mol Biol* 45:459–469.
107. To K, A Fotovati, KM Reipas, JH Law, K Hu, J Wang, A Astanehe, AH Davies, L Lee, et al. (2010). Y-box binding protein-1 induces the expression of CD44 and CD49f leading to enhanced self-renewal, mammosphere growth, and drug resistance. *Cancer Res* 70:2840–2851.
108. Rountree CB, S Senadheera, JM Mato, GM Crooks and SC Lu. (2008). Expansion of liver cancer stem cells during aging in methionine adenosyltransferase 1A-deficient mice. *Hepatology* 47:1288–1297.
109. Yamamoto H, JR Masters, P Dasgupta, A Chandra, R Popert, A Freeman and A Ahmed. (2012). CD49f is an efficient marker of monolayer- and spheroid colony-forming cells of the benign and malignant human prostate. *PLoS One* 7:e46979.
110. Mulholland DJ, L Xin, A Morim, D Lawson, O Witte and H Wu. (2009). Lin-Sca-1+CD49fhigh stem/progenitors are tumor-initiating cells in the Pten-null prostate cancer model. *Cancer Res* 69:8555–8562.
111. Chan RW, EH Ng and WS Yeung. (2011). Identification of cells with colony-forming activity, self-renewal capacity, and multipotency in ovarian endometriosis. *Am J Pathol* 178:2832–2844.
112. Schober M and E Fuchs. (2011). Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. *Proc Natl Acad Sci U S A* 108:10544–10549.

Address correspondence to:

Dr. Luis G. Villa-Diaz

Department of Biological Sciences

Oakland University

Rochester, MI 48309

E-mail: luisvilladiaz@oakland.edu

Received for publication November 10, 2016

Accepted after revision May 11, 2017

Prepublished on Liebert Instant Online May 11, 2017