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COMPREHENSIVE REVIEW

The Role of Integrin α6 (CD49f) in Stem Cells: More than a Conserved Biomarker

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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 α6, 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 α6 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 α6 in stem cell biology.

Keywords: integrin α6, 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)

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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 α6, 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 (~10%) of cells in neonatal human foreskin with the phenotype $\alpha6^{bri}10G7^{dim}$ exhibit highest regenerative capacity compared to other basal cells and were quiescent at the time of isolation [10]. Since then, the expression of integrin α6 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 α6 in some of these populations is conserved among several mammalian species. Furthermore, integrin α6 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 α6

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 α6 (CD49F)

Cardiac stem cells	$CD117^{(+)}$/Laminin$1^{(+)}$/**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-$Kit^{(-)}$/$Thy1.1^{(-)}$ Fetal: c-$Met^{(+)}$/**CD49f**$^{(+/low)}$/c-$kit^{(-)}$/$CD45^{(-)}$/$TER199^{(-)}$	[86,87]
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	$SSEA1^{(+)}$/$SSE3^{(+)}$/$SSEA4^{(+)}$/EMA-$1^{(+)}$/$CD29^{(+)}$, **CD49f**$^{(+)}$	[99]
Prostate stem cells	$CD45^{(-)}$/$CD31^{(-)}$/$Ter119^{(-)}$/Sca-$1^{(+)}$/**CD49f**$^{(+)}$; Trop$2^{(hi)}$/**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/$^{(+)}$PDGFR$a^{(+)}$/PDGFR$b^{(+)}$/$CD29^{(+)}$/$CD44^{(+)}$/**CD49f**$^{(+)}$/$CD90^{(+)}$	[105]
Tracheal epithelial stem cells	**CD49f**$^{(bri)}$/Sca-$1^{(+)}$/$ALDH^{(+)}$	[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 β1 (CD29) or integrin β4 (CD104), integrin α6 functions as a receptor for the following laminins: LAMA2, LAMA3, LAMB1, LAMB2, LAMB3, LAMC1, and LAMC2. In addition, integrin α6 has two isoforms with distinct cytoplasmic variants, α6A and α6B, which are generated by alternative mRNA splicing [15,16]. Thus, the signaling directed from integrin α6 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 α6 is found in early stages of development and throughout adult life. Embryonic deletion of integrin α6 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 α6 in development and formation of organs.

Expression of integrin α6 in stem cells: from germ cells to somatic stem cells

The expression of integrin α6 has been identified in somatic stem cells and germ cells from early developmental stages through adulthood. The heterodimer of integrin α6β1 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 α6 and integrin β1. 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 α6$^+$ cells compared to β1$^+$ cells. These findings suggest that integrin α6 selects a pure population of spermatogonial stem cells; while because integrin β1 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 α6 and β1 are expressed, and their expression is continuous during all stages of oocyte development [24]. It has been demonstrated that the presence of integrin α6β1 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 α6β1 has been detected at the mRNA level throughout preimplantation development. At the protein level, α6 is detected from two-cell embryos to the late morula/early blastocyst stage. At the early and late blastocyst stage, α6β1 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 α6, while trophoblast outgrowing cells are α6$^-$ [26]. In the appropriate in vitro conditions, the ICM outgrowths (integrin α6$^+$ cells) become what are known as ESCs [27,28], which have integrin α6β1 in their undifferentiated and pluripotent state [29,30].

As shown in Table 1, integrin α6 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 α6 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 α6 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 α6 significantly inhibits the homing of mouse HSCs to the bone marrow (BM) and impairs long-term multilineage reconstitution after competitive repopulation assays. Integrin α6 is also found in human primitive HSCs, suggesting a conserved interspecies functional role during hematopoiesis [31]. More recently, further evidence that integrin α6 (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 α6 in human hematopoietic cells distinguishes HSCs from multilineage progenitor populations [32].

Mammary Stem Cells

Integrin α6 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 α6 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 (ClinicalTrails.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 α6 -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 $PODLX^{hi}/CD49f^{hi}$ is less likely to result in lethal pulmonary emboli and these cells survive longer in the lung compared to $PODLX^{low}/CD49f^{low}$ 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 α6β1; and selection of $\alpha6^{high}$ or $\beta1^{high}$ cells leads to enrichment of NSCs from neurospheres [48]. The expression of β1 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 $\alpha6^{high}$ cells. Reports show that $\beta1^{high}$ cells have upregulated expression of Sox2, Sox3, Musashi1, and Bmi1 compared to $\beta1^{low}$ cells and are able to differentiate into neurons and astrocytes [48]. A similar pattern of $\alpha6^{high}/\beta1^{high}$ 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 α6 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 α6 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 α6 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 α6 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 $\alpha6^{high}$ cells demonstrates a significant increase in tumor formation with lower numbers of transplanted cells and in a shorter time compared to integrin $\alpha6^{low}$ cells. Knockdown of *integrin α6* 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 α6 has been used to enrich CSC populations from four different cervical uterine cancer cell lines: HeLa, SiHa, Ca Ski, and C-4 l. The integrin $\alpha6^{+}$ 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 α6 in the Mechanisms Used to Maintain Self-Renewal in Stem Cells

Despite the common expression of integrin α6 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 α6 and support its transcription [45]. It has also been shown that KLF9 represses the transcription of integrin α6 by binding its promoter region [52]. Interestingly, this repressive action of KLF9 on integrin α6 inhibits both glioblastoma cell stemness and tumorigenicity [52].

Our current understanding of the molecular mechanisms by which integrin α6 regulates self-renewal of stem cells comes largely from studies in ESCs and breast CSCs. We recently reported a critical role of integrin α6 in the self-renewal of human ESCs and identified the molecular mechanisms involved in this process [30]. The heterodimer of integrin α6β1 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 α6 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 α6, and it has been reported that the A isoform is able to prevent the activation of integrin β1 [53]. Indeed, it was observed that β1 signaling in hESCs is inactive, since focal adhesion kinase (FAK) is not phosphorylated in undifferentiated cells. Activation of integrin β1 signaling by activating antibodies induces the phosphorylation of FAK and the differentiation of human ESCs. Furthermore, during differentiation integrin α6 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 α6 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 α6 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 α6, prevent integrin β1/FAK activation, and maintain the expression of transcription factors involved in self-renewal and pluripotency (Fig. 1) [30,54].

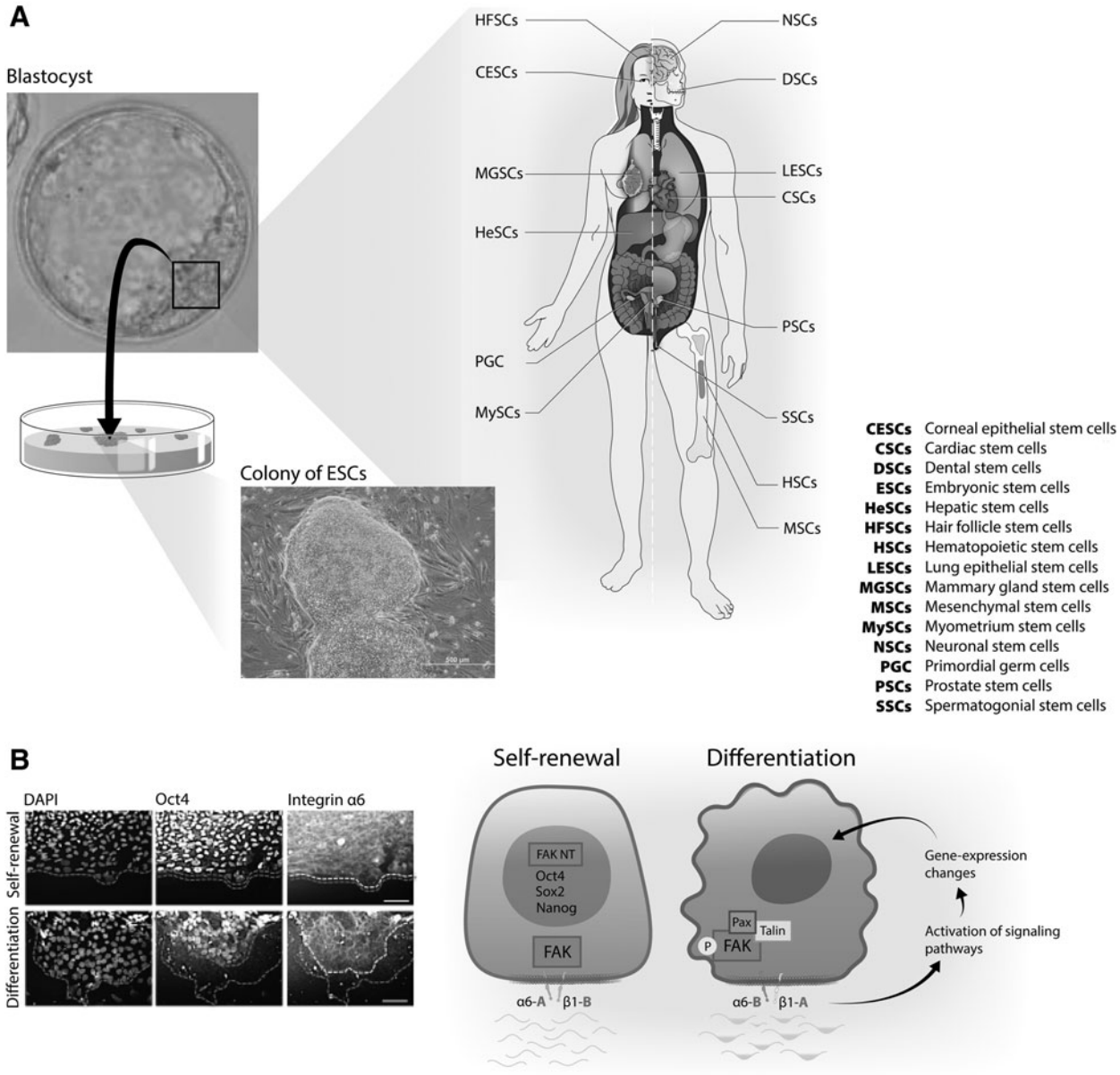

FIG. 1. Integrin α6 (CD49f) is a conserved biomarker of stem cells involved in their self-renewal. **(A)** Illustration showing the presence of integrin α6 in diverse stem cell populations. From pluripotent stem cells, the inner cell mass of the blastocyst embryo and ESC to adult stem cells (for a complete list of stem cell types refer to Table 1). **(B)** Representative micrographs showing coexpression of integrin α6 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 α6, respectively. Notice tightly coexpression of Oct4 and integrin α6 in both undifferentiated and differentiating colonies. A schematic model illustrating a mechanism by which integrin α6 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 α6Aβ1B. This protein configuration maintains the FAK inactive and localized in the cytoplasm and the nuclei of undifferentiated cells. Change in translation of integrin α6β1 to α6Bβ1A 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 α6 is consistently found in breast CSCs [55–57] and recently it has been shown that the B isoform, rather than the A isoform of α6, defines more precisely the breast CSC population [17]. Phenotypically the integrin α6B-dominant population has a mesenchymal cell morphology compared to the epithelial cell morphology of the α6A-dominant population, and they form heterodimers differently: the α6B heterodimer with integrin β1, while α6A population expresses integrin β4. 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 α6B population. Furthermore, α6B cells have a greater capacity to form mammospheres and to initiate tumors.

The expression of A and B isoforms of integrin α6 is regulated by alternative splicing mechanisms [58], and the ESRP1-splicing factor has been identified as a key regulator of α6A while it also functions to repress α6B [17]. Interestingly, VEGF signaling also promotes the initiation of triple negative breast cancer tumors through suppression of ESRP1 and, consequently, by the induction of α6B expression [17,57]. Supporting the concept that integrin α6B 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 α6B [59]. The interaction between laminin511 and integrin α6B 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 α6 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 α6, 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 α6 and Oct4 [30]. Breast CSCs produce laminin511, which functions as a ligand for α6Bβ1 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 α6 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 α6 (CD49f). We synthesized data from several lines of evidence in a broad range of stem cells to support the case that integrin α6 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.

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Author Disclosure Statement

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