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Review

Integrin Signaling and ECM Proteins in hPSC Maintenance and Differentiation

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Abstract

Integrin signaling serves as a fundamental regulator in human pluripotent stem cell (hPSC) biology, mediating adhesion, survival, and pluripotency through interactions with extracellular matrix (ECM) components. Specific integrins, including $\alpha6\beta1$, $\alpha v\beta5$, and $\alpha3\beta1$, engage ECM ligands such as laminin-511/521 and vitronectin (VTN) to sustain hPSC self-renewal. These engagements trigger essential downstream pathways, including PI3K/AKT, MAPK/ERK, focal adhesion kinase (FAK)-Src, and RhoA/Rho-associated protein kinase (ROCK), thereby maintaining the expression of pluripotency factors like OCT4, NANOG, and SOX2 while integrating mechanotransductive cues. FAK and Src convert ECM-derived mechanical signals into biochemical responses, regulating cytoskeletal reorganization, YAP/TAZ nuclear translocation, and context-dependent gene expression. For scalable, xeno-free culture, recombinant substrates such as truncated vitronectin (VTN-N) and laminin-511/521 E8 fragments, paired with defined media (e.g., Essential 8 or mTeSR1), support robust hPSC expansion under good manufacturing practice (GMP) conditions. Extending to differentiation, integrin-ECM crosstalk directs lineage commitment across diverse fates, including hematopoietic, cardiovascular, neural, hepatic, epithelial, endodermal, and oligodendroglial lineages, by fine-tuning signaling specificity and ECM composition. This review focuses on recent advances in the mechanistic interplay between integrin signaling and ECM proteins in hPSC maintenance, mechanotransduction, and lineage-directed differentiation, emphasizing defined culture systems and their translational potential in regenerative medicine.

Keywords: human pluripotent stem cells; integrins; extracellular matrix (ECM); pluripotency; differentiation; mechanotransduction

1. Introduction

Integrin–extracellular matrix (ECM) interactions are central to realizing the translational potential of human pluripotent stem cells (hPSCs) in regenerative medicine, disease modeling, and cell-based therapy. By providing biochemical and biomechanical cues that regulate adhesion, survival, and lineage specification, integrin signaling enables the development of defined culture systems that support large-scale expansion and controlled differentiation of hPSCs into clinically relevant cell types [1]. These integrin-guided processes are essential for generating functional derivatives for cell replacement therapies, constructing physiologically relevant *in vitro* disease models, and advancing drug discovery platforms. However, key challenges remain for clinical translation, including reducing cultivation costs, eliminating animal-derived components, and optimizing xeno-free ECMs and media formulations compatible with good manufacturing practice (GMP) standards. Addressing these challenges through a deeper mechanistic understanding of integrin–ECM signaling will be critical for achieving reproducible, safe, and scalable hPSC-based therapies.

The goal of this review is to elucidate how integrin signaling orchestrates hPSC behavior, from adhesion and survival to self-renewal and lineage specification, through dynamic interactions with defined ECM components. We aim to summarize current understanding of integrin–ECM-mediated signaling pathways, including PI3K/AKT, MAPK/ERK, focal adhesion kinase (FAK)–Src, and RhoA/Rho-associated protein kinase (ROCK), and their integration with mechanotransductive regulators such as YAP/TAZ. By focusing on how specific integrin–ligand pairs govern hPSC pluripotency and differentiation, we seek to define the molecular principles that enable xeno-free, scalable hPSC culture and guide lineage-specific differentiation. Ultimately, this review aims to advance strategies for engineering ECM-based microenvironments that enhance the safety, efficiency, and translational potential of hPSC-derived cell therapies.

2. Unique Characteristics of ECM-Integrin Signaling in hPSCs

In hPSCs, highly expressed integrins, such as $\alpha6\beta1$, $\alpha v\beta5$, $\alpha3\beta1$, and to a lesser extent $\alpha5\beta1$ and $\alpha7\beta1$, mediate key interactions with ECM proteins including laminin-511/521, vitronectin (VTN), and fibronectin (FN) [2,3]. These interactions activate downstream signaling cascades, including PI3K/AKT, MAPK/ERK, FAK–Src, and RhoA/ROCK, to sustain pluripotency or induce lineage differentiation. Among these, $\alpha6\beta1$–laminin and $\alpha v\beta5$–

VTN interactions are particularly critical for maintaining pluripotency [3,4]. Compared with somatic cells and adult stem cell types, such as mesenchymal stem cells (MSCs) or hematopoietic stem cells (HSCs), hPSCs display unique integrin expression profiles and mechanosensitivity tailored to their pluripotent nature. For example, fibroblasts primarily utilize $\alpha5\beta1$–FN interactions to support migration and tissue repair [5,6], whereas HSCs depend on $\alpha4\beta1$ for Vascular Cell Adhesion Molecule-1 (VCAM-1)-mediated niche anchoring [7,8]. In contract, hPSCs rely on $\alpha6\beta1$ and $\alpha v\beta5$ to engage laminin-511/521 and VTN, activating PI3K/AKT, FAK-Src, and RhoA/ROCK signaling [2,4,9–11]. In particular, $\alpha6\beta1$–laminin interactions are essential for single-cell survival and clonal propagation through Fyn–RhoA/ROCK signaling [9,12,13]. These signaling cascades are crucial for regulating key pluripotency genes such as OCT4, NANOG, and SOX2.

hPSCs also are highly sensitive to ECM stiffness, with FAK-Src signaling tuned to softer substrates (e.g., laminin-based matrices) compared to MSCs, which respond to a broader range of stiffness via $\alpha2\beta1$–collagen interactions [14–16]. This heightened mechanosensitivity and reliance on specific integrin-ligand pairs (e.g., $\alpha6\beta1$–laminin, $\alpha v\beta5$–VTN) enable hPSCs to maintain a delicate balance between self-renewal and differentiation, distinguishing them from somatic cells and adult stem cells with more lineage-restricted signaling profiles.

3. Integrin Structure and Functional Classification

Integrin signaling governs hPSC behavior by transducing ECM-derived cues into intracellular responses that regulate adhesion, survival, pluripotency, and differentiation. Integrins are transmembrane heterodimers composed of one α (120–180 kDa) and one β (90–120 kDa) subunit, forming 24 distinct heterodimer receptors from 18 α and 8 β subunits [17]. They are functionally categorized into four groups: (1) arginine–glycine–aspartic acid (RGD)-binding, (2) leukocyte cell-adhesion, (3) collagen-binding, and (4) laminin-binding integrins (Table 1) [18,19]. While integrins primarily mediate cell-ECM adhesion, they also support direct or indirect cell-cell interactions. For instance, $\alpha4\beta1$ on hematopoietic stem/progenitor cells (HSPCs) binds to VCAM-1 on endothelial or stromal cells, contributing to niche interactions [20–22], whereas fibroblast-derived fibronectin (FN) can serve as bridges to indirectly link integrins on neighboring cells to promote intercellular communication [23].

The functional versatility of integrins arises from their ability to pair with multiple subunits, bind diverse ligands, and activate overlapping intracellular pathways. Some integrins occupy more than one functional group or have overlapping ligand specificities. For example, $\alpha4\beta1$, classified as a leukocyte integrin, also binds specific sites on FN in an RGD-independent manner. This complexity reflects: (1) certain α subunits pairing with multiple β subunits (e.g., $\alpha4$ with $\beta1$ and $\beta7$); (2) certain β subunits pairing with multiple α subunits (e.g., $\beta2$ with αL, αM, and αX); (3) individual integrins interacting with multiple ligands (e.g., $\alpha4\beta1$ to VCAM-1 and FN); and (4) individual ECM ligands binding multiple integrins (e.g., FN to $\alpha v\beta3$, $\alpha v\beta6$, $\alpha5\beta1$, and $\alpha4\beta1$) [24].

4. Mechanotransduction and Integrins

Integrins are crucial mediators of mechanotransduction, the process by which cells sense and respond to mechanical forces from their ECM and neighboring cells [25]. In hPSCs, integrins bind to ECM components to create a physical and biochemical link between internal cellular cytoskeleton and its external surroundings, and act as mechano-sensors, detecting cues like substrate stiffness, shear stress, or ECM topography. These signals are transduced through pathways such as FAK and Src, modulating cytoskeletal organization, gene expression, and cell fate decisions (Fig. 1) [26–28]. For example, integrins (e.g., $\alpha6\beta1$, $\alpha v\beta5$) binding to ECM proteins, laminin-511 or VTN, promote adhesion, proliferation, pluripotency, or differentiation into specific lineages such as neural or mesodermal. This process integrates physical cues with molecular signaling to regulate hPSC identity and function. Kim *et al*. [29] demonstrated that ECM stiffness regulates hPSC behavior through mechanotransduction. By culturing hPSCs on tunable cell-derived matrices, they showed that matrix stiffness modulates adhesion, growth, migration, and pluripotency, identifying an optimal stiffness range for maintaining the pluripotent state [29].

4.1 FAK-Src Complex Activation

FAK activation is one of the major integrin-mediated signaling events. Mechanical forces or ECM binding induces integrin clustering, recruiting FAK to focal adhesions through interactions with talin and paxillin. FAK undergoes autophosphorylation at tyrosine 397 (Y397), creating a docking site for blinding Src Homology 2 (SH2) domain of Src family [28,30]. Src binding to FAK-Y397 disrupts autoinhibitory conformation of Src, enabling phosphorylation of additional FAK sites (e.g., Y576/Y577) for full kinase activation. The FAK-Src complex recruits downstream effectors like Grb2, PI3K, and p130Cas, amplifying downstream signaling [31–34]. In hPSCs, $\alpha6\beta1$-laminin interactions activate FAK-Src, promoting PI3K/AKT signaling to sustain pluripotency markers and prevent apoptosis [10,35].

4.2 Cytoskeletal Dynamics Regulation of RhoA/ROCK Signaling

The FAK-Src complex activates RhoA, promoting actin polymerization and actomyosin contractility via ROCK-mediated myosin light chain phosphorylation. This enhances cytoskeletal tension, critical for cell migration and adhesion maturation. In hPSCs, $\alpha6\beta1$-laminin inter-

Table 1. Integrin categories.

Category	Examples	Binding specificity
RGD-binding integrins	$\alpha5\beta1$, $\alpha v\beta3$, $\alpha v\beta5$, $\alpha IIb\beta3$	Recognize the arginine-glycine-aspartic acid (RGD) motif found in many extracellular matrix (ECM) proteins, such as fibronectin (FN), vitronectin (VTN), and fibrinogen.
Leukocyte cell-adhesion integrins	$\alpha L\beta2$, $\alpha M\beta2$, $\alpha X\beta2$, $\alpha D\beta2$	Primarily expressed on white blood cells and mediate cell-to-cell adhesion by binding to intercellular adhesion molecules (ICAMs) and other counter-receptors on endothelial cells. The $\beta2$ subunit is a defining feature of many of these integrins.
Collagen-binding integrins	$\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$, $\alpha11\beta1$	Recognize specific GFOGER (O = hydroxyproline) sequences in collagen, the most abundant protein in the ECM.
Laminin-binding integrins	$\alpha3\beta1$, $\alpha6\beta1$, $\alpha7\beta1$, $\alpha6\beta4$	Mediate cell adhesion to laminin, a major component of the basement membrane. Some of these integrins, like $\alpha6\beta4$, are particularly important for forming hemidesmosomes, which anchor epithelial cells to the basement membrane.

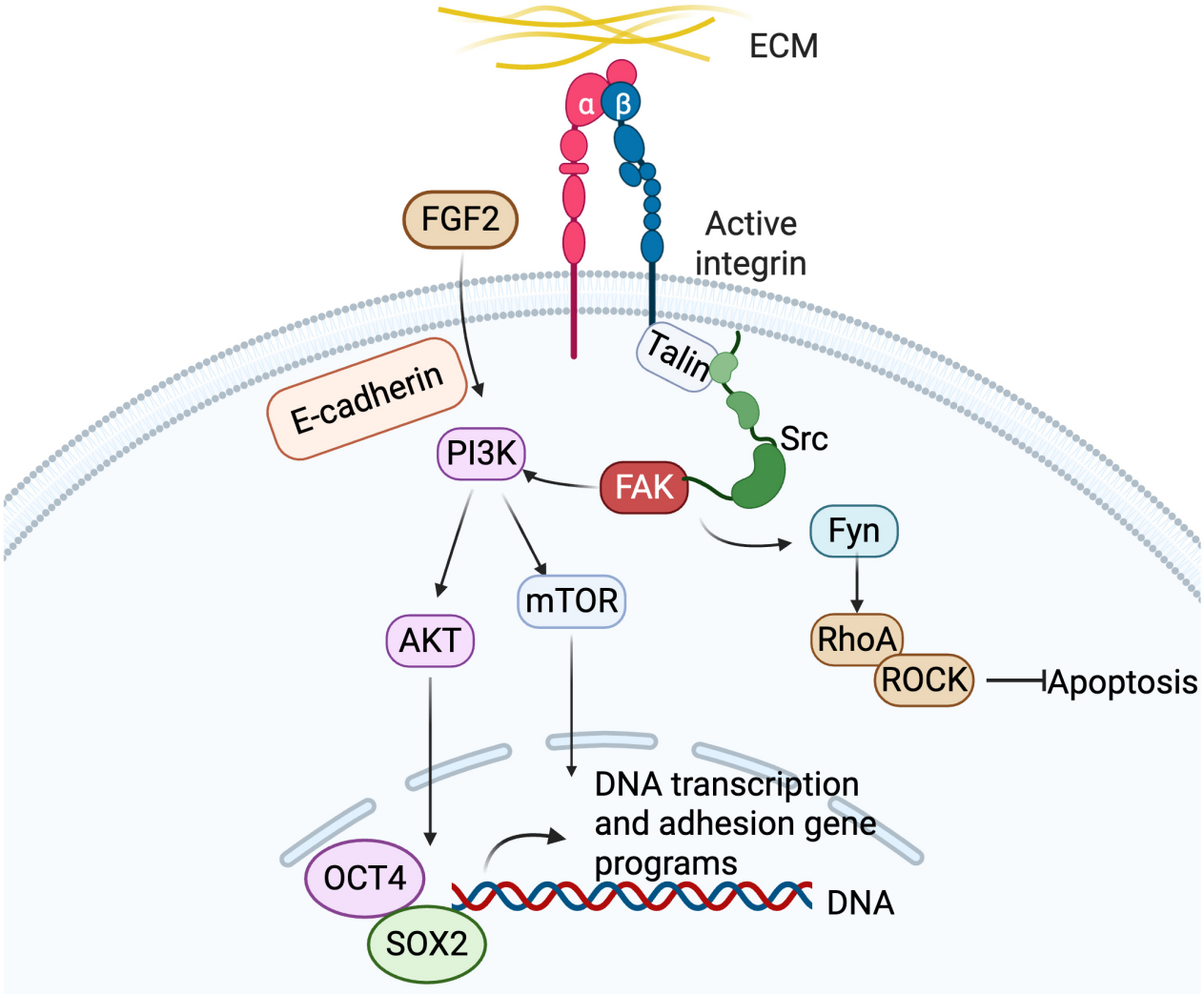

Fig. 1. Activation of integrin signaling by ECM proteins to regulate hPSC adhesion, pluripotency and differentiation. ECM, extracellular matrix; hPSC, human pluripotent stem cell. Created in BioRender (https://BioRender.com/b1mqg26).

action activates Fyn, a Src family kinase, which synergizes with RhoA/ROCK signaling pathway. Fyn-RhoA-ROCK signaling pathway inhibits Rho-associated protein kinase (ROCK) activity to maintain pluripotency [9,36]. The

FAK-Src complex phosphorylates paxillin and p130Cas, and strengthens interactions with Crk and Nck adaptors, stabilizing focal adhesions, while vinculin and α-actinin reinforce integrin-actin linkages under tension, enabling force transmission [37]. ROCK inhibitors, such as Y-27632, are typically used to support hPSC survival and pluripotency during single-cell passaging [36].

4.3 Gene Expression Modulation

Cytoskeletal tension through the engagement of $\alpha 6\beta 1$-laminin and $\alpha v\beta 5$-VTN inhibits Hippo pathway kinases (LATS1/2), preventing YAP/TAZ phosphorylation. Dephosphorylated YAP/TAZ translocate to the nucleus, binding TEAD transcription factors to drive proliferation/survival genes (e.g., CTGF, CYR61) via Src and Rac [38,39]. FAK-Src activation of MAPK/ERK is critical for stem cell proliferation via Grb2-SOS and PI3K-Akt via PIP3 pathways, promoting transcription factors like FOXO1 for cell cycle progression and pluripotency maintenance [40,41]. $\alpha 6\beta 1$-laminin and $\alpha v\beta 5$-VTN signaling also synergizes with EGFR/HER2 to amplify ERK and AKT activation, sustaining OCT4 and NANOG expression [3,42,43]. These pathways collectively ensure hPSC self-renewal by integrating mechanical and biochemical cues.

4.4 Functional Outcomes

FAK activation protects cells from anoikic [44], making integrins natural targets for regulating anoikis resistance. This resistance is essential for hPSC survival during single-cell passaging and differentiation [45,46]. Anoikis, a form of apoptosis induced by detachment from ECM, is a major contributor to low post-transplantation survival rates in stem cell therapy. For example, anoikis limits the efficiency of hESC-derived cardiomyocyte (hESC-CM) engraftment in cardiac repair [47]. Co-transplantation with biocompatible ECM materials can mitigate anoikis by providing structural and biochemical cues that support cell survival [48].

FAK-Src signaling drives cytoskeletal remodeling, focal adhesion turnover, and directional motility. FAK phosphorylation is required for MSC differentiation [49], while in cancer stem cells, FAK promotes migration and epithelial-to-mesenchymal transition (EMT), enhancing invasive potential [50]. In hPSCs, integrin-mediated activation of $\alpha 6\beta 1$ and $\alpha v\beta 5$ protects against anoikis through FAK-Src and downstream PI3K/AKT signaling, supporting single-cell survival and clonal propagation, particularly via $\alpha 6\beta 1$-laminin interactions [13,44–46]. Concurrently, YAP/TAZ and ERK/AKT pathways suppress apoptosis and promote proliferation [38,40]. ECM stiffness further modulates Src activity, tuning hPSC responses to matrix rigidity and influencing self-renewal versus differentiation [51–53]. Inhibition of Src accelerates differentiation across all germ layers, linking FAK-Src signaling to cell cycle control and the maintenance of pluripotency [51,53,54]. These studies highlight a link between FAK-Src signaling, cell cycle regulation, and hPSC differentiation potential.

5. Integrin Signaling for iPSC Pluripotency

Mouse embryonic fibroblasts (MEFs) were initially used as feeder cells for derivation and maintenance of hESCs [55]. Human foreskin fibroblasts later provided a xeno-free alternative culturing system to replace MEFs [56]. Matrigel, derived from mouse sarcoma tumors, served as feeder-free system for hESC growt [2,57]. However, those feeders and Matrigel are allogeneic or xenogeneic origin with potential source of variability and viral or bacterial contamination. Matrigel is a complex and undefined mixture of ECM proteins, including laminins, collagen IV, fibronectin, and other basement membrane proteins, triggering complex integrin signaling. The undefined composition of Matrigel complicates standardization of hPSC culture and differentiation.

Integrin-ECM interactions are essential for hPSC maintenance and lineage specification (Table 2, Ref. [3,4,58–65]). High levels of integrin $\alpha 6$ and $\beta 1$, moderate levels of $\alpha 2$, and low levels of $\alpha 1$, $\alpha 2$, and $\alpha 3$, and $\beta 4$ are expressed in hPSCs [2]. The predominant $\alpha 6\beta 1$ integrin binds to laminins, suggesting that laminins play a critical role in hPSC maintenance. A study by S. Rodin *et al.* [3] demonstrated that human recombinant laminin-511 can effectively support the long-term self-renewal and pluripotency of hPSCs, establishing a defined, animal-product-free culture system for hPSCs. Laminin-511 promotes hiPSC survival through $\alpha 6\beta 1$ integrin activation and downstream Fyn–RhoA–ROCK signaling, providing a mechanistic basis for its ability to support cell viability during single-cell passaging [9]. Villa-Diaz *et al.* [10] further demonstrated that integrin $\alpha 6\beta 1$ sustains hPSC self-renewal by maintaining FAK in an inactive state. Perturbation of FAK signaling promotes hPSC differentiation, revealing a regulatory mechanism in which integrin-ECM interactions modulate intracellular signaling to control stem cell fate in response to the microenvironment [10]. Truncated laminin-511 or -521 E8 fragments (~200–300 kDa) contains integrin-binding regions, supporting feeder-free hPSC culture [58,66]. Recombinant human laminin-511/521 E8 fragments, iMatrix-511 and iMatrix-521, ensures defined, xeno-free feeder-free hPSC culture. VTN, particularly its truncated vitronectin form (VTN-N), also support hPSC growth via its RGD-dependent binding to $\alpha v\beta 5$ in hPSCs with Essential 8 (E8) medium [4,67,68].

5.1 Laminin-511/521 Interacting With $\alpha 6\beta 1$ and $\alpha 3\beta 1$

Laminin isoforms are widely used for the maintenance of hPSC pluripotency, particularly in defined, feeder-free culture systems. Laminin-511 and laminin-521 are heterotrimeric glycoproteins, composed of α, β and γ chains [69]. They are key ECM components for hPSC culture, supporting pluripotency and cell survival by binding to $\alpha 6\beta 1$

Table 2. Key integrins involved in hPSC maintenance and differentiation.

Integrin receptor	ECM ligands	hPSC pluripotency	hPSC differentiation	signaling	References
$\alpha6\beta1$	Laminin-511, Laminin-521, Laminin-111	Major role	Neural, cardiac, hepatocyte	PI3K/Akt, FAK and ILK	[3,58,61]
$\alpha v\beta5$	VTN (via RGD sequence)	Major role	Hemogenic endothelial, endothelial, hematopoietic	FAK and ILK	[4,59,60]
$\alpha v\beta3$	VTN, FN (RGD)	Secondary role	Hemogenic endothelial, hematopoietic	PI3K and FAK	[59,60]
$\alpha5\beta1$	FN (RGD)	Less effective	Mesenchymal, endothelial, cardiac	MAPK/PI3K	[59,63]
$\alpha3\beta1$	Laminin-511, -521, -111	Low affinity	Neural, epithelial, hepatocyte		[58,61,62]
$\alpha4\beta1$ (VLA-4)	VCAM-1, FN (CS-1 region)	Minimal role	Hematopoietic, endothelial	MAPK/PI3K	[64,65]
$\alpha7\beta1$	Laminin-111, -211	Minor role	Myogenic, neural, hepatocyte		[58,61,62]

Table 3. Integrins in hPSC lineage differentiation.

Lineage	Key integrins	Role	Key pathways	References
Cardiac	$\alpha3\beta1$, $\alpha6\beta1$, $\alpha4\beta1$, $\alpha v\beta1$, $\alpha2\beta1$, $\alpha5$	Facilitate cardiomyocyte differentiation; FN promotes mesoderm via $\alpha4\beta1$ and $\alpha v\beta1$; $\alpha2\beta1$ enhances maturation; Laminin-111/-421 support organoid formation; laminin-521 inhibits it.	PI3K/AKT (ILK inhibition reduces AKT phosphorylation).	[83–86,107]
Endothelial	$\alpha v\beta3$, $\alpha v\beta5$	Drive endothelial commitment with VTN; Laminin-411 and Collagen IV enhance CD31 expression; FN inhibits via TGFβ.	VEGF via PI3K/AKT and MAPK/ERK.	[87–89]
Hematopoietic	$\alpha v\beta3$, $\alpha v\beta5$, $\alpha4\beta1$, $\beta1$	Promote hematopoietic transition with VTN; $\alpha4\beta1$ maintains stem cells; $\beta1$ aids homing and engraftment.	PI3K/AKT and MAPK/ERK enhance VEGF signaling.	[60,90,91]
Neural	$\alpha6\beta1$, $\alpha5$, $\beta1$	Support dopaminergic neurons with laminin-521/-511; mediate progenitor adhesion/migration; aid Schwann cell differentiation and myelination.	PI3K and MAPK.	[92–95]
Hepatic and Cholangiocyte	$\alpha3\beta1$, $\alpha v\beta5$, $\alpha5\beta1$	Drive hepatocytes and definitive endoderm with laminin-521/-511; laminin-411 E8 promotes cholangiocytes.	PI3K/AKT and MAPK regulate growth.	[61,62,96,99,103,104]
Corneal Epithelial	$\alpha3\beta1$, $\alpha6\beta1$	Enhance adhesion with laminin-332 E8.	Supports wound healing and mechanotransduction.	[97,98]
Definitive Endoderm	$\alpha5\beta1$, $\alpha3\beta1$ and $\alpha v\beta5$	Enhance endoderm transition with RGD peptides and matrix stiffness; $\beta1$ crucial for specification.	MEK-ERK and PI3-kinase regulated by integrin-ECM.	[99–102]
Oligodendrocyte	$\alpha v\beta3$, $\alpha v\beta5$, $\beta1$	Support maturation with VTN and laminin; aid myelination.	PI3K, MAPK, Fyn kinase, Cdc42/Rac1.	[105,106]

and $\alpha3\beta1$ integrins in hPSCs [3,13,70]. Other integrins, such as $\alpha7\beta1$ and $\alpha6\beta4$, may also contribute to laminin-511 and laminin-521 binding for supporting cell adhesion [69]. Compared to $\beta1$ chain of laminin-511, $\beta2$ chain of laminin-521 enhances $\alpha6\beta1$ binding affinity, improving adhesion, single-cell survival and clonal propagation via PI3K/Akt signaling [13].

E8 fragments of laminin-511 and laminin-521, containing the C-terminal E8 region (LG1-3 domains of the $\alpha5$ chain, parts of $\beta1/\beta2$, and $\gamma1$ chains), retain integrin-binding activity, ensuring robust adhesion, survival, and pluripotency [58,66]. By excluding non-essential domains, E8 fragments reduce complexity, improve purity, and maintain the critical cell-binding activity of full-length laminins [13]. When paired with defined media (e.g., E8 or mTeSR1), iMatrix-511 and iMatrix-521 support good manufacturing practice (GMP)-compliant, cost-effective, and scalable hPSC culture.

5.2 Vitronectin Interacting With $\alpha v\beta3$ and $\alpha v\beta5$

VTN, a monomeric glycoprotein (75 kDa in ECM, or 65 kDa and 10 kDa fragments in plasma), contains an RGD (Arg-Gly-Asp) motif in its somatomedin B domain for integrin binding, and domains for binding heparin, collagen, and plasminogen activator inhibitor-1 (PAI-1). The RGD motif of VTN primarily binds to $\alpha v\beta3$ and $\alpha v\beta5$ integrins to mediate cell adhesion, migration, survival and pluripotency via PI3K/AKT and MAPK/ERK pathways [4,59]. The truncated VTN-N form, defined recombinant human protein, supports xeno-free culture conditions and is effec-

tive at concentrations of approximately 500 ng/cm^2 in conjunction with Essential 8 or mTeSR1 medium [67,71].

mTeSR1 contains BSA, which acts as a carrier for lipids and other components and is known to bind to non-specific surfaces, whereas Essential 8 (E8) medium does not contain BSA. E8 medium is specifically designed as a simplified, defined alternative to mTeSR1, with the goal of reducing variability by removing undefined components like BSA. However, a study comparing two feeder-free culture systems for deriving hiPSCs from fibroblasts found the Matrigel/mTeSR1 combination to be significantly more efficient than the Vitronectin/E8 system in generating hiPSC colonies and expressing pluripotency markers [72]. It provides a key insight into how specific culture conditions can influence the efficiency of generating and maintaining high-quality hiPSCs, which is crucial for their eventual use in therapeutic applications and disease modeling.

6. Integrin Signaling in hPSC Differentiation

Laminin-511 and laminin-521 are the most abundant laminin subtype expressed in hPSCs [73–76]. The crosstalk between laminins and hPSCs not only supports hPSC pluripotency, but also hPSC differentiation since differentiation protocols of hPSCs often require single-cell dissociation, which can induce apoptosis in hPSCs [77]. Laminin are expressed in early stage of embryos, suggesting a role of guiding the earliest steps of embryonic development [78–81]. During hPSC differentiation, the expression pattern of laminin isoforms changes. The expression of laminin-511, but not laminin-521, is increased by retinoic acid (RA)-induced mesodermal and endodermal differentiation in hESCs, indicating that laminin isoforms are involved in lineage differentiation [82]. Other Integrin ligands, such as VTN and FN, also have profound influences on differentiation when combined with specific growth factors (Table 3, Ref. [60–62,83–107]).

6.1 Mesoderm Differentiation

The interaction of specific ECM proteins with integrins such as $\alpha5\beta1$ and $\alpha6\beta1$ in hPSCs modulates key signaling pathways, including BMP and Wnt, to promote early mesoderm formation and the subsequent development of cardiac, vascular, and hematopoietic lineages.

6.1.1 Cardiac Differentiation

In human cardiac tissue, laminins (e.g., laminin-211, -221, -411, -421, -511, and -521) are highly expressed during fetal cardiac development and in adult cardiac tissue [108]. Their interactions with $\alpha3\beta1$ and $\alpha6\beta1$ integrins are involved in cardiac-ECM interactions for normal cardiac function. The combination effects of laminin-521 and laminin-221 on cardiovascular differentiation in hPSCs have been demonstrated [109,110]. Laminin-111 and -421 promote the generation of hPSC-derived heart organoids [83,111]. FN is essential for hPSC differentiation into cardiomyocytes. Endogenous FN accumulation in ECM is critical for mesoderm formation, particularly towards cardiac lineage differentiation. Specific knockdown FN in hPSCs prevented mesoderm formation and subsequent hPSC-CM generation [83]. Antibody blocking study showed that integrins $\alpha4\beta1$ and $\alpha v\beta1$, but not $\alpha5\beta1$, are key receptors mediating the binding of FN to cells to regulate cardiac differentiation of hPSCs [83]. The downstream signaling molecule integrin-linked kinase (ILK) is activated by these integrins. Inhibiting ILK leads to a decrease in phosphorylated AKT, a crucial molecule involved in cell survival and proliferation. This reduction in phosphorylated AKT is associated with increased apoptosis, ultimately inhibiting cardiac differentiation [83,84]. Stimulation of collagen integrins ($\alpha2\beta1$) at the progenitor stage using vitronectin-collagen I matrices accelerates hiPSC-derived cardiomyocyte maturation, yielding a ~3-fold increase in cardiac troponin I expression, enhanced sarcomere development, and improved beating potency. Integrin inhibition experiment to block the function of the $\alpha2\beta1$ integrin confirms the essential role of integrin stimulation in the process [85]. Additionally, integrin $\alpha5$ subunit is critical for the early stages of hPSC-cardiac differentiation, by establishing the correct cell identity during the development of mesodermal progenitors [86].

6.1.2 Endothelial Differentiation

Laminin-411 is important for increasing hPSC-derived endothelial differentiation via the activation of multiple signaling pathways, including Focal Adhesion Kinase (FAK), Integrin-Linked Kinase (ILK), Notch, and β-catenin pathways. Activating Wnt signaling through small molecules improves laminin 411-guided endothelial differentiation [87]. While Laminin 411 alone increase endothelial differentiation of hPSCs, an optimized ECM formulation consisting of collagen I, collagen IV, and laminin 411 significantly enhances hPSC endothelial differentiation, compared to laminin 411 + FN or Matrigel. Collagen IV and laminin 411 enhance the expression of endothelial markers like CD31, while TGFβ signaling inhibits endothelial differentiation [88]. VTN also promotes endothelial lineage commitment through synergistic $\alpha v\beta3$ and $\alpha v\beta5$ signaling, enhancing VEGF signaling via PI3K/AKT and MAPK/ERK pathways [60,89]. $\alpha v\beta3$ and $\alpha v\beta5$ integrins, acting as vitronectin receptors, are involved in endothelial cell adhesion and migration, which are crucial for normal vascular development and function. Blocking $\alpha v\beta3$ and $\alpha v\beta5$ integrins by antagonists inhibits endothelial cell invasion and differentiation induced by angiogenic factors like VEGF and FGF-2. Integrin $\alpha v\beta3$ is a significant player in endothelial cell survival and angiogenesis, especially in tumor development. Blocking $\alpha v\beta3$ results in inhibiting tumor angiogenesis. The interaction between VEGFR2 and $\alpha v\beta3$ is required for VEGFR2 phosphorylation and the activation of mitogenic pathways involving FAK [112–114].

6.1.3 Hematopoietic Differentiation

VTN-integrin signaling directs hPSC differentiation toward hematopoietic lineages. As in embryonic development, hematopoietic differentiation from hPSCs proceeds through three major developmental stages: (1) mesodermal induction from hPSCs, (2) specification of hemogenic endothelium from mesodermal progenitors, and (3) endothelial-to-hematopoietic transition (EHT) to generate hematopoietic stem and progenitor cells. VTN promotes the development of hematopoietic-fated mesoderm and hemogenic endothelial cells, upregulating key hematopoietic transcriptional factors, including GATA2, PU.1, GATA1, GFI1B, and KLF1 [60]. Synergistic $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin signaling supports hematopoietic development, with $\alpha v\beta 3$ specifically enhancing VEGF signaling to promote endothelial lineage commitment [60,115,116].

Integrins also modulate ECM organization to regulate cell signaling during hematopoiesis, with $\alpha 4\beta 1$ integrin playing a key role in HSC maintenance [117,118]. Importantly, Michaels *et al*. [90] demonstrated that co-presentation of DLL4 with VCAM1 during EHT increased production of T-cell-competent hematopoietic progenitors by more than 80-fold compared with DLL4 alone, implicating $\alpha 4\beta 1$-VCAM1 engagement as a pro-hematopoietic cue during EHT.

Beyond αv and $\alpha 4$ integrins, $\beta 1$ integrin plays essential roles in hematopoiesis, including HSC homing and engraftment [119]. Yuzuriha *et al*. [91] further reported that laminin-421 or laminin-121 regulates the hematopoietic potential of hPSC derivatives through a $\beta 1$-integrin to ILK to β-catenin/JUN pathway, identifying a specific $\beta 1$-integrin-centered mechanism controlling hPSC hematopoietic induction.

6.2 Ectoderm Differentiation

The ectoderm is the outermost of the three primary germ layers in early embryogenesis. During development, it differentiates into neuroectoderm and non-neural ectoderm. Given the difficulty of isolating adult stem cells from neural tissues, generating neuroectodermal lineages from hPSCs has become a central focus in current ectoderm differentiation studies.

6.2.1 Neural Differentiation

Both laminin-511 and laminin-521 provide essential signals that support the survival and self-renewal of hPSCs while priming them for subsequent differentiation. These laminins also facilitate the differentiation of hPSCs into dopaminergic neurons [92]. The laminin $\alpha 5$ chain in both laminin-521 and -511 is crucial for providing a controlled environment for hPSC-derived neurons by cross-talking with integrins and ECM proteins such as collagens and FN [93].

Laminin-511, in particular, promotes the survival and differentiation of midbrain dopaminergic neurons by engaging integrin $\alpha 3\beta 1$ on the neuronal surface, which activates YAP signaling and its nuclear translocation [120]. Laminin-521 is widely used as a supportive matrix for culturing and differentiating hPSCs into various neuronal subtypes, owing to its ability to mimic the native cellular environment and enable robust expansion and directed differentiation in a defined, xeno-free system.

The interaction between laminin-521 and integrin $\alpha 6\beta 1$ plays a major role in neural progenitor adhesion and migration during central nervous system (CNS) development [94,95,121]. In the peripheral nervous system and in hPSCs, $\beta 1$ integrin signaling is critical for Schwann cell differentiation and myelination. Studies using Schwann cell-specific knockout models and *in vitro* system have demonstrated that $\beta 1$ integrin is essential not only for early nerve development but also for initiating myelination [96,122–124].

6.2.2 Corneal Epithelial Differentiation

The surface ectoderm gives rise to the corneal epithelium, conjunctival epithelium, lens, and the epidermis of the eyelids. Among ECM components, laminin-332 is an essential constituent of the basement membrane of corneal epithelial cells and plays a central role in supporting their growth. Specifically, E8 fragments from laminin-332 markedly enhance adhesion and expansion of iPSC-derived corneal epithelial cells compared with fragments from other laminins. This difference in adhesive properties across laminin substrates enables the selective enrichment of iPSC-derived corneal epithelial cells over other ocular cell types [97]. Integrin $\alpha 3\beta 1$, which exhibits high affinity for laminin-332, is a major determinant of corneal epithelial adhesion. Binding of $\alpha 3\beta 1$ integrin to laminin-332, including its E8 fragment, activates several intracellular signaling pathways, including FAK/ERK, PI3K/Akt, Rho GTPase and Src kinases in corneal epithelial cells. These pathways regulate fundamental cellular processes like proliferation, migration, and survival [98,125–128].

6.3 Endoderm Differentiation

6.3.1 Definitive Endoderm

Definitive endoderm is an epithelial cell layer formed during early embryonic development that gives rise to multiple internal organs and tissues, including the digestive tract (stomach, intestines, liver, pancreas), respiratory system (lungs, trachea, bronchi) and thyroid gland. Definitive endodermal progenitors are characterized by the expression of key transcription factors, such as SOX17, GATA4, and FOXA2 [129].

Integrin signaling, in coordination with other pathways, plays a crucial role in guiding the differentiation of definitive endoderm into specific organ lineages. FN and VTN are major ECM components that promote definitive endoderm specification by interacting with integrins $\alpha 5\beta 1$, $\alpha 3\beta 1$ and $\alpha v\beta 5$ [99–101,130,131]. Specifically, the dif-

ferentiation process was associated with an increase in FN-binding integrin α5 (ITGA5) and VTN-binding integrin αv (ITGAV). Blocking α5 and αv integrins with shRNA disrupted endodermal differentiation, while cell isolation based on α5 and αv expression enriched for endoderm cells, indicating that these interactions are critical for endodermal differentiation [99].

Synthetic PEG-based hydrogel functionalized with cyclic RGD peptides have been shown to support hPSC attachment and promote transition to definitive endoderm. In this context, matrix stiffness emerges as a key mechanical cue, with FAK signaling serving as a downstream intracellular mediator of integrin-ECM interactions [102]. During the early stages of definitive endoderm differentiation, integrin-mediated traction forces are required for SMAD2 phosphorylation and nuclear translocation, which enhances the expression of definitive endoderm markers, such as SOX17, thereby linking mechanical signaling to TGF-β pathway activation [100].

6.3.2 Hepatic and Cholangiocyte Differentiation

Laminin-521 and -511, interacting with primarily $\alpha3\beta1$ integrin, support hPSC differentiation into definitive endoderm and subsequently into hepatocytes. The use of recombinant laminin-511 and -521, either alone or in combination, provides a defined, xeno-free substrate that significantly improves the efficiency and quality of hPSC-derived hepatocytes [61,62,103]. During the differentiation of hPSCs into definitive endoderm, there is a shift in integrin expression. The pluripotency-associated integrins ($\alpha3\beta1$, $\alpha6\beta1$) are downregulated, while definitive endoderm cells show high expression of other integrins, such as $\alpha v\beta5$ binding to VTN and $\alpha5\beta1$ binding to FN [96,99]. Integrin-linked kinase (ILK) is an intracellular protein that acts at the cell-matrix interface to integrate signals from integrins. In primary hepatocytes, ILK is involved in matrix-induced differentiation. Hepatocytes lacking ILK undergo incomplete maturation, suggesting its importance in achieving full hepatocyte function, a mechanism likely shared by hPSC-derived cells [132]. To understand the precise control of the ECM for cell fate decisions, E8 fragments from different laminins were used to induce iPSC differentiation [104]. While E8 fragments from other laminin isoforms do not, E8 fragments from laminin-411 and laminin-511 specifically promote the differentiation of iPSCs into cholangiocyte-like epithelial cells, increasing in the expression of cholangiocyte-specific markers and formation of larger and more numerous cholangiocyte cysts in a three-dimensional (3D) collagen gel, mimicking the bile duct structures found *in vivo* [104].

6.3.3 Oligodendrocyte Differentiation

VTN-integrin signaling, combining with signaling molecules sonic hedgehog (Shh), retinoic acid (RA), and noggin, supports hPSC differentiation into oligodendrocytes, mediated by αv-containing integrins, such as $\alpha v\beta3$ and $\alpha v\beta5$, via both PI3K/AKT and MAPK/ERK pathways [105,106,133]. While $\alpha v\beta3$ primarily activates the MAPK pathway to promote proliferation, $\alpha v\beta5$ preferentially activates the PI3K pathway, which is essential for the oligodendrocyte differentiation, specifically for the transition from oligodendrocyte progenitor cells to mature oligodendrocytes. [133–136]. However, the roles of PI3K/AKT and MAPK/ERK pathways are not always straightforward, with timing and coordination being critical for successful differentiation and myelination.

Besides VTN, other ECM proteins may interact with integrins in oligodendrocytes to initiate intracellular signaling cascades, influencing crucial functions like proliferation, survival, and maturation that are essential for CNS myelination [137,138]. Laminin-2, found on the surface of axons, binds to the $\alpha6\beta1$ integrin on oligodendrocytes to enhance membrane formation, promotes survival, and is critical for myelination [139]. While laminin promotes myelination, FN can inhibit the differentiation and maturation of oligodendrocytes [138]. Elevated FN is often found in CNS lesions and can negatively regulate oligodendrocyte differentiation, making it an antagonist to laminin in this process. However, the roles of FN and laminin-2, as well as downstream signaling in hPSC differentiation into oligodendrocytes remain unknown.

6.4 The Effect of 3D Culture on Integrin Signaling in hPSC

Transitioning from conventional 2D monolayer cultures to 3D systems profoundly alters integrin signaling in hPSCs, particularly during lineage differentiation, such as organoid formation. Enhanced cell–cell and cell–ECM interactions in 3D systems create a more physiologically relevant microenvironment than 2D cultures, thereby enhancing the fidelity of hPSC differentiation and tissue morphogenesis [140]. For example, cardiomyocytes derived from 2D hPSC cultures fail to fully recapitulate the electrophysiological and contractile properties of native heart tissue [141–143]. In contrast, 3D organoid systems have been successfully established to generate diverse tissue types from hPSCs, including the brain, eyes, kidney, lung, stomach, intestine, inner ear, skin, thyroid, and liver [144–153].

Synthetic 3D matrices, such as polyethylene glycol (PEG)-based hydrogels, offer defined and tunable microenvironments that improve reproducibility and control compared to conventional organoid methods. For instance, Chow *et al*. [154] demonstrated that encapsulating hPSC-CMs in a 3D PEG hydrogel improved cardiac function and tissue integrity following myocardial infarction in rats, suggesting indirect paracrine or remodeling effect despite limited long-term cell survival. Within such systems, integrins mediate cell–matrix attachment and transduce essential “outside-in” signals that regulate survival, proliferation, and mechanosensation. Functionalization of hydrogels with integrin-binding peptides, particularly RGD mo-

tif, enhances cell adhesion [155]. Through RGD–integrin interactions, cells form focal adhesions that physically couple the cytoskeleton with the ECM, promoting dynamic cell–matrix crosstalk and activating intracellular signaling pathways that govern adhesion, spreading, proliferation, migration, differentiation, and mechanotransduction. During vascular differentiation of iPSCs, 3D PEG hydrogels have been shown to upregulate ECM components and integrins relative to 2D cultures, facilitating the formation of functional vascular networks [156].

Despite these advances, fully recapitulating human physiology in 3D hPSC cultures remains challenging, largely due to the absence of a vascular network capable of delivering oxygen and nutrients while removing metabolic waste. Nevertheless, the integration of 3D culture systems with defined xeno-free ECMs controlled mechanical properties represents a promising direction for future studies aimed at modeling human development and disease and advancing hPSC-based tissue engineering and therapeutic applications.

7. Summary and Future Perspectives

Integrin-ECM interactions are fundamental to hPSC maintenance and lineage specification. The transition from xenogeneic feeders such as MEFs and Matrigel to defined substrates like laminin-511/521 and VTN has enabled xeno-free, reproducible culture systems suitable for clinical translation. In the pluripotent state, $\alpha 6\beta 1$-laminin and $\alpha v\beta 5$-VTN interactions sustain adhesion, survival, and self-renewal through PI3K/AKT, MAPK/ERK, FAK–Src, and RhoA/ROCK signaling, maintaining expression of pluripotency genes OCT4, NANOG, and SOX2. FAK serves as a key signaling hub integrating these cascades to promote adhesion, survival, and p53 degradation [157].

hPSCs exhibit distinct integrin profiles and heightened mechanosensitivity compared to somatic and adult stem cells, relying primarily on $\alpha 6\beta 1$ and $\alpha v\beta 5$ for pluripotency maintenance. During differentiation, specific integrin-ligand pairs direct lineage commitment: laminin-$\alpha 6\beta 1/\alpha 3\beta 1$ interactions favor cardiac and neural lineages; VTN-$\alpha v\beta 3/\beta 5$ supports endothelial, hematopoietic, and oligodendrocyte specification; and FN-$\alpha 5\beta 1$ promotes mesoderm and endoderm differentiation. Recombinant laminin E8 fragments and VTN-N provide defined, scalable platforms that preserve stem cell integrity and enable controlled lineage induction. Collectively, these findings underscore integrin signaling as a central regulator of hPSC biology, from self-renewal to lineage-specific differentiation.

Despite these advances, challenges remain in translating integrin–ECM biology into fully defined, GMP-compliant hPSC platforms. Current recombinant ECM proteins remain costly and variable, and the complexity of integrin signaling networks complicates the dissection of ligand-specific effects on pluripotency and lineage choice. Furthermore, conventional 2D culture systems often fail to capture the spatial, mechanical, and topographical cues that regulate integrin clustering and focal adhesion dynamics *in vivo*.

Future research should focus on engineering synthetic ECMs with tunable biochemical and biomechanical properties to systematically dissect integrin-mediated mechanisms under controlled conditions. Integration of bioengineered hydrogels, 3D microenvironments, and advanced imaging and omics approaches will enable high-resolution mapping of integrin signaling networks. Additionally, combining these systems with CRISPR-based perturbation and mechanobiological modeling may reveal how integrin signaling integrates with pathways such as Wnt, Notch, and TGF-β to orchestrate hPSC fate.

Ultimately, the development of cost-effective, scalable, and xeno-free ECM platforms, together with a deeper mechanistic understanding of integrin–ECM crosstalk, will be essential for achieving reproducible, safe, and efficient hPSC-based applications in regenerative medicine, disease modeling, and cell therapy manufacturing.

Author Contributions

TW: Contributed to drafting the manuscript and preparing the figures. ZZW: Involved in study design, manuscript writing, and final approval of the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, Google Gemini 3 and OpenAI GPT-4.1 were used for language editing and grammar checking. The authors reviewed and edited all text and take full responsibility for the content of the published work.

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