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COMPREHENSIVE REVIEW

Reviewing and Updating the Major Molecular Markers for Stem Cells

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Stem cells (SC) are able to self-renew and to differentiate into many types of committed cells, making SCs interesting for cellular therapy. However, the pool of SCs in vivo and in vitro consists of a mix of cells at several stages of differentiation, making it difficult to obtain a homogeneous population of SCs for research. Therefore, it is important to isolate and characterize unambiguous molecular markers that can be applied to SCs. Here, we review classical and new candidate molecular markers that have been established to show a molecular profile for human embryonic stem cells (hESCs), mesenchymal stem cells (MSCs), and hematopoietic stem cells (HSCs). The commonly cited markers for embryonic ESCs are Nanog, Oct-4, Sox-2, Rex-1, Dnmt3b, Lin-28, Tdgf1, FoxD3, Tert, Utf-1, Gal, Cx43, Gdf3, Gtcm1, Terf1, Terf2, Lefty A, and Lefty B. MSCs are primarily identified by the expression of CD13, CD29, CD44, CD49e, CD54, CD71, CD73, CD90, CD105, CD106, CD166, and HLA-ABC and lack CD14, CD31, CD34, CD45, CD62E, CD62L, CD62P, and HLA-DR expression. HSCs are mainly isolated based on the expression of CD34, but the combination of this marker with CD133 and CD90, together with a lack of CD38 and other lineage markers, provides the most homogeneous pool of SCs. Here, we present new and alternative markers for SCs, along with microRNA profiles, for these cells.

Introduction

Stem cells (SC) are defined as a class of undifferentiated cells capable of self-renewal, perpetuating their population and giving rise to many types of committed or more specialized cells through differentiation [1]. SCs can be found during all stages of development from the embryo to the adult organism, and they consist of cells with varying differentiation potential.

Cells taken from the zygote to as far as the blastocyst stage are considered totipotent because they have the potential to generate a whole organism [2]. By the blastocyst stage, the cells become more specialized and are considered pluripotent. Embryonic stem cells (ESCs) are obtained at this stage and can generate tissues from the 3 germ layers [3], but they are not able to originate a whole individual [2]. Fetal and adult tissues also have several sources of SCs. These cells, however, have a limited differentiation potential that is less than that of ESCs. Mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) are found among this type of SCs.

The discovery of SCs has brought new possibilities to the scientific and clinical area, as they have the potential to be applied in cell replacement therapy, gene therapy, drug discovery, disease modeling, and developmental biology [4–6]. Nevertheless, the pool of SCs obtained from in vivo and in vitro conditions is not homogeneous; rather, the cells are in several stages of differentiation. Therefore, identifying unambiguous markers is essential for isolating the most primitive cells and for clearly identifying the different stages of undifferentiated and committed cells.

In this context, the aim of this review is to construct a molecular profile, including classical and new candidate molecular markers, of the 3 most studied human SCs: ESCs, MSCs, and HSCs.

Molecular Markers for ESC Characterization

ESCs are commonly isolated from the inner cell mass (ICM) during the blastocyst stage and possess the capacity to self-renew and to originate all cell types of an organism [7]. Since the first cultures of ESCs were established [8,9], considerable effort has been made to characterize a unique ESC-associated molecular signature. In 2007, the International Stem Cell Forum created the so-called "International Stem Cells Initiative" to establish an ESC molecular identity [10]. A total of 59 human ESC (hESC) lines were analyzed for cell-surface antigens and gene expression as potential markers

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for ESCs [10]. In the same year, a consensus ESC gene list and a consensus differentiation gene list were proposed by Assou and coworkers [11] based on 38 publications regarding ESC transcriptomes. They also created an online database [http://amazonia.montp.inserm.fr] where the transcriptome dataset is available.

The set of molecular markers commonly applied to identify ESCs consists of cell-surface proteins and genes specifically expressed in ESCs (Table 1). The characteristic cell-surface markers of ESCs were first detected in human embryonic carcinoma [12–14]. Among them are stage-specific embryonic antigen-3 (SSEA-3) and 4 (SSEA-4) and the tumor rejection antigens (TRA-1-60 and TRA-1-81) [9,15]. These surface markers are observed in the ICM, but they are absent in the 2–8 cell and morula stages [16]. When ESCs are induced to differentiate, these antigens are downregulated, and SSEA-1 is upregulated [16,17]. Moreover, GCTM2, GCTM343, alkaline phosphatase, CD90, CD24, and CD9 are other surface molecules identified in hESCs [9,10,15,16, 18,19].

In addition to surface molecules, there are some genes whose expression is characteristic of ESCs. Classically, the 3 transcription factors Nanog, Oct-4, and Sox-2 are used as indicators of the uncommitted status of an ESC [15,20]. Alternatively, other molecules (Table 2) are cited in the scientific literature as putative markers of ESCs, and all of them have their expression downregulated when these cells are induced to differentiate [9,15,18,19,21–26]. Below, we discuss the genes most commonly used to confirm ESC identity. It should be noted that some of the genes listed in Table 2 are not discussed because there are none or very few studies about their roles in ESCs.

Classical Molecular Markers for ESC

Nanog

Named after the mythological Celtic land of the ever-young *Tir nan Og*, Nanog was first described in 2002 by 2 groups independently [27,28]. This transcription factor is a homeodomain protein whose expression is observed in the morula and ICM but is absent from unfertilized oocytes, 2- to 16-cell embryos, early morula, and trophectoderm [27,29]. Nanog is downregulated when organogenesis is initiated at the time of embryo implantation [27]. The silencing of the Nanog gene leads to the differentiation of ESCs into trophoectoderm and extraembryonic endodermal lineages, along with a downregulation of Oct-4 [29]. In murine ESCs (mESCs), the overexpression of Nanog can maintain these cells in an undifferentiated state even without LIF, likely by the inhibition of Gata4 and Gata6 [28]. The expression level of Nanog seems to be regulated by the inhibitor of differentiation 1 (Id1) protein [30], which acts as a negative regulator of helix-loop-helix DNA-binding proteins [31]. ESCs in which Id1 is knocked down display Nanog expression levels that are 35% lower than wild-type ESCs and exhibit a loss of the capacity to self-renew [31].

Oct-4

Oct-4, also known as Oct-3, Oct-3/4, POU5f1, OTF3, or NF-A3 [32], is another transcription factor that has roles in controlling the pluripotency of ESCs. It is expressed in unfertilized oocytes [7,32] and after fertilization as far as the 10-cell stage the observed transcripts are mainly of maternal origin and were expressed before zygote formation [32]. After the 10-cell stage, Oct-4 expression stabilizes, indicating the beginning of the embryonic production of Oct-4. During the blastocyst stage, Oct-4 can be observed in both the ICM and trophoectoderm, with Oct-4 levels higher in the former [32]. However, Oct-4 is highly expressed in the ICM of the early blastocyst but is absent from the trophoectoderm in mice [33]. The levels of Oct-4 determine the fate of ESCs because its downregulation leads to ESC differentiation into trophoectoderm [33,34], and an upregulation of less than 2-fold leads to ESC differentiation into extraembryonic endoderm and mesoderm [33].

An important point that Oct-4 alone is not sufficient to maintain an undifferentiated phenotype. The withdrawal of LIF from mouse ESCs leads to their differentiation despite the expression of Oct-4 [33].

Sox-2

Sox-2 is included in the SOX B1 group of transcription factors and has a single high-mobility group DNA-binding domain [35]. Together with Oct-4 and Nanog, Sox-2 plays a role in the maintenance of ESC pluripotency [36]. Its expression is first observed during the morula stage, followed

Table 1. The Most Common Molecular Markers Used for Embryonic Stem Cells, Mesenchymal Stem Cells, and Hematopoietic Stem Cells Characterization

SC	*Molecular markers*
ESCs	
Positive markers	SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, alkaline phosphatase, Nanog, Oct-4, and Sox-2.
Negative markers	SSEA-1.
MSCs	
Positive markers	CD13, CD29, CD44, CD49e, CD54, CD71, CD73, CD90, CD105, CD106, CD166, and HLA-ABC.
Negative markers	CD14, CD31, CD34, CD45, CD62E, CD62L, CD62P, and HLA-DR.
HSCs	
Positive markers	CD34, CD90, and CD133.
Negative markers	CD38 and lineage markers[a].

[a]A detailed list of negative lineage markers can be found on Table 8.

SC, stem cell; ESCs, embryonic stem cells; MSCs, mesenchymal stem cells; HSCs, hematopoietic stem cells; SSEA, stage-specific embryonic antigen; TRA, tumor rejection antigens.

Table 2. The Most Cited Candidate Embryonic Stem Cell Maker Genes in Literature

Gene abbreviation	*Gene name*	*Biochemical function*[a]
Cx43	Connexin 43	Component of connexons.
DNMT3B	DNA (cytosine-5) methyltransferase 3β	Required for genome-wide de novo methylation and is essential for the establishment of DNA methylation patterns during development.
FOXD3	Forkhead box D3	Required for maintenance of pluripotent cells in the pre-implantation and peri-implantation stages of embryogenesis.
GAL	Galanin	Contracts smooth muscle of the gastrointestinal and genitourinary tract, regulates growth hormone release, modulates insulin release, and may be involved in the control of adrenal secretion.
GDF3	Growth differentiation factor 3	Control differentiation of ESCs in mice and humans. This molecule plays a role in mesoderm and definitive endoderm formation during the pre-gastrulation stages of development.
PODXL	Podocalyxin-like	Pro-adhesive molecule, enhancing the adherence of cells to immobilized ligands, increasing the rate of migration and cell-cell contacts in an integrin-dependent manner.
LEFTYA	Left-right determination factor A	Required for left-right (L-R) asymmetry determination of organ systems in mammals.
LEFTYB	Left-right determination factor A	Required for left-right axis determination as a regulator of LEFTY2 and NODAL.
LIN28	Cell lineage protein 28	Acts as a "translational enhancer," driving specific mRNAs to polysomes and thus increasing the efficiency of protein synthesis. Its association with the translational machinery and target mRNAs results in an increased number of initiation events per molecule of mRNA and, indirectly, in stabilizing the mRNAs.
NANOG	Nanog	Transcription regulator involved in inner cell mass and ESCs proliferation and self-renewal. Imposes pluripotency on ESCs and prevents their differentiation towards extraembryonic endoderm and trophectoderm lineages.
OCT4	Octamer binding protein 4	Forms a trimeric complex with SOX2 on DNA and controls the expression of a number of genes involved in embryonic development
REX1	Zinc finger protein 42	Involved in self-renewal property of ESCs.
SOX2	SRY-related HMG box 2	Transcription factor that forms a trimeric complex with OCT4 on DNA and controls the expression of a number of genes involved in embryonic development.
TDGF1	Teratocarcinoma-derived growth factor 1	Play a role in the determination of the epiblastic cells that subsequently give rise to the mesoderm.
TERF1	Telomeric repeat binding factor 1	Component of the shelterin complex (telosome) that is involved in the regulation of telomere length and protection.
TERF2	Telomeric repeat binding factor 1	Component of the shelterin complex (telosome) that is involved in the regulation of telomere length and protection.
TERT	Telomerase	Catalytic component of the teleromerase holoenzyme complex whose main activity is the elongation of telomeres by acting as a reverse transcriptase that adds simple sequence repeats to chromosome ends by copying a template sequence within the RNA component of the enzyme.
UTF-1	Undifferentiated embryonic cell transcription factor-1	Acts as a transcriptional coactivator of ATF2, a transcriptional activator, probably constitutive, which binds to the cAMP-responsive element.

[a]Extracted from GeneCards (www.genecards.org).

by the ICM, epiblast, and cells from the extraembryonic ectoderm [36]. In addition, Sox-2 was also detected in the precursor cells of the developing central nervous system and in both male and female germ cells [37]. An up to 2-fold increase in the expression of Sox-2 leads to the differentiation of ESCs to ectoderm (mainly neuroectoderm), endoderm, and trophoectoderm lineages, likely due to an observed downregulation of genes (Oct-4, Nanog, FGF-4, UTF-1, Lefty-1) controlled by Sox-2 [36].

Sox-2-disrupted mice embryos fail to survive after implantation. Cultured cells from the entire blastocyst or ICM of these embryos differentiate into trophoblast cells and extraembryonic endoderm [37], thus suggesting that Sox-2 has a role in the maintenance of the undifferentiated status of epiblast cells.

The transcription factors detailed above act together in the maintenance of the SC status of ESCs. The knockdown of Sox-2 downregulates Oct-4 and Nanog, indicating that these transcription factors act together to maintain SC status [38]. Additionally, Sox-2 is unable to activate its target genes alone and must have a partner protein, which in ESCs is Oct-4 [35]. These 2 transcription factors bind to DNA as a heterodimer [33], and their targets include their own coding genes and Nanog [39–41]. Nanog also regulates the expression of Sox-2 and Oct-4 [37,38] in a feed-forward manner.

Rex-1

Rex-1, also named zinc finger protein 42 (Zfp-42), is a transcription factor that was first identified in mice teratocarcinoma [12]. It has been studied primarily in mESCs, but its expression has also already been reported in hESCs [16,25]. In mice, Rex-1 is downregulated when the cells from the ICM differentiate into embryonic ectoderm, but it remains expressed in trophoblast cells [42]. In ESCs, Rex-1 appears to inhibit their differentiation, which is evidenced by the increased susceptibility of Rex-1$^{-/-}$ cells to differentiate after exposure to retinoic acid [43]. The expression of this transcription factor is regulated by Sox-2, Nanog, and Oct-4 [44]. An Oct-4-binding site has already been reported in the promoter of Rex-1, and its expression seems to be regulated by the levels of this transcription factor [45].

Dnmt3b

Dnmt3b is a de novo methyltransferase detected in oocytes, 2- to 4-cell embryos, and in the blastocyst stage in humans [46]. In mice, it is expressed in the ICM, epiblast, and embryonic ectoderm in a pattern similar to that observed for Oct-4 [46]. It presents 4 splicing variants, but only the Dnmt3b1 isoform is observed at these stages. This variant is observed in ESCs and, upon differentiation, its expression shifts to the Dnmt3b3 variant [47]. In mESCs, Dnmt3b interacts physically with Dnmt3a and stimulates its reciprocal activities [48]. Dnmt3a$^{-/-}$/3b$^{-/-}$ mESCs show a progressive decrease in the levels of methylation together with an increasing inability to differentiate [49]. The impairment in the methylation levels affects the promoters of Oct-4 and Nanog; consequently, abnormal expression of these transcription factors during differentiation is observed [48]. In contrast, Dnmt3b does not seem to have a role in ESC self-renewal [50].

Foxd3

Foxd3, initially termed *Genesis*, belongs to the HNF-3/Forkhead transcriptional regulatory family [51]. In mice, Foxd3 is first detected during the blastocyst stage. It is not observed in either oocytes or during the first cleavage stages [52]. After ESCs differentiate, Foxd3 can still be detected in neural crest cells [53]. A lack of Foxd3 in mESCs increases the number of apoptotic cells [54]. Foxd3$^{-/-}$ mice embryos die during the gastrulation stage because of a loss of epiblast cells and the expansion of extraembryonic tissues [52]. However, the expression of the genes necessary for ESC maintenance (Oct-4, Sox-2, and Nanog) is not altered in Foxd3$^{-/-}$ embryos [52,54]. Thus, Foxd3 seems to be important for the survival and self-renewal of ESCs and to repress their differentiation [54]. There are studies reporting the interaction of Oct-4 and Nanog with Foxd3, although mRNA levels are not altered in cells lacking Foxd3 [54]. Oct-4 was shown to act as a corepressor of Foxd3 [55], and it is regulated by Foxd3 together with Nanog, which counterbalance the inhibitory effect of Oct-4 on its own promoter [56].

Tdgf1

Tdgf1, also termed Cripto, is a member of the EGFP/TGFα growth factor family [57]. It is first detected at the blastocyst stage; during later stages of embryonic development, it is observed in the developing heart in mice [58]. It appears to have a role in blocking neural differentiation and in allowing ESCs to differentiate into cardiac cells [59]. Cripto$^{-/-}$ embryos die before birth and exhibit aberrant development and a lack of cardiogenic differentiation. Tdgf1 suppression in ESCs leads to their differentiation into neuronal lineages [60]. However, the proliferation levels of Crypto$^{-/-}$ ESCs are not different from wild-type ESCs [61].

Lin-28

Lin-28 is an RNA-binding protein that is highly expressed in hESCs [62] and is important for their growth and survival [63]. ESCs lacking Lin-28 show a decrease in their proliferation and a higher number of apoptotic cells when compared to wild-type ESCs [63,64]. High levels of Lin-28 expression at low cell densities slow the cell cycle and leads to differentiation toward an extraembryonic endoderm lineage [62]. Regarding its roles in ESCs, Lin-28 is involved in enhancing mRNA translation and the inhibition of some microRNA (miRNAs). Lin-28 acts on the let-7 miRNA family to block the processing of pri-let-7a and 7g in vitro. When Lin-28 is knocked down, the levels of mature let-7 family members are increased and are accompanied by decreasing in Oct-4 and Nanog expression. [65]. Lin-28 also regulates Oct-4 at the translational level, as its knockdown leads to a reduction in Oct-4 protein levels but not of its mRNA [63,64,66]. Oct-4 is also observed in Lin-28-associated polysomes, indicating that Lin-28 might be involved in the active translation of this transcription factor [66]. Other targets for translational activation are Cdk4 and cyclins A and B [64].

UTF-1

UTF-1 is a transcription factor that is stably associated with chromatin and acts as a transcriptional repressor

[67,68]. During embryonic development in mice, UTF-1 cannot be observed in the morula but is upregulated at the blastocyst stage, specifically in the ICM. Recently, it has been observed in the primitive ectoderm and extraembryonic ectoderm [69]. ESCs with reduced levels of UTF-1 were delayed in differentiation and experienced perturbed EB formation [67,68], but their self-renewal was not affected, which resulted in increased expression levels of several genes. The explanation for this phenotype is that UTF-1 promotes chromatin condensation of its target genes, preventing their aberrant expression [68]. Moreover, it has been suggested that UTF-1 might maintain an ESC chromatin state that is susceptible to differentiation stimuli [67].

UTF-1 is bound by Oct-4 and Sox-2 in regulatory regions located at 3′ position of its gene, as demonstrated by in vitro assays [70,71]. There is an overlap between genes regulated by UTF-1 and those that are targets of Nanog, Sox2, Dax1, Nac1, Oct-4, Klf4, Zfp-281, Rex1, and c-Myc [69].

Novel Cell Markers for ESCs

Applying ESCs to cellular therapy is not feasible for many reasons. First, ESCs display a high potential for generating tumors in vivo. Moreover, the isolation of a pool of ESCs requires the destruction of human embryos, which raises ethical concerns about their use in cell therapy.

For clinical applications, the determination of markers that identify undifferentiated ESCs from a pool of cells ready for transplantation is desirable because this would allow tumor induction to be avoided [72]. Additionally, it is important to achieve a homogeneous pool of ESCs for basic and applied studies in vitro, allowing for the better characterization of cellular and molecular properties of those cells [72].

In addition to the classical ESC markers discussed above, surface proteins and highly expressed genes have been proposed as new ESC markers. Regarding surface proteins, ESCs have been reported to be positive for CD24, CD30, CD49f, CD50, CD90, CD133, CD200, and CD326. From this set of markers, CD133 and CD326 have been proposed as ESC markers due to their downregulation after the induction of neuronal differentiation [73,74]. On the other hand, CD24 may not be proposed as a marker due to its detection in differentiated cells [75]. CD30, although expressed in ESCs and downregulated under differentiation, was reported to be present on ESCs plasma membrane as a consequence of the culture media, specifically in serum-free condition [76]. Together with those proteins, a new surface marker was recently added to the list of the SSEA-associated membrane molecules of ESCs. Named SSEA-5, its expression is detected in the ICM of human blastocist and it was reported to be 5-fold lower when ESCs were induced to differentiate. Moreover, SSEA-5 was suggested as a marker for removing remaining undifferentiated ESCs in conditions of differentiation induction [74]. Additionally, the combined analysis of SSEA-5 together with CD9, CD50, CD90, and CD200 was more effective in detecting potential teratoma cells within differentiating ESCs [74].

In this sense, it is important to note that additional surface markers can be discovered from plasma membrane proteomics studies (for more details on ESCs plasma membrane proteomics, see the Ref. [77]).

Within ESCs, other highly expressed genes and putative new markers include line-type transposase domain containing 1 protein (L1TD1), Forkhead box O1 (FOXO1), and E1B-AP5. L1TD1 is highly expressed in ESCs and is absent from most adult tissues. *In silico* analysis revealed that it is restricted to the blastocyst stage, where its expression is downregulated during differentiation in a pattern similar to that observed for Oct-4, Nanog, and Sox-2. In addition, L1TD1 is a downstream target for Nanog protein [78].

FOXO1 is also expressed at higher levels in ESCs, is downregulated during ESC differentiation, and has been implicated in the regulation of ESC pluripotency. Knockdown of FOXO1 does not alter the self-renewal of ESCs but is accompanied by the downregulation of Oct-4, Nanog, and Sox-2, leading to the spontaneous differentiation of ESCs into mesoderm and endoderm lineages. Moreover, FOXO1 appears to act by activating Sox-2 and Oct-4 expression [79].

Adenovirus early region 1B-associated protein 5 (E1B-AP5) is a nuclear RNA-binding protein observed in the nucleus and cytoplasm, where its phosphorylated form is presented at the ESC surface and can be used as a specific marker for ESCs. Cells positive for this marker are also positive for the classical ESC markers (ie, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81, Nanog, Oct-4, and Sox-2). Moreover, this surface protein was not detected after cell differentiation [72].

Transcriptome studies are also a potential source of new ESC markers [18,19,21–26]. These reports have revealed many genes that are enriched in ESCs and also downregulated during cell differentiation. All of these genes can be considered putative markers of an undifferentiated state, but only a few have had their roles in ESCs investigated. Table 3 summarizes the results from transcriptome studies comparing the expression profiles of ESCs and differentiated cells.

miRNAs associated with ESCs

Studies of mESCs have revealed that null Dicer cells display a pronounced loss in proliferative capacity [84]. The lack of DGCR8, another important member of the miRNA generation pathway [85], appears to cause alterations in mESCs; these cells exhibited extended population doubling, increased numbers of cells in the G_1 phase, and differentiation impairment [86]. These results indicate that miRNAs possibly have important roles in the biology of ESCs, and several have been identified as being exclusive to those cells, such as clusters miR-302-367, miR-520, and miR-371/372/373.

The miR-302-367 cluster is located on chromosome 4 and is composed of 9 miRNAs disposed in a polycistronic manner: miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-367, and miR-367* [87]. This cluster is highly expressed in ESCs [88,89], more than 20-fold when compared to adult cells or hMSCs, and its expression is rapidly downregulated when cells are induced to differentiate. It has also been detected in induced pluripotent cells [90]. In addition, putative binding sites for Oct3/4, Nanog, Rex1, and Sox-2 have been identified within its promoter sequence, proving its function in ESCs [88]. Indeed, Card et al. [91] showed that Oct-4 and Sox-2 bind to the promoter of this cluster.

Table 3. Genes Expressed at Higher Levels in Embryonic Stem Cells When Compared to Differentiated Cells Based in Transcriptomic Data

ESC lineage	*Differentiated cells*	*ESC-associated transcripts differentially expressed*	*Reference*
HES3, HES4	Cells from fetal brain, fetal liver, adult brain, placenta, adult testis, adult kidney, adult lung, adult heart	CCNB1, CFL1, CGI-48, CHK2, CKS1B, CLDN6, Cx43, DNMT3B, ERH, FAM60A, FAS, FLJ10713, FZD7, GDF3, GTCM1, HMGA1, LIN-28, NCL, Numatrin, PCNA, PNF1, POU5F1, REX-1, SFRP1, SOX-2, TDGF-1, TERF-1, TMSB15A.	[25]
BG02	Embryoid body	ARL8, BRIX, C15orf15, C20orf1, C20orf129, C20orf168, CCNB1, CCNC, CCT8, CDC2, CER1, CRABP1, CRABP2, CYP26A1, DDX21, DNMT3B, EIF4A1, ELOVL6, EPRS, FABP5, GAL, GDF3, GJA1, GSH1, GTCM-1, HDAC2, HMGB2, HMGIY, HNRPA1, HNRPAB, HSSG1, IDH1, IFITM1, IMP-2, IMPDH2, Jade-1, KIAA1573, KIF4A, KPNA2, Laminin receptor, LAPTM4B, LDHB, LEFTB, Lin-28, LRRN1, MAD2L2, MGST1, MTHFD1, MTHFD2, Nanog, NASP, NBR2, NME2, NPM1, NS, Numatrin, POU5F1, PPAT, PSMA2, PSMA3, PTTG1, RAMP, RPL24, RPL4, RPL6, RPL7, SEMA6A, SET, SFRP2, SLC16A1, SMS, SNRPF, SOX2, SSB, STK12, TD-60, TDGF1, TK1.	[26]
GE01/GE07/GE09 (pooled RNA)	Embryoid body	C20orf129, CCNB1, CCNC, CRABP1, CYP26A1, ELOVL6, FABP5, FLJ12581/Nanog, HDAC2, HSPA4, JADE-1, KIAA1573, KPNA2, LEFTB, MGC27165, GST1, NASP, NS, PSMA3, PTTG1, RAMP, RPL17, SEMA6A, SFRP2, SLC16A1, TDGF1, TNNT1, ZNF257.	[80]
H1	Embryoid bodies	AK3, DUSP6, E2IG5, FLJ10713, FRAT2, GAL, LEFT B, MYO10, PLP1, POU5F1, PROML1, PSIP2, SPS, STRIN, VRK1, VSNL1.	[81]
CH3, CH4	Embryoid bodies	A2ML1, AASS, ADCY2, ADD2, AK5, ARTN, C14orf115, C1orf182, C9orf61, CABYR, CACNA1G, CAMKV, CDCA7L, CHST4, CKMT1, CNTN1, CRABP1, CTGF, CXCL6, DCAMKL1, DDX25, DEPDC2, DNMT3B, DPPA2, DPPA4, FBXL16, FGF2, FLJ12505, FLJ12684, FLJ30707, GABRA5, GABRB3, GAP43, GPC4, GPR19, GPR23, GRPR, HESX1, INA, INDO, INHBE, ITGB1BP3, LEFTY1, LOC168474, LOC283174, MDN1, NALP4, NANOG, NAP1L2, NEF3, NEFL, NELL2, NMNAT2, NMU, NPTX2, OLFM1, OSBPL6, PCSK9, POU5F1, PTHB1, PTPRB, PTPRZ1, RAB39B, RARRES2, RASL11B, RDH12, RET, RNF182,SAMHD, SCG3, SCGB3A2, SEPHS1, SLC10A4, SLC7A3, SOX2, SYT1, TAC1, TAF4B, TDGF1,TERF1, TIMP4 TNFRSF8, USP44, WIF1, ZIC3.	[82]
H1, H7, H9	Embryoid bodies, immature hepatocytes and putative neural cells	FLJ35207, FOXH1, FOXO1A, GABRB3, GAP43, GRPR, PHC1, PODXL1, POU5F1, PRDM14, PTPRZ1, SALL1, SALL2, SZF1, THY1, ZIC2, ZIC3, ZNF206.	[21]
BG01, BG02, BG03	Embryoid bodies	CKMT1, DIAPH2, DNMT3B, EBAF, GABRB3, GDF3, GYLTL1B, IFITM1, LCK, LIN28, MIBP, NTS, PMAIP1, POU5F1, TDGF1, UTF1, ZNF206, ZPF42.	[83]

FOXO1, forkhead box O1.

Another typical ESC miRNA cluster is miR-520. It is located on chromosome 19 and is composed of 21 miRNAs: 515-5p, miR-517a, miR-517b, miR-517c, miR-518b, miR-518c, miR-519b, miR-519c, miR-519e, miR-520a, miR-520b, miR-520c, miR-520d, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-524*, miR-525-3p, and miR-526b* [89]. The roles of these 2 clusters in ESCs include (i) cell growth arrest, (ii) negative regulation of cellular metabolic processes, (iii) negative regulation of transcription, and (iv) small GTPase-mediated signal transduction [89].

The miRNAs miR-371-372-373 also comprise a cluster located on chromosome 19. This cluster is highly expressed in ESCs [88], and as observed for other miRNA clusters, its expression is rapidly downregulated when the cells are induced to differentiate [92].

Moreover, miRNAs initially observed in MSCs were also identified in ESCs, such as miR-9, miR-28, miR-29, miR-42, miR-63, and miR-89 [93]. These miRNAs are downregulated during the transition from ESCs to endothelial cells. Interestingly, miR-9 and miR-28 act by inhibiting CDH5 and

endoglin translation, respectively [94]. In addition to the miRNAs identified in ESCs, low levels of piRNA, small noncoding RNAs that interact with Piwi proteins, were also observed in these cells [94]. The occurrence of 3′ modifications and RNA editing in miRNAs observed in ESCs have also been reported [94].

The roles of miRNAs in ESCs are still not fully understood. Research in mice has revealed that miRNAs typical of mESCs have roles in cell proliferation, being implicated in promoting G1 to S phase transition through the suppression of the G1/S inhibitors [95]. Similar results were observed in hESCs; the miR-302 cluster was shown to be involved in the progression from G1 to S phase. Cell cycle regulators, cyclin D1, and possibly Cdk4 are targets of the miRNAs from this cluster [91]. Recently, NR2F2 (nuclear receptor subfamily 2, group F, member 2) was reported as a target for miR-302 [96]. Oct-4 induces the miR-302 expression and it is inhibited by NR2F2 [96].

Although little is known about the function of the miRNAs that are enriched in ESCs, some of them have already been reported as good reprogramming factors for the induced pluripotent stem cells (iPSCs) generation. Among the miRNAs successfully applied on reprogramming of fully differentiated cells into iPSCs, there are the cluster miR-302-367 [97], the miR-302b alone [96,98], the combination of miR-302, miR-369, and miR-200c [99], the miR-302b associated with miR-372, and the miR-372 alone [98]. These miRNAs are generally associated with factors as Oct-4, Sox-2, Klf4, Nanog, and c-Myc [96–98].

Published miRNA profiles of hESCs are summarized on Table 4. The data displayed correspond to the highly expressed miRNAs as listed by the authors of each article.

Interestingly, a comparison between ESCs and iPSCs showed that both cells have similar groups of upregulated miRNAs. Among them there are the clusters of miR-302-367 and miR-17-92 (composed by miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92). However, members of the miR-371-372-373 and miR-520 clusters were downregulated in iPSCs [90]. For more details on iPSCs miRNA profile, see Ref. [90].

Molecular Markers for MSC Characterization

MSCs are multipotent, adherent SCs capable of differentiating into osteoblasts, adipocytes, and chondroblasts [104] and also to nonmesenchymal lineages such as pancreatic islands [105], hepatocytes [106,107], and neuron-like cells [108]. MSCs were first isolated from bone marrow by A.J. Friedenstein [109]; nevertheless, many researchers have been able to isolate them from other regions of the human body, including adipose tissue [110,111], heart [111], trabecular bone [112], vessels [113], peripheral blood [114], skin [115], deciduous teeth [116], and many others. MSCs are also found in fetal tissues and fluids, such as amniotic fluid, umbilical cord blood [117], amnion, and placenta [118].

When MSCs are isolated from different tissues and organs, a heterogeneous pool of cells with several differentiation potentials can be obtained [119]. In addition, MSCs are not present in high quantities in tissues. For example, in the bone marrow, MSCs comprise 0.01% to 0.001% of the total cell number [96]. Therefore, the definition of markers that make it possible to isolate the most primitive MSCs and to identify those subpopulations with different potentials to generate mature cells is necessary to improve the cellular and

Table 4. microRNAs Highly Expressed in Embryonic Stem Cells, When Compared to Embryoid Bodies or Differentiated Cells

Lineage	*Highly expressed miRNA profile*	*Reference*
hES-T3	miR-20b, miR-26b, miR-200c, miR-302a*, miR-302b*, miR-302c*, miR-302d, miR-367, miR-371, miR-372, miR-373.	[100]
H9, I6,BG01v	miR-96, miR-127, miR-141, miR-200b, miR-200c, miR-299-3p, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302d, miR-324-3p, miR-367, miR-369-3p, miR-372, miR-515-5p, miR-517a, miR-517b, miR-517c, miR-518b, miR-518c, miR-519b, miR-519c, miR-519e, miR-520a, miR-520b, miR-520c, miR-520d, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-524*, miR-525, miR-525*, miR-526b*, miR-550-2, miR-612.	[89]
WA09, WA01, HSF6, HUES7, HUES13	miR-302 cluster, miR-371/372/373 cluster, primate-specific placenta associated cluster (54 miRNAs), miR-17, miR-106a.	[101]
SNU-hES3	miR-154*, miR-200c, miR-302a*, miR-302a, miR-302b*, miR-302b, miR-302c, miR-302c*, miR-302d, miR-371, miR-372, miR-373, miR-373*, miR-368.	[97]
Cyt25, Cyt203, HES2, HES 3, HES4	miR-21, miR-200c, miR-222, miR-296, miR-302a, miR-302c, miR-367, miR-371, miR-372, miR-373, miR-320d, miR-494.	[102]
H1	miR-18b, miR-20b, miR-92b, miR-154, miR-184, miR-187, miR-302a, miR-302b, miR-302c, miR-302d, miR-324-3p, miR-363*, miR-512-3p, miR-518b, miR-518c, miR-519d, miR-520g, miR-524*, miR-1323, miR-1901, miR-1908, miR-1910, miR-1911.	[103]
H9	Let-7a, Let-7b, Let-7c, Let-7d, Let-7g, Let-7i, miR-7, miR-92, miR-106b, miR-155, miR-181d, miR-184, miR-185, miR-187, miR-211, miR-222, miR-296, miR-302a, miR-302d, miR-331, miR-424, miR-484, miR-486, miR-503, miR-519c, miR-520, miR-518c, miR-519a, miR-574, miR-594, miR-744, miR-760, miR-766, miR-766*, miR-874, miR-877, miR-941, miR-1298, miR-1308, miR-1246, miR-1254, miR-1261, miR-1266, miR-1268, miR-1272, miR-1275, miR-1301, miR-1306, miR-1307, miR-1308.	[98]

Asterisk indicates miRNA strands less commonly found associated with Argonauta complex.
miRNA, microRNA.

molecular characterization of MSCs. Thus, the focus of this section will be on information about classical markers for MSCs, recently reported or alternative markers, and the miRNA profile of MSCs.

In 2006, The International Society for Cellular Therapy published the minimal criteria to identify a human SC as an MSC [120]. Among these are the expression of the surface proteins CD73, CD90, and CD105 together with the lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR [120]. However, many other markers have been identified and used by researchers (Table 5).

Some of the markers listed above appear to be dependent on the original tissue where the MSCs were isolated, but many are common among all MSCs. Based on the scientific literature, we suggest a list of common positive and negative surface markers found in MSCs (Table 1).

Together with these surface markers, several articles have reported the expression of some ESC-associated markers in MSCs from different sources (Table 6). The expression levels of some of these markers are downregulated when MSCs are induced to differentiate followed by an increase in SSEA-1 [122,124]. These changes in MSC marker expression recapitulate what is observed during ESC differentiation.

The real function of the ESC-associated markers in MSCs is not completely understood, and their presence has been considered as a primitive phenotype and an indication of the stem potential of the cells [141]. On the other hand, the expression of Nanog in MSCs could be due to a transition from in vivo to in vitro conditions, from the quiescent to the proliferative state [111]. In fact, Nanog seems to have roles in the maintenance and differentiation of MSCs in vitro. Studies with murine MSCs reported that the expression of this transcription factor is downregulated during differentiation. In addition, Nanog overexpression or knockdown leads to an increase or a reduction in cell proliferation, respectively [152]. In vitro, the knockdown of *NANOG* also resulted in the elevation of osteocalcin expression, a marker of osteogenic differentiation. In vivo, during the healing of an induced bone injury, Nanog expression was detected early in the process, preceding the expression of osteogenic differentiation markers. The timing of Nanog expression can be explained by the necessity of MSC population expansion, whose cells will be recruited for the healing process [152]. When the same healing experiment was repeated and Nanog expression was blocked, osteogenic differentiation was impaired, and adipogenic cells were observed [152]. In fact, Nanog seems to be related to favoring MSC differentiation to an osteogenic rather than an adipogenic fate. A decrease in Nanog expression is observed during adipogenic differentiation [153], and when Nanog is overexpressed in MSCs induced to adipogenic differentiation, there is a decrease in the expression of adipogenic markers and weaker Oil red staining [154].

Novel and Alternative MSC-Associated Markers

Although great progress has been made regarding the definition of MSC markers, we are still far from defining a specific molecular signature for these cell types. Here, we discuss novel and alternative markers reported for MSCs.

A study of plasma membrane surface proteins of MSCs derived from bone marrow detected 113 transcripts, including 20 CDs, expressed by MSCs but not by hematopoietic cells. From this group, 8 markers (CD49b, CD73, CD90, CD105, CD130, CD146, CD200, and integrin $\alpha V/\beta 5$) allowed for the isolation of MSCs from bone marrow mononuclear cells. CD200 has been proposed as a molecular marker to isolate bone marrow MSCs because cells isolated using this marker display a high enrichment in colony-forming units-fibroblasts when compared to the total mononuclear fraction before sorting and were able to differentiate into osteogenic, adipogenic, and chondrogenic lineages [155].

Battula et al. [151] have proposed FZD9 (Frizzled-9 or CD349) as a marker for primitive MSCs. The cells isolated from placenta that display FZD9 exhibited high clonogenic potential, which was enhanced when FZD9 was combined with CD10 and CD26 [156]. Additionally, this fraction expressed high levels of Oct-4, Nanog, and SSEA-4 [147]. However, Tran et al. [157] proposed that FZD9 is a good marker for the isolation of MSCs specifically for arterio/angiogenic therapy but not for discriminating between MSCs and non-MSCs. The expression of this marker was also observed in MSCs isolated from the periodontal ligament and was downregulated after the second passage [157].

Kaltz et al. [158] reported NOTCH-3 as a marker for the enrichment of MSCs capable of both osteogenic and adipogenic differentiation from bone marrow. In addition, the same research group demonstrated that ITGA11 and MSCA-1 could be used as markers for bone marrow MSC-derived cells that are mainly unipotent: osteogenic or adipogenic, respectively.

Using an antibody against the nerve growth factor receptor (NGFR or CD271), Quirici et al. [159] were able to isolate highly proliferative MSCs that were prone to osteogenic and adipogenic differentiation. Moreover, the authors observed that these cells were able to support the growth of hematopoietic progenitors [159]. The $CD271^{bright}$ subpopulation had been reported to contain a fraction of highly clonogenic bone marrow MSCs [160]. The $MSCA\text{-}1^{+}CD56^{+}$ fraction of this subpopulation demonstrated a high clonogenic potential and osteogenic, chondrogenic, and pancreatic differentiation, but it was unable to generate adipose cells [161]. These cells also present the capacity to inhibit T-cell proliferation and the differentiation of monocytes to dendritic cells [161]. Bühring et al. [160] observed that combining CD271 with CD140b, W8B, HEK-3D6, FZD-9, and CD56 makes it possible to isolate highly clonogenic MSCs.

GD2, a disialoganglioside, is another proposed MSC marker. It is detected in $CD45^{-}$ and $CD73^{+}$, $CD90^{+}$ MSCs isolated from bone marrow, but it is not observed in other cells from the same compartment, such as leukocytes, myeloid cells, T-lymphocytes, B-lymphocytes, or hematopoietic progenitors [162]. MSCs isolated from adipose tissue also present GD2 at the same levels as detected in MSCs from the bone marrow [162]. MSCs isolated from the umbilical cord also present GD2, and it can be detected as far as the 10th passage [163]. Cells isolated based on this marker were able to differentiate into adipocytes, osteoblasts, chondrocytes, and neuronal cells [162–164]. However, it has been reported that the $GD2^{-}$ and $GD2^{+}$ fractions have the same MSC marker profile and a similar potential to differentiate. Moreover, $GD2^{-}$ cells show higher proliferation rates than $GD2^{+}$ cells [164]. Nevertheless, the inhibition of GD2 synthesis leads to a block of neuronal differentiation [164].

Table 5. Surface Markers for Different Mesenchymal Stem Cells Reported in Scientific Literature

Source	*Positive markers*	*Negative markers*	*References*
	Adult origin		
Bone marrow	CD29, CD44, CD71, CD73, CD90, CD105, CD106, STRO-1.	CD13, CD14, CD34, CD45, CD133, CD144.	[106,117,121]
Deciduous teeth	CD13, CD73, CD105, CD146, STRO-1.	CD34, CD43, CD45.	[116,122]
Permanent teeth	STRO-1.	No negative markers reported.	[122]
Periodontal ligament	CD9, CD13, CD29, CD44, CD49a, CD49b, CD49c, CD49d, CD49e, CD51/61, CD71, CD73, CD90, CD105, CD106, CD119, CD120a, CD146, CD166, STRO-1, HLA-ABC.	CD2, CD3, CD8a, CD11a, CD14, CD16, CD18, CD19, CD20, CD24, CD25, CD31, CD33, CD34, CD38, CD41a, CD45, CD50, CD54, CD56, CD62E, CD62L, CD62p, CD66b, CD104, CD117, CD121a, CD123, CD124, CD126, CD133, CD235a, CD318, HLA-DR, STRO-1.	[124–126]
Apical papilla	CD146, STRO-1.	No negative markers reported.	[127]
Skin	CD13, CD29, CD44, CD49d, CD71, CD73, CD105, CD166.	CD10, CD11b, CD14, CD31, CD34, CD45, CD133, CD106, HLA-DR.	[128–130]
Endometrium	CD29, CD44, CD73, CD90, CD105, CD146.	CD31, CD34, CD45, STRO-1.	[131]
Adipose tissue	CD9, CD10, CD13, CD29, CD34*, CD44, CD49d, CD49e*, CD54, CD55, CD59, CD71, CD73, CD90, CD105, CD166.STRO-1, HLA-ABC.	CD11a, CD11b, CD11c, CD14, CD16, CD18, CD31, CD34, CD45, CD50, CD56, CD61, CD62e, CD104, CD106, CD133, CD144, HLA-DR.	[110,117,132]
Periferal blood	CD44, CD105, STRO-1.	CD3, CD14, CD20, CD45, CD68, CD106, HLA-DR.	[114]
Menstrual blood	CD9, CD29, CD41a, CD44, CD59, CD73, CD90, CD105.	CD14, CD31, CD34, CD38, CD45, CD133, STRO-1.	[133]
Saphenous vein	CD13, CD29, CD44, CD54, CD90, HLA-I	CD14, CD31, CD34, CD45, CD133.	[134]
Limbal stroma–eye	CD29, CD54, CD71, CD90, CD105, CD106, CD166.	CD11a, CD11c, CD14, CD31, CD34, CD45, CD138.	[135]
Lung	CD13, CD73, CD90, CD105, CD166, HLA-I, STRO-1.	CD14, CD34, CD45, HLA-DR, HLA-DQ.	[136,137]
	Fetal origin		
Amniotic fluid	CD13, CD29, CD44, CD49e, CD73, CD90, CD105, CD166, HLA-ABC.	CD1a,CD10,CD11a,CD11b,CD14, CD31,CD34,CD45, CD49d,CD50, CD54,CD62E,CD62P,CD117,CD133, HLA-DR,HLA-DP,HLA-DQ.	[138,139]
Bone marrow	CD29, CD44, CD49e, CD54, CD58, CD73, CD90, C105, CD106, CD123, CD166, HLA-ABC.	CD14,CD25,CD31,CD34,CD40,CD45, CD49d,CD50, CD62E,CD62L,CD62P, CD68,CD80,CD86,CD106, CD120a, CD120b,CD127,HLA-DR.	[140,141]
Peripheral blood	CD29, CD44, CD73, C105, CD106.	CD14,CD31,CD34,CD45,CD68,HLA-DR	[140]
Liver	CD29, CD44, CD49e, CD54, CD58, CD73, CD90, CD105, CD106, CD123, CD166, HLA-ABC.	CD14,CD25,CD31,CD34,CD40,CD45, CD49d,CD50, CD62L,CD62P,CD68, CD80,CD86,CD106,CD120a, CD120b, CD127,HLA-DR.	[140,141]
Lung	CD44, CD49e, CD54, CD58, CD73, CD90, CD105, CD123, CD166, HLA-ABC.	CD14,CD25,CD31,CD34,CD40,CD45, CD49d, CD50,CD62E,CD62L,CD62P, CD80,CD86,CD106, CD120a,CD120b, CD127,HLA-DR.	[141]
Pâncreas	CD13, CD29, CD44.	CD34,CD45,HLA-DR.	[142]
Placenta	CD29, CD44, CD49e, CD54, CD73, CD90, CD105, CD166, HLA-ABC.	CD31,CD34,CD45,CD49d,CD123, CD133,HLA-DR.	[118,143]
Spleen	CD44, CD49e, CD54, CD58, CD73, CD90, CD105, CD123, CD166, HLA-ABC.	CD14,CD25,CD31,CD34,CD40,CD45, CD49d, CD50,CD62E,CD62L,CD62P, CD80,CD86,CD106, CD120a,CD120b, CD127,HLA-DR.	[141]
Umbilical cord vein	CD29, CD73, CD90, CD105, CD166.	CD14,CD31,CD34,CD45.	[105]
Umbilical cord blood	CD29, CD44, CD49b, CD49d, CD51, CD58, CD71, CD73, CD90, CD105, CD106, HLA-ABC.	CD3,CD7,CD11a,CD11b,CD14,CD19,CD33, CD34, CD45,CD62L,CD62P,CD117, CD133,CD135,CD31, CD144, LA-DR.	[118,144,145]
Wharton's jelly	CD10, CD13, CD29, CD44, CD49e, CD51, CD68,CD73, CD80, CD90, CD105, CD117, CD166, HLA-ABC.	CD14,CD31,CD33,CD34,CD45,CD56, CD86,CD163, HLA-DR.	[146]

*Less than 28% of positive cells for this marker. The authors considered this percentage as positive.

Table 6. Major Embryonic Stem Cells Markers Observed in Mesenchymal Stem Cells

ESC marker detected	*Source of cells*	*Reference*
Oct-4, Nanog, Sox2, SSEA-4	Bone marrow, adipose tissue, dermis, and heart	[115]
OCT-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, low level of cells were TRA-1-80	Pulp of deciduous teeth	[122]
OCT-4, Nanog, SSEA-4	Amniotic fluid	[139,147]
OCT-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81	Fetal bone marrow, liver, and blood	[148]
SSEA-4	Dermis	[149]
SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT-4, Nanog, Sox-2, REX-1	Periodontal ligament	[124,150]
Nanog	Bone marrow, adipose, and cardiac tissue	[111]
Oct-4	Menstrual blood	[133]
Oct-4, Nanog, SSEA-4	Bone marrow and placenta	[151]

SSEA-4, a classical ESC marker, has been demonstrated to be useful for isolating MSCs with potential to differentiate into adipocytes, chondroblasts, osteoblasts, pancreatic, and neuronal cells, together with the capacity for forming organized bone tissue in vivo [124,151,165].

miRNAs associated with MSCs

Little is known about the miRNAs involved in the regulation of MSCs. The majority of the reports about miRNAs expressed in MSCs have focused on molecules with roles in the osteogenic [166–168], adipogenic [169–171], and chondrogenic [172,174,175] differentiation pathways. There are few reports exploring miRNA profiles and their functions associated with the maintenance of the stem state of MSCs (Table 7).

Molecular Markers for the Characterization of HSCs

HSCs are adult SCs found in the bone marrow [178], umbilical cord blood [179], fetal liver [180], and peripheral blood after mobilization [181,182]. A true HSC must be able to self-renew and give rise to all of the mature cells that comprise the hematopoietic system [183]. One approach to check the stemness of a candidate HSC is to test its capacity for performing a long-term reconstitution of hematopoiesis in recipient animals subjected to myeloablative treatment [183].

The identification of molecular markers that can characterize true primitive HSCs will make their isolation from the heterogeneous pool of cells where they are located easier. Below, we list the classical molecular markers reported by the scientific literature for the enrichment of highly primitive HSCs (Table 1), together with new candidate markers and the miRNA profiles of these cells.

Classical and Alternative HSC-Associated Markers

$CD34^+$

$CD34^+$ is a member of the sialomucin family of surface molecules [184]. It is the classical marker for HSCs, although its functions are not completely understood. It has been speculated that CD34 plays roles in cell adhesion and/or HSC differentiation [184]. Murine cells constitutively expressing this surface protein failed to completely

Table 7. microRNA Profile Reported for Mesenchymal Stem Cells from Different Sources

MSC source	*miRNAs*	*Reference*
Bone marrow	miR-15b, miR-16, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-27a, miR-27b, miR-145, miR-29a, miR-30d, miR-99a, miR-100, miR-103, miR-107, miR-125a, miR-130a, miR-143, miR-181a, miR-191, miR-193a, miR-193b, miR-199a, miR-199a*, miR-210, miR-214, miR-221, miR-222, miR-320.	[166]
Bone marrow	miR-18b, miR-21, miR-122a, miR-132, miR-140, miR-143, miR-145, miR-181a*, miR-181a-2, miR-181c, miR-335*, miR-337, miR-340, miR-409-5p, miR-431, miR-491, miR-519b, miR-520f, miR-520e, miR-520g, miR-652.	[173]
Adipose tissue	miR-16, miR-19b, miR-20a, miR-21, miR-24, miR-26a, miR-26b, miR-29a, miR-30c, miR-31, miR-92, miR-93, miR-99a, miR-119a, miR-125a, miR-125b, miR-127, miR-140, miR-146a, miR-146b, miR-152, miR-106b, miR-181d, miR-186, miR-190, miR-19, miR-214, miR-221, miR-270, miR-320, miR-339, miR-342, miR-365, miR-376a, Let-7a, Let-7b, Let-7g, Let-7i.	[176]
Bone marrow	miR-16, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-27a, miR-27b, miR-29a, miR-30a-5p, miR-31, miR-100, miR-125a, miR-125b, miR-143, miR-145, miR-152, miR-191, miR-199a, miR-199a-AS, miR-214, miR-221, miR-222, miR-320, Let-7a, Let-7c, Let-7d, Let-7e, Let-7f, Let-7i, Lrt-7b.	[177]

Asterisk indicates miRNA strands less commonly found associated with Argonauta complex.

differentiate [185]. Nevertheless, Nielsen and McNagny [186] argue that this blockage of differentiation may be due to inhibitory effects of CD34 on cell adhesion.

The bone marrow fraction positive for this molecule is known to be capable of hematopoietic reconstitution in recipients that underwent myeloablative therapy [187,188], indicating the presence of primitive HSCs. Interestingly, this marker is also expressed in vascular endothelial cells [189].

Nevertheless, there is no consensus regarding whether the most primitive HSCs are located in the $CD34^+$ fraction. In 1996, Osawa et al. [190] observed that murine cells negative for this marker were able to engraft and reconstitute hematopoiesis in irradiated animals more efficiently than $CD34^+$ cells. Further studies using human HSCs confirmed that cells lacking CD34 are able to engraft and differentiate into multilineage hematopoietic cells in vivo [191–193]. These cells can be isolated from the fetal liver, fetal blood, umbilical cord blood, peripheral blood, and bone marrow [191]. Analysis of $CD34^-$ cells isolated from the bone marrow and umbilical cord blood revealed that they lack the HLA-DR and CD90 antigens and are rarer than $CD34^+$ cells [191].

Some authors have raised the possibility of $CD34^-$ cells being a more primitive precursor than $CD34^+$ [191] based in the fact that $CD34^+$ cells were identified in myeloablated receptors that received $CD34^-$ cells [190–193]. Dao et al. [194] proposed that cells can interconvert between $CD34^+$ and $CD34^-$ based on their observation that $CD34^+$ grafts give rise to $CD34^-$ cells, and when these cells were transplanted to secondary recipients, they were able generate $CD34^+$ cells. However, the issue about what kind of cell ($CD34^+$ or $CD34^-$) is the most primitive HSC is a long way from being solved. In contrast to the previously mentioned observations, Gao et al. [195] utilized 3 different sources of HSCs and 4 purification methods and observed no engraftment in mice by the $CD34^-$ fraction. The same results were observed by Bhatia et al. [196], who reported that no engraftment was achieved even when 2.6×10^6 $CD34^-$ cells were injected into recipient mice [196].

Nevertheless, some interesting findings can possibly help to explain these contradictory results. Nakamura et al. [197] observed that, when cultured with murine stromal cells as feeders, $CD34^-Lin^-$ cells turn from nonproliferative to proliferative, generate $CD34^+$ cells, and exhibit colony-forming activity. Additionally, these cells were only able to engraft into nonobese diabetes/severe combined immunodeficiency (NOD/SCID) mice when cultured under these conditions. Interestingly, the range of engraftment was positively related to the level of $CD34^+$ cells. In the reports showing $CD34^-$ engraftment in irradiated recipients, the cells or the mice were exposed to human interleukin-3 (IL-3) and granulocyte/macrophage colony-stimulating factor [191–193]. Moreover, Gallacher et al. [198] isolated different subfractions of $CD34^-CD38^-Lin^-$ and observed that only $CD133^+$ cells were capable of engrafting in NOD/SCID mice. This fraction represents only 0.2% of the $CD34^-CD38^-$ cells. It is possible that the $CD34^-$ fraction contains primitive HSCs, and the negative results for hematopoietic multilineage differentiation in vitro and lack of engraftment in vivo may be due to the low levels of the true primitive $CD34^-$ cells and/or lack of pre-stimulation.

Although a consensus has not been established, HSCs are still being commonly obtained based on their expression of CD34. However, the enrichment of HSCs based on this marker results in a heterogeneous pool of cells [199] in which only a small number of cells can be considered SCs [200]. Therefore, other markers must be applied to subdivide this fraction and identify the most primitive HSCs.

CD133

CD133, also known as AC133, is an HSC surface marker that is restricted to the $CD34^+$ fraction. The percentage of $CD133^+$ cells is generally lower than that of $CD34^+$ cells, comprising 20%–60% of the $CD34^+$ cells isolated from the adult bone marrow, umbilical cord blood, peripheral blood, fetal liver, and bone marrow [201]. The $CD133^+$ fraction of the $C34^+$ HSC pool exhibits a high capacity for expansion, is enriched for megakaryocytic progenitor cells, and is able to differentiate into a larger number of erythroid cells [202]. These cells can home to and engraft in the bone marrow of myeloablated mice and sheep, primary and secondary recipients in the last case [201,203].

However, CD133 is not a marker that is exclusive to HSCs. In mice ESCs, CD133 is upregulated in committed and early progenitor cells and has been proposed to be a molecular marker of this stage of differentiation [204]; CD133 is also found in fetal and adult hepatic SCs [205] and in neural SCs [206]. Moreover, CD133 has been proposed to be a common marker for 2 cell fractions, $CD34^+$ and $CD34^-$, which may be enriched for the most primitive SCs [199]. The engraftment of the latter fraction in NOD/SCID mice is only observed when they are enriched for CD133.

Thy-1

Thy-1, also known as CD90 or CDw90, is observed in cells lacking or with low levels of expression of Lin^-, *c-kit*, CD38, CD45RA, CD71, and HLA-DR [207, 208].

Studies using umbilical cord blood [209], peripheral blood [210], and fetal [207] and adult bone marrow-derived cells have shown that the $CD34^+Thy\text{-}1^+$ subset is capable of generating long-term cultures and can give raise to multilineage differentiated cells in vitro. This type of cells is also able to engraft in radiation-ablated recipients and generates myeloerythroid and lymphocyte B lineages more effectively than the $Thy\text{-}1^-$ fraction [207]. However, it has been suggested that both $Thy1^+$ and $Thy1^-$ subsets have self-renewal potential and are capable of engrafting in recipient mice and that the unique difference between them is the HSC frequency. The levels of chimerism of these 2 subset fractions are similar under nonlimiting cell transplantation conditions, but a limiting dilution analysis revealed that HSCs are 5 times more abundant in the $Thy\text{-}1^+$ fraction [211].

There are also reports demonstrating the generation of $Thy\text{-}1^+$ cells from $Thy\text{-}1^-$ cells [211,212]. However, Majeti et al. [213] were able to establish a cell hierarchy, where $Thy\text{-}1^+CD45RA^-$ cells give rise to $Thy\text{-}1^-CD45RA^-$ cells that are, in turn, upstream of $Thy\text{-}1^-CD45^+$ cells.

Although it is known that the $Thy\text{-}1^+$ fraction includes primitive HSCs, the function of this surface protein has not yet been elucidated. A reduction in the number of hematopoietic colonies was observed when an anti-Thy-1 antibody was added to cultures of $Thy\text{-}1^+$ cells, suggesting that it may be involved in the development of HSCs [209]. Thy-1 has

also been proposed as a cell cycle status indicator based on the observation that only the Thy-1$^+$ fraction enters the S/G2/M-phases when cells are stimulated by cytokines [212].

Kinase insert domain receptor

Kinase insert domain receptor (KDR), also known as vascular endothelial growth factor receptor 2 (VEGFR2) or fetal liver kinase-1 (Flk1) in the mouse, is a less-known HSC marker. In vitro and in vivo studies have revealed that primitive HSCs are enriched in the CD34$^+$KDR$^+$ fraction, whereas the KDR$^-$ subset is composed mainly of lineage-committed hematopoietic progenitor cells. KDR$^+$ cells are very rare in the CD34$^+$ fraction, comprising 0.1 to 0.5% of the cells [214]. It has also been proposed that the CD34$^+$KDR$^+$ fraction comprises 5% to 6% of hemangioblasts, cells with bilineage differentiation potential, that are capable of generating hematopoietic and endothelial cells [215].

Cub domain protein 1

Cub domain protein 1 (CDCP1) is co-expressed with CD34 and CD133 in HSCs and is absent from mature cells. The HSC fraction isolated based on this marker and subsequently injected into the NOD/SCID mouse is able to engraft and generate various mature hematopoietic lineages [216].

HSC-Associated Negative Markers

CD38

This surface molecule is considered a negative marker for HSCs. It is expressed in differentiated erythroid, myeloid, B-lymphoid precursors, and T-lymphoid lineages [199]. The CD34$^+$CD38$^-$ fraction consists of a highly primitive set of cells that are able to generate progeny in long-term cell culture [199,217] and can engraft NOD/SCID mice to produce differentiated hematopoietic cells [196]. These cells are quiescent, and the increase in CD38$^+$ cells correlates with the beginning of cycling and differentiation [199].

Even when the CD34$^-$ cells are enriched, the fraction that demonstrates a greater engraftment and proliferation capacity is CD38$^-$ [191].

HSC-Associated Lineage Markers

Uncommitted HSCs lack characteristic markers that distinguish them from differentiated lineages [196] (Table 8). When HSCs are purified, it is usually by negatively selecting for these markers.

Table 8. Differentiated Hematopoietic Lineage Markers Absent from the Surface of Hematopoietic Stem Cells[a]

Cell type	*Marker*
Lymphocytes T	CD2, CD3, CD4, CD5, CD7, CD8.
Lymphocytes B	CD10, CD19, CD20, CD24.
Myeloid	CD14, CD15, CD16, CD33, CD41.
Erythroid	CD71, glycophorin A.
Natural killer	CD56.
Granulocyte	CD66b.

[a][200,208,198,220, http://pathologyoutlines.com].

New HSC-Associated Markers

Many molecular markers have been established for HSCs. However, HSCs exist within a highly heterogeneous pool of cells, which makes it difficult to isolate the most primitive SCs. Therefore, the discovery of new HSC markers will help to obtain a more homogeneous pool of HSCs. Below, we discuss some of the potential new markers for these cells.

CD49f, also known as integrin α6, yielded positive results when applied together with CD34 and Thy-1 to sort HSCs. The CD34$^+$ Thy1$^+$/Thy1$^-$CD49f$^+$ fractions showed high levels of chimerism in receptor mice [211].

Complement component 1 q subcomponent receptor 1 (C1qRp), or CD93 and a human homolog of the murine protein AA4, has been proposed to be a marker for the simultaneous isolation of primitive HSCs found in both CD34$^+$ and CD34$^-$ fractions. In vitro and in vivo assays have demonstrated that sorting cells using C1qRp leads to the isolation of primitive hematopoietic progenitors. This molecule has also been proposed to be a positive sorter for the HSCs found in the CD34$^-$ fraction, as the isolation of these cells is difficult due to the lack of a characteristic positive marker [219].

The isolation of primitive HSCs based on the activity of aldehyde dehydrogenase (ALDH) has also been reported. ALDH consists of a group of enzymes that are involved in the oxidation of aldehydes to carboxylic acids [220]. Cells positive for ALDH are negative for lineage markers but enriched for CD34$^+$ cells and for cells with short- and long-term activities [221,222]. This is a simple and inexpensive method for isolating HSCs; the fluorescent substrate for ALDH can also be combined with other markers [223].

Some additional HSC markers have been established in mice and are interesting for future studies to confirm their status as HSC markers in humans. Among them are ecotropic viral integration site 1 (Evi1), endothelial cell-selective adhesion molecule 1 (Esam1) and Flk2. Evi1 is a transcription factor exclusively expressed in HSCs and is downregulated during their differentiation [214]. Evi1 expression identifies HSCs with long-term repopulating activity, which are considered the most primitive HSCs [223]. Evi1-positive cells exhibit significant self-renewal and differentiation potential in vitro [223]. In studies in vivo, Evi1$^+$ cells showed the potential to engraft in irradiated recipients and to give rise to differentiated lineages, as evidenced by the presence of myeloid, B, and T cells in the peripheral blood of recipients [223]. The same was observed in secondary transplantations, demonstrating the in vivo long-term multilineage repopulating potential of Evi1-positive HSCs, suggesting that Evi1 is needed to maintain long-term HSC activity [223]. Evi1 overexpression blocks the differentiation of HSCs and induces their expansion. Therefore, Evi1 appears to regulate the transition from HSC to a more committed progenitor. In other words, it controls the balance between self-renewal and differentiation [223].

Esam1 is a transmembrane protein that is highly expressed in both human and murine HSCs and is downregulated when these cells become committed to differentiate [224]. Mature hematopoietic cells, with the exception of megakaryocyte progenitors, do not express this marker. Higher levels of chimerism were observed when mouse cells were sorted using an Esam1 antibody [224]. It has been

proposed that Esam1 may be a more effective marker and could be a substitute for other markers in the isolation of HSCs [224].

Flk2 has been proposed as a negative marker for HSCs with long-term repopulating capacity. Murine cells isolated from bone marrow and fetal liver that are negative for this marker show a greater capacity to repopulate irradiated recipients. Flk2 is present in short-term HSCs and is upregulated as the cells become more mature [225].

miRNAs Associated with HSCs

It is known that miRNAs also have a role in SC mechanisms [226]. However, little research has focused on the miRNAs related to the most primitive HSCs. Most of the work has focused on miRNAs related to more committed cells or to diseases associated with the hematopoietic system [227]. Below, we summarize the latest reports on miRNAs in HSCs from the adult bone marrow, umbilical cord blood and mobilized peripheral blood (Table 9).

The miRNAs differentially expressed in the $CD133^+$ fraction of the cells isolated from bone marrow (miR-146a, miR-146b-sp, miR-99a, miR-10a, miR-125b, miR-551b, miR-125a-sp) are involved in the inhibition of differentiation, apoptosis, and cytoskeletal remodeling. miRNAs expressed in the $CD34^+CD133^-$ subset (miR-142-3p and miR-425) were observed to have negative effects on cell proliferation [229].

Merkerova et al. [232] detected differences in the miRNAs expressed by HSCs obtained from bone marrow and umbilical cord blood. Bone marrow-derived $CD34^+$ cells differentially express let-7b, miR-1, miR-34a, miR-195, miR-203, miR-214, miR-545, and miR-548d, and umbilical cord blood cells express the set of miRNAs that is clustered on chromosome 19q13: miR-517c, miR-518a, miR-519d, and miR-520 h.

The miRNA miR-155 seems to block myeloid and erythroid differentiation in hHSCs [230]. Moreover, it is thought that miR-17, 24, 146, 155, 128, and 181 may also maintain hematopoietic cells at an early stem-progenitor stage by blocking their differentiation [230].

Table 9. microRNAs Expression Observed in Different Hematopoietic Stem Cells Fractions from Different Tissues

HSC origin	*Cell fraction*	*miRNAs*	*Reference*
Adult bone marrow[a]	$CD34^+CD133^-$	miR-15a, miR-15b, miR-16, miR-17, miR-19a, miR-19b, miR-20a, miR-20b, miR-21, miR-23a, miR-25, miR-26a, miR-26b, miR-30b, miR-92a, miR-92b, miR-101, miR-106a, miR-126-3p, miR-142sp, miR-142-3p, miR-142-5p, miR-144, miR-181a, miR-191, miR-221, miR-222, miR-223, miR-451, miR-663, miR-638, Let-7a, Let-7c, Let 7f, Let-7g.	[228,229]
	$CD133^+$	miR-15a, miR-16, miR-17, miR-19a, miR-19b, miR-20a, miR-20b, miR-21, miR-26a, miR-26b, miR-29c, miR-30b, miR-92a, miR-92b, miR-101, miR-126-3p, miR-142-3p, miR-142-5p, miR-144, miR-221, miR-222, miR-223, miR-451, Let-7a, Let-7f.	[229]
	$CD34^+$	miR-9-3, miR-16a, miR-16b, miR-17, miR-20, miR-23a, miR-23b, miR-24-1, miR-24-2, miR-25, miR-26a, miR-26b, miR-27a, miR-29a, miR-29c, miR-30a, miR-30b, miR-30d, miR-32, miR-33, miR-92, miR-93, miR-95, miR-96, miR-100-1/2, miR-102, miR-103, miR-103-2, miR-106, miR-107, miR-122a, miR-123, miR-128a, miR-128b, miR-130a, miR-135-2, miR-146, miR-155, miR-181a, miR-181b, miR-181c, miR-182, miR-183, miR-190, miR-191, miR-193, miR-192-2/3, miR-194, miR-197, miR-198, miR-199a, miR-202, miR-203, miR-204, miR-205, miR-206, miR-212, miR-213, miR-221, miR-222, miR-223, Let-7b, Let-7c, Let-7d.	[230]
Umbilical cord blood	$CD34^+$	miR-10a, miR-10b, miR-15a, miR-16, miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a, miR-20b, miR-21, miR-27a, miR-27b, miR-93, miR-99a, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-125a, miR-125b, miR-126, miR-129, miR-130a, miR-142-3p, miR-142-5p, miR-144, miR-155, miR-181a, miR-181c, miR-181d, miR-196b, miR-222, miR-451, miR-519d, miR-551b, miR-520h, Let-7e.	[231–233]
	$CD34^+CD38^-$	miR-127, miR-365, miR-452, miR-520h, miR-526b*.Predicted miRNAS: miR-100, miR-105, miR-149, miR-209.	[231]
Peripheral blood	$CD34^+CD133^{+b}$	miR-10a, miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a, miR-20b, miR-34a, miR-93, miR-106a, miR-126, miR-130a, miR-146a, miR-221, miR-363, miR-599.	[233]
	$CD34^+$	miR-10a, miR-15a, miR-16a, miR-16b3, miR-17, miR-19a, miR-19b, miR-20, miR-20a, miR-23a, miR-23b, miR-24, miR-24-1, miR-25, miR-26a, miR-26b, miR-27a, miR-30b, miR-30c, miR-30d, miR-30e, miR-92, miR-93, miR-103, miR-103-2, miR-106, miR-107, miR-123, miR-126, miR-130a, miR-140, miR-142, miR-143, miR-146, miR-155, miR-181a, miR-191, miR-193, miR-196b, miR-197, miR-213, miR-221, miR-222, miR-223, miR-320, miR-363, miR-424/322, Let-7a, Let-7b, Let-7c, Let-7d, Let-7f, Let-7g.	[230,234]

[a]The miRNAs cited here are the listed by the article's authors as the highly expressed in the cells.
[b]Common miRNAs found in 2 pools of HSCs, either $CD34^+$ or $CD133^+$.
Asterisk indicates miRNA strands less commonly found associated with Argonauta complex.

Conclusions

ESCs are generally identified by a set of surface markers and the expression of 3 transcription factors (Nanog, Oct-4, and Sox-2). However, transcriptome assays have revealed a set of genes that are highly expressed in ESCs and are not found in their differentiated counterparts. The commonly cited markers are Rex-1, Dnmt3b, Lin-28, Tdgf1, FoxD3, Tert, Utf-1, Gal, Cx43, Gdf3, Gtcm1, Terf1, Terf2, Lefty A, and Lefty B. Nevertheless, these putative markers are far less studied than Nanog, Oct-4, and Sox-2. Some lack studies in hESCs, and others have not even been studied in ESCs.

MSCs are usually identified by the expression of CD73, CD90, and CD105 along with the absence of CD34. Although there is variation among the MSCs isolated from many different regions, it is possible to establish a common set of markers for these cells in which the cells are positive for CD13, CD29, CD44, CD49e, CD54, CD71, CD73, CD90, CD105, CD106, CD166, and HLA-ABC expression and negative for CD14, CD31, CD34, CD45, CD62E, CD62 L, CD62P, and HLA-DR expression. Moreover, many articles have reported the expression of ESC markers; SSEA-4 has been proposed as a new marker for isolating MSCs. Nevertheless, there is no consensus about the expression of these molecules, and little is known about their roles in MSCs.

HSCs are primarily isolated based on the expression of CD34, but the pool of cells obtained is composed of cells with many degrees of differentiation. Therefore, the combination of this marker with other surface molecules, such as CD133 and CD90, along with the lack of CD38 and lineage markers provides the most homogeneous pool of SCs. In addition, the $CD34^-$ fraction has also been reported as containing true HSCs, but there is no consensus regarding this finding.

Regarding miRNAs, profiles are being established for each type of SC. However, their functions in the status of SCs have not been completely elucidated. Although there has been considerable progress in the study of SC markers, it is still far from being fully understood.

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Author Disclosure Statement

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