Chapter 5
Stemness and Stem Cell Markers

There are no shortcuts in evolution.

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Abstract Stemness is still a contraversive entity and the definition is evolving. It can roughly be defined as the most primitive cell state capable of transdifferentiating into divergent functional cell lines. Different stem cells express different stem cell markers which are hallmarks of these cells together with adequate functionality. Sometimes the cells possess/express the markers (phenotype) but the function is lacking and therefore they cannot be considered stem cells. Markers are protein products of clonal expansion, during self-renewal of stem cells, where the entire energy is invested in their multiplication. They are permanent labels of stemness and different in different stem cell types from different sources. Here we are presenting the examples of stem cell markers known so far.

5.1 Introduction

Mutant analysis and transcriptional profiling experiments determined that stem cell markers are genes and their protein products used in scientific purposes to isolate and identify stem cells, using magnetic bead technology [1, 2]. We now know that many different types of stem cells exist in animal world, but they all are found to participate in very small percentage/populations in the human body. Thus, in some cases one stem cell could be found in 100,000 cells in circulating blood [1]. That is why it is so hard to detect and identify them. As we know, they inhabit the specific parts of the organs known as niche, which is already defined as a hypoxic region of the body under great influence of circulatory, neural, paracrine, endocrine, cytokine, and other factors [3, 4]. Signaling and cross-talk between the elements of niche and stem cells are of critical importance for their perpetuation of stemness. Thus, for example, Paneth cells constitute the niche for Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5)—we can find them in intestinal crypts [5, 6]. We also know that BM is the source of three different types of adult stem cells: HSC, MSC, and VSELs. The markers are mostly receptors which can be bound to specific ligands

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causing signaling mechanisms propagation within the cell. Labeled with fluorescent dyes, they can be detected, and isolated/segregated with the help of Flow Cytometry (FC) and Fluorescence Acquired Sorting analysis (FACS) [3–5]. Currently, the marker-based flow cytometry (FCM) technique and magnetic cell sorting (MACS) are the most effective cell isolating methods, and a detailed marker list will help to initially identify, as well as isolate ESCs using these methods [7]. Some functional assays have also been developed for stem cell marker identification and detection [3, 4]. Therefore, we were able to classify stem cells into distinctive categories.

5.2 Human Embryonic Stem Cell Markers and Pluripotency

ESC derives from inner cell mass of the blastocyst of embryo and are totipotent in their full capacity to transdifferentiate into any kind of mature body cell [8]. ESCs retain pluripotency and self-renewing ability due to both their inherent properties and the culture conditions in which they are propagated [7, 8]. The ability to differentiate into all cell lineages in living bodies while maintaining an undifferentiated state during in vitro culture makes ESCs prior to clinical transplantation [7]. Their specific receptors/markers depend on how old the embryo is. However, the crucial markers are: octapeptide4 (**Oct 4**), homeobox protein **Nanog**, **Tra1-60**, sex determining region Y-box 2 **Sox-2/SRY**, and stage-specific embryonic antigen-4 (**SSEA-4**). They can be detected by fluorescently labeled antibodies [7]. The pluripotent status of stem cells can be also characterized by a high level of alkaline phosphatase (AP) expression, along with the expression of multiple pluripotency markers. The epiblast (EPI) is the origin of all somatic and germ cells in mammals, and of pluripotent stem cells in vitro. To explore the ontogeny of human and primate pluripotency, comprehensive single-cell RNA sequencing for pre- and post-implantation EPI development in cynomolgus monkeys (*Macaca fascicularis*) was performed [1]. The group has shown that after specification in the blastocysts, EPI from cynomolgus monkeys (cyEPI) undergoes major transcriptome changes on implantation [1]. Thereafter, while generating gastrulating cells, cyEPI stably maintains its transcriptome over a week, retains a unique set of pluripotency genes, and acquires properties for "neuron differentiation" [1]. Human and monkey pluripotent stem cells have shown the highest similarity to post-implantation late cyEPI, which, despite coexisting with gastrulating cells, bears characteristics of pre-gastrulating mouse EPI and epiblast-like cells in vitro [1]. The authors concluded that these findings not only reveal the divergence and coherence of EPI development, but also identify a developmental coordinate of the spectrum of pluripotency among key species, providing a basis for better regulation of human pluripotency in vitro [1].

In recent years, a wide range of cell surface markers and generic molecular markers have been reported to be indicative of undifferentiated ESCs, especially for human species (Fig. 5.1) [8–10]. Proteins involved in several signal pathways are also known to have important functions in cell fate decision. Lectins and other

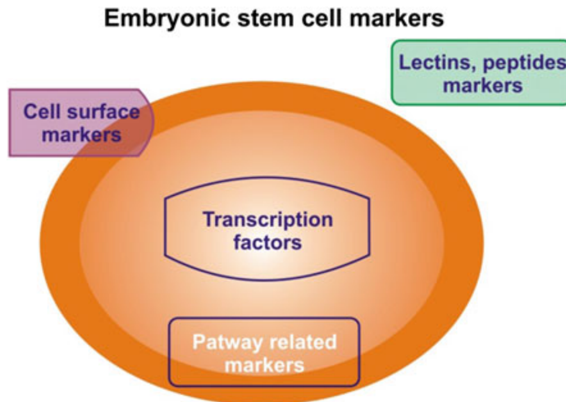

Fig. 5.1 Categories of embryonic stem cell markers

similar peptides have been found to specifically bind to ESCs. Unfortunately, many ESC markers overlap with those of tumor stem cells, making the problems when these markers are used for ESC identification and isolation [8]. In addition, understanding the mechanisms that regulate the pluripotency of human ESCs (hESCs) remains a major challenge, as recent studies have shown that human and mouse ESCs differ in these mechanisms despite their similar embryonic origins [8]. Further knowledge of these markers is critically needed for the proper uses of ESCs and elucidation of the mechanisms governing the pluripotency and self-renewal of ESCs.

5.3 Fetal Stem Cell Markers

FSCs are intermediary stadium between MSCs and ESCs. They originate from different fetal and extraembrional tissues during the fetal life. Growth kinetics, morphology, immunophenotype, potential for differentiation and incorporation in vivo, depend on the origin. They are more primitive and have bigger multipotential from their "adult" analogs (hematopoietic cells of fetal blood-HSCs, and they have bigger proliferative capacity from HSCs from the cord blood and HSCs from bone marrow of adults [11–16]. Certain subpopulations are showing pluripotent potential. These cells show lower immunogenic features and more seldom cause the graft versus host reaction (GvHR), which makes them potentially good cancnicates for transplantation.

Table 5.1 summarizes classification of fetal stem cells, showing that markers are either the markers of HSCs or the markers of MSCs.

Table 5.1 FSCs—classification and distribution of fetal stem cells

Fetal tissues	Extraembrional tissues
Blood (MSCs, HSCs)	Cord Blood (HSCs, MSCs, similar ESCs—CBEs, VSEL, endothelial progenitors, iPSCs)
Liver (MSCs, HSCs)	Tissue of cord Wharton's jelly, (MSCs)
Bone Marrow (MSCs, HSCs)	Umbilical blood vessels (HUVEC)
Lungs (MSCs)	Amniotic Fluid ((MSCs, AFCs, VSELs)
Pancreas (MSCs)	Placenta (Epithelial cells of amnion, MSCs of amnion, MSCs of chorion, and HSCs
Brain cortex	
Mesonephros	

(compilation from different sources)

5.4 Cord Blood Stem Cell Markers

These cells are considered to be multipotent—they can develop into more than one cell type, but are more limited than pluripotent ESCs [17–19]. Cord blood stem cells were reported to be successfully used in the treatment of acute myocardial infarction (AMI) [20]. As well as HSCs they have **CD34+ marker** as the essential for recognition and identification when collected, counted, and prepared for conservation.

5.5 Placental Stem Cell Markers

Given the fact that placenta originates partly from baby and partly from mother, as we can see from Table 5.1. Placental markers are from amnion (markers of epithelial cells of amnion, MSCs of amnion) and chorion and markers of HSCs, mostly CD34+.

5.6 Adult Stem Cell Markers

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell-hematopoietic stem cell (HSC) in the bone marrow (BM) normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the BM cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. On the other hand, it has been shown that there are stem cells in the brain [19]. Great curiosity of these dividing cells is that they have receptor for Zika virus which explains why the virus

Table 5.2 Markers of different adult stem cells (compilation from many sources)

Cell type	Markers
Hematopoietic Stem Cell (HSC)	CD34, CD45, CXCR4
Endothelial Progenitor Cells (EPC)	CD34, CD73, CD133, CXCR4, KDR, anti-M IgG
Very Small Embryonic Like Cells (VSEL)	CD34, CD133, CXCR4, SSEA4, anti-M IgG
Mesenchymal Stem Cells (MSC)	CD34, CD45, CD90, CD105, CD106, CD44

can cause undeveloped brain or different degree of microcephaly [20]. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue. However, we believe that our group has shown that HSC are playing significant role in AMI by trans-differentiating into myocardial cells, which suggests that HSCs can be at least multipotent [21]. Yet, this remains an area of great debate within the research community. There are still scientists who think that pluripotent adult stem cells do not exist in humans at all (as they do in plants) and that it is the reason that they cannot regenerate the whole organism, namely entire organ such as it is the case with amphibia. This controversy demonstrates the challenges of studying adult stem cells and suggests that additional research using adult stem cells is necessary to understand their full potential as future therapies [22].

However, given the fact that many researchers have got the data with adult stem cells, there are three solid candidates with distinct markers that we can consider pluripotent: HSC, MSC, and VSEL stem cells. They have different morphology and different markers presented on Table 5.2. Otherwise, each tissue has its own stem cell markers and they vary with propagation in culture. Additional research on regulation of chromatin factors and genome organization, crucial for stem cell maintenance, and management of pluripotency is needed to consolidate and interpret the data [22–25].

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