EMBRYONIC STEM CELL MARKERS: AN OVERVIEW

Abstract

Molecules that are particularly expressed in ES cells are called embryonic stem cell (ESC) markers. For ESC pluripotent maintenance and self-renewal processes to be characterised and clarified, as well as to facilitate clinical applications of ES cells, it is essential to understand how they function. A crucial therapeutic difficulty is separating ES cells from other cell types, particularly tumour cells that share similar markers. To address this, the most effective cell separation techniques currently available—marker-based flow cytometry (FCM) and magnetic cell sorting—are used to identify and extract ESCs. Here, we go over many molecular indicators of undifferentiated ESCs on the cell surface and in general. It also lists additional compounds, including as lectins and peptides, that bind to ESCs with different degrees of specificity and affinity. The review also looks at markers that overlap with tumour stem cells (TSCs), which raises questions regarding whether they should be used singly or in combination for cell isolation and identification. This chapter introduces ESC markers, including lectins and peptides, as well as cell surface and general molecular markers. The intricacy of marker selection is brought to light, especially in light of potential TSC overlap. By using this knowledge, researchers can improve cell separation procedures and advance the use of ES cells in clinical settings.

Keywords: Embryonic Stem Cells (ESCs); Stem Cell Markers; Pluripotency Maintenance; Cell Isolation Techniques; Tumor Stem Cells (TSCs).

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I. INTRODUCTION

Embryonic stem cells (ESCs) are a collection of pluripotent stem cells that are obtained from the inner cell mass of the blastocyst, which is an embryo in its early developmental stage [1]. These cells exhibit an extraordinary capacity to sustain both pluripotency and self-renewal, which is a crucial characteristic for their prospective clinical uses [1] **(Fig 1).**

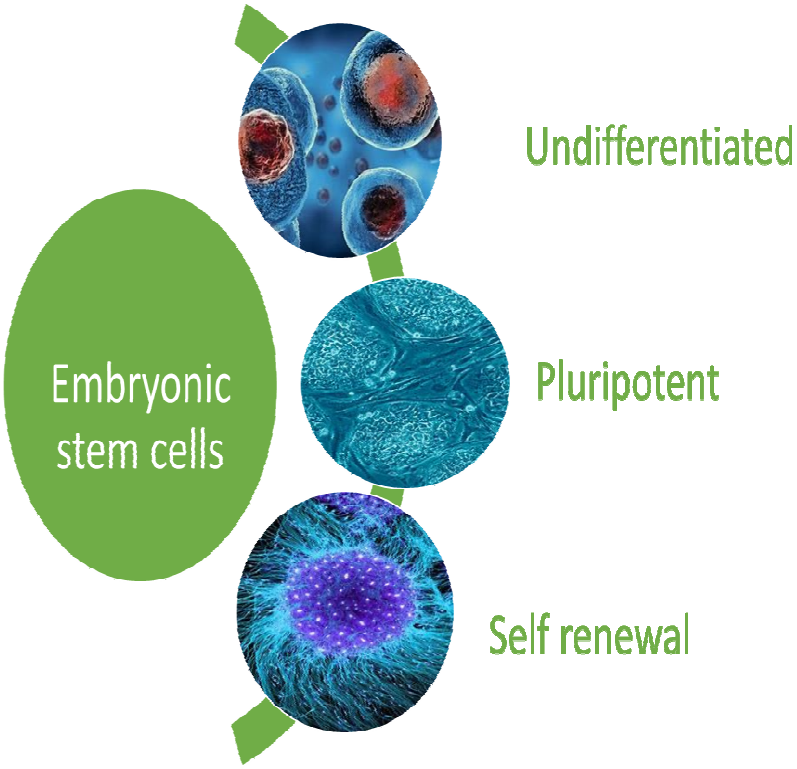

Figure 1: Depiction of the different properties of Embryonic stem cells A) Undifferentiated cells C) Pluripotency C) Self –renewal property.

Pluripotency is the inherent potential of embryonic stem cells (ESCs) to undergo differentiation into several cell lineages that exist within living animals, while simultaneously maintaining an undifferentiated state when cultivated in vitro. The aforementioned characteristic renders ESCs highly sought-after candidates for a range of therapeutic and regenerative medicine strategies [1].

Nevertheless, a significant obstacle in the therapeutic application of embryonic stem cells (ESCs) pertains to the accurate and consistent differentiation between ESCs and other cellular entities, including tumour cells, in order to mitigate possible hazards and complexities [1]. Therefore, it is crucial to comprehend the precise gene expression patterns and discern unique molecular markers linked to embryonic stem cells (ESCs). Markers have a vital role in facilitating the identification, separation, and subsequent examination of embryonic stem cells (ESCs).

Considerable advancements have been achieved by researchers in the identification of many cell surface markers and generic molecular markers that can function as indications of undifferentiated embryonic stem cells (ESCs), specifically within the human species [1]. Furthermore, the significant contributions of proteins engaged in many signalling pathways towards the determination of cellular destiny have been acknowledged. It is noteworthy that several lectins and peptide analogues have exhibited a distinct affinity for embryonic stem cells (ESCs), hence expanding the range of identifying methods available [1]. Nevertheless, it is crucial to recognise the presence of a hindrance that occurs as a result of the convergence of embryonic stem cell (ESC) markers with those present in tumour stem cells. Therefore, it is imperative to take caution when employing these markers for the purpose of identifying and isolating embryonic stem cells [1].

Moreover, the understanding of the processes that regulate the pluripotency of human embryonic stem cells (hESCs) has become a prominent and formidable task in recent times. Recent findings have revealed that human and mouse embryonic stem cells (ESCs), despite originating from similar embryonic sources, demonstrate variations in their regulation mechanisms [2]. Hence, it is imperative to further explore the understanding of these molecular markers, as it holds significant value in not only optimising the application of embryonic stem cells (ESCs), but also in unravelling the complex mechanisms governing their ability to differentiate into many cell types and sustain their capacity for self-renewal [1]. In order to make significant progress in the fields of regenerative medicine and developmental biology, it is crucial to possess a thorough comprehension of embryonic stem cell (ESC) markers and their function in the maintenance of pluripotency and self-renewal. Smith et al. (2020) did a study wherein they identified a novel surface marker, SALL4, as a dependable sign of undifferentiated human embryonic stem cells (hESCs) [3]. The utilisation of this particular marker, in combination with well-established markers such as OCT4 and NANOG, has proven to be effective in precisely identifying and isolating human embryonic stem cells (hESCs). This approach successfully addresses the issue of overlapping tumour stem cell markers [3].

Furthermore, numerous research have been conducted to examine the importance of distinct signalling pathways in the regulation of pluripotency and self-renewal in embryonic stem cells (ESCs). The Wnt signalling pathway has been recognised as a significant factor in regulating the destiny of embryonic stem cells (ESCs) [4]. Upon initiation, this signalling pathway initiates a cascade of intracellular processes that ultimately result in the preservation of embryonic stem cells' undifferentiated phenotype. In contrast, the suppression of the Wnt pathway facilitates the differentiation of embryonic stem cells, underscoring its importance in regulating the determination of cell destiny [4]. The Hedgehog (Hh) pathway is another significant signalling system that has been involved in the maintenance of pluripotency in embryonic stem cells (ESCs). The study conducted by Li et al. (2019) demonstrated that the Hedgehog (Hh) pathway has a crucial function in preserving the pluripotency of embryonic stem cells (ESCs) via the modulation of GLI transcription factors [5]. The aforementioned discoveries offer significant contributions to our understanding of the complex signalling networks that govern the differentiation of embryonic stem cells (ESCs), thereby opening up possibilities for future therapeutic interventions. In conjunction with surface markers and signalling pathways, alternative molecules have demonstrated potential in their capacity to function as indicators of undifferentiated embryonic stem cells (ESCs). For example, it has been shown that short non-coding RNAs, specifically microRNAs (miRNAs), have a substantial role in influencing the pluripotency and differentiation of embryonic stem cells

(ESCs) [6]. The regulatory mechanism of gene expression is facilitated by tiny RNA molecules that selectively bind to messenger RNAs (mRNAs), thereby exerting an influence on crucial physiological activities. Certain microRNAs (miRNAs), including miR-145, have been recognised as inhibitors of embryonic stem cell (ESC) pluripotency, facilitating their specialisation into distinct cell lineages [6]. On the other hand, the functions of miR-302 and miR-367 have been acknowledged in the preservation of embryonic stem cell (ESC) pluripotency and the suppression of differentiation [6]. The aforementioned findings underscore the complex regulatory network that governs the behaviour of embryonic stem cells (ESCs) and offer vital insights into their potential therapeutic uses.

Although these developments provide a substantial contribution to our comprehension of embryonic stem cell (ESC) markers and their regulatory processes, there are still hurdles in distinguishing the disparities between human and mouse ESCs. Research has demonstrated discernible patterns of gene expression and epigenetic changes in both human and mouse embryonic stem cells (ESCs), underscoring the necessity for additional inquiry [2]. An example may be found in a study conducted by Chen et al. (2018), which illustrated disparities in the regulation of X-chromosome inactivation between human and mouse embryonic stem cells (ESCs). This process is of utmost importance as it plays a vital role in preserving pluripotency [7]. The existence of such inequalities underscores the importance of taking into account species-specific variations when extrapolating findings from mouse embryonic stem cell (ESC) studies to human ESCs.

Embryonic stem cells (ESCs) hold significant potential for a range of therapeutic and regenerative medicine strategies owing to their unique characteristics of pluripotency and capacity for self-renewal. The successful clinical application of undifferentiated embryonic stem cells (ESCs) relies heavily on the identification and characterization of unique molecular markers associated with these cells. Considerable progress has been achieved by researchers in the identification of cell surface indicators, generic molecular markers, and signalling pathways that exert an influence on embryonic stem cell (ESC) pluripotency and self-renewal. However, it is important to use caution in order to differentiate between markers of embryonic stem cells (ESCs) and those of tumour stem cells. Moreover, the comprehension of disparities between human and mouse embryonic stem cells (ESCs) poses a considerable obstacle, hence requiring additional exploration of regulatory mechanisms specific to each species.

Future research endeavours should prioritise the expansion of our comprehension regarding embryonic stem cell (ESC) markers and their respective roles. This will aid in the advancement of safer and more efficient therapeutic applications. Through the utilisation of embryonic stem cells (ESCs) and the elucidation of the underlying processes that govern their ability to differentiate into various cell types and sustain their own population, we have the opportunity to facilitate significant progress in the fields of regenerative medicine and developmental biology. Consequently, this development presents the potential to revolutionise the field of contemporary medicine and enhance the well-being of numerous persons.

II. CELL SURFACE MARKERS

Cell surface proteins play a crucial role in recognizing and differentiating cell types due to their selective binding with signal molecules. These specialized membrane proteins

can act as markers, aiding in the identification of specific cell types. However, it is important to note that certain membrane markers overlapping with tumor cell types pose challenges in distinguishing embryonic stem cells (ESCs) without compromising the integrity of the cell membrane [1] **(Fig 2).**

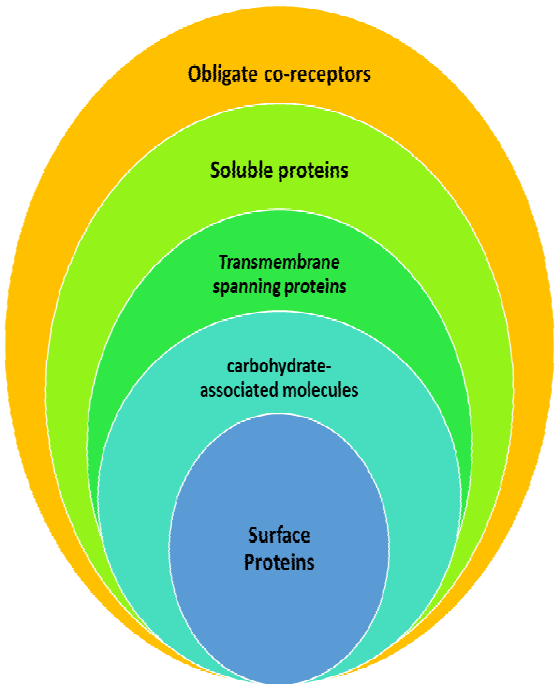

Figure 2: Depicting the nature of different embryonic stem cell markers .Some markers are surface proteins ,Some are carbohydrate based molecules ,while others are transmembrane based proteins , Soluble type of proteins and others are obligate co-receptors.

1. **Stage Specific Embryonic Antigens (SSEA):** SSEA indicators, which are identified by three monoclonal antibodies that target distinct carbohydrate epitopes linked to lacto- and globo-series glycolipids, specifically SSEA-1, SSEA-3, and SSEA-4, have crucial functions in regulating cellular contacts on the surface during developmental processes [3]. The SSEA-1 antigen, also known as CD15/Lewis x, is prominently present on the outer membrane of pre-implantation stage murine embryos, as well as in germ cells and teratocarcinoma stem cells in both mice and humans. Nevertheless, it is not present in human embryonic stem cells (hESCs) and human embryonic cancer cells, as indicated by previous research [4]. In addition to its presence during embryonic development, the expression of SSEA-1 has been detected in multiple adult tissues, including the oviduct epithelium, endometrium, epididymis, as well as particular parts of the brain and kidney tubules [5]. It is noteworthy that the expression of SSEA-1 exhibits an upward trend during the process of differentiation in human cells, but it demonstrates a downward trend during differentiation in mouse cells.

   In contrast, SSEA-3 and SSEA-4 are produced during the process of oogenesis and are present in the cell membranes of oocytes, zygotes, and early-stage embryos undergoing cleavage [6]. Both SSEA-3 and SSEA-4 markers are observed to be present in undifferentiated monkey embryonic stem cells (ESCs). The presence of SSEA-4 is not observed in mouse embryonic stem cells (ESCs), but it becomes evident after the process

of differentiation. This is particularly notable in human embryonic germ (EG) cells, human teratocarcinoma stem cells, and ESCs [7]. The significance of these SSEA markers as effective tools for identifying and characterising particular cell populations during embryonic and tissue development is underscored by their presence and absence patterns across different stages of development and in various cell types.

2. **Cluster of Differentiation (CD) Antigens:** CD antigens are a collection of surface proteins that fall into diverse classes, including integrins, adhesion molecules, glycoproteins, and receptors. These proteins play a crucial role in recognising and characterising distinct cell types [8]. Numerous CD antigens have been linked to both mouse and human embryonic stem cells (ESCs). Pluripotent human embryonic stem cells (ESCs) exhibit the expression of CD9, CD24, CD133, CD90, and CD117, with CD133 additionally serving as a marker for hematopoietic stem cells [9-11].

Integrins, which belong to the subclass of CD antigens, are cell surface receptors that exist as α/β heterodimers and play a crucial role in facilitating cell adhesion to adjacent tissues [12]. The aforementioned receptors are vital in several cellular processes such as cell adhesion, signalling, migration, growth, and survival [12]. Integrins cooperate with several proteins, including cadherins, immunoglobulin superfamily cell adhesion molecules, selectins, and syndecans, to promote cellular connections and communication both between cells and between cells and the extracellular matrix [13]. Integrins, which are capable of binding to several constituents of the extracellular matrix (ECM) such as fibronectin, vitronectin, collagen, and laminin, facilitate bidirectional signalling known as outside-in and inside-out signalling. The process of outside-in signalling involves the transmission of information from the extracellular matrix (ECM) to the cell. On the other hand, inside-out signalling refers to the activation of additional integrins, which enables the cell to respond quickly and adaptively to alterations in its surrounding cellular environment [14].

The integrin family is comprised of many α and β subunits that interact to create a wide range of integrin types, each exhibiting unique patterns of distribution within tissues and overlapping specificities for ligands [16]. The maintenance of stemness in undifferentiated mouse embryonic stem cells (ESCs) has been shown to be influenced by several integrins, namely α5β1, αvβ5, α6β1, and α9β1 [17]. Integrin α6 (CD49f/CD29), a protein weighing 120 kilodaltons and consisting of two splice variants (α6A and α6B), serves as a receptor for laminins and facilitates cellular adhesion processes on the basal membrane [18]. The involvement of integrin α6 (CD49f/CD29) has been shown to be crucial in the homing of hematopoietic stem and progenitor cells to the bone marrow [19]. Similarly, in human prostate cancer cells, integrin α6 has been found to be significant in influencing cell behaviour [20].

The significance of these integrins in embryonic stem cells (ESCs) is underscored by their presence and functional activities, which contribute to the establishment and preservation of ESC niches [15]. Gaining a comprehensive comprehension of the complexities inherent in integrin-mediated signalling pathways and their intricate interactions with the extracellular matrix (ECM) and other molecules present on the cell surface would significantly contribute to the advancement of our current understanding of stem cell behaviour. Moreover, such understanding will yield useful insights that may be used to the fields of regenerative medicine and tissue engineering, facilitating the

development of innovative therapeutic approaches. Integrins have the potential to function as viable targets for the regulation of embryonic stem cell (ESC) behaviour, differentiation, and destiny determination. This might potentially lead to the development of therapeutic interventions and improvements in the field of regenerative medicine.

3. **TRA-1-60 and TRA-1-81:** The antigens TRA-1-60 and TRA-1-81 are found on the cell surfaces of human embryonal carcinoma (EC) cells and human pluripotent stem cells. These antigens are useful indicators for the identification and isolation of embryonic stem cells (ESCs) [21]. Furthermore, it should be noted that these antigens are also seen to be present in teratocarcinoma and embryonic germ cells, as indicated by previous research [22]. The TRA-1-60 antibody is capable of identifying a specific epitope of a proteoglycan that is susceptible to neuraminidase. On the other hand, the TRA-1-81 antibody is able to attach to a different epitope of the same molecule, which is not affected by neuraminidase. It has been proposed that this particular epitope may be a variation of the protein podocalyxin [23]. Nevertheless, it is imperative to acknowledge that the presence of TRA-1-60 can also be identified in the serum of individuals afflicted with germ cell tumours. This presents a significant obstacle in utilising TRA-1-60 as an exclusive indicator for embryonic stem cells [24].

4. **Frizzled (Fzd):** Fzd, belonging to the G-protein-coupled receptor (GPCR) superfamily characterised by seven transmembrane-spanning domains, serves as a pivotal mediator in the transmission of Wnt signals [25]. The N-terminal section of the protein is characterised by its significant size and extracellular location. Within this region, there is a domain known as the cysteine-rich domain (CRD), which plays a crucial role in enabling the binding of the protein to Wnt proteins [26]. The transmission of Wnt signals occurs via the Fzd receptor family. Upon binding of Wnt proteins, Fzd receptors form a complex with co-receptors LRP5 or LRP6. This complex activates the canonical Wnt/β-catenin pathway by blocking the phosphorylation of β-catenin by GSK3-β. Furthermore, it should be noted that specific Wnt proteins have the ability to initiate the Fzd/Ca2+ and Fzd/PCP (planar cell polarity) pathways, hence enhancing the range of signal transduction processes [27].

   The PDZ domain of Dvl proteins, a crucial downstream signalling component, interacts with the intracellular C-terminus of Fzd, thereby establishing a connection between Fzd and many intracellular signalling pathways [27]. The Fzd subfamily in mammals consists of ten members, namely Fzd1 to Fzd10. The expression of these members in both mouse and human embryonic stem cells (ESCs) underscores their importance in facilitating several signalling pathways [28].

   In addition, several Fzd receptors have the ability to engage in interactions with other secreted proteins, including Norrin and R-Spondin, thereby introducing intricacy to their physiological activities [27]. These interactions have a role in the refinement of Wnt signalling, therefore impacting the determination of cell destiny and the progression of developmental processes in embryogenesis. The wide range of Fzd receptors and their participation in several pathways highlights their crucial role in coordinating cellular reactions to Wnt signalling stimuli. A thorough comprehension of the complexities associated with Fzd signalling in embryonic stem cells (ESCs) could potentially serve as a critical factor in harnessing their whole capabilities for the advancement of regenerative medicine and tissue engineering endeavours. Additional investigation of the distinct

functions and governing mechanisms of unique Frizzled (Fzd) receptors will enhance our comprehension of Wnt signalling in embryonic stem cells (ESCs) and facilitate the development of novel treatment approaches.

5. **Stem Cell Factor (SCF or c-Kit Ligand):** Stem Cell Factor (SCF), alternatively referred to as kit-ligand, KL, or steel factor, is a cytokine that engages in interactions with the c-Kit receptor (CD117) [29]. The SCF protein can be found in two distinct states: as a transmembrane protein and as a soluble protein. The soluble stem cell factor (SCF) is present in the form of a homodimer that is related through non-covalent interactions. This dimer is characterised by glycosylation and exhibits notable secondary structural elements, such as alpha helices and beta sheets. In each monomer of the stem cell factor (SCF), there are two disulfide bridges that exist throughout the chain. Additionally, the functional core of SCF, namely the N-terminal 141 residues, has been identified as significant. This core is denoted as SCF1-141. The region under consideration encompasses both the dimer interface and the segments responsible for binding and activating the receptor Kit [29].

   The transmission of signals by SCF occurs through the process of ligand-mediated dimerization of its receptor, Kit. Kit is classified as a type III receptor protein-tyrosine kinase and is closely associated with other receptors, including those for platelet-derived growth factor (PDGF), macrophage colony-stimulating factor, Flt-3 ligand, and vascular endothelial growth factor (VEGF). The binding of SCF to Kit results in the initiation of receptor dimerization and the activation of protein kinase activity [29]. Stem cell factor (SCF) is observed to be present in diverse fibroblast-like cell populations and locations associated with hematopoiesis, including the foetal liver and bone marrow. The cytokine is of significant importance in the processes of hematopoiesis, spermatogenesis, and melanogenesis. The survival of differentiating embryonic stem cells is reliant on the SCF-KIT pathway, which underscores its importance in governing the fate of stem cells [30]. Considering the crucial functions of SCF in hematopoiesis and the maintenance of stem cells, comprehending its regulatory mechanisms and interactions with the c-Kit receptor presents significant potential for the advancement of regenerative medicine and therapeutic interventions. Additional investigation into the SCF-KIT pathway and its influence on the behaviour of stem cells will enhance the advancement of innovative approaches aimed at harnessing the inherent capabilities of stem cells for the purpose of tissue repair and regeneration. Furthermore, the comprehensive investigation of the complex network of signalling pathways associated with the SCF receptor holds the potential to enhance our comprehension of cellular fate determination and differentiation mechanisms. This, in turn, may pave the way for novel therapeutic strategies in diverse medical domains.

6. **Cripto (TDGF-1):** The Cripto gene, alternatively referred to as teratocarcinoma-derived growth factor-1 (TDGF-1), is responsible for encoding a newly discovered human growth factor that exhibits structural similarities to epidermal growth factor. During the process of embryonic development, Cripto plays a crucial role as a necessary co-receptor for various transforming growth factor β (TGF-β) ligands. These ligands include nodals, growth and differentiation factor 1 (GDF1), and GDF3. In addition to its pivotal role in embryogenesis, Cripto serves as an oncogene, displaying elevated expression levels in tumours and facilitating carcinogenesis via various mechanisms, including the stimulation of mitogenic signalling pathways and the inhibition of activin signalling [31].

III. TRANSCRIPTION FACTORS

Nuclear genes are of utmost importance in essential biological processes, wherein transcription factors serve as crucial modulators of gene expression. In typical circumstances, certain transcription factors remain in a dormant state until specific signal transduction events occur, prompting their interaction with corresponding recognition sequences. The presence and role of distinct genes within the nucleus are indicative of the cellular response to particular circumstances. Therefore, the monitoring of gene expression can be utilised as helpful indicators for distinct biological conditions. The transcription factors expressed in embryonic stem cells (ESCs) are presented in **Table 1.**

Table 1: Nuclear Transcription Factors and Their Characteristics. Embryonal Carcinoma (EC) Cells, Neural Stem (NS) Cells

Nuclear transcription factors	**Characteristics**
Oct-3/4	Mouse & human ES cells, EC cells
Sox2	Mouse & human ES cells, EC cells, NS cells
KLF4	Mouse & human ES cells, EC cells
Nanog	Mouse & human ES cells, EC cells
Markers	
Rex1 (Zfp42)	Mouse & human ES cells, EC cells
UTF1	Mouse & human ES cells, EC cells
ZFX	Murine ES cells, human ES cells, hematopoietic stem cells, EC cells
TBN	Mouse, human inner cell mass
FoxD3	Murine ES cells, human ES cells, EC cells
HMGA2	Mouse & human ES cells
NAC1	Mouse & human ES cells
GCNF (NR6A1)	Mouse & human ES cells, EC cells
Stat3	Murine ES cells, Human ES & EC cells
LEF1, TCF3	Mouse & human ES cells, EC cells
Sall4	Mouse & human ES cells, EC cells
Fbxo15	Mouse ES cells, early embryos, and testis tissue, EC cells
ECAT genes	
ECAT11 (FLJ10884/ L1TD1)	Human & EC cells
Ecat1	Mouse oocytes, EC cells
ECAT9 (Gdf3)	Human & EC cells
Dppa genes	
Dppa5 (ESG1)	Mouse & human ES cells, EC cells
Dppa4	Mouse & human ES cells, EC cells
Dppa2 (ECSA)	Mouse & human ES cells, EC cells
Dppa3 (Stella)	Mouse & human ES cells, EC cells, primordial germ cells, oocytes, preimplantation embryos

1. **CORE Nuclear Transcription Factors:** In the year 2006, Yamanaka et al. conducted an experiment wherein they successfully produced pluripotent stem cells from mouse embryonic fibroblasts. This was achieved by introducing four specific factors, namely Oct4, c-Myc, Sox2, and Klf4 [32]. After this significant advancement, induced pluripotent stem (iPS) cells have been effectively generated from different somatic cells through the overexpression of a specific group of genes. Nevertheless, there were apprehensions over the potential tumorigenicity linked to the reactivation of c-Myc, as around 20% of the children of induced pluripotent stem cells (iPS cells) developed tumours [33]. As a response, scholars have devised an altered procedure for the development of induced pluripotent stem (iPS) cells, which obviates the requirement of the Myc retrovirus. This modification has led to a notable reduction in the presence of non-iPS background cells and the consistent production of iPS cells of superior quality [34].

   Subsequent inquiries have indicated that the quantity of reprogramming factors can be diminished in instances where somatic cells exhibit adequate levels of endogenous complementing factors. In this particular case, it was shown that adult mouse neural stem cells (NSCs) exhibited elevated amounts of endogenous Sox2 and c-Myc in comparison to embryonic stem cells (ESCs). As a result, the inclusion of Oct4, in combination with either Klf4 or c-Myc, proved to be enough in inducing the formation of induced pluripotent stem (iPS) cells from neural stem cells (NSCs) [35].

   In recent scientific developments, researchers have made a significant discovery indicating that the only expression of the transcription factor Oct4 is sufficient to directly reprogram adult mouse neural stem cells (NSCs) into a pluripotent state. This discovery emphasises the essential role of Oct4 in the direct conversion of neural stem cells into pluripotent stem cells, as it is both necessary and capable of inducing this reprogramming process [36]. Comparable findings were achieved through the utilisation of human neural stem cells (NSCs) [37].

   The advancements in induced pluripotent stem cell (iPS cell) synthesis carry significant ramifications for the fields of regenerative medicine and disease modelling. Through a comprehensive comprehension of the fundamental elements implicated in the process of converting somatic cells into pluripotent stem cells, scholars are able to investigate novel paths for prospective therapeutic applications and acquire significant knowledge regarding the field of developmental biology and the aetiology of diseases. In addition, the ongoing improvement of reprogramming techniques guarantees a heightened level of safety and dependability in the production of induced pluripotent stem (iPS) cells, thereby enhancing their suitability for prospective therapeutic uses.

   - **Octamer-binding Protein 4 (Oct4):** Oct4, alternatively referred to as Oct3/4 or POU5F1, is a member of the POU family of transcription factors. It holds significant importance in the regulation of stem cell pluripotency and differentiation [38]. The functionality of these transcription factors relies on the POU domain, whereas areas outside the POU domain show minimal sequence conservation and do not play a crucial role in DNA binding [39]. The orthologous genes of Oct4 exhibit a high degree of structural organisation and conservation throughout several mammalian species, including humans, bovines, mice, and rats [40].

Oct4 expression during early embryonic development is predominantly limited to pluripotent and germ line cells, and it is sustained within the inner cell mass (ICM) of blastocysts [41]. The differential expression of Oct4 is detected during the differentiation of the inner cell mass (ICM) into epiblasts (primitive ectoderm and embryonic ectoderm) and hypoblasts (primitive endoderm and embryonic endoderm) around 4.5 days post-coitum (dpc). The expression of Oct4 remains present in the epiblast, but as hypoblast cells undergo differentiation into visceral and parietal endoderms, there is a temporary increase in Oct4 protein levels followed by a subsequent decrease to levels that cannot be detected. At 7.5 days post coitum (dpc), Oct4 expression undergoes gradual repression in the epiblast during the process of gastrulation [41].

The expression of Oct4 is also observed in pluripotent cell lines that originate from the inner cell mass (ICM), epiblasts, and primordial germ cells (PGCs), including embryonic stem cells (ESCs), embryonic carcinoma (EC) cells, and embryonic germ (EG) cells, as long as these cells maintain their undifferentiated state [41].

Moreover, Oct4 assumes a crucial function in the regulation of gene expression in the initial stages of development, encompassing Sox2, Fgf4, Rex1, hCG, and Utf1. This involvement contributes to the preservation of pluripotency and the appropriate cellular differentiation [38].

A comprehensive comprehension of the regulatory roles played by Oct4 in the realm of stem cell biology and the initial stages of embryonic development is of utmost importance in order to make significant progress in the fields of regenerative medicine, disease modelling, and reproductive technology. A comprehensive understanding of the mechanisms underlying Oct4-mediated gene regulation and its intricate connections with other pivotal transcription factors would greatly facilitate the manipulation of stem cell destiny and the advancement of innovative treatment approaches for diverse disease contexts. Ongoing investigation into the intricate biochemical networks associated with Oct4 holds the potential to unveil novel findings that carry significant significance for both fundamental scientific knowledge and practical medical applications.

- **Sry-related High-mobility Group (HMG) Box-containing (Sox) Family:** Sox2, which is a constituent of the Sox gene family, is classified as one of the HMG box transcription factors that engage in functional interactions with POU domain proteins [42]. Just like Oct3/4, the Sox gene family plays a role in the preservation of pluripotency. However, Sox2 is uniquely linked to multipotent and unipotent stem cells, whereas Oct3/4 is solely expressed in pluripotent stem cells. It is worth mentioning that Sox2 was among the initial genes employed by multiple research groups to induce induced pluripotent stem (iPS) cells [43].

The Sox2 gene harbours a minimum of two distinct regulatory domains that exhibit unique activity within pluripotent embryonic cells. The reported expression pattern of this gene closely resembles that of Oct4, which has been documented in pre-implantation embryos of both humans and mice, as well as in several cell lines

such as mES, hES, mEC, and hEC. In subsequent stages of development, Sox2 and Oct4 are concurrently expressed in post-migratory primordial germ cells [44]. In addition, it has been observed that in the initial stages of mouse embryo development, there is a simultaneous expression of Oct4, Sox2, and osteopontin within the identical cellular population. While Sox1 exhibits comparable efficacy to Sox2 in the generation of induced pluripotent stem (iPS) cells, it is worth noting that other members of the Sox gene family, namely Sox3, Sox15, and Sox18, also contribute to the production of iPS cells, albeit with diminished efficiency [42].

Gaining a comprehensive understanding of the unique roles played by Sox2, as opposed to Oct4 and other Sox genes, is crucial for knowing the complexities involved in the control of pluripotency and the differentiation of stem cells. The elucidation of crucial regulatory elements and their interactions with other transcription factors will yield significant knowledge regarding the molecular mechanisms that govern cell fate determination. Moreover, this understanding will present new prospects for utilising stem cells in regenerative medicine and therapeutic interventions. The continuous investigation in this particular domain exhibits significant potential in enhancing our comprehension of stem cell biology and transforming medical therapies for diverse diseases and disorders.

- **Krupple-like Factor (Klf) Family:** The family of transcription factors known as Krüppel-like factors (Klf) is of significant importance in the regulation of diverse biological processes, encompassing cell proliferation, differentiation, development, and apoptosis. The Klf family is distinguished by the presence of three Cys2 His2 zinc fingers positioned at the C-terminus, which are separated by a highly conserved H/C link. These factors exhibit comparable preferences for various DNA binding sites that are high in GC content, and they can engage in competition with one another to occupy these sites. In addition, it is worth noting that Klf proteins exhibit a considerable level of homology with the specificity protein (Sp) family of zinc-finger transcription factors, resulting in their comparable binding patterns across many genes.

  Klf5, alternatively referred to as intestine-enriched Krüppel-like factor or Bteb2, is a pioneering member of this gene family that plays a crucial role in developmental processes. According to a report, it has been observed that Klf5 plays a direct role in regulating the transcription of Oct3/4 and Nanog, two crucial factors responsible for the renewal of embryonic stem cells (ESCs) and the preservation of their pluripotency [45]. In contrast, it has been observed that Klf4 and Klf2 exhibit functional redundancy in their regulation of the self-renewal and pluripotency of embryonic stem cells (ESCs). In addition, they exert regulatory control over the transcriptional activity of many pluripotency-associated factors in embryonic stem cells (ESCs), such as Nanog, Tcl1, Esrrb, Sall4, Tcf3, Mycn, and Fbxo15. Nevertheless, it has been observed that individual Klfs are not essential for the self-renewal of embryonic stem cells (ESCs), indicating that they may collectively collaborate to regulate shared targets [46]. Within embryonic stem cells (ESCs), it has been observed that these Krüppel-like factors (Klfs) exhibit a high degree of colocalization within distinct genomic regions. This finding provides additional

evidence to support the notion that these Klfs work together in a cooperative manner to regulate genes associated with pluripotency [47].

The complex interaction among members of the Klf family in the preservation of pluripotency and the control of crucial transcription factors underscores their importance in the field of stem cell biology. The comprehension of the intricate connections and regulatory mechanisms of Klfs aids in the elucidation of the underlying processes involved in the self-renewal and differentiation of embryonic stem cells (ESCs). The aforementioned observations possess significant potential in the advancement of regenerative medicine and therapeutic approaches through the manipulation of pluripotency and stem cell destiny for diverse clinical applications. Further exploration of the Klf family and its interconnectedness with other regulatory variables will augment our understanding of stem cell biology and propel advancements in medical research and therapeutic interventions.

- **Nanog:** The transcription factor Nanog is of utmost importance in the preservation of pluripotency and self-renewal in embryonic stem cells (ESCs) of both mice and humans [48]. The study conducted by Chambers et al. (49) provided evidence supporting the notion that Nanog plays a pivotal role in the hierarchical organisation of transcription factors, which ultimately determines the identity of embryonic stem cells (ESCs). The presence of Nanog mRNA has been seen in pluripotent embryonic stem (ES) cells and embryonic germ (EG) cells, as well as in both mouse and human embryonic carcinoma (EC) cells. However, the expression of this gene is suppressed at an early stage of embryonic stem cell (ESC) differentiation, which aligns with its close connection to the identity of pluripotent stem cells. The limited expression of Nanog corresponds to the temporary capacity for embryonic stem cell (ESC) formation that is detected in the inner cell mass (ICM) during the initial stages of embryonic development, but is no longer present after implantation [49]. Mitsui (50) provided more evidence to support the importance of Nanog in the preservation of pluripotency in mouse epiblasts and embryonic stem cells (ESCs). The role of Nanog in facilitating embryonic stem cell (ESC) self-renewal is not influenced by the LIF/Stat3 pathway. ICMs lacking Nanog were unable to create epiblasts and instead only produced cells resembling parietal endoderm. The loss of Nanog resulted in a loss of pluripotency in embryonic stem cells (ESCs), leading to their differentiation into the extraembryonic endoderm lineage. This highlights the critical role of Nanog in determining the identity of ESCs [51].

  It is worth noting that Nanog mRNA is observed in pluripotent mouse and human cell lines, but its presence is missing in differentiated cells. Nanog expression is confined to founder cells during the process of preimplantation embryo development, from which embryonic stem cells (ESCs) can be produced. The induction of embryonic stem cell self-renewal is facilitated by the simultaneous action of endogenous Nanog and cytokine activation of Stat3. The clonal proliferation of embryonic stem cells (ESCs) can be achieved solely through the overexpression of Nanog from transgenic constructs, without the requirement of Stat3 activation, while still maintaining Oct4 levels. The restoration of cytokine dependency, multilineage differentiation potential, and embryo colonisation capacity occurs when the transgene

is excised, highlighting the significant role of Nanog in regulating pluripotency and determining the destiny of embryonic stem cells [51].

The crucial role of Nanog in the preservation of embryonic stem cell (ESC) identity and the ability to self-renew is a subject of significant study within the fields of stem cell biology and regenerative medicine. The comprehension of the molecular mechanisms and regulatory networks associated with Nanog presents potential opportunities for the manipulation of pluripotency and the improvement of somatic cell reprogramming into induced pluripotent stem cells (iPSCs). Understanding the mechanisms of Nanog-mediated signalling pathways and its interaction with other essential transcription factors has significant importance in leveraging the capabilities of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) for diverse clinical purposes such as tissue engineering, disease modelling, and personalised therapeutic interventions. Further investigation into Nanog and its regulatory mechanisms is expected to significantly enhance our understanding of stem cell biology and facilitate the translation of this information into groundbreaking medical therapies.

2. **Reduced Expression 1 (Rex1 or Zfp-42):** The gene Rex1 (Zfp42) is responsible for encoding a transcription factor belonging to the zinc finger family. This transcription factor is known to be produced at high levels in both mouse and human embryonic stem cells (ESCs). The Rex1 protein is composed of four zinc finger motifs and an acidic domain, as stated in reference 52. Rex1 was initially discovered in F9 embryonal carcinoma (EC) cells, and its expression is reduced upon exposure to retinoic acid (RA) in order to promote cellular differentiation. It exhibits resemblance to Yy1, a conserved evolutionary constituent of the polycomb-related complex [53]. Although the precise mechanism of action of Rex1 remains elusive, it is well recognised as a pluripotency marker in several types of stem cells, such as multipotent adult progenitor cells and amniotic fluid cells [54]. Previous research utilising traditional gene targeting techniques has shown that Rex1 is not necessary for the maintenance of embryonic stem cell pluripotency or embryonic development [55]. Therefore, Rex1 is predominantly recognised as a pluripotency marker lacking functional importance, similar to alkaline phosphatase activity. The investigation of Rex1's involvement in ESC differentiation was conducted by generating Rex1 double knockout ESC lines. These lines exhibited heightened expression of ectoderm, mesoderm, and endoderm markers in comparison to wild-type cells. This finding suggests that Rex1 plays a restrictive role in retinoic acid-induced differentiation in ESCs [56].

3. **Undifferentiated Embryonic Cell Transcription Factor (UTF1):** The transcriptional co-activator UTF1 is known to engage in an interaction with the metal-binding motif of activation transcription factor-2 (ATF-2), hence exerting a crucial influence on the initiation of embryonic stem cell (ESC) differentiation. The inhibition of UTF1 in embryonic stem (ES) and cancer cells leads to a significant impediment or complete obstruction of the differentiation process [57]. The expression of this gene is predominantly observed in pluripotent embryonic stem cells (ESCs), where it exhibits a strong association with chromatin in both mouse and human ESCs. This association potentially plays a role in preserving the essential epigenetic conditions required for maintaining pluripotency [58]. The gene UTF1 harbours a regulatory element that exhibits preferential interaction with a complex consisting of Oct3/4 and Sox-2. Previous

studies have demonstrated that Oct4 and Sox2 play a role in regulating the expression of UTF1 [60]. The efficacy of induced pluripotent stem cell (iPSC) formation is greatly enhanced by the co-expression of UTF1 with reprogramming factors c-Myc, Oct4, Sox2, and KLF4, in addition to the siRNA knockdown of p53 [61]. The presence of UTF1 mRNA has been observed in various regions of mouse embryos, including the inner cell mass, primitive ectoderm, and extra-embryonic tissues [62]. The expression of this gene is predominantly limited to pluripotent cells, specifically the inner cell mass (ICM) cells, within mouse blastocysts. Furthermore, its levels exhibit a rapid decline when the cells undergo differentiation [63].

4. **X-linked Zinc Finger Protein (ZFX):** The ZFX gene is transcribed from the silenced X chromosome and shares a similar structure with its counterpart on the Y chromosome, known as ZFY [64]. The transcripts of ZFX and ZFY genes are responsible for encoding proteins that possess amino-terminal domains characterised by high acidity, as well as carboxy-terminal zinc-finger motifs that are related with the binding of nucleic acids. Both ZFY and ZFX have the potential to act as transcriptional activators that play a role in the process of sex determination. Several alternatively spliced transcript variants of ZFX, which encode various isoforms, have been discovered and are potentially associated with unique functionalities [65]. Previous research conducted on mice using conditional gene targeting techniques has provided evidence indicating that the presence of ZFX is necessary for the process of self-renewal in both embryonic and hematopoietic stem cells [66]. Furthermore, it has been suggested that ZFX may play a role in the proliferation and expansion of B-cells, hence contributing to the maintenance of lymphocyte homeostasis [67].

5. **Taube Nuss (Tbn):** The Tbn protein, which exhibits a high degree of conservation across humans and mice, serves as the prototype for a distinct group of proteins that play critical roles in several developmental processes. The restriction of this phenomenon is limited to cells within the inner cell mass (ICM) and is crucial for the viability of those ICM cells [68]. The presence of Tbn expression has also been seen in human embryonic stem cells [69]. When Tbn is not present, the inner cell mass (ICM) undergoes programmed cell death, known as apoptosis. This disrupts the equilibrium between cell death and cell survival in the early stage of embryos, ultimately leading to the death of pluripotent ICM cells. However, the trophectoderm cells manage to survive this process.

6. **Forkhead Box D3 (FoxD3):** FoxD3, belonging to the Forkhead box family, exhibits a winged-helix DNA-binding conformation and assumes a pivotal function in the process of embryogenesis [70]. The transcriptional regulator in question plays a crucial role in maintaining pluripotency during the pre-implantation and peri-implantation stages of embryonic development in mice [71]. Additionally, it is implicated in the process of trophoblast production [72]. FoxD3 is an essential factor for the preservation of the mammalian neural crest. Mouse embryos lacking FoxD3 (FoxD3 (-/-)) exhibit failure during the implantation stage, resulting in the absence of structures derived from the neural crest [73]. The study conducted by FoxD3 et al. (74) demonstrates the collaborative role of Oct4 and Nanog in maintaining pluripotency in embryonic stem cells (ESCs).

7. **HMGA2:** The architectural transcription factor, HMGA2, does not possess inherent capability for direct transcriptional activation. In contrast, it exerts control over gene expression via modifying the structure of DNA through its interaction with AT-rich areas and direct engagement with other transcription factors. The HMGA2 gene has high levels of expression and widespread distribution, and it serves as a vital component in embryonic development [75]. The promotion of self-renewal in stem cells is facilitated by it, and a decrease in its expression has been associated with the ageing of stem cells [76]. The expression of HMGA2 is often observed to be modest in normal adult tissues; nevertheless, its overexpression or rearrangement has been found to be related with the development of several malignancies [77].

8. **Nucleus Accumbens-1 (NAC1):** NAC1, a nuclear factor classified within the Pox virus and zinc finger/bric-a-brac tramtrack broad complex (POZ/BTB) domain family, was originally discovered within a distinct neuronal forebrain structure associated with reward motivation and addictive behaviours [78]. The recruitment of HDAC3 and HDAC4 by NAC1 is responsible for the repression of gene expression in neuronal cells, with a specific focus on co-repressing other POZ/BTB proteins in the central nervous system [79]. The expression of NAC1 is increased in several forms of tumours, such as breast, renal cell, and hepatocellular carcinoma, as well as high-grade ovarian serous carcinoma. In these tumour types, NAC1 has been associated with the development of chemoresistance [80]. NAC1 is a constituent element of an expanded transcriptional network within embryonic stem cells (ESCs), which encompasses Oct4, Sox2, Nanog, Sall1, KLF4, and Sall4 [82].

9. **Germ Cell Nuclear Factor (GCNF):** GCNF, alternatively referred to as nuclear receptor subfamily 6 group A member (NR6A1), is classified as an orphan member within the superfamily of nuclear receptor genes [83]. The expression of this gene occurs during the development of the nervous system, as well as at particular stages of germ cell maturation in the adult ovary and testis. The involvement of GCNF in several biological processes such as gametogenesis, neurogenesis, and proper embryonic development during gastrulation has been suggested [89]. The inactivation of GCNF in mice results in aberrant posterior development, compromised midbrain development, inadequate closure of the neural tube, and eventual embryonic mortality [90]. The GCNF protein functions as a transcriptional repressor for the Oct4 and protamine genes, and it plays a crucial role in regulating gene expression during embryonic development and the formation of sperm cells [91].

10. **Stat3:** The protein Stat3, which plays a critical role in signalling for various cytokines and growth factor receptors, is of utmost importance in the developmental process of murine foetuses [94]. Stat3 activation in mouse embryonic stem cells (ESCs) occurs through the binding of leukaemia inhibitory factor (LIF) to the LIF receptor. This binding event triggers the translocation of Stat3 into the nucleus, subsequently resulting in the activation of many downstream genes such as Sall4, Myc, and KLF4 [95]. The induction of ESC differentiation is observed when Stat3 is suppressed [96], but the continuous activation of Stat3 keeps ESCs in an undifferentiated state, even in the absence of LIF [97]. The constitutive activation of Stat3 has been observed in a range of human tumours [98], and it has been found to have carcinogenic properties [99] as well as anti-apoptotic activities [100]. The process of transcriptional activation is governed by the phosphorylation event occurring at the tyrosine residue 705, which subsequently triggers

dimerization, facilitates nuclear translocation, and enables DNA binding [101]. The process of phosphorylation at the serine 727 residue, facilitated by either the MAPK or mTOR pathways, seems to have an impact on the regulation of transcriptional activity [102]. The levels of Stat3 isoforms, namely Stat3α (86 kDa) and Stat3β (79 kDa), exhibit variability based on factors such as cell type, exposure to ligands, or stage of cell maturation [103].

11. **LEF1 and TCF:** LEF1 and TCF are members of the HMG DNA-binding protein family of transcription factors, which encompasses lymphoid enhancer factor 1 (LEF1), T-cell factor 1 (TCF1), TCF3, and TCF4 [104]. Initially characterised as regulators of early lymphoid development (reference 105), LEF1 and TCF1 function as downstream effectors in the Wnt signalling pathway. The binding of these molecules to specific regions known as Wnt response elements facilitates the formation of docking sites for β-catenin. Upon activation of Wnt signalling, β-catenin is then transported to the nucleus, where it plays a crucial role in promoting the transcription of target genes. The expression of LEF1 and TCF proteins undergoes dynamic changes throughout the process of development, and the Wnt signalling pathway is abnormally activated in several cancer types, such as colon cancer [106]. The protein TCF3, often referred to as TCF7L1, plays a pivotal function in the integration of Wnt signalling with the regulation of stem cell differentiation [107].

12. **SALL Family:** The SALL gene family, commonly referred to as Hsal, is known to exert significant influence on the regulation of developmental processes across diverse animals. The set of genes known as SALL1, SALL2, SALL3, and SALL4 were first isolated from a DNA sequence that showed similarity to the sal gene found in Drosophila [108]. SALL4 plays a crucial role in the regulation of Oct4 and is necessary for maintaining pluripotency in embryonic stem cells [109]. The depletion of Sall4 in mouse embryonic stem cells (ESCs) leads to their redirection towards the trophoblast lineage when cultured in an environment without feeder cells. Although Sall4 plays a crucial role in stabilising embryonic stem cells (ESCs), it is not necessary for the maintenance of pluripotency [110]. SALL4 and Oct4 play a crucial role in maintaining the equilibrium of gene expression within the SALL gene family, specifically in relation to Sall1 and Sall3, which are expressed in both murine and human embryonic stem cells (ESCs). The elimination of Sall1 and Sall3 in mice results in neonatal mortality as a consequence of developmental abnormalities [111].

13. **F-box 15 (FBXO15):** FBXO15, which belongs to the F-box protein family and is distinguished by a 40-amino acid F-box motif, has been identified as a newly discovered target of Oct3/4. Nevertheless, it has been established that ESC self-renewal, development, and fertility are not essential [112]. The expression of FBXO15 is mostly observed in undifferentiated mouse embryonic stem cells (ESCs), and its expression diminishes quickly following the deactivation of Oct3/4. The expression profile of this gene closely resembles that of Oct3/4 and is predominantly limited to embryonic stem cells (ESCs), early-stage embryos, and testicular tissue.

14. **ESC Associated Transcript (ECAT) Genes:** The ECAT genes are key components in the field of stem cell biology. The gene ECAT1 is responsible for encoding an RNA-binding protein that has a K homology (KH) domain. This protein is produced exclusively in oocytes of mice [113]. The study identified ECAT4 as Nanog, a key regulator involved

in the maintenance of both mouse embryonic stem cells (mESC) and human embryonic stem cells (hESC) [114]. The study identified ECAT5 as ERas, an oncogene similar to Ras, which plays a role in regulating the proliferation of embryonic stem cells [115]. The growth and differentiation factor 3 (GDF3), known as ECAT9, was discovered to have a crucial role in maintaining pluripotency in mouse embryonic stem cells (mESCs) by blocking the signalling of bone morphogenetic protein (BMP) [116]. The gene ECAT11, alternatively referred to as FLJ10884 or L1TD1, exhibits high levels of expression in undifferentiated human embryonic stem cells (hESC). Research findings have indicated that L1TD1 is a downstream effector of Nanog and can be employed as a valuable indicator for the identification of undifferentiated human embryonic stem cells [117].

15. **Developmental Pluripotency-associated (DPPA) Genes:** The DPPA molecules, including a cluster of five proteins, are designated as a collection of Oct4-related genes and function as indicators for pluripotent cells in the early stages of embryonic development and germline formation. DPPA5, alternatively referred to as ESG1, is a protein that has a KH domain and is expressed in both EG cells and ESCs. This characteristic renders it a promising candidate as a marker for ESCs [118]. The gene DPPA3, commonly referred to as Stella, exhibits expression in primordial germ cells, oocytes, preimplantation embryos, and pluripotent cells [119]. The protein in question functions as an indicator of pluripotency and is involved in various cellular processes such as transcriptional repression, cell division, and the preservation of pluripotency in both mice and humans. Germ cell tumours have been shown to exhibit intron-less loci that are closely associated. This finding has been documented in a study [120]. According to the literature, DPPA4 has been identified as a nuclear factor that is connected with active chromatin. It has a role in controlling the differentiation of embryonic stem cells into a primitive ectoderm lineage [121].

IV. SIGNAL PATHWAY-RELATED INTRACELLULAR MARKERS

Multiple intracellular signalling channels are of utmost importance in the preservation of embryonic stem cell (ESC) self-renewal and pluripotency. Consequently, these pathways serve as significant indicators of ESC destiny. The fundamental signalling pathways that govern the self-renewal and pluripotency of embryonic stem cells (ESCs) encompass LIF-STAT3, BMP-SMAD, TGF-β/Activin/Nodal, IGF-IR, FGFR, and Wnt-β-catenin [122]. The importance of LIF-STAT3 and BMP-SMAD in maintaining the self-renewal of mouse embryonic stem cells (ESCs) has been well-documented [123]. However, it should be noted that LIF-STAT3 is not found to be active in undifferentiated human embryonic stem cells (hESCs) [124]. The BMP signalling pathway has been found to have a substantial impact on both mouse and human embryonic stem cells (ESCs) [125]. However, it is worth noting that the upstream effectors and resultant consequences of this system typically exhibit variations between the two species. As an illustration, BMP4 has been observed to sustain pluripotency in mouse embryonic stem cells (mESCs), while prompting trophectoderm development in human embryonic stem cells (hESCs) [126, 127]. The transduction of BMP signals is facilitated by SMAD proteins, which regulate the expression of downstream genes by interacting with other DNA-binding proteins within the nucleus. Notably, SMAD1/5/8 have elevated expression levels, making them potential markers for embryonic stem cells [128].

The Wnt and TGF-β/Activin/Nodal signalling pathways play a critical role in promoting self-renewal in both mouse and human embryonic stem cells (ESCs). The Wnt/β-

catenin signalling pathway, which plays a crucial role in cellular proliferation and embryonic development [129], exhibits significant expression in embryonic stem cells (ESCs) and governs their capacity for pluripotency [130]. Therefore, it might be regarded as an indicator for embryonic stem cells (ESCs). Members of the transforming growth factor-beta (TGF-β) family are involved in determining the fate of human embryonic stem cells (hESCs) [131]. The maintenance of human embryonic stem cell (hESC) pluripotency and the upregulation of Oct4 and Nanog transcription require the activation of Smad2/3 and Smad4 through the Activin/Nodal signalling pathway [132]. Therefore, it is possible that Smad2/3 and Smad4 could function as indicators in human embryonic stem cells (hESC). Table 2 provides a summary of the potential markers associated with these pathways.

Table 2: Different Markers, Characteristics and Classification of Embryonic Stem Cells

Markers	Characteristics	Classification
SMAD1/5/8	Mouse ES cells, embryonal carcinoma (EC) cells	Smad proteins ((R-Smad), BMP signalling pathway
SMAD4	Mouse ES cells, human ES cells, embryonal carcinoma (EC) cells, early embryos, and testis tissue	Smad proteins (Co-SMAD), TGF- β /Activin/Nodal signalling pathway, BMP signalling pathway
SMAD2/3	Human ES cells, embryonal carcinoma (EC) cells	Smad proteins ((R-Smad), TGF-β /Activin/Nodal signaling pathway
β-catenin	Mouse ES cells, human ES cells, embryonal carcinoma (EC) cells	Transcription activators, Wnt/β-catenin signaling pathway

V. ENZYMATIC MARKERS

Both mouse and human embryonic stem cells (ESCs) demonstrate increased amounts of alkaline phosphatase and telomerase. The expression of alkaline phosphatase is highly pronounced on the cellular membrane of embryonic stem cells (ESCs). In the context of humans, the antibodies TRA-2-49 and TRA-2-54 have the capability to identify and detect alkaline phosphatase. Enzymatic-based reactions are commonly employed for detection in murine cells [133]. Therefore, the utilisation of alkaline phosphatase labelling has proven to be a dependable technique for the identification and evaluation of pluripotency in embryonic stem cells (ESCs). The National Institutes of Health (NIH) stem cell resource website has an extensive compilation of various markers, which can be accessed at the following link: http://stemcells.nih.gov/info/scireport/appendixe.asp#eii.

VI. OTHER MARKERS I

In recent investigations, scholars have undertaken an examination of the utilisation of diminutive compounds, such as lectins or abbreviated peptides, which exhibit a targeted affinity towards surface receptors on embryonic stem cells (ESCs). By employing quantum dots (QD) or fluorescence dyes, these compounds can serve as markers for the purpose of labelling, identifying, and isolating embryonic stem cells (ESCs).

1. **Lectins :** Lectins are a class of proteins that has the ability to bind to carbohydrates, specifically recognising a wide range of sugar structures. This characteristic renders them highly useful in the field of recognising and characterising glycosylation patterns on the surface of cells [134]. The utilisation of their application has played a role in the demarcation of embryological developmental phases in certain species and has aided the examination and recognition of distinct cell types through the analysis of cell surface carbohydrate presentation [135]. During the preimplantation and implantation periods of development, lectin receptors, which are glycans that are regulated by development, are observed on the cell surfaces of mouse embryonic stem cells (ESCs) [136]. Lectins have demonstrated their utility as markers in the identification of retinal progenitor cells produced from mice embryonic stem cells (ESCs) for transplantation therapy [137]. Additionally, lectins have been employed in the investigation of differentiated human ESCs [138]. Furthermore, lectins have been utilised as indicators to delineate several phases of mouse embryogenesis and to characterise distinct subsets within colonies of adherent human embryonic stem cells (hESCs).

2. **Peptides Specific for ES Cells:** The fundamental importance of identifying ligands that bind to specific cell targets lies in the fact that receptor-ligand interactions are involved in a wide range of cellular biological processes. This has significant implications for the creation of drugs, biomaterials, and diagnostic tools [139]. The utilisation of phage display technology has proven to be a remarkably effective approach in the identification of previously unknown biomarkers [140]. The technology encompasses the fusion of nucleotide sequences of arbitrary polypeptides with a phage coat protein, facilitating the exhibition of chimeric proteins on the surface of the phage. By employing a process of targeted selection, a collection of phages can be generated that exhibit progressively enhanced affinity for the desired target. Ligands that have been found by phage display screens has the ability to selectively bind to particular places on target cells, hence functioning as markers that facilitate the recognition and isolation of those cells. Previous studies have documented the existence of various short peptides that are specifically designed for Rhesus Monkey Embryonic Stem Cells (R-ESCs) and mouse ESCs. These peptides have been chemically linked with quantum dots, resulting in a targeted approach for ESCs [141]. Specific peptides targeting human embryonic stem cells (hESCs) and human embryonal carcinoma cells (ECs) have also been discovered (Reference 142). When embryonic stem cells (ESCs) were cultivated on self-assembled monolayers that presented certain peptide sequences, they exhibited the expression of pluripotency markers at levels similar to those observed when cultured on Matrigel [142].

VII. MARKERS OVERLAPPING WITH TUMOR STEM CELLS

Adult stem cells possess distinct attributes that set them apart, such as their extended lifespan, ability to regenerate themselves, and capability to differentiate into many cell lineages. These distinctive features render adult stem cells indispensable in both typical physiological processes and pathological states [142]. When the ability of stem cells to differentiate is compromised and their capacity to proliferate becomes unregulated, these altered stem cells may develop tumorigenic characteristics, leading to the formation of cancer stem cells (CSCs) or tumour stem cells (TSCs) that have substantial involvement in the process of carcinogenesis. Cancer stem cells (CSCs) have been successfully extracted from a range of bodily organs, such as the breast, brain, blood (specifically leukaemia), skin (melanoma), head and neck, thyroid, cervix, and lungs [141]. In recent research, a variety of

CSC markers have been employed to differentiate tumour cells from normal tissues [142]. It is noteworthy that embryonic stem cells (ESCs) and cancer stem cells (CSCs) exhibit a considerable overlap in marker gene expression. This observation gives rise to potential apprehensions regarding the use of ESC transplants.

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