A SHORT INTRODUCTION TO MARKERS OF STEM CELLS AND THEIR NEURAL DERIVATES

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Stem cells are primal cells common to all multi-cellular organisms that retain the ability to renew themselves through cell division (1). These cells can differentiate into a wide range of specialized cell types including cells of endoderm, mesoderm and ectoderm origin (2; Figure 1). The three broad categories of mammalian stem cells exist: embryonic stem cells, derived from preimplantation early embryos, adult stem cells, which are found in adult tissues, and cord blood stem cells, which are found in the umbilical cord. Embryonic stem cells (ESC) are pluripotent i.e. able to differentiate into all of the specialized embryonic tissues while adult stem cells and progenitor cells act as a repair system for the body, replenishing specialized and degenerated cells (3). Therefore, characterization of undifferentiated and differentiated stem cells is crucial for their application. In recent years, scientists have discovered a wide array of stem cells that have unique capabilities to self-renew, grow indefinitely, and differentiate or develop into multiple types of cells and tissues. Researchers now know that many different types of stem cells exist but they all are found in very small populations in the human body, in some cases one stem cell in 100,000 cells in circulating blood. So, how do scientists identify these rare types of cells found in many different cells and tissues-a process that is much similar to finding a needle in a haystack? The answer is rather simple thanks to specific stem cell markers. This review describes some of stem cell markers and their differentiation neural derivatives.

1) EMBRYONIC STEM CELL MARKERS

Embryonic stem cells are stem cells derived from the early stage preimplantation embryo (3). Embryonic stem cells are pluripotent and able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes ESC from multipotent progenitor cells found in the adult since these only form a limited number of cell types. The presence of pluripotent adult stem cells remains a subject of scientific debate, however research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures (4). The markers of most frequently used pluripotency markers are listed below. Alkaline phosphates (ALPL) also known as tissue-non specific isozyme precursor, is related to, but distinct from, intestinal/ALPI, placental/ALPP, and placental-like/ALPPL2 alkaline phosphates. An Alkaline phosphate is expressed at very high levels in undifferentiated human and mouse ESC, embryonic carcinoma (EC) cells, and embryonic germ (EG) cells (1-3). Interestingly, mutations in this enzyme have been linked directly to hypophosphatasia, a disorder that is characterized by hypercalcaemia and skeletal defects (5). Nanog is one of the most crucial pluripotency factors and this molecule is a member of the homeobox family of DNA binding transcription factors that has been shown to maintain pluripotency of ESC (6). Its expression is high in undifferentiated ESC and is down regulated during ESC differentiation, concomitant with loss of pluripotency (2). The second key factor of pluripotency is Oct-3/4. This is a 34 kDa POU transcription factor that is expressed in ESC and EG cells, and its expression is required to sustain cell self-renewal and pluripotency. Oct-3/4 is the most recognized marker for pluripotent ESC and reprogrammed (iPS) somatic cells (1, 2). Rex-1 is a zinc finger family transcription factor that is highly expressed in ESC (7) and one of several gene markers used to identify undifferentiated stem cells since its expression is down regulated upon stem
and differentiation is accompanied by an up regulation of SSEA-1 and down-regulation of SSEA-3 and SSEA-4. The SSEA 1, 3 and 4 are globoseries glycolipids recognized by monoclonal antibodies originally raised to distinguish early stages of mouse development. Primate pluripotent cells express SSEA-3 and SSEA-4 (the epitope recognized by the latter is more readily detected than that seen by the former), and express SSEA-1 only upon differentiation (2, 9). Other cell-surface markers are TRA-1-60, GTCM-2 and TRA1-80. TRA1-60 epitope is a sialidase-sensitive epitope associated with this proteoglycan; the antibody GCTM-2 reacts with its core protein, and antibody TRA-1-80 reacts with other unknown epitopes on the same molecule (10). Human ESC, as well as monkey ESC reacts with TRA1-60, TRA1-80 and GCTM-2. Although GCTM-2 and TRA1-60 do not label mouse ESC or EC cells, it is not clear whether the mouse cells lack the surface proteoglycan or whether the antibodies are species specific.

II) HEMATOPOIETIC STEM CELL MARKERS

Hematopoietic stem cells (HSC) are stem cells found in bone marrow that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutro-
philos, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). However, the definition of HSC has undergone considerable revision in the last two decades since there are numerous evidences that these cells might be pluripotent in their primitive nature (11). In reference to phenotype, HSC are identified by their small size, lack of lineage (lin) markers, low staining with vital dyes such as rhodamine 123 or Hoechst 33342 and presence of various antigenic markers on their surface, many of which belong to the cluster of differentiation series. The most used markers are: CD34, CD38, CD45, CD90, CD133, CD105, and also c-kit-, the receptor for stem cell factor. CD34 remains the main marker of the HSC population but also a marker for multipotent stem cells present on lineage-committed hematopoietic progenitors from bone marrow and a subpopulation of immature thymocytes (12). CD133 (Prominin-1) is a HSC marker but also neural marker which is expressed in adult human differentiated cells and certain types of kidney cancer (13). Meanwhile CD133 is a novel marker for human prostatic epithelial stem cells (14). During the purification of HSC by FACS method, a group of up to 14 different mature blood-lineage marker could be identified: CD13 and CD33 for myeloid, CD71 for erythroid, CD19 for B cells, CD61 for megakaryocytic (human) and CD45 for B cells, Mac-1 (CD11b/CD18) for monocytes, Gr-1 for Granulocytes, Ter119 for erythroid cells, IL7Ra, CD3, CD4, CD5, CD8 for T cells (mice). These antibodies are used as a mixture to deplete the lin+ cells or late multipotent progenitors (12-14).

III) NEURAL CELLS

Stem cells are extremely useful for furthering our understanding of both normal and abnormal human development, providing a human cell preparation that can be used to screen for new reagents and generating large numbers of differentiated cells that can be used for transplantation purposes. Critical among the applications for the latter are diseases and injuries of the nervous system, medical approaches to which have been, to date, primarily palliative in nature. Differentiation of human pluripotent stem cells into cells of the neural lineage therefore has become a central focus which has resulted in the description of several numerous methods for neural cell differentiation from human pluripotent stem cells (15, 16). Among these are methods for the generation of such divergent neural cells as dopaminergic neurons, retinal neurons, ventral motoneurons, and oligodendroglial progenitors (Figure 2). Some of the markers frequently used for identification of neural cells are listed below. While acetyl cholinesterase is a marker of early neuronal development (17), Musashi-1 is an evolutionally conserved marker for central nervous system (CNS) progenitor cells including neural stem cells (NCS; 18).

As a putative NCS marker Nestin is expressed in different areas of the adult mammalian brain that are known to support mitotic activity (19). Widely used marker of NCS is also Sox1 which is a transcription factor from the SoxB1 subgroup (20) while SOX2 is a persistent marker for multi potential NCS derived from ESC (21). A marker of axonal sprouting in mid stages of embryonic development is ELF which probably plays important role in NSC development (22). One of the earliest markers to signal neuronal commitment in primitive neuroepithelium is beta-tubulin (23). There are seven classes of beta-tubulin gene, one of which, class III (TUJ-1), is neuron specific (23) and was found in medulloepithelial rosettes. MAP2 is a marker of neuronal differentiation as a neuron-specific protein that stabilizes microtubules in the dendrites of post mitotic neurons (24). This marker is essential for development of early neuronal morphology and maintenance of adult neuronal morphology and appears early in neuronal maturation of the neocortex, particularly in the sub plate region (25). Additional markers of developing neocortex are Pax6, Tbr2 and Tbr1 which are sequentially expressed by radial glia, intermediate progenitor cells, and post mitotic neurons (26). A Neuronal nucleus (NeuN) is a 46/48-kD nuclear phosphoprotein antigen used widely in research and diagnostics to identify post mitotic neurons (27, 28). This is a neuron-specific protein which is present in most neuronal cell types of vertebrates and a marker of neuronal maturation in early human fetal nervous system (29). NeuN expression per se is a reliable marker of proliferate capacity but levels of NeuN expression may also be indicative of the physiological status of a post mitotic neuron (28). One of the most important neurotransmitters is the neurotransmitter acetylcholine which is synthesized by choline acetyl transferase (ChAT). Choline acetyltransferase is a cholinergic neuron-specific marker

Figure 2: Differentiated human embryonic stem cells express TUJ-1 (green) and TH (tyrosine hydroxylase, marker for catecholamine-synthesising neurons; red) markers. Nuclei stained with DAPI (blue). Scale bar: 10 μm.
The improvement of the protocols for in vitro differentiation of stem cells has lead to the development of numerous markers for identification of diseased or damaged tissue/cells. Neuron specific enolase (NSE) a common marker for both endocrine cells and enteric nerves has been also used as a marker of in vitro neuronal damage (31) and N-cetlylaspartate (NAA), a marker of cellular dysfunction, neuronal loss or damage has been used to study acute brain injury (32). Neuron-specific enolase CSF (CSF-NSE) could be used as a quantitative marker of ischemic damage or as a non-disease specific marker for the neuronal degeneration in dementia disorders providing useful adjuncts in the assessment of neuroprotective drugs in stroke (33). There are several human neural disorders which are evident by certain reduced activities of different proteins. For instance, ChAT activity is markedly reduced in the affected brain areas and TG-1 has been identified as a marker for neuronal nuclei in Alzheimer’s disease (34). In addition, neuroendocrine-specific protein C (NSP-C) a marker of neuronal differentiation is reduced in brain of patients with Down syndrome and Alzheimer’s disease. One of the frequently used markers of human brain tumors is calcineurin (35) while protein kinase C (PKC) is an early and sensitive marker of ischemia-induced progressive neuronal damage in gerbil hippocampus (36). Taken together, there are numerous cell markers and for sure we mentioned some of them. The list of the markers is useful especially where differentiated or damaged tissue/cells need to be identified. However, not all markers are specific (for instance see CD133). Additionally, Nestin may not be a suitable marker solely for the identification of neuronal cells but also to identify endocrine precursor cells in the pancreas (37). Therefore, additional analysis of cell type(s) should be demanded including specific intracellular markers and where possible the specific functionality of the undifferentiated/differentiated cells.

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REFERENCES


