

A Reliable Technique for Stem Cell Marker Identification

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ABSTRACT

Stem cells have emerged to give a promising result in regenerative medicine. Stem cell derived growth have been studied in various animals. These stem cell are identified by specific markers. Various methods have been used to identify these markers. Some of the methods were been used last decades to identify the stem cells. This article presents a concise review on the methods used for identifying markers and discuss the very most reliable technique of the identification of stem cell markers.

Keywords: IHC-immunohistochemistry, ICC-immunocytochemistry, FC-flow cytometry, stem cell, stem cell markers, MSC- mesenchymal stem cell, HSC-haematopoietic stem cell, ESC- embryonic stem cell, CSC- cancer stem cell

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INTRODUCTION

Stem cells, the building block for organ development and tissue repair, stem cells have a unique and wide ranging capabilities, thus delineating their potential application to regenerative medicine[2]. Stem cell markers are the key to identify stem cell. In recent years considerable effort has been invested in detection and characterization of stem cell markers, variety of methods were used for the detection of these markers. The major challenges in stem cell research was analysis of heterogeneous cell population. This article focuses on one that analysis the heterogeneous cell population. As there are various technique like IHC, ICC, ELISA, WESTERN BLOT etc.,[1] for identification of stem cell markers one of the facing challenge was to identify

heterogeneous cell population to sort viable cells to purify for use in later experiments. One must know the cell surface signature for the particular cell of interest, characterization of cell surface signature enable identification of subpopulation, isolation of pure population, development of quality control assay for cell preparation.

Stem cell

Stem cells are unspecialized precursor cells characterized by 2 unique abilities.

- Self-renewal
- Differentiation[1]

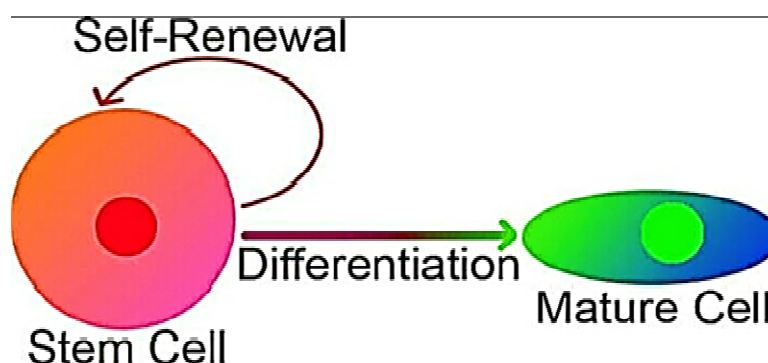


figure1: stem cell differentiation and self renewal
Source: www.allthingsstemcell.com

Stem cell markers

These are the genes or their protein products used by a researcher to isolate and identify the stem cell [1, 4].

Types

- EMBRYONIC STEM CELL MARKERS
- INDUCED PLURIPOTENT STEM CELL MARKERS.
- MESENCHYMAL STEM CELL MARKER
- HAEMATOPOIETIC STEM CELL MARKER
- CANCER STEM CELL MARKER

DERIVATIVES FROM EACH CLASS OF STEM CELL

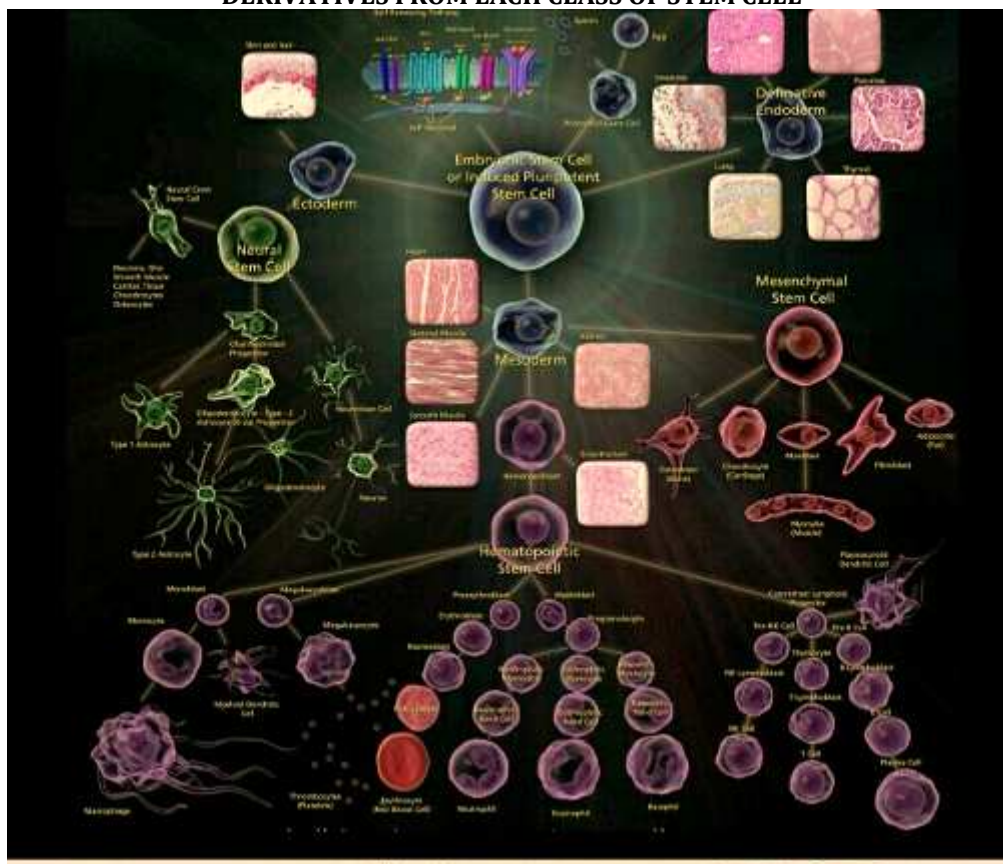


figure2: derivatives of various forms of cells from pluripotent stem cells
Source: www.bdbiosciences.com

Here are the some of the examples for each type of markers [1,3,4]

EMBRYONIC STEM CELL MARKERS: NaNog, nuclostemim, oct4, SSEA1, SOX2, TELEOMEREASE, CRIPTO.

HAEMATOPOITIC STEM CELL MARKERS: CD-133, CD-34, SOX-2, CD44, ABCG2, podocalyxin, thrombopoietin.

MESENCHYMAL STEM CELL MARKER: CD-105, STRO-1, TNAP etc.,

NEURONAL STEM CELL MARKERS: Nestin.

PULPAL STEM CELL MARKERS: CD-29, CD-44, CD-105, CD-117, CD-146, STRO1

STEM CELL	NERVE STEM CELL	BONE STEM CELL	PULPAL STEM CELL	Cancer stem cell
MARKERS	SOX-2	CD-146,CD-105	CD-44,CD-105,	Al p, aldh-1
	CD-44	STRO-1	STRO-1	CD-44
	NESTIN	CD-73	CD-117	Etc.,
	DCX etc.,	CD-44 etc.,	CD-29 etc.,	

Table1: type of stem cell and few examples of their respective markers.

EXAMPLES OF A NORMAL STEM CELL MARKER WHICH IS ALSO A CANCER STEM CELL MARKER: ALDH-1, CD-34, CD-44, CD-117, CD-13, Nestin, CD-77, CD-176. [1, 3, 4]

DIFFERENTIATING BETWEEN NORMAL AND CANCER STEM CELL

During the process of malignant transformation from a normal SC to CSC

- Stem cell marker (glycoprotein) under glycolysation.
 - Cancer stem cell marker carry a specific glycan.
 - Changes in stem cell marker glycoprotein lead to altered behaviour of cells[3].
- Cancer stem cell marker express the following antigen TF, TN, LEWIS X(CD147) [3, 5].

TECHNIQUES OF STEM CELL MARKER IDENTIFICATION

WB – WESTERN BLOTTING

ICC – IMMUNOCUTOCHEMISTRY

IHC – IMMUNOHISTOCHEMISTRY

IP – IMMUNOPRECIPITATION

ELISA – ENZYME LINKED IMMUNO SORBENT ASSAY[1, 4]

ESCM- FC, WB, ICC, IP, IHC

MSCM – ICC, FC, WB, ELISA

HSCM – ICC, ELISA, IHC, WB, BP

NSCM – FC, ICC, IHC [1]

The most reliable technique in terms of sensitivity, specificity, economic and time is

FLOW CYTOMETRY [7, 8, 9, 10]

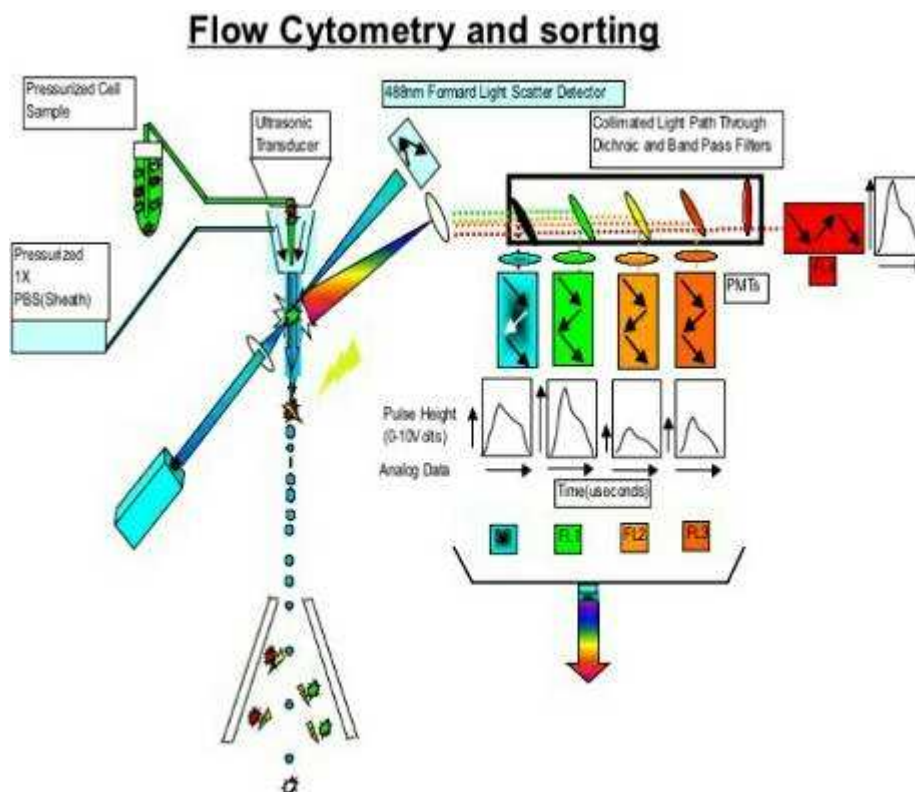


figure3: flow cytometry
Source: cellbiology.med.unsw.edu.au

COMPONENTS: Fluid system – consist of a

- Pressurised cell sample
- Ultrasonic transducer

LASER – light source for scatter and fluorescence

OPTICS – collimated pathway through dichroic and band pass filters.

DETECTORS – which receive light from the optics

ELECTRONICS N COMPUTER SYSTEM: Convert signal from the detector into digital data and perform analysis[9].

In Flow cytometry fluorescence technique is used to identify and enumerate surface markers. Stream of fluid passing through an electronic detection apparatus. Different population of molecules, cells or particles can be differentiated by size and shape using forward or right angle scatter. Cells made to flow in a single stream in a flow cell by hydrodynamic focussing. Sample stream containing cells are focussed by a surrounding layer of the sheath fluid. a argon laser (488nm) is focused on walls.

Parameters measured by flow cytometry

FS – FORWARD SCATTER (measure of the cellular size)

SS – SIDE SCATTER (measure the granularity of the cell)

FL-1, FL-8 – FLUORESCENCE SENSOR

The multiple parameters measured by flow cytometry includes size, granularity, nucleic acid (DNA or RNA), receptor level[7, 8].

Steps

1. Sorting of cells by FACS
2. Stem cell differentiation
3. Stem cell surface screening

Sorting of fluorescence activated cells: flow sorting extends flow cytometry with the additional capacity to divert and collect the cells exhibiting an identifiable and set of characteristics either mechanically or by a electrical means. FLOW CYTOMETRY SORTING SCHEMATIC: the nozzle /flow cell is vibrated by a transducer (converts electrical energy into mechanical energy) so it produces a stream breaking into droplets. Laser interrogation and signal processing followed by sort decision: sort right, sort left or sort no electronic delay until cell reaches break off point then the stream is charged: + or -. Charged droplets deflected by electrostatic field from plates held at high voltage (+/-3000volts). Besides tubes can sort onto slides or multi-well plates [7, 10].

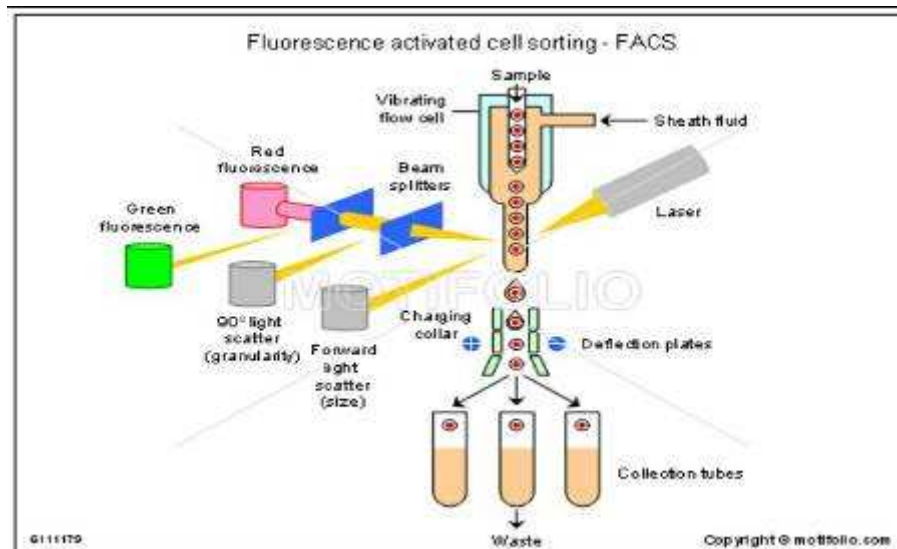
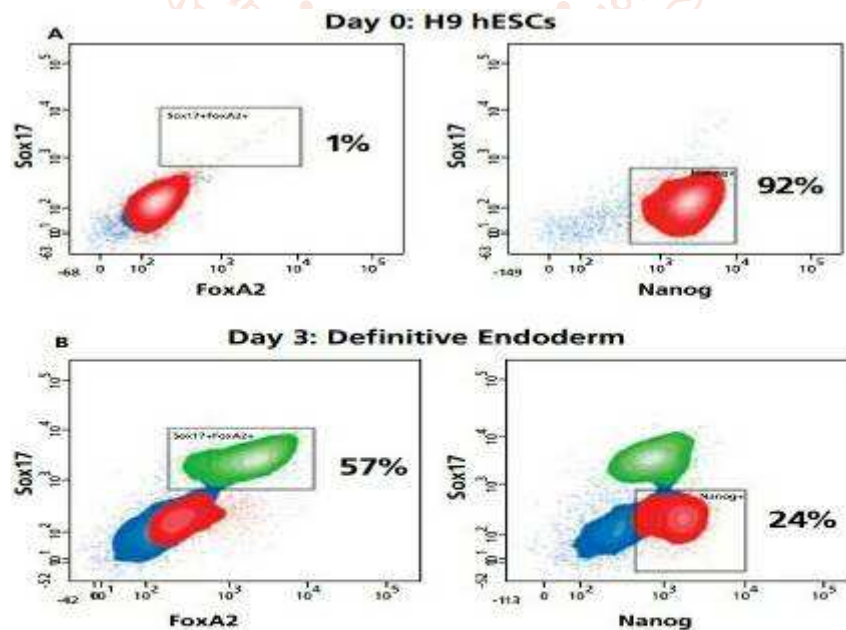


Figure4: cell sorting Source: www.motifollo.com

Stem cell differentiation: The ability of the human pluripotent cells to differentiate into various cell lineage is a central topic in developmental biology and has applications of regenerative medicine and cellular therapy [13]. Multiparametric flow cytometry is an excellent method for determining the relative numbers of cells expressing markers of interest.[8] Definitive endoderm generates the liver, pancreas, and intestine. During lineage specification into definitive endoderm, the levels of the transcription factor SOX17 AND FOX A2 increase, while pluripotency marker such as nanog decrease [15].



Changes in transcription factors in definitive endoderm development.

H9 hESCs (WiCell) were differentiated into definitive endoderm according to D'Amour, et al⁶ and monitored on a BD LSRFortessa flow cytometer using the BD Stemflow™ definitive and pancreatic endoderm analysis kit (Cat. No. 562496). As embryonic stem cells (A) differentiated toward definitive endoderm (B), more cells expressed both Sox17 and FoxA2, while Nanog expression decreased.

figure5: stem cell differentiation by changes in transcription factors

Source: BD biosciences (stem cell research)

Neuronal cell differentiation: differentiation of neural stem cell to neural lineage monitored using flow cytometry NSC express markers -less nestin and more DCX. A subpopulation of cells that continues to express nestin further delineates into glial cell population that express CD44 [14,15]

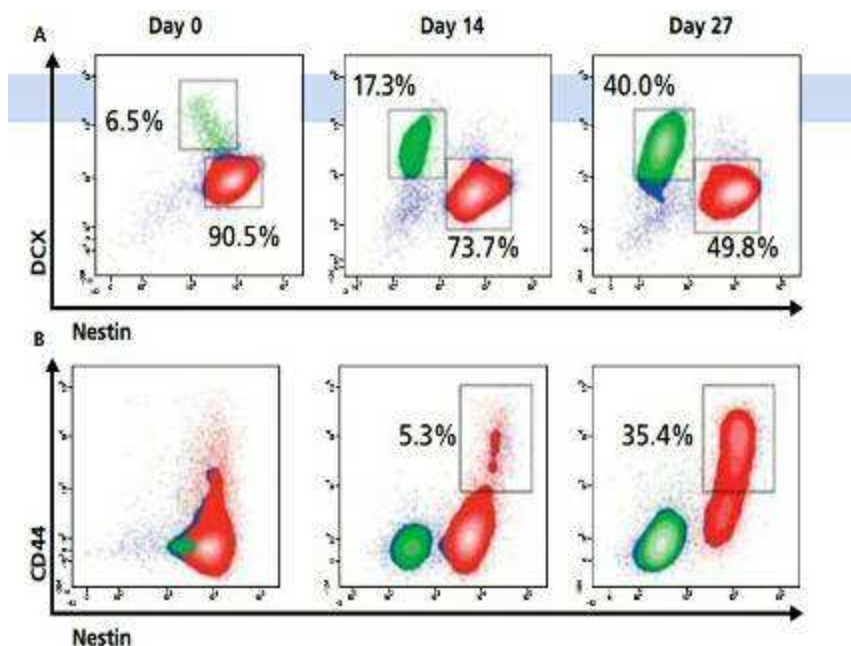


Figure 6: changes in intracellular and surface markers in neuronal cell differentiation.

Source: BD biosciences (stem cell research)

Different types of related cell may share marker BD-Lyolplate cell surface marker screening panel provide a effective solution for profiling stem cell. Purified NSC when screened with (CAT NO: 560747) and on imaging potential hits were verified [11, 15].

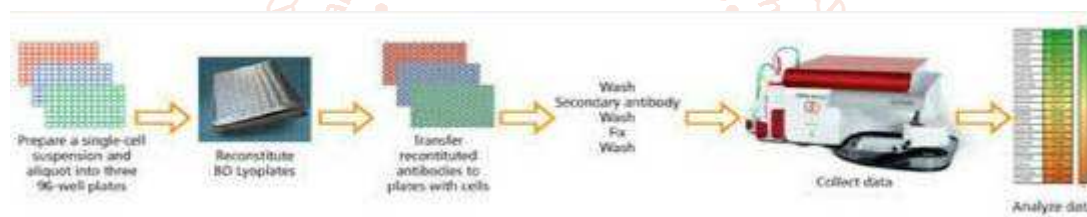


figure7: BD biosciences (stem cell research)

We're as the western blot technique is cumbersome costly, if antigen is degraded quickly, WB wont detect it. Longer process, Time consuming. In IHC disadvantageous aspect tend to stem from its lack of specificity, difficulty of interpretation and low percentage of reproducibility. ELISA time consuming and cumbersome costly [12].

Conclusion:

Faster is the soul for every business. On considering the factors like realibility, rapidity, sensitivity and specificity the unique technique is flow cytometry because it investigates large number of cell population at single cell level, analysis heterogeneous cell population and its subpopulation and is the powerful methodology of characterizing and isolating stem cell and their derivative [12, 15]. Flow cytometry has been used for decades by the biologists to address the challenge of heterogeneity. New methods and tools are enabling researchers to employ this powerful technique to make key discoveries of stem cell types and their respective lineages.

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