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STEM CELLS- AN OVERVIEW

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ABSTRACT

Stem cells are specialized cells that have the potential to differentiate into various tissues with different functions. Based on the formation of development, stem cells are classified into totipotent, pluripotent and multipotent cells. Their chief sources include adult body tissues such as Skin, Bone, Cartilage, Muscle, Intestine etc, from the germ line tissues, from the blood in the umbilical cord at the time of birth, from peripheral blood stem cells isolated from blood samples, embryonic stem cells harvested from the inner cell mass of the blastocyst (seven to ten days after fertilization) etc. Recently stem cell therapy has gained tremendous popularity in treating blood disorders, leukemia, CVDs, Nervous disorders etc. They find their use in treating cancers, Testing New Drugs, Screening Toxins and new methods of gene therapy to treat genetic illness. Preservation of Stem Cells has been a challenging task since the viability of the cells should remain till use. Cryopreservation using various solvents like DMSO at various concentrations is being used. However the research is still going on in this arena. Now a days various Stem Cell banks are available across the country marking a milestone. In this present article we'll discuss about various sources, clinical applications, preservation of stem cells and a note on Stem Cell Banks and their preservation cost.

KEYWORDS

Stem cells, Preservation of Stem Cells, Sources, Stem cell banks.

INTRODUCTION:

Stem cells are specialized cells that can differentiate in various tissue types. [13]The best understood example of a stem cell in humans is fertilized egg or zygote. It is a single cell formed by the fertilization of a sperm and ovum. Both sperm and the ovum carry half of the genetic material to form a fertilized egg. Once the zygote starts dividing, it is known as an embryo. Then the cell starts doubling rapidly to form a sophisticated organism. That sophisticated organism consists of many billions of cells with functions as those of eyes, heart, immune system, the color of skin, brain, etc. All of the specialized cells that make up these body systems are descendants of the original zygote, a stem cell with the ability to develop into different kinds of body cells. The cells of a zygote are said to be totipotent (capacity to develop into any type of cell).

By the regulation of gene expression the stem cells become specialized cells. Recently stem cell therapy has gained tremendous popularity has it plays a critical role in the treatment of numerous incurable diseases like transplantation therapies and leukemia.

Based on their developmental formative, stem cells may be *Totipotent cells*- which is capable of generating every cell body or fully furnished organism, *Pluripotent cells*-capable of generating any 'virtual tissue type' but not the organism on the whole, *Multipotent cells*- set of differentiated cells giving many tissues. E.g.: Mesenchymal Stem Cells (MSC) are used to generate bones, connecting tissues, muscle, and cartilage. [1]

STEM CELLS VS PROGENITOR CELLS:

Stem cells are the specialized cells with unlimited self renewal and 'multilineage capacity'. While Progenitors or 'Precursors' have limited renewal and regeneration capacities. Examples include: Hemopoeitic Progenitor cells (HPCs), Endothelial Progenitor Cells (EPCs), Mesenchymal Progenitor Cells (MPCs). These can be identified in the blood/tissue using following markers given in **Table 1**.

Table 1. Common markers used to identify EPC, MPC, HSC, and HPC[16]

Cell type	Human	Mouse
EPC (Alternative terminology: ECFC, OEC)	Positive expression	EPC are exceedingly rare in mice and are yet to be isolated and culture expanded.
	CD31, vascular endothelial cadherin (VE-cadherin), vWF, VEGF-R2, VEGF-R1, CD34, CD146, CD105, eNOS, UEA-1 lectin (binding), Ac-LDL (uptake)	
	Negative expression	
	CD45, CD14, CD11b, CD133,[a] CD90[a]	
MPC (Alternative terminology: MSC)	Positive expression	Positive expression
	CD90, CD44, CD29, CD105, NG2, platelet-derived growth factor (PDGF)-Rβ	CD90, CD44, CD29, CD105, NG2, PDGF-Rβ
	Negative expression	Negative expression
	CD45, CD14, CD11b, CD31, VE-cadherin	CD45, Gr-1, CD11b, CD31, VE-cadherin
HSC	Positive expression	Positive expression
	CD34, CD133	c-Kit, Sca1
	Negative expression	Negative expression
	Lineage-specific markers	Lineage-specific markers
HPC	Positive expression	Positive expression
	Common antigen	Common antigen
	CD45	CD45, c-Kit, Sca1
	Lineage-specific markers	Lineage-specific markers
	Myeloid: CD11b, CD14, Ac-LDL (uptake), UEA-1 lectin (binding), CD31, VEGF-R2, VEGF-R1	Myeloid: CD11b, Gr-1, Ac-LDL (uptake), BS-1 lectin (binding), CD31, TIE2, VEGF-R2, VEGF-R1
	Lymphoid: CD3, CD4, CD8	Lymphoid: CD3, CD4, CD8
	Erythroid: Glycophorin A, Ter-119	Erythroid: Ter-119
	Megakaryocytic: CD41, CD42	Megakaryocytic: CD41, CD42
	Negative expression	Negative expression
	CD133	CD133

[a]Culture-expanded EPC are negative for CD133 and CD90.
Abbreviations: Ac-LDL, acetylated low density lipoprotein; ECFC, endothelial colony-forming cell; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; MPC, mesenchymal progenitor cell; OEC, outgrowth endothelial cells; NG2, chondroitin sulfate proteoglycan; PDGF-Rβ, platelet-derived growth factor receptor beta; TIE2, TEK tyrosine kinase with Ig and EGF homology domains-2; UEA-1, *Ulex europaeus* agglutinin-1; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

SOURCES OF STEM CELLS:

Typical sources for Stem Cells include

Adult Stem Cells:

The adult body tissues such as Skin, Bone, Cartilage, Muscle, Intestine etc have stem cells which can regenerate respective cells once they worn or die out. Hence Adult Stem Cells are termed to be 'tissue specific'. However presence of Stem Cells in Heart is still unclear.

Adult Stem Cells are multipotent as they generate 'few cells' which regenerate into specific tissues or organs. They are also referred to as Somatic Stem Cells. They can be isolated from adult tissues, umbilical cords and other non-embryonic sources. However the term 'adult stem cells' seems to be misnomer since they can be isolated even from infants.[7] Characteristic examples include bone marrow transplant and mesenchymal Stem Cells. In the former, Stem cells are collected from the heamopoietic system of the individual and transplanted. In the latter, mesenchymal stem cells are used to regenerate bone, cartilage, fat, muscle. They are commonly used in

osteoarthritis and bone surgeries and fractures. [3]

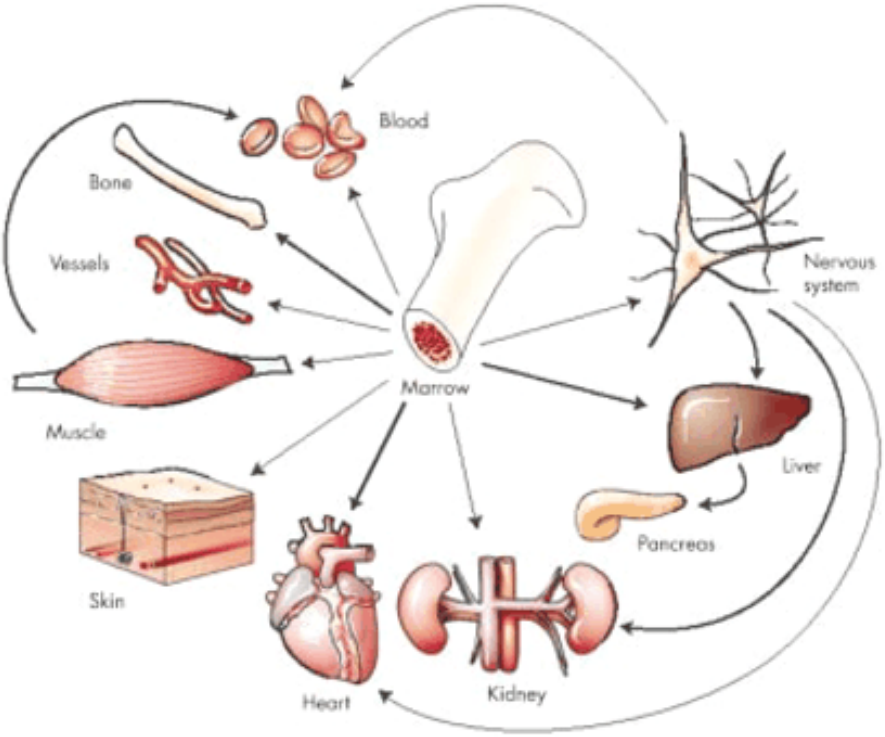

Fig.1: Explaining various possibilities in regeneration using Adult Stem Cells. [8]

Fetal stem cells:

Fetal stem cells are taken from the germline tissues that will generate the gonads of aborted fetuses. Like adult stem cells, they are also tissue-specific, and regenerate the mature cell types within the particular tissue or organ.

Umbilical Cord blood stem cells:

At birth the blood in the umbilical cord is rich in blood-forming stem cells. The applications of cord blood are similar to adult bone marrow and are used to treat diseases or to restore the blood system after treatment for specific cancers. Cord blood stem cells are also tissue-specific. These are multipotent stem cells that are able to differentiate into certain, but not all, cell types.

Peripheral Blood Stem Cells:

Peripheral blood stem cells (PBSCs) can be isolated from blood sample. The blood stem cell is capable of giving rise to a very large number of very different cells that generate the blood and immune system, including red blood cells, platelets, granulocytes, and lymphocytes.

Embryonic Stem Cells:

These are harvested from the inner cell mass of the blastocyst seven to ten days after fertilization and can give rise to all cell types. Embryonic stem cells have the risk of transforming into cancerous tissue after transplantation. Cells taken from one section of an embryo that might have become part of the eye can be transferred into another section of the embryo which may develop into blood, muscle, nerve, or liver cells.

These hES cells can differentiate into Embryoid bodies (EBs) in vitro. The EBs contains 'cardiomyocytes' which have an inbuilt beating capacity thus finding their use cardiac disorders. [6]

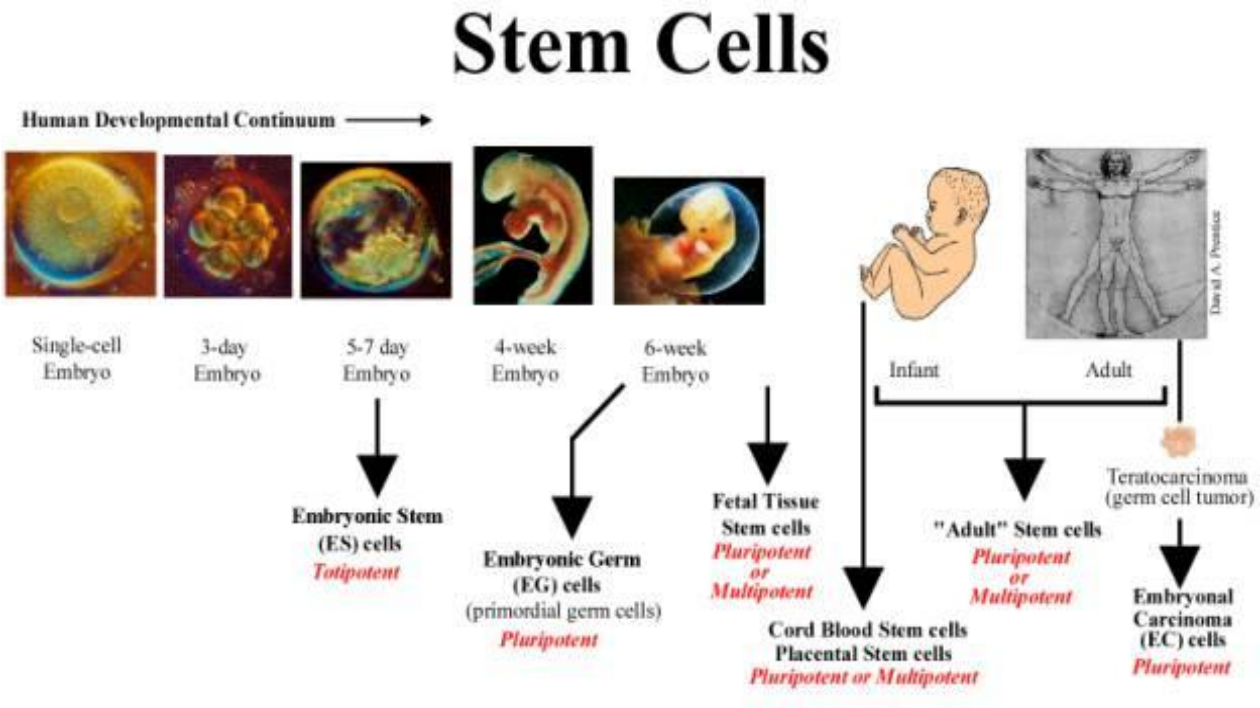

Fig 2: Isolation of different Stem Cells during developmental stages of Human Embryo.

The human embryoid bodies (EBs) isolated from hESCs has a characteristic feature of expressing gastrula organizer which paved new path in hESC discovery. [11]

Induced Pluripotent Stem Cells (iPS cells):

Induced pluripotent stem cells (iPSCs) were first invented for human cells in 2007. Adult cells are genetically converted to an embryonic stem cell–like state. In animal studies, iPSCs have similar characteristics of pluripotent stem cells. Human iPSCs can differentiate and generate multiple different fetal cell types.

There are two types of Stem Cells in Bone Marrow namely, Mesenchymal Stem Cells(MSC) and Hemopoietic Stem Cells(HSC). The MSCs are used in regeneration of chondrocytes, bones, muscles, cartilages etc. While HSCs may be classified into long term renewing HSCs and short term renewing HSCs. Both the cells are multipotent and give rise to progenitors- CLP (Common Lymphoid Progenitor) and CMP (Common Myeloid Progenitor) from which various cells like B-cells, T-cells, Dendrites, Natural Killer cells, RBCs, Platelets are formed as shown in Fig-3[5]

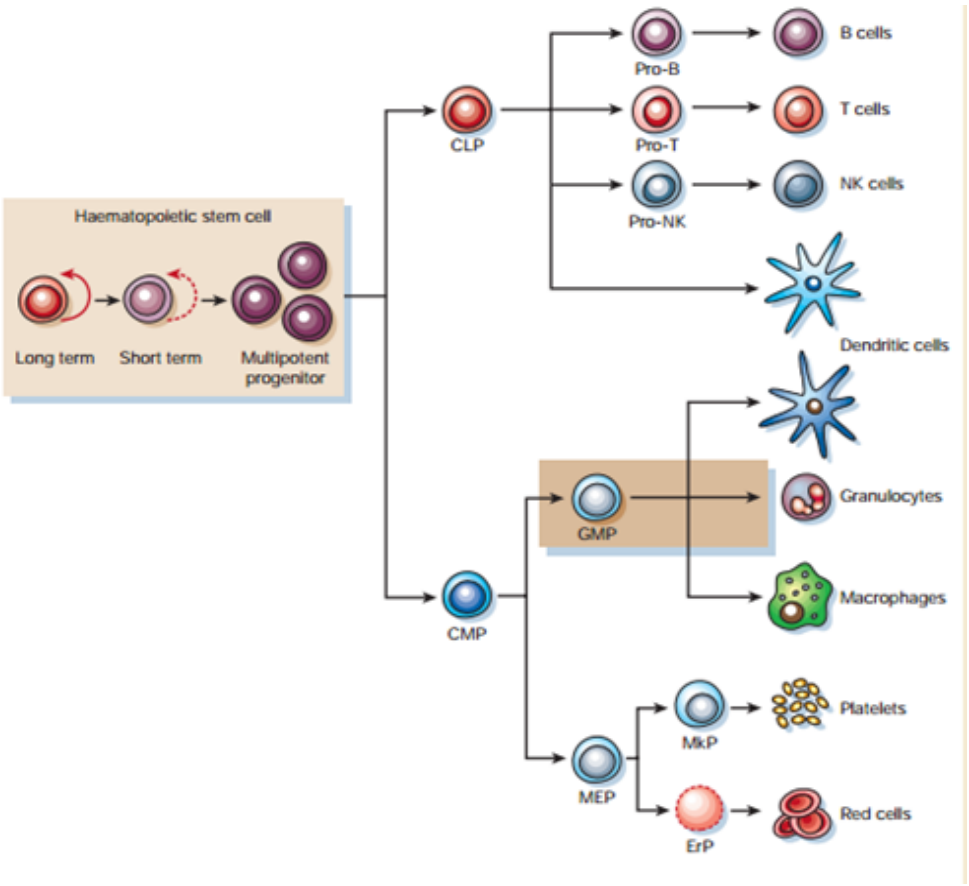

Fig.3: Explaining the formation of various cells from Hemopoietic Stem Cells of Bone Marrow.

It was reported that Limbal Stem Cell Deficiency (LSCD) leads to severe ocular disturbances resulting in loss of vision. The common and successful treatment for LSCD is the transplantation of epithelial cell sheets from a healthy individual. However studies are being carried out to investigate the therapeutic efficiency of murine vibrissiae hair follicle bulge derived stem cell (HFSCs) as a source for reconstruction of epithelial sheet in patients with LSCD. [12]

IMPORTANCE OF STEM CELLS:

Science has developed rapidly in the recent past. Many innovations resulted in the best outcomes in clinical, therapeutic and diagnostic fields. Stem cells, both cells have found use in treating blood disorders, leukemia, CVDs, Nervous disorders etc. Let us deal some of the prime clinical uses of Stem Cells. [1]

In Type I Diabetes in children: Type I Diabetes is an autoimmune disorder characterized by deficiency of Insulin producing cells in pancreas i.e. Beta cells. Pluripotent cells capable of differentiating into beta cells can overcome this shortage. Recent investigations revealed the use of MSCs in treating diabetic nephropathy, diabetic cardiomyopathy, diabetic retinopathy, and diabetic polyneuropathy. [9]

Nervous system disorders: The advancement of science somehow failed to understand the complete functioning of Brain! All the literatures, texts, articles we are being presented cover half among the halves. Common CNS disorders include Parkinsonism, a disorder characterized by deficiency of Dopamine, Alzheimer's disease where the production of certain neurotransmitter dies out. Pluripotent cells which can generate a new nerve fiber restoring its function may be used for treatment. Studies showed that Nestin, a

filament protein is essential for self renewal of Neural Stem Cells (NSCs). [14]

Immunodeficiency disorders: 'Bubble boy', 'Wiskott-Aldrich syndrome' is the most common combined Immunodeficiency disorders. Transplantation of stem cells reconstituted with the responsible gene can nullify the effect leading to normalization of individual covering an average life span.

Bone and Cartilage diseases: Mesenchymal Stem Cells (MSC) can be used in treating diseases relating bone and cartilage. Now a day's MSCs are used in severe cases of osteoporosis where they are used in osteoporosis, osteoarthritis and in filling large gaps after fractures and surgeries.

Cancers: Particular cases like Bone Marrow Cancers may be best treated by 'properly differentiated stem cells' which can restore normal functioning of bone marrow.

Testing New Drugs: Stem cells grown in the laboratory may be useful for testing drugs and chemicals rather than using animals. It is also safer, cheaper and more ethically acceptable.

Screening Toxins: Stem cells are useful for screening potential toxins in substances such as pesticides before they are used in the environment.

Testing Gene Therapy Methods: Stem cells are useful in the development of new methods of gene therapy which help people suffering from genetic illnesses.

Immunosuppressive property: Studies on MSCs have shown dose dependant ability to suppress lymphocyte proliferation in response to allogenic or xenogenic antigens. [10]

Antibacterial activity: MSCs have found to treat sepsis caused by pathogenic bacteria. Human MSCs isolated from bone marrow were grown on Conditioned Medium and tested on Gram negative bacteria (*E.coli and P.aerugenosa*) and Gram positive (*S.aureus*). MSCs have shown significant results and this inhibition was attributed to expression of a protein LL-37 in MSCs. [15]

Spinal Cord Injury: Mesenchymal Stem Cells from bone marrow modify the inflammatory environment reducing the effect of inhibitory scar tissue in the spinal cord injuries, providing a healthy environment for axonal extension resulting in a better therapy for CNS injured patients. [17]

PREPARATION AND CRYOPRESEVATION OF MSCs:

Patients in Nara Medical University along with Tissue Engineering Research Center came up with an interesting approach in isolating Mesenchymal Stem Cells used in hard tissue regeneration. [3]

1. 3ml of fresh marrow cells were isolated from anterior iliac crest and placed in tube containing 3ml of 10U/ml heparinized Phosphate buffer saline.
2. The tube was then set to centrifugation for 10mins at 4^0C. The supernatant containing the fat layer was discarded. The mono nuclear cells at the interface were collected and strained through 100µm nylon cell and washed twice with PBS containing 0.5% HSA (Human Serum Albumin) [4]
3. The residue was then equally distributed in two T-75 flasks. 15ml of Eagle's Minimum Essential medium alpha containing 15% of fetal bovine serum and antibiotics was added to it.
4. The flasks were then cultured in a "humidified atmosphere" of 95% air and 5% CO2 at 37^0C.
5. After 2-3 days of incubation, 13ml of fresh medium was added. The medium must be changed two-three times a week and the floating cells like RBCs were removed each time.
6. After 7-11 days of cultivation, the adherent cells were released from the flasks using 0.05% trypsin or 0.53mM EDTA.
7. The cells were centrifuged to concentrate the cells and suspended in Cell Banker. The cells are cryopreserved at -80^0C before use.

PRESERVATION OF STEM CELLS:

In the recent times, application of Stem Cells in various arenas has gained prominence. With the advent of Stem cell technology treatment to many incurable diseases like Blood Cancer are now available. At the same time there is real difficulty in procuring these Stem Cells since

their preservation needs utmost attention. The safety principle should remain viable until use. Development of 'Cryopreservation protocol' includes following steps: [2]

- Pre-freeze processing;
- Introduction of a cryopreservation solution;
- Freezing protocol;
- Storage conditions;
- Thawing conditions and
- Post thaw assessment.

STEM CELL BANKS IN INDIA:

Some major Stem Cell Banks in India include

- Cryobanks International India Pvt Ltd http://www.cryobanksindia.com/
- LifeCell International - India's first cord blood bank www.lifecellinternational.com/
- Babycell Cord Cell Bankin- www.babycell.in/
- Relicord- Cord Blood Banking Services by Reliance Life sciences http://www.relicord.com/
- Stemade- Dental Stem Cell Banking- http://www.stemade.com/
- Jeevan Stem Cell Bank, Chennai- http://www.jeevan.org/stemcell/index.html
- Stem One Biologics- http://www.stemone.co.in/

PRESERVATION COST OF STEM CELL IN INDIA:

With the bumping awareness of Stem Cells in India, many Stem Cell banks cropped up across the country. While the price for preservation is sky touching for a layman. For example, the cost of Umbilical cord cell preservation in India ranges from Rs.79, 000-Rs.1, 19,000. [18]

SUMMARY:

A stem cell is a special kind of cell that has a unique capacity to renew itself and to give rise to specialized cell types. Stem cells which do not raise any ethical questions are available from adults and children, umbilical cord blood, and even placentas. From various body tissues, including blood, bone, muscle, brain, fat, nasal/sinus, skin, and even baby teeth have obtained success in present research, more frequently bone marrow. Obtaining stem cells from umbilical cord blood is showing great promise in finding treatments for some cancers, including leukemia. Stem cells are undifferentiated cells that can become various specialized cells needed by specific organs of the body. There is significant interest in finding ways to use stem cells to repair diseases that originate in the brain, heart, liver, lungs, spinal cord, immune deficiencies, etc including diseases like Parkinson's, diabetes, heart diseases, spinal cord injuries, cancer, some birth defects, etc. Hence it is proved that the 'health' future of kids is in parent's hands by preserving their stem cells by a specialized process "cryopreservation" and storing them in Stem Cell Banks.

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FURTHER READING:

URL	Description
http://www.closerlookatstemcells.org/Stem_Cell_Types.htm	This site is maintained by International Society for Stem Cell Research (ISSCR). It gives concise information regarding Stem Cells.
http://www.medicalnewstoday.com/info/stem_cell/	This link is a generalized discussion on various types of Stem Cells.
http://www.lifecellinternational.com/stem-cell-banking-affordable.aspx	This site is maintained by Life Cell International Ltd. It gives information about storage of cord stem cells, price in India etc.

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