A miracle cell: Stem Cell- Friend or Foe? Review

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Profile:
Dr. Rashmi K Ambasta finished her Masters from Banaras Hindu University (BHU), Varanasi. She has qualified national level exam for BHU RET-JRF, ASRB-lecturership, CSIR-JRF, ICMR-JRF. Her PhD was awarded from Johann Wolfgang. Goethe University, Frankfurt am Main, Germany. During her PhD she received the scholarship from DFG BAT IIA/2 staff position. After finishing her PhD in 2004, she went to Boston, USA for her postdoctoral training. She has done two postdoctoral trainings in Boston. First one was in Boston Biomedical Research Institute (BBRI) in the field of cancer progenitor cells and second in Caritas St. Elizabeth Medical Centre in the field of Stem cell. Both the postdoctoral trainings were sponsored by NIH, USA. Further, she has been selected for Centenary Research Associate (RA) fellowship from Indian Institute of Science (IISc), Bangalore, India. Later she moved to India as an Assistant Professor in VIT University, Vellore, India. She was soon promoted to the post of Associate Professor in VITU, Vellore, India. During her four year tenure in VITU, she established independent lab and research facility, where several research students were guided under her guidance. Most of students trained with her went abroad for their further studies. In VIT, she was recognized for her excellent teaching and research in the form of award from VIT University. She has received DST Fast track award for young scientist from SERB-DST, Government of India for her work on cancer research. Dr. Ambasta was also one of the finalists for DBT-Innovative Young Biotechnologist Award (IYBA) 2010. Dr. Ambasta worked at Delhi Technology University (Former Delhi College of Engineering), Delhi as a Guest faculty. She is an active member of Society for Biotechnologists (SBTI), Society for Neurochemistry India (SNCI), Indian Association for Cancer Research (IACR) and ISSCR(International society for stem cell research). She was one of the finalists for DBT Women scientist award for her work on diabetes and stem cell from DBT-Bio-CARE grant, Government of India. She has published sixteen international/national research/ review article and several conference abstract. Her high impact research work has been heavily appreciated and cited all over the world. Her current and future research interest is in the field of cancer drug targeting and diabetes therapy using regenerative medicine.

Abstract:

Regenerative medicine is a promising field and it is due to identification of a miracle cell, i.e stem cell. Currently, stem cell has elicited interest and excitement for therapeutical purpose. Stem cell can be characterized as cells that can self-renew and differentiate into several lineage. Stem cell has been isolated from several types of tissue in the body and it has immense proliferation and differentiation capacity. There are different types of stem cell and characterization of each is important for its application in regenerative medicine. Recently, we have seen an information explosion in the area of stem cell research. Stem cells are likely to revolutionize the entire health care system. The moment has certainly arrived for all of us to introduce ourselves with the following: what are stem cells, their characteristics, their potential applications, current research translating to therapy, and possible barriers of its application. Evidences from pioneering studies have proved that stem cell can be harnessed to offer a mode of therapy to various life threatening disease. The disease can be cardiovascular disease, diabetes, skin regeneration, bone transplantation, cancer etc. In order to transplant a damaged organ, scientist uses a scaffold along with stem cell to grow an organ in the lab. These transplanted organs however can lead to immune rejection of the organ in some cases and the patient life might collapse. This review takes you on a sojourn of origin, isolation and therapeutic potential of stem cell. It also focuses on various barriers for the application of stem cell therapy.

Keywords: Stem cell, Scaffold, Regenerative medicine

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Introduction:

Stem cells are no more a scientific fiction. Scientific research success has promised a new mode of therapy in the form of a stem cell therapy. The identity of a cell is hidden in its nucleus and i.e its DNA. The DNA characterizes a cell and differentiates one cell from another. Stem cell is one such cell, which might have same DNA but the expression of DNA is unique in different cells. The final product of expression of DNA can be a protein. The millennium of proteomics has arrived which illuminates us with different protein in stem cell. The expression of protein is regulated by signaling pathway. Therefore, it will be right to state that signaling pathway is unique in stem cell.

The journey of science has revealed and understood that the secret of life lies in the "complicated signaling network within the cell". In our attempt to understand the signalling process, we have realized that scientific discoveries in cellular and molecular biology have truly revolutionized our collective understanding of the biological processes that could greatly effect and dramatically change our lives in the future. In the new era, where biotechnology have replaced traditional biology, chemistry, bio-physics and bio-chemistry, we are exploring "cell therapy for cellular problems". Therefore, identification of signaling process in each stem cell is important, so that stem cell signaling can be controlled and regulated.

Although stem cell technology is just emerging, the regeneration of body parts is hardly a new concept. The story for regeneration reminds us about hydra and sea star regenerating arms, a lizard could grow back the lost tip of its tail etc. Since then, there have been slow and steady attempts at understanding the regenerative capabilities of human being. Stem cells are likely to revolutionize the entire health care system. The peak time has certainly arrived for all of us to introduce ourselves with the following: what are stem cells, their characteristics, their potential applications, current research translating to therapy, and possible barriers of its application.

Stem Cell: These cells are specialized cells with ability to self renew and differentiate into one or more specialized types for tissue repair. In case of an injury, stem cell self renews, divides and give rise to daughter stem cell and progenitor cell. A progenitor cell is an intermediate cell type formed before complete differentiated stage. Progenitor cells are committed to differentiation for a particular lineage. There are different types of stem cells like, embryonic stem cell (ESC) and adult/somatic stem cell. Adult stem cell can be further classified into HSC (Hemopoetic stem cell) and MSC (Mesenchymal stem cell).

Embryonic stem cell: are derived from 2-11 day old blastocysts. These cells have the highest potential to regenerate. However there are moral, ethical and scientific concerns regarding use to these miracle cells. Even scientists are worried about teratoma(cancer) formation from these cells. Therefore, use of ESC cells[1] has been restricted in research these days.

Adult stem cells: HSCs are obtained from cord blood or peripheral blood. MSCs reside in bone marrow stem cells (BMSCc), limbal stem cells, hepatic stem cells, dermal stem cells, etc. Adult stem cell are undifferentiated cells found throughout the body after development that can replenish damaged cells via its self renewal capacity. Plasticity of stem cells can be governed by its capacity to transdifferentiate to lineage of its choice. The therapeutic potential of stem cell is due to its unique ability to be harvested from the patients.

Source of stem cell:

Stem cells can be derived from either peripheral blood, cord blood, bone marrow, or any adult tissue transported in the right medium to the laboratory. It is centrifuged, trypsinized, and propagated under ideal conditions and stored in the cell bank. The cell bank is further passaged to yield colonies of stem cells, given the right inductive signals using appropriate growth factors to allow them to differentiate into required cell types. These are injected or implanted into a patient as cell-based therapy. Homing will ensure that the stem cells reach the site of injury/tissues.

Below you will find the detailed protocol for isolation of MSC from BMSCs:

Isolation of stem cell:

Animal bones are either crushed or flushed to flush out the bone marrow cells and then washed several times. These cells are treated with ammonium chloride to get rid of RBC. Then separted on ficoll gradient and then bone marrow mononuclear cells are isolated according to Asahara protocol[2-4]. The bone marrow mononuclear cells can be grown in conditioned medium for MSC for MSC cells to grow and divide.
Bone marrow is a complex tissue containing stem cells for hematopoietic cells and stem cells that are precursors of non-hematopoietic tissues. The precursors of non-hematopoietic tissues are capable of serving as a feeder layer that supports hematopoietic stem cell growth, self-renewing without differentiation, and becoming one of a number of phenotypes. They were initially named plastic-adherent cells or colony-forming-unit fibroblasts and subsequently named either marrow stromal cells or mesenchymal stem cells (MSCs). Extensive experimentation has defined conditions for their isolation, propagation, and differentiation in vitro and in vivo.
Bone marrow cells could be collected from bone either by crush or flush method. The cells should be diluted with phosphate buffer saline in the ratio 1:1 and centrifuged at 900g for 10 minutes at room temperature. The washed cells should be resuspended in PBS to a final volume of 10 ml and layered over equal volume of 1.073g/ml of Histopaque solution. After centrifugation at 900 *g* for 30 minutes, the mononuclear cells (MNCs) could be recovered from the gradient interface and washed with PBS. Histopaque fractionated MNCs or non-fractionated bone marrow cells should be suspended in Dulbecco's modified Eagle's medium containing 1 g/l of glucose (DMEM-LG; GIBCO) supplemented with10% fetal bovine serum (FBS; GIBCO), 100 U/ml penicillin,100 µg/ml streptomycin, and 25 µg/ml amphotericin B. All cells should be plated in 20 ml of medium in a culture device. The cultures could be maintained at 37°C in 5% CO2 in air,with an initial medium change at 7 days after initial plating and then medium changes every 3 or 4 days. Once the cells gain more than 80% confluency, they could be recovered with trypsin /EDTA and replated at a ratio of 1:3. The expanded cells could be used for characterization of their renewal capacity and their specific response to a consistent set of surface marker antibodies.
Once the cells has been isolated and characterized, then they can be delivered to the patients.

Methods of cell delivery:
There are two common methods of cell delivery i.e intravenous injection and cell encapsulation system. Intravenous injection (direct delivery of cells) and cell encapsulation systems (indirect delivery of cells using a carrier). The cell encapsulation approach uses a biocompatible, biodegradable material that is seeded with cells and implanted into defective patient in order to regenerate the lost tissue.These cells can be cryopreserved and stored for future use in the stem cell bank.

Characteristics of stem cell: Stem cells can be totipotent(total potential to differentiate into all specialized cells), pluripotent (potential to differentiate into many specialized cells) [5], multipotent(potential to differentiate into more than one lineage). The plasticity[6,7] of an adult stem cell is described as its ability to expand beyond its potential irrespective of the parent cell from which it is derived.

Organ Culture: is the culture of part/whole organs from cells and scaffold(support for organ growth) in the *in vitro* condition so that these organs can replace actual organs in case of damage. Plasma clot, agar gel, raft and grid method are some of the modes of organ culture. In plasma clot drops of plasma and embryo extract are prepared in a watch glass in a moist cotton pad and then piece of tissue is installed on top of plasma clot. The technique has been modified and instead of cotton raft and grids can also be used. In case of agar gel, agar is used for organ culture. The proper growth of organs require right kind of scaffold. Scaffold is a temporary support for the growth of organs. It is very critical to choose the exact type and right material of scaffold.

Scaffold: There are different types of scaffold[8-11] like, alginate, carbon nanotube, titanium with or without hydroxyapetite coating etc. It is important that the scaffolds are biodegradable and does not induces immune system.

Signalling network in stem cell:

Stem cells are undifferentiated cells that can give rise to several lineage. The pluripotent nature of stem cell is governed by several complicated transcriptional network. The signaling process in the embryonic stem cell is tightly regulated by several transcription factors(TFs). In order to understand the transcriptional regulatory network, expression of Nanog, Oct4, Smad1, Sox2, Zfx, c-myc, n-myc and Klf4 etc were studied by scientists. Amongst the thousand of different type of TFs, Oct4-Nanog and Sox2 were identified to play a critical role in maintaining the pluripotent nature of stem cell.

Oct4 (Octamer Binding Transcription Factor-4),

SOX2 (SRY (Sex Determining Region Y) Box-2)

Nanog (Nanog Homeobox)

Besides forming the regulatory circuit, the three core factors Oct4, Nanog and SOX2[12-15] contribute to the hallmark characteristics of ESCs by activation of target genes that encode pluripotency and self-renewal mechanisms and repression of signaling pathways that promote differentiation. In total, 352 genes are bound by Oct4, Nanog and SOX2 simultaneously in undifferentiated Human ESCs, which may be expressed or repressed. These factors are known to play different roles in ES-cell biology. Nanog, Oct4, and Sox2 are the core regulators of mouse ESC pluripotency. Although their basic importance in human ESCs has been demonstrated, the mechanistic functions are not well defined. The mapping of transcription factor binding sites will identify the transcriptional regulatory network that defines human embryonic and other stem cell identity in future.

Application of stem cell for therapy in different kind of disease, a Boon:

Despite of stem cells holding promising candidacy for advanced clinical research, there still are many technical hindrances to overcome before the theoretical promises become reality. Scientific understanding so far indicates that the biggest challenge of cancer and genetic defects amongst the population is due to aberrancies in cell cycle process. Need for an elaborate understanding of molecular pathways and functional genetics is felt to design better strategies for cure. Understanding the signals of turning on and off in genes that control differentiation of stem cells have become crucial.

The field of functional genomics is also closely associated with stem cells. Gossler et al, in 1986 reported use of mouse embryonic cells to produce transgenic animals. This followed with publications in the area of mouse genetics reporting the possibility of tinkering selective genes in embryonic cells. Implication of these findings could lead to introduction of a desired gene in embryonic cells for transgenic mice production, which as well can be transferred to

its progeny. This also entails researchers to study mammalian gene's functioning and expression in mouse via introduction of human histocompatibility genes

Preliminary phases of clinical trials of novel drugs can be done using stem cells such as pluripotent cell lines of human origin. Cancer cells are already known to be used for anti-tumor drug screening. However, to hold any relevance, these trials need to be done under near identical conditions which require precise control of stem cells differentiating into various types of cells later on. Mimicking the same differentiation conditions there and after is the limitation of current research that needs to be overcome. Cell based therapeutics is considered to be the forte for stem cells. Owing to its importance, this particular field is termed as regenerative therapy. If the stem cells could be signaled to differentiate into desired specific cell types, they may hold cure for problems like neurodegenerative disease (Alzheimer's and Parkinson's syndrome), injuries (like spinal injury, burns) and other genetically susceptible disease (like diabetes, rheumatoid arthritis).

To regenerate tissues/organs, the prerequisites are:

- Stem cells
- Signaling molecules – to induce differentiation into the required tissue type
- Immunomodulatory effect
- Biodegradable scaffold
- Scaffold material, design and delivery

Scaffold material to support and harvest cellular proliferation. Once the tissue or organ is ready for transplantation to replace defective organs, then a major challenge has to be addressed and i.e immune-acceptance of the transplanted tissue/organ.

Immune rejection of transplanted stem cell, a threat to life:

Transplant rejection is a major challenge to be addressed before and after transplantation. This happens when the recipient body's immune system rejects the transplanted tissue and destroys them. The first successful transplant was performed in 1954 by Joseph Murray for identical twins. Certain prediction tests can be done to avoid rejection problem. The problem can be solved to some extent by the use of immunosuppressant drug[16]. Major Histocompatibility complex, a cell surface molecule can be correlated with the intensity of transplant rejection. There are different types and degrees of rejection like, acute and chronic. Upon diagnosis of rejection immunosuppressive agents can be given. The antibodies used till date are, monoclonal interleukin receptor, basiliximab, daclizumab, polyclonal T cell antibodies, anti thymocyte globulin, anti-lymphocyte globulin and monoclonal anti-CD20 antibodies rituximab. Improper use of these transplanted tissues[17,18] and immunosuppressive drug[19-21] can be fatal for life.

Conclusion:

Stem cell is a boon in all aspects as it can offer a mode of therapy for the damaged tissues. Tissue cultured in vitro can be transplanted to replace the damaged tissues. Upon transplantation between unrelated patients, sometime transplant rejection cases can originate, which can be life threatening. Bone marrow transplant from the same and related patient can be an alternative to transplant rejection problem. Despite of all odds and solution, cases of transplant rejection are common. Regardless of all failures, success rate also has been reported, establishing the fact that stem is a friend but due to transplant rejection cases it can be fatal and can act as a foe. The demand of the time is to transplant tissues in a planned and organized manner so that transplanted organs can be accepted by the body for normal functioning of life.

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