

Stem Cell Engineering: The New Generation of Cellular Therapeutics

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Abstract

Hematopoietic stem cells (HSCs) supply all blood cells throughout life by making use of their self-renewal and multilineage differentiation capabilities. Over the last few years, transplantation of hematopoietic stem and progenitor cells from mobilized blood stem cells, umbilical cord blood and selected CD34⁺ cells has been used for treatment of patients with hematologic and non-hematologic malignancies. The techniques have become available that allow the extensive proliferation, orderly differentiation, functional activation and gene transfer of hematopoietic stem/progenitor cells in *ex vivo* culture systems. These techniques have now developed to the point at which clinical trials are now underway in a variety of settings for the applications of hematopoietic stem cell transplantation, hematopoietic support after high-dose chemotherapy, immunotherapy of cancers, and gene therapy. The article will discuss the characteristics, detecting assays, surface markers, expansion, orderly differentiation, cell therapy and gene therapy of hematopoietic stem/progenitor cells. Except that, it will also discuss some advanced achievements of stem cell research, such as the multilineage differentiation of marrow-derived mesenchymal stem cells. Some results of different gene expression between stem cells and induced cells by gene chip analysis will be reported.

Cell therapy has emerged as a strategy for the treatment of many human diseases. The aim of cell therapy is to replace, repair or enhance the biological function of damaged tissue or organs. This can be achieved by the transplantation of isolated and characterized cells to a target organ in sufficient number and quality for them to survive long enough to restore function. So to choose appropriate cell for each specific disease is a very important task. Recently, the regenerative potential of adult stem cells has been under intense investigation. Adult stem and progenitor cells possess the capability of self-renewal and differentiation into at least 1 or more mature cell types. Such properties make it possible to use adult stem cells to regenerate damaged or senescent cells throughout life. And it could also circumvent the immunologic problems and ethical issues correlated with embryonic stem cells (ES cells). So adult stem cells offer an ideal cell type for cell therapy.

Our stem cell lab. has carried out stem cell research, especially hematopoietic stem cell (HSC) research, for more than ten years. We have carried out work in the following four aspects:

1. HSC isolation from different sources, *ex vivo* ex-

pansion and orderly differentiation

2. Mesenchymal stem cell (MSC) isolation, induced differentiation *in vitro* and *in vivo*
3. HSC gene therapy
4. Mechanism study related with stem cell development

In fact, cellular transfusion therapies began with mature blood cells, first with whole blood and then evolving to the use of fractionated blood components. The second phase of cellular therapies was stem cell transplantation from bone marrow, mobilized peripheral blood, and umbilical cord blood. Over the last few years, techniques have become available to allow the extensive proliferation and orderly differentiation of hematopoietic stem/progenitor cells (HS/PCs) in *ex vivo* culture systems.

To elucidate the role of recombinant human hematopoietic growth factors (HGFs) for expanding CD34⁺ HS/PCs, these cells were isolated using a high-gradient magnetic cell sorting system (MACS), expanded with individual or combined HGFs (rhFL, SCF, TPO, IL-3, IL-6, GM-CSF, G-CSF and EPO, etc.) in a 21-day li-

quid culture systems. A combination of HGFs increased total nucleated cells more than 500.0~1400.0-fold, hematopoietic progenitor cells by 94.1±28.6-fold and CD34⁺ cells by 24.0±3.8-fold, respectively. With some techniques including the addition of some hematopoietic inhibitors (such as TGF-β, LIF and MIP-1α, IL-8, PF4 of chemokines), the use of stromal cell and continuous perfusion culture, significant expansion of primitive hematopoietic cells (LTC-IC, CD34⁺CD38⁻ cells, CFU-GEMM) has been demonstrated.

CD34⁺ cells were also induced to different committedly to granulocytes, erythrocytes, megakaryocytes, T/B lymphocytes and dendritic cells (DC) using different cytokines combination. Mixed cord blood plasma was used to substitute for fetal bovine serum in the consideration of future clinical use. Differentiated cells were detected by FACS analysis.

We began with MSC research in recent years. MSC is more powerful in its differentiation potential. It can not only contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, adipose and stroma, it can also differentiate into neural cells, which belong to a different germinal layer. In our lab, MSC was isolated from adult and fetus human bone marrow and cord blood. After passaging for 15 times, cell number increased 1.36x10⁷ folds. The cultured MSC comprised a single phenotypic population by FACS analysis of expressed surface antigens, which is positive for CD29, CD44, CD71, CD90 and negative for CD11a, CD11b, CD34, and the leukocyte common antigen CD45. The cells did not differentiate spontaneously during culture expansion.

Using lineage-specific culture conditions, the expanded cells can be induced to differentiate into osteocytes, chondrocytes, adipocytes and neural cells *in vitro*. To test its potential usefulness in clinical, animal model of Parkinson's Disease (PD) was constructed and MSC was injected into the brain of the PD mouse. Differentiated cells originated from donor were detected by fluorescent microscope and *in situ* hybridization. And decreased symptoms in the PD mice was observed. It demonstrated that MSC can differentiate into dopamine-producing neurons *in vivo* under certain microenvironment. In another experiment, rat skin was cut to construct a model of dermal trauma, MSC was implanted onto the collagen membrane to create a dermal substitute, and then the membrane was implanted onto the affected part to reconstitute the derma. Differentiated cells originated from donor were detected using *in situ* hybridization.

As to gene therapy, we have concentrated on transferring HGFs into bone marrow stromal cells to promote HSCs proliferation or protect them from radiation injury. Egr-1 is a radiation-inducible gene, its promoter is very sensitive to radiation. Upon radiation, the promoter can induce the downstream gene(s) to express strongly. Based on these, we inserted the FL gene under the control of Egr-1 promoter, and transfected the plasmid into the bone marrow stromal cell line HFCL. It demonstrated that FL could express in response to radiation, protect the co-cultured CD34⁺ cells from radiation injury, and

its expression reached peak 16 hour after radiation, then began to decrease, so it won't interfere with the normal microenvironment except for the protection effect. We have also transfected hGM-CSF into the same stromal cell line to observe its function in promoting HSC proliferation.

The suicide gene system of HSV-tk/GCV was studied for its function in killing tumor cells by apoptosis and enhanced DC function was identified after exposing to the apoptotic tumor cells. It suggested that HSV-tk/GCV system, combined with DC-based cell therapy would be a promising therapeutic strategy for tumor.

The last aspect we have involved in is mechanism study related with stem cell development. Using suppression subtractive hybridization (SSH) technique, combined with the gene chip as a powerful screening method, the profiles of different gene expression for fetus MSC and adult MSC, CD34⁺ HSC and CD34⁺ HSC, fetus kidney and adult kidney, fetus muscle and adult muscle were identified. And some interesting genes are under intense investigation.

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