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FUNCTIONAL CELL-PROLIFERATION AND DIFFERENTIATION BY SYSTEM MODELING FOR CELL THERAPY

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Abstract: ***When primary-cells including non-modified and modified human cells which are used to produce a special substance are infused into patients, it would be defined as cell therapy. A good cell performance with its good proliferation model for cell engineering and cell therapy should maintain its characterization and efficacy with ethical acceptation and safe application. Because efficacy of cell therapy maybe will be decreased in vivo special microenvironment after infusion, moreover because safety of cell therapy would be challenged by DNA genetically modified human cell, a functional induction/inhibition of some genes expressions used in the primary-cell growth without DNA genetic modification has been increasingly reported. Here, the cell therapy of cell engineering based on system modeling for a special function and/or still retaining a special function in vivo microenvironment is called as functional cell-therapy. Nowadays, following research and development (R&D) of primary-cell engineering techniques and system modeling with this computational simulation performance, the novel techniques of primary-cell culture based on system biology will be progressively studied for stem cell which scientists designed and induced into special functional cells for regeneration medicine or for quiescent T-cell which oncologists apply for personalized immunotherapy for tumor disease.***

Key words: Primary cell culture, cell therapy, T-cell, stem cell, genomic analysis, system modeling, quantitative pathway, gene expression signature.

I. INTRODUCTION

Cells that are cultured directly from clinical specimens are known as primary cells[1]. The primary cells isolated from living tissues can be cultured *ex vivo* or *in vivo* environments. The primary-cell culture technique can be used in stem cells, lymphocytes, cancer stem cell and so on[2]. Accompanying the research and development (R&D) of stem cells for regenerative medicine and application of leukocytes (including T-cell, macrophage and other leukocytes) for adoptive cell immunotherapy to tumor disease and erythroid cell for different anemia as well, primary cell culture technique regarding stem cells and leukocytes harvested from clinical specimens can play an increasingly important role in modern cell therapy[3]. Although *ex vivo* differentiation of stem cells into some designed terminal cells has a well-established model by cell genetic modification such as induced pluripotent stem cells (iPS cells or iPSCs), these safety challenge of genetically modified cells has obviously decelerated their clinical application[4]. Moreover, even if non-modified human cells would be expanded very well *ex vivo*, efficacy of the non-modified human cells would be decreased *in vivo* special microenvironment after infusion back to patients. Fortunately, when human genome was decoded after 2003, it will give scientists and physicians a novel idea to develop some new models to increase or decrease gene expression during the primary-cell culture[5]. For example, many genomic databases from primary cells have been established in Gene Expression Omnibus (GEO), such as database from stem cell into different terminal cells or the database from T-cell, macrophages and leukocytes. More importantly, some gene regulations related with network and bioinformatics platform using system modeling have been extensively reported. Based on the R&D of cell proliferation and differentiation for primary cell culture, relied on gene expression related to system biology, it is good time to set up some new strategies for functional cell-proliferation contributing the primary-cell engineering and functional cell-therapy for clinical application.

II. CONCEPT FOR FUNCTIONAL CELL PROLIFERATION WITH ITS CELL THERAPY

The history of cell therapy experiences through three stages in more than one hundred years according to R&D of primary-cell culture techniques and cell therapy: cellular therapy, cell therapy and functional cell therapy.

Cellular therapy-also called as live cell therapy, early refers to various procedures in which cells or tissue from animal embryos, fetuses or organs is injected into human beings. The first recorded attempt at cellular therapy in 1912 when German physicians attempted to treat hypothyroid children using thyroid cells[6]. In 1970, Dr. Kühnaubegan used

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cellular therapy to treat cancer patients in Tijuana, Mexico[7]. It is also claimed to build the immune system and help patients with Down's syndrome, Alzheimer's and AIDS[8]. In 1987, Australian researchers reported a result that studied children with Down's syndrome who received cellular therapy with similar children who did not. The study demonstrated no any evidence between two groups[9]. First malpractice case involving cellular therapy was filed for Dr. Cousens for a *Clostridium perfringens* infection (gas gangrene)[10]. After then, live cell therapy from animal sources into human beings has been almost completely discontinued.

Cell therapy-the modern cellular therapy, now called as cell therapy, early applied for bone marrow transplantation[11]. The first breakthrough was performed by Dr. Dausset in 1952, who won Nobel Prize in Physiology/Medicine (1980) by discovering an HLA antigen for an immunological rejection and thus set up allogeneic bone marrow transplantation by HLA match test[12]. Now, cell therapy broadly describes the process of introducing a group of human cells such as stem cell, T-cell, macrophage or other leukocyte into patients. Recently, because stem cells have been tremendously researched in clinical application, now stem cell therapy, narrowly and directly, is called as cell therapy[13]. In the manual, we still utilize a broad terminology, or cell therapy including stem cell, T-cell, macrophage and leukocyte. As we all know, cell therapy can be divided into Autologous Cell Therapy and Allogeneic Cell Therapy. Autologous cell therapy should be harvested from a patient and then introduced back into the same patient[14]. This autologous method is first priority due to non-immunologic matching assay requirement. Allogeneic cell therapy approach involves the harvesting of cells from one or universal donors followed by large scale expansion after immunologic matching[15]. This allogeneic approach utilizes cell types that do not elicit immune responses on implantation therefore have the potential to treat hundreds of patients from a single manufactured lot of cells. The allogeneic cell therapy adapts to the manufacturing because the product can be readily available in a cell bank[16].

Functional Cell Therapy-if a cell needs to produce a special substance, genetically modified human cells is routinely used to produce the special substance, called as cell-based gene therapy (Fig. 1A)[17].This kind of cell therapy has obvious challenges in clinical application. For example, eighteen years ago, we have used retroviral vector carrying TNF-α gene to transfect into tumor infiltrating lymphocytes (TIL) to treat a patient with advanced liver tumor. We need very prudent performance due to safe reason for clinical patient treatment. Currently, some challenges are still remained in genetically modified human cells for cell therapy: (A) long-term culture after genetically modified primary cell is a great challenge to reach enough cell number for therapeutic efficacy; (B) safety question cannot be addressed by transfected stem cell or immune-cells[18]. After 2003, decoded human gnomic profiles give scientists and physicians a new platform to develop some new strategies. Up to date, workflows from genomic profile to system modeling with the biotechnology and cell engineering have been increasingly reported[19]. For example, while data of **genomic profile** are observed in a cell, several enzymatic reactions, called as **pathways,** are discovered to keep the cell function. Because of numerous distinct pathways co-exist within cell, this collection of pathways is called as **network**. Computational reconstructing network (or simulation) allows an in-depth insight into the molecular mechanisms called as **system modeling** from the network. These models correlate the genome with molecular mechanisms and compound action. System modeling also can identify significant genes linking different compounds by a quantitative integrated network and this mathematical topology model (significant genes previously called as therapeutic targeting identification, TI, and now called as **gene expression signature**, GES)[20-21]. Hitherto, this knowledge has been increasingly applied to create novel biotechnology and **cell engineering**. Here, the cell using the novel biotechnology to produce/induce several proteins is called as **functional cells**. The cell therapy with a special function and/or function in special microenvironment is called as **functional cell therapy**(Fig.1B). Nowadays, based on known system biology with this analysis of targeting compounds[22], it is a good time to develop functional cell-proliferation and differentiation in the 21st century cell-therapy.

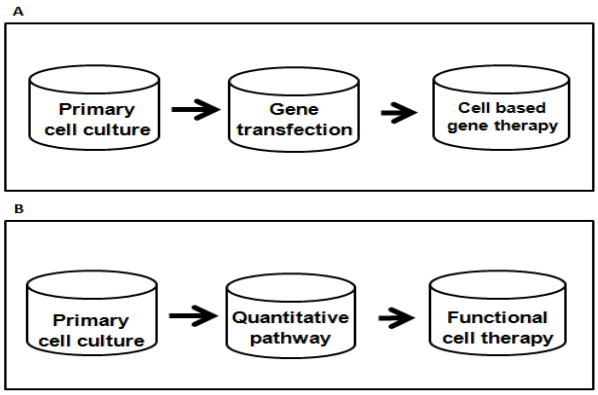


Fig. 1 Strategies of genetically-modified cell therapy (A) and functional cell therapy by therapeutic targeting identification with compound or growth factor inducing (B)

III. CELL BIOPROCESSING RELATED WITH FUNCTIONAL CELL-PROLIFERATION

Cell bioprocessing is a process from primary cell isolation to expansion until the expanded cell harvest, which belongs to cell engineering process including products quality as that of biopharmaceutical drugs and clinical/commercial quantities of therapeutic cells[23]. Therapeutic cell manufacturing processes can be divided into upstream processes and downstream processes. The upstream process is defined as the entire process from early cell isolation and cultivation until final harvest including the availability of GMP grade fetal bovine serum[24]. Downstream Bioprocessing focuses on the final harvest with their subsequent process such as concentration of the harvested cells, clarification of the harvested cells, formulation of the cells into an appropriate solution for bio-preservation and filling cells into final container for cryopreservation, storage and delivery to clinics[25]. When the cell therapy is needed for a patient, it must be shipped under appropriate conditions to the clinical site, prepared for delivery to the patient and then administered by a trained medical doctor or trained medical

center[26]. We experienced these eighteen years to perform cell bioprocessing by using basic facilities until now using cGMP facilities (current Good Manufacturing Practice). As functional cell-proliferation and differentiation based on system modeling, most of bioprocessing is involved in upstream including inducing and inhibiting genes during culture stage.

IV. CELL THERAPY AND NEW MODEL FOR FUNCTIONAL CELL-THERAPY

Accordingly, there are two main mechanisms by which cells employ therapeutic purposes: a) cells (such as macrophage. T-cell or all leukocyte) have the capacity to directly act or release soluble factors such as cytokines and growth factors which act in self-cleaning in a tumor microenvironment with their retaining viability for several weeks; b) cells (such as stem cell) are subject to the cell engraftment and differentiation replacing damaged tissue.

Leukocyte based-leukocytes can be routinely classified in two main groups: granular leukocytes and non-granular leukocytes, or lymphocyte and monocyte. Up to now, lymphocyte, macrophages/monocyte and granular leukocyte have been all reported to be feasible in cell therapy as Table 1. Because this cell therapy supports immune mechanisms to defense tumor disease, we also call the cell therapy as cell-based immunotherapies or adoptive cell immunotherapy[27].

Table-1 Comparison of cell therapy for tumor disease using leukocyte

Cell types	Names	Animal trial	Human trial	Pros	Cons
T-cell	LAK/DC-CIK	Yes	Yes	Culture easy	Non-specific
				Enough cell number	Middle effect
	NK	Yes	Yes	Culture easy	Non-specific
				Enough cell number	Middle effect
	TIL	Yes	Yes	Higher specific	Difficult culture
				Higher effect	Cell number limitation
Macrophage	TAM1	Yes	Yes	Higher effect	Difficult culture
					Cell number limitation
Leukocyte	TAN1	Yes	Yes	Culture easy	Not-many human data
				Enough cell number	
	CKA	Yes	Initiating	Non-culture	Screening efficacy of donor
				Enough cell number	Not many human data

A. T-cell

In the late 1980s, Rosenberg and his colleagues initially used T cell-based cytotoxic responses to attack cancer cells (called as tumor-infiltrating lymphocyte (TIL) or adoptive T-cell therapy) to treat malignant melanoma, it achieved encouraging clinical results[28]. After more than twenty years development, the cell expansion method has been developed from IL2 induction into anti-CD3 and allo-reactive feeder cells[29]. These T cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anti-cancer activity. Although TIL has successfully reported to treat malignant melanoma and B-cell leukemia/lymphomas with an advantage, higher population CD8+cell by TCR (T-cell receptor) recognizing tumor-Ag associated MHC-I peptide complex[30], TIL therapy has several challenges: A. efficacy of TIL therapy is different from different tumors, for example, most of laboratories reported only efficacy in malignant melanoma and B-cell leukemia/lymphomas[31]; B. cell bioprocessing standard is varied, such as to isolate TIL in different location in tumor mass, varied expand methods and changeable cell number harvest; C. biopharmaceutical and clinical/commercial quantities of therapeutic cells is not easy to set up for different patients and diseases. We have been working for TIL R&D more than twenty years for several hundred clinical patients. In 1993, we discover that efficacy of TIL therapy is related to enzyme digestion and is associated to TIL harvested different location in tumor mass so that we set up criteria method with cold enzyme digestion to isolate TIL from different solid tumors[32]. Our data demonstrated that TILs have very good response to some solid tumors, especially in lung cancer (NSCLC) and liver cancer although brain tumor and kidney cancer still have faced some challenges[33]. Recently, we utilized genomic analysis to study TIL features from liver tumor. Results show that TIL is inhibited in both tumor tissue and tumor-surround microenvironment such as hepatic sinus. We concluded that TIL immune will be influenced by multiple factors, including toxic substances in micro-environments[34].

Genetically modified techniques have been largely developed in T-cells, such as genetic modification of tumor antigen, engineered T cells with higher affinity and engineered T-cell with some special substance by TNF-α, TGF-β, IL-2, IL12 and IFN-γ[35]. In 1995 we successfully utilized retroviral vector to transfect TNF-α for a patient with liver tumor[36]. Although retroviral vectors work very well for genetic modification, the safety of transfected T-cells is still unknown. Due to T-cell efficacy and transfected T-cell safety for clinical application, we begin to study some new strategies by genomic profiles related novel biotechnology and cell engineering. After more than ten years efforts, according to clonal selection theory of lymphocyte, selected lymphocytes response to specific antigens in immune system. Even if TILs are heterogeneous cells, some CD8+cells have already contacted tumor cells in tumor mass so that some TILs should be clonally selected and recognize tumor Ag. In order to set up good system modeling for TIL or TIL CD8+cell, we first used single-cell technique to isolate the T-cells attaching tumor cells. Theoretically, the TIL CD8+cells have been selected by the tumor specific antigen or tumor associated Ag. Following uncovering genomic profiles from TIL CD3+cells and TIL CD8+cells, now we establish system modeling related cell engineering to study induce or inhibit some specific genes[37]. This system modeling can be developed into personalized immunotherapy for patients.

B. Macrophage

Macrophages are a major component of the leukocyte infiltrate in the tumor microenvironment. During the neoplastic progression, macrophage, dendritic and NK cells are attracted into the tumor site and initiate the immune response against tumor cells. They activate and present tumor Ag into T-cell, which are then activated to kill tumor cells[38]. In 1998, culture human macrophages have been achieved in hydrophobic plastic, gas-permeable bags so that this process enables collection of non-adherent macrophages and is well adapted to the safety requirements of cell therapy.

In most clinical trials, macrophages activated by interferon-gamma (IFN-γ) remain unknown. *In vitro*, macrophages are as efficient as monocyte-derived dendritic

cells (MDDCs) in stimulating cytotoxic T lymphocyte (CTL) clones or circulating CTL precursors. However, tumor cells are often capable of escaping the immune function. If the immune surveillance is not sufficient, macrophage in tumor tissue (tumor-associated macrophage, TAM) also contributes to tumor progression by growth factors and neovascularization. After several years researches, TAM has been discovered two groups, M1-macrophages exposed in IFN-γ have antitumor activity and M2-macrophages activated by IL-4 or IL-13 have oriented to tissue repair, tissue remodeling[39]. Most of evidences have demonstrated M1 switch to M2 under local hypoxia, low glucose level and low pH with regulating by CCL families, TGF-β, VEGF, PDGF, and M-CSF. This switch eventually appears tumor dissemination and invasion characteristics[40]. A recent study revealed that activation of macrophages by the infusion of antibodies against CD40 may induce macrophage-mediated tumor regression in both a mouse model for pancreatic cancer and patients with pancreatic cancer[41]. IL-6 stimulates tumor macrophage infiltration in ovarian cancer. This action can be inhibited by the neutralizing anti-IL-6 antibody (siltuximab) in clinical studies[42]. Since TGF-β is responsible for tumor infiltration by macrophages, depletion of TGF-β has been shown to enhance tumor vaccine efficacy[43]. Because CCL2 plays a major role in the recruitment of TAMs, anti-CCL2 would be a blocking step in preventing this recruitment. As all shown above, if we can use system modeling related biotechnology and cell engineering to induce some genes with M1 antitumor mechanism and to inhibit M2 some genes helping-tumor function in tumor microenvironment, functional T-cell therapy *in vitro* inducing M1or *in vitro* inhibiting M2 based on system modeling will be encouraged for future application *in vivo*.

C. Leukocyte

Traditionally, granulocyte mainly functions as response for different infection. Now, a new field emerges in tumor disease, or tumor associated neutrophil (TAN) and CKA (granulocyte with cancer killing activity, CKA)[44], so that cell therapy extends cell based immunotherapy from T-cell and macrophage into granulocyte. The two kinds of granulocytes have been involved in clinical researches. TAN can be divided into two types, TAN1 with an anti-tumor function and TAN2 with a pro-tumorigenic function. Anti-tumor activities of N1 include expression of more immuno-activating cytokines and chemokines, lower levels of arginase and lower levels of TGF-β with capability killing tumor cells *in vitro*[45]. As we know as above, therapeutic targeting identification from quantitative network with its genomic analysis is very good tool to block TGF-β for functional cell therapy *in vitro* and *in vivo*, such as inducing N1 *in vitro* or inhibiting N2 *in vivo* from therapeutic targeting identification. CKA is secondly discovered for some scientists to study granulocytes anti-tumor cell[46]. Some scientists used spontaneous regression/complete resistant (SR/CR) mice to uncover leukocytes infiltration (by innate immunity) into tumor tissue to regress tumor mass. CKA have been uncovered as a group of cells including PMN, NK and other T-cell and macrophages.

Stem-cell based-numerous clinical trials are utilized for stem cells at present, such as adult stem cell (autologous or allogeneic), fetal stem cell, human embryonic stem cells (hESCs) and iPSCs by viral or non-viral transduction with OCT4, KLF4, SOX2, and/or c-MYC[47]. During the last 20 years, adult stem cells (ASC) obtained from different tissues such as blood, bone marrow, fat tissue and skin have been characterized for their functions. Stem cells from cord blood (FSC) were successfully transplanted in 1988, when it was first used to regenerate blood and immune cells on a 6-year-old boy suffering from Fanconi anemia[48]. In tumor research field, a successful cord blood transplant was performed on chronic myelogenous leukemia (CML) in 1997[49]. Manufacturing challenges of cell therapy of ASC and FSC mainly focus on cell number. Large numbers of cells (which may exceed 10^6 cells/kg/dose in human beings) must be produced per lot to meet the larger dosage[50]. The cells must be also satisfied the number with maintaining their characterization and efficacy. Because of ASC and FSC number limitation, human embryonic stem cells (hESCs) are emerged in cell therapy stage in 1998, or pluripotent cells derived from the inner cell mass of the blastocyst. They have the ability to "indefinitely" renew themselves and to differentiate into different cell types as an "indefinite" source of therapeutic cells. Due to the ethical controversy associated with the use of embryonic cells, a new stem cell, iPSCs, appeared in development phase of cell therapy. In 2006, Dr. Yamanaka first generated iPSCs from mouse fibroblasts[51]. In the second year, Dr.Thomson reported iPSCs from adult human cells[52]. However, iPSC technique need cell genetic modification; these kinds of cells have a great safety challenge so that physicians take very careful steps into clinical application. In order to overcome this kind of challenge, in 2011, protein-based human iPS cells have emerged so that it would give functional cell therapy based on system modeling a great opportunity in stem cell replacement therapy. Here we will introduce some stem cell based cell therapy and some new ideas for the functional stem cell therapy based on system modeling as well.

A. Neural Stem Cell Therapy

The developments of neural stem cell therapy (NSCs) focus on blindness, stroke, neurological disorders, and cancer disease. In stroke disease, StemCells Inc. has proposed a clinical trial to use human central nervous system-stem cells (HuCNS-SCs) to treat stroke patient. Now it has reached the stage of a first-in-man clinical trial[53]. In neurological disorders and neurodegenerative diseases, after early evidence from a mouse model to be treated by NSC for Alzheimer's and Parkinson's disease, one of cell therapy is Parkinson's disease by using hESCs[54]. Before hESCs can be used to treat PD patients, they must differentiate into midbrain dopamine (mDA) neuron-like cells, an effective model with low risk of side effects has been established. Reprogramming of human somatic cells to a pluripotent state has been achieved. The cell engineering of hiPSCs with embryonic stem cell properties become a powerful tool for biomedical research. As we discuss before, the cell therapy will be faced a safety challenges. In the brain tumor diseases, a report demonstrated that NSCs can track tumor cells and deliver cytosine deaminase, which converts a non-toxic pro-drug into a chemotherapeutic agent. This treatment has a very strong advantage to specifically target and kill the cancer cells. The clinical trial for such a treatment is performed for

recurrent brain tumors[55]. Although functional cell-therapy is just beginning for neural stem cell therapy (NSCs)[19], the cell model of NSCs would make great contributions for stem cell replacement therapy.

B. Mesenchymal Stem Cell Therapy

Mesenchymal Stem Cell Therapy (MSCs) is involved in immune modulatory treatment and multi-potent proliferation for regeneration, such as bone/cartilage regeneration, skeletal/myocardium regeneration. In immune-modulatory therapy, MSC therapy for graft-versus-host disease (GVHD) and Crohn's disease has been successfully reported[56]. For bone regeneration, scientists have successfully used transplanted bone marrow cells from HLA-identical siblings to patient suffering from osteogenesis imperfecta (OI) to discover normal osteoblast formation[57]. A second clinical trial for bone formation is that allogeneic fetal MSCs transplanted in utero in patients with severe OI can differentiate fetal MSCs into bone in a human fetus[58]. For cartilage regeneration, Medipost Co. Ltd is currently studying an efficiency and safety by using fetal stem cell to treat articular cartilage defect in old patients[59]. Moreover, cardiomyocyte regeneration is also very popular topics. Autologous BM MSCs introduced into myocardial infarction (MI) have resulted in significant reduction of damaged regions. Clinical trials for treatment of acute MI are underway by Osiris Therapeutics. Although functional cell-therapy is still vacuity for MSCs, concept of MSCs would give functional cell therapy a great opportunity to study.

C. Hematopoietic Stem Cell Therapy

Hematopoietic Stem Cells (HSCs) are earliest stem cell therapy to treat blood and immune disorders. Now there are three types of HSC to use in blood and immune disorders since 1952: syngeneic (identical twins), autologous, and allogeneic transplants. Enormous syngeneic marrow infusion and allogeneic marrow grafting were largely performed in clinical patients. HSCs therapy can effectively reconstitute damaged blood-forming cells and immune system after high-dose chemotherapy. To lower the risks of transplant by graft rejection and GVHD, allogeneic HSCT must satisfy compatibility at the HLA loci. Mismatch of HLA loci would result in treatment-related mortality and higher risk of acute GVHD. In addition to BM derived HSCs, the use of alternative sources such as stem cell from umbilical cord blood (UCB) and peripheral blood stem cells (PBSCs) has been increasingly reported. Although the time of engraftment is longer and graft failure rate is higher, the use of UCB requires less stringent HLA loci matching.

Because hiPSCs have great challenges in ethical problem and safety in clinical application, several laboratories are going to set up non-DNA genetic modification techniques to address the question. In 2011, protein-based human iPS cells efficiently generating functional dopamine neurons was reported in rat model of Parkinson disease[60]. It would bring a great opportunity in stem cell replacement therapy based on system modeling, As Fig.1B demonstrated, a new field will emerge in the near future for functional stem cell therapy by using system modeling.

V. MANAGEMENT, MANUFACTURING AND FUTURE OF CELL THERAPY

Management of cell therapy

The genetic manipulation of cell adoptive immunotherapy *in vitro* is much safer than that of stem cell so that most of management focuses on stem cell therapy, especially hESC cell therapy. According to current management system with their files, an international coordination organization, International Society for Stem Cell Research (ISSCR), has established some guidelines, for examples, informed consent should have privileges to obtain from fully autonomous individuals information and donor privacy must be maintained at all times. The ISSCR also requires regulatory reviews to be conducted before any embryo cells are obtained. Scientists who want to use hESC from human embryos are required to submit proposals to a stem cell research oversight (SCRO) for approval and give sound scientific explanation for the need. The ISSCR only allow that qualified researchers can use hESCs with their document management for their cell line banking and distribution. International efforts known as the International Stem Cell Banking Initiative (ISCBI) have also been set up similar standards in cell banking and storage distribution. Because functional cell-proliferation based on system modeling is not involved in vector-based genetic modification, it will give patients very good news with very safe performance for cell therapy.

Manufacturing

According to current publications[61], therapeutic cell manufacturing is more interesting in hESC because hESC can be easily managed at large scale of manufacturing processes with drug tests and toxic screening although other cells such as ASC, FSC and hiPSC have been largely studied. For examples, myocardiocytes from hESC are researched *in vitro* models to test drug responses; Hepatocytes derived hESC are also useful models in the preclinical stages of drug discovery; dopamine-producing cells derived hESC into neurons by *in vitro* model could be used in Parkinson's disease; some laboratories were able to differentiate hESC into insulin producing cells to study control diabetes. Now several companies have been performed clinical trials for human diseases. For examples, Geron Company in California has been performing human subjects to treat spinal cord injuries by hESC derived Oligodendrocytes (GRNOPC1) and Advanced Cell Technologies (ACT) has been processing clinical trials in human subjects using hESC derived retinal epithelial pigment (RPE) cells for Stargadt's Macular Dystrophy (SMD) and age related macular degeneration. Geron Company has been developing several hESC-derived products to treat spinal cord injury, cardiac and other chronic degenerative diseases. Currently, GRNOPC1, one of their products for spinal cord injury are ologodendrocyte progenitor cells differentiated from hESCs. In this stem cell process, several growth factors are applied to induce hESCs into oligodendrocyte precursors. The precursors can repair the myelin insulation around the nerve cells so that the nerve cells may transmit signals. Upon injection of GRNOPC1 into the spinal cord injury site, restoration of motor and load-bearing functions were successfully improved in rat models. In 2009, the Food and Drug Administration (FDA) granted

Geron clearance for a phase I clinical trial and also conducted them to further assess the safety and effectiveness of GRNOPC1 in patients affected in the thoracic and cervical regions. Advanced Cell Technology (ACT) is the second company in the United States to begin clinical trials on their hESC-based therapy targeting dry acute macular degeneration (AMD). ACT aims provide treatment to the disease via transplantation of hESC-derived RPE cells. Significant improvements to visual recovery were observed without any adverse effects in rat models. Moreover, ACT also utilized a multi-center trial to determine the safety and tolerability of the RPE cells transplanted into patients with dry AMD. ACT results have demonstrated that these RPE cells will be able to improve visual acuity in patients. Although hiPSC and hESC have been reported to clinical trial for few patients, large numbers of hiPSC and hESC reports are focused on animal models or drug screening so that eventually, the functional cell proliferation based on system modeling would increase a great opportunity to clinical application.

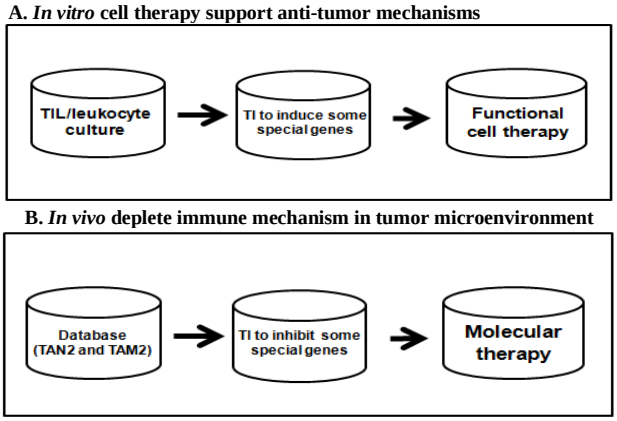


Fig. 2 Strategies of adoptive cell immunotherapy for functional cell therapy in special tumor microenvironment

Future Potential

In cell engineering for immune-cell therapy, the cells have the capacity to directly-act or release soluble factors to interact with other cells, such as cell adoptive immunotherapy. On the other hand, cell engineering for stem cells, called as stem cells replacement therapy of the target organ, has begun to clinical trial such as stem cell into neuron to treat spinal cord injury and AMD although replacement of complete organ structures is still in early period. The near future of cell therapy may still more focus on the immune cells for tumor disease and autoimmune diseases as well as stem cell (such as NSC, MSC and HSC for neuron replacement, myocardiocyte replacement and stem cell transplantation, respectively). Although genetically modified cell therapy has successfully involved in treatment of several clinical diseases, it is related with viral or non-viral vector to genetic manipulation *in vitro* so that these cells are required a strictly regulated environment such as the Food and Drug Administration (FDA) to ensure the highest requirement of clinical safety. As the manual mentioned, cell engineering based on system modeling will give us a good tool to work in clinical adoptive immunotherapy and cell replacement therapy. At least four advantages are involved in functional cell therapy: (a) after *in vivo* infusion, the primary cell can support a special function. For example, as Fig. 2 and Fig. 3 demonstrated when special functional TIL inducing some specific genes (such as increasing TNF-α), it will play a special function to perform cell therapy for cancer patients.

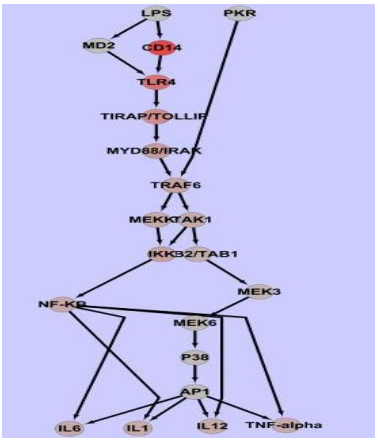
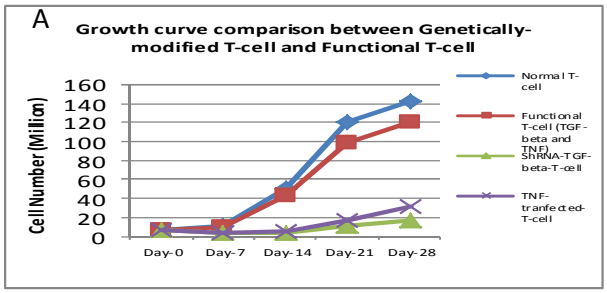


Fig. 3 Strategies of functional cell therapy by quantitative network to identify and induce TNF-α expression

(b) Because of e*x vivo* bioprocessing without any genetic modification performance, primary cell can keep very good proliferation rate and very good differentiation situation as Fig. 4A so that it can support enough cell to treat patients. Moreover, (c) because of depleting some genes (such as inhibiting TGF-β as Fig. 4B, it should more regress tumor disease by inhibiting TAM2 and TAN2 *in vivo* microenvironment mechanism. Furthermost, (d) because of e*x vivo* bioprocessing without any viral or non-viral vector genetic-modification performance, the functional primary cell can support much safer cells than genetically-modified cells to treat patients.



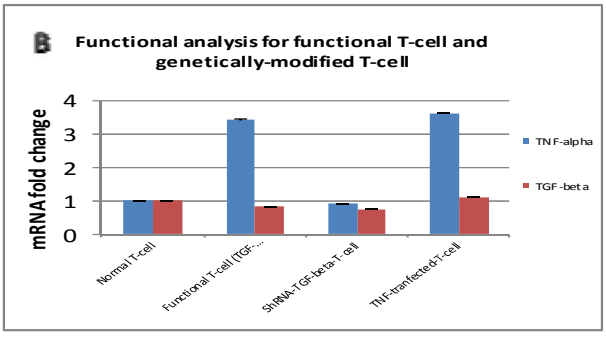


Fig. 4 Growth curve difference among ShRNA-TGF-β T-cell, Retroviral vector-TNF-α T-cell, normal TIL and functional T-cell (A) and gene expression fold change among genetically-modified-T-cell and Functional T-cell (B)

Author's contributions

BL conceived, designed and guided the work and edited the manuscript. BL, HH, JD, LMY performed some of their experiments for cell therapy with their study over 20 years and DY performed QA and QC for cell therapy.

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The inclusion of trade names or commercial products in this article was solely for the purpose of providing specific information and does not imply recommendation for their products.

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