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EDITORIAL

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Combining Stem Cells and Genes for Effective Therapeutics

Gene therapy has suffered from skepticism from both the scientific community and the pharmaceutical industry. In addition to the risk of insertional mutagenesis/tumorigenesis, the widespread clinical application of gene therapy is hampered due to inefficient systemic delivery. However, in recent years, new approaches, including stem cell-based gene therapy, have boosted the potential comeback of gene therapy.[1] This theme issue discusses the opportunities and challenges of combining gene and stem cell therapy.

Stem cells possess unique properties of self-renewal and pluripotency in differentiating into diverse specialized cell types. Self-renewal confers the feasibility to manufacture a sufficient number of cells for therapeutic applications, and these expanded cells are robust enough to be genetically manipulated. In addition, there exists a large array of stem cell types such as embryonic stem cells (ESCs) and stem cells derived from adult tissues such as bone marrow, amniotic fluid and adult adipose tissue. Therefore, stem cells may eventually change the modes of administration of gene medicine from direct injection to *ex vivo* manipulation. Implanted stem cells find their targets via an intrinsic mechanism (e.g., homing property and tumor tropism), which presents an advantage over current gene delivery vehicles. A great example is hematopoietic stem cells (HSCs), which have the ability to replenish themselves and differentiate into progenitor cells and mature blood cells. Repopulation of bone marrow as well as blood cells through the infusion of HSCs, which are subjected to *ex vivo* gene correction, has been successfully illustrated to treat inherited diseases in human subjects.[2−4] In this issue, potential clinical application of HSCs in treating autoimmune diseases, such as type 1 diabetes and rheumatoid arthritis, by inducing immune tolerance has been discussed by Alderuccio et al.[5]

On the contrary, tumorigenicity resulting from stem cell-based therapy also remains a concern. As such, a mechanism to remove transplanted cells from the body when necessary is of great advantage. Stem cells are different from conventional therapeutic modalities in that they can integrate into the tissues and become indistinguishable from resident cells. Strikingly, genetic modification can offer an appealing strategy in labeling cells and facilitating *in vivo* tracking as well as elimination of cells that have gone awry.[6] Hence, the marriage between gene therapy and stem cell-based therapy would definitely broaden their applications in clinical settings and offer new therapeutic modalities.

The safety issue is of great concern for both gene therapy and stem cell therapy. Viral vectors have been extensively applied for the *ex vivo* transduction of stem cells, and improving their safety and efficiency is of great interest to the researchers. In this issue, Chen et al. tested the biosafety profile of human mesenchymal stem cells (MSCs) transduced by a hybrid baculovirus vector.[7] The vector was developed by exploiting the FLP/Frt-mediated recombination for circular episome formation, thus conferring the long-term transgene expression without integration into the host genome. Various aspects of the transduced MSCs were examined including viability, proliferation, differentiation, gene integration, karyotype, tumorigenesis-associated genes and *in vivo* tumorigenesis. The authors found that while transduction slightly inhibited proliferation of MSCs, it did not affect their viability or differentiation potential. As opposed to the insertional mutagenesis and tumorigenesis associated with retroviruses and adeno-associated virus (AAV), the hybrid baculovirus was much safer, without inducing tumorigenicity of MSCs in nude mice. In addition, with an objective to improve the safety profile of viral vectors, Avedillo Diez et al. have developed a series of novel gammaretro- and lentiviral vectors with self-inactivating (SIN) configuration for the gene therapy with potential clinical applications in treating Wiskott−Aldrich Syndrome (WAS).[8] SIN vectors have a lowered risk of activating cellular proto-oncogenes. Importantly, these novel vectors were demonstrated to be as effective as the clinically used LTR-driven vector in the transgene expression of WAS protein (WASP) in both WASP deficient murine HSCs and human $CD34^+$ cells as well as in their differentiated myeloid progeny.

The tumor tropism of adult stem cells such as MSCs and neural stem cells (NSCs) confers on them the ability to serve as gene delivery vehicles for targeted cancer therapy. Both targeting specificity and therapeutic efficacy of MSCs and NSCs in preclinical models have been established. More attractive are the induced pluripotent stem cells (iPSCs) due to their ESCs like infinite expansion potential, compared to the limited *ex vivo* expansion capacity of MSCs and NSCs. In addition, iPSCs can be generated from somatic cells, thus conveniently providing fully syngeneic cells for implantation and minimizing potential immunogenicity. In a proof-of-concept study, Lee et al. derived NSCs from mouse iPSCs, transduced them with a baculoviral vector containing the herpes simplex virus thymidine kinase (HSVtk) suicide gene, and utilized them for treatment in a mouse intracranial human glioma xenograft model.[9] The iPSCs-derived NSCs could be maintained *in vitro* for up to at least 6 months, which is very advantageous for therapeutic applications. Importantly, these cells could migrate to tumor tissues upon injection into the striatum contralateral to the tumor site. The suicide gene, HSVtk, phosphorylates ganciclovir (GCV), which in turn exhibits strong bystander effect to interfere with DNA replication, thereby killing tumor cells. Thus, genetic modification of these iPSC derived NSCs with HSVtk transgene inhibited tumor growth upon *in vivo* transplantation. In another study, by Ahmed et al., the performance of NSCs and MSCs on the delivery of an oncolytic adenovirus in a rodent orthotopic glioma model was compared, showing that NSCs had the superior therapeutic efficacy in intracranial tumors.[10] Hence, a selection of optimal cell candidates for certain therapeutic applications should be considered.

Cihova et al. have reviewed stem cell-based cancer gene therapy, particularly the stem cell mediated suicide gene therapy (also called enzyme prodrug therapy).[11] In this case, the gene for

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an enzyme (e.g., HSVtk and cytosine deaminase) is delivered and targeted to the tumor by transduced stem cells and the enzymatic activity of gene product converts a less toxic prodrug (e.g., GCV and 5-fluorocytosine) into a highly cytotoxic substance at the tumor site. MSCs have also been used to deliver many therapeutic genes including interferon β (IFN-β), interleukin-12 (IL-12) and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) for cancer therapy. However, it is critical to note that MSCs themselves may have either inhibitory or stimulatory effects on tumor growth. To gain further understanding on these effects, in this issue, Uppalapati et al. identified the expression of several tumor suppressor genes including sulfatase-1 (SULF-1), glucose phosphate isomerase (GPI), and adipocyte differentiation-related protein (ADRP) in rat umbilical cord matrix stem cells, which had significant inhibitory effects on rat mammary adenocarcinoma (Mat B III) cell proliferation.[12] Additionally, although it is generally accepted that the tumor tropism of MSCs is mediated through both chemokines and growth factors, the role of recruiting MSCs by tumor microenvironment is still not fully elucidated. In this issue, Garcia et al. studied how the microenvironment of liver carcinoma modulated the tumor tropism of MSCs.[13] It was found that, in addition to factors produced by hepatocellular carcinoma cells, the specific microenvironment of liver fibrosis also contributed to the tumor-tropic migration of MSCs.

Furthermore, genetic modification of stem cells is an attractive strategy in treating many other different diseases. For example, islet transplantation to treat type 1 diabetes has limited clinical application due to primary graft rejection. MSCs can play a favorable role in islet transplantation as they can not only promote immune tolerance but also secrete growth factors essential for rapid islet revascularization. Wu et al. have described the mechanisms of MSCs and different strategies, including genetic modification in improving islet transplantation.[14] MSCs can potentially transdifferentiate into β cells, thereby representing another source of insulin-producing cells. In addition, the immunomodulatory and trophic effects of MSCs play major roles in generating therapeutic effects. Moreover, MSCs could be transduced to express insulin gene enhancer protein (ISL-1), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) to promote angiogenesis as well as β cell survival. Gene and stem cell combinatory therapy has also been applied to skin wound healing. Cutaneous wound healing is a well orchestrated integration of the complex biological and molecular events, including cell migration and proliferation, extracellular matrix deposition, angiogenesis, and remodeling. In this issue, Gauglitz et al. have reviewed the current gene and stem cell therapy in improving wound healing capacities and discussed the application of stem cell-based skin engineering in conjunction with gene recombination.[15]

Bone tissue repair still represents a major challenge. However, transplanting genetically modified stem cells expressing bone induction factors such as bone morphogenetic proteins (BMPs) can be advantageous. Sheyn et al. have discussed the repair of bone defect using adipose-derived MSCs expressing BMP-6 in a rodent model of vertebral compression fractures (VCFs).[16] Notably, in this model, *in situ* osteogenic differentiation of MSCs was confirmed and significant bone repair with morphological and structural similarities to the native tissue was observed.

Coronary artery disease leading to myocardial infarction is a major cause of mortality and morbidity worldwide. The hallmark of myocardial infarction is the massive loss of functioning cardiomyocytes. In the review article by Haider et al., current advances in heart regeneration through implanting genetically modified stem cells have been surveyed.[17] While stem cells have been previously tested for cardiac regenerative therapy, genetic modification can further enhance their survival, engraftment and cardiovascular differentiation in infarcted myocardium. Notably, iPSCs represent a unique product generated through genetic manipulation of cells, often fibroblasts, and hold great promise for myocardial repair. However, in two different articles by Yan et al. and Singla et al., iPSCs were derived from cardiac ventricular specific cell types such as H9c2 cells, originally isolated from embryonic cardiac ventricular tissue.[18,19] These cells could successfully differentiate into different cardiovascular cells (cardiac myocytes, vascular smooth muscle cells and endothelial cells), contribute to the myocardial regeneration and eventually improve cardiac function in rodent models of infarct heart.

In some cases, *in situ* gene transduction of transplanted cells may be necessary, especially for those local treatments. Implantation, when assisted with biomaterials such as hydrogels, can constrain stem cells within the target sites. Alternatively, the use of biomaterials can also make it possible to implement gene delivery to encapsulated stem cells *in situ*. Gojgini et al. examined this concept and aimed to identify the critical parameters influencing transfection efficiency of cationic polymers into MSCs encapsulated in hydrogels.[20] Plasmid DNA was formulated with poly(ethyleneimine) (PEI) into nanoparticles in a hydrogel made of hyaluronic acid (HA) and coembedded with mouse MSCs. The transfection efficiency was dependent on plasmid DNA dose, concentration of bioconjugated cell-adhesion peptide motif (RGD) as well as hydrogel mechanics, providing rationales for *in situ* optimizing gene delivery to implanted stem cells in the complex tissue microenvironment.

Nevertheless, challenges exist in translating gene therapy into clinical applications. The combination of gene delivery and stem cell technology is emerging as a novel avenue to expand gene therapy. A collection of different original studies and reviews in this theme issue thus provides a platform for researchers to further advance gene therapy into clinical applications as well as more diverse disease conditions. Biotechnological refinements in genetic modification to facilitate sophisticated modulating stem cells will continue to be of great interest. Recent understanding of biology of stem cell niches and cancer stem cells may generate even more opportunities for gene therapy. As such, we envision that stem cells can help in translating genetic materials into new therapeutic and diagnostic modalities. In the end, we would like to offer our sincere thanks to all the contributors to this issue of *Molecular Pharmaceutics*. We hope these contributions will provide greater insights into combining stem cells and gene therapy for effective therapeutics. We would also like to thank Dr. Saurabh Singh for critically reviewing this editorial. We hope this issue provides stimulation for innovative approaches and development of novel therapies by gaining insights into this fascinating field.

Zhaoyang Ye*,† and Ram I. Mahato*,‡

†State Key Laboratory of Bioreactor Engineering, School of Bioengineering, East China University of Science and Technology, Shanghai 200237, China

‡Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, Tennessee 38103, United States

AUTHOR INFORMATION

Corresponding Author

*Z.Y.: East China University of Science and Technology, 130 Mei-Long Road, P.O. Box 309, Shanghai 200237, P. R. China; tel, +86-21-64251570; fax, +86-21-64252250; e-mail, zhaoyangye@ecust.edu.cn. R.I.M.: Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 19 S Manassas, RM 224, Memphis, TN 38103-3308, USA; tel, (901) 448-6929; fax, (901) 448-2099; e-mail, rmahato@uthsc.edu.

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