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Short Communication

Transcriptional regulation of the genes involved in skin-regeneration using protein delivery

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Abstract

Photoaging caused by UVB-irradiation leads to extracellular matrix damage. Most of the skin aging phenomenon is due to the loss of collagen and elastin fibers in dermal layer. ICE-1 and ICE-2 are important transcription factors involved in type collagen synthesis. To increase type collagen synthesis by regulating the activity of these transcription factors, we designed the intranuclear transcription modulation domains (TMD) of ICE-1 and ICE-2 which can be delivered effectively into the nucleus by being conjugated with protein transduction domain (PTD). Overexpressed ICE-1 and ICE-2 gene through transient transfection and treatment of purified recombinant proteins, pICE-1and pICE-2 upregulated type collagen synthesis on UVB-damaged human dermal fibroblast. In conclusion, transcriptional regulation of type collagen gene by using transcription modulation domains of ICE-1 and ICE-2 may have a significant anti-photoaging effects in human dermal fibroblast.

Keyword:

skin aging, photoaging, type collagen, TMD, PTD

Introduction:

Mesenchymal stem cells (MSCs) are multipotent stem cells that can be isolated and expanded from many tissues, and are being investigated for use in cell therapies. Though MSC therapies have demonstrated some prosperity, none have been FDA approved for clinical use. MSCs lose stemness ex vivo, decrementing therapeutic potential, and face supplemental barriers in vivo, decrementing therapeutic efficacy. Culture optimization and genetic modification of MSCs can surmount these barriers. Viral transduction is efficient, but circumscribed by safety concerns cognate to mutagenicity of integrating viral vectors and potential immunogenicity of viral antigens. Nonviral distribution methods are safer, though constrained by inefficiency and toxicity, and are flexible and scalable, making them alluring for engineering MSC therapies.

Human mesenchymal stem cells (hMSCs) Human mesenchymal stem cells (hMSCs) are multipotent adult stem cells that can be facilely isolated and expanded from many tissues, including bone marrow (hBMSCs), adipose (hAMSCs), and umbilical cord (hUCMSCs) . hMSCs are tri-lineage multipotent in vitro (osteogenic, adipogenic, and chondrogenic), and home to sites of inflammation in damaged tissues in vivo after transplantation, where they can facilitate tissue repair through differentiation for cell repopulation, and promote tissue remodeling and modulation of the immune replication through secretion of magnification factors, cytokines, and exosomes hMSCs are immune evasive, enabling allogenic transplantation for cell therapies that make utilization of the aforementioned properties. Supplementally, MSCs can be efficiently reprogrammed to engender induced pluripotent stem cells (iPSCs). Consequently, hMSCs are being widely investigated for use in cell therapies for treatment of many diseases. Over 200 hMSC cell therapy clinical tribulations have been consummated, and another 81 are currently active, according to the US National Library of Medicine clinical tribulation database. These clinical tribulations include therapies for treatment of autoimmunity, graft versus host disease, ischemia, injury of central nervous system, and cancer. Though clinical tribulations have demonstrated some measures of prosperity, no hMSC therapy has been approved by the FDA for clinical use. hMSC therapies have not resulted in widespread prosperity, in part due to challenges associated with maintenance of stemness during expansion ex vivo, resulting in progressive loss of self-instauration, differentiation potential, and immunomodulatory capacity that decrease hMSC therapeutic potential, as well as adscititious challenges after distribution in vivo, including transplantation survival and target engraftment. To surmount the barriers that limit their performance in therapies, and enhance their properties, hMSCs can be modified by optimization of culture conditions and exogenous gene transfer, ex vivo. In expansion, stemness maintenance can be enhanced by inclusion of media factors and tuning of substrate properties or culturing in 3-D in order to suppress cell sene scence. Loss of proliferative capacity, pluripotent gene expression, and differentiation potential in MSCs are, in part, due to senescence linked to low telomerase. Through gene distribution, MSCs have been prosperously immortalized by induced expression of human telomerase reverse transcriptase (hTERT), which signifi cantly elongates MSC expansion afore replicative senescence, while retaining expression of pluripotency genes, immunosuppressive properties, and differentiation potential. hMSCs can withal be engineered ex vivo to enhance therapeutic function in vivo, through induced expression of pro-survival genes, adhesion ligands targeting cell membrane receptors line age-concrete genes for directed differentiation, or genes that encode for engenderment and secretion of gro wth factors, cytokines and miRNA in exosomes. Thus, many researchers are investigating methods to efficiently transfer genes to MSCs.

Many studies have genetically modified MSCs, which naturally

home to tumors to secrete anti-tumo rigenic factors, or to express suicide enzymes that cleave pro-drugs, inducing cytotoxicity in tumors. For secretion of an anti-tumorigenic factor, Mangraviti et al. engineered hAMSCs to treat glioblastoma by transfecting with PBAE complexed with pDNA encoding for secreted BMP-4, which significantly suppressed magnification of encephalon tumor initiating cells (BTIC) in vitro. In additament to achieving 75% transfection efficiency and high viability, AMSCs transfected with PBAEs exhibited significantly higher motility and incursion in vitro than AMSCs transduced with lentivirus . When engineered hAMSCs were administered intranasally to mice with glioblastoma, survival was protracted, relative to mice that received control hAMSCs . Another commonly investigated anti-tumorigenic factor for distribution to tumors by MSCs is tumor necrosis factor cognate apoptosis-inducing ligand (TRAIL). In a categorical example, Jiang et al. transfected hAMSCs with PBAE complexed with TRAIL-expressing pDNA, achieving 68% transfection efficiency and about 90% viability, which was 5.5-fold more efficient that LF2000. Transfected hAMSCs were injected into encephalons of patient-derived tumor xenograft (PDTX) glioblastoma NCr unclad mouse model, and migrated to tumor margins. Compared to untransfected hAMSCs, TRAIL-expressing hAMSCs decremented tumor size 2.5-fold and incremented survival time. Alternatively to anti-tumorigenic factor secretion, MSCs have withal been transfected for suicide gene therapy. For example, Zhang et al. transfected rat BMSCs with spermine-pullulan complexed with pDNA encoding thymidine kinase (TK). BMSCs were injected into a mouse B16F10 pulmonary melanoma metastasis model and migrated to tumor nodules. Upon systemic treatment with pro-drug ganciclovir, TK secreted from transfected BMSCs cleaved ganciclovir to its cytotoxic form within tumors, truncating the number of metastatic lung nodules by 70%, and decrementing lung weight by 30%. In a different novel suicide gene approach that increments radioiodine uptake in tumors, Schug et al. stably transfected hBMSCs with slumbering resplendency transposon encoding for sodium iodide symporter (NIS) driven by a TGFβ-1-responsive promoter, to induce expression of NIS when hBMSCs are within tumor stroma that secretes TGFβ-1. Engineered hBMSCs sequestered iodine when stimulated with TGFβ-1 in vitro, and were consequently tested further in vivo, injected systemically into mouse liver cancer models. Mice that received radioiodide therapy, exhibited delayed tumor magnification and elongated survival, relative to mice that did not receive radioiodide therapy, suggesting prosperous tumor-localized, hBMSCs sequestration of radioiodide. To summarize, MSCs can be engineered to secrete anti-tumorigenic factors and to facilitate suicide gene therapy utilizing nonviral gene distribution, with demonstrated efficacy in animal cancer models that may translate to efficacious human cancer therapies.

Intercellular transfer of exosomes, which contain organelles, proteins, and RNAs, is thought to be a mechanism by which MSC therapeutic effects are conferred. Isolated MSC exosomes have been utilized to treat pre-clinical models of cardiovascular, neurological, musculoskeletal, and immune system diseases. However, the therapeutic effects of MSC exosomes can be enhanced by nonviral gene distribution. For example, to promote survival and function of transplanted islet cells in a diabetic mouse model, Wen et al. transfected hMSCs with pDNA encoding for siRNA against genes involved in pancreatic islet graft failure, Fas and miR-375. In co-culture with human islet cells in vitro, hMSCs transferred transgenic siRNA to islet cells via exosomes, promoting islet cell survival and rescuing islet cell function decremented by inflammatory cytokines. These transfected hMSCs were then co-transplanted with human pancreatic islets into diabetic mice with humanized immune systems, which resulted in incremented islet survival and function, and suppressed islet immune repudiation in comparison to islets co-transplanted with untransfected hMSCs. In additament to passive loading of hMSC exosomes with overexpressed oligonucleotides, nonviral gene distribution to increment exosome engenderment and actively load exosomes with transgenic mRNA has additionally been demonstrated in hMSCs by Kojima et al. To increment exosome engenderment, hMSCs were electroporated with pDNA encoding for three proteins involved in exosome biogenesis, engendering 10-fold more exosomes than untransfected hMSCs, and similarly, through transgenic expression of CD63 fusion proteins with targeting ligands or mRNA- binding petides, hMSC exosomes were engineered to present targeting ligands and load mRNA cargoes. Thus, with efficient nonviral gene distribution, hMSC exosomes can be engendered in immensely colossal quantities, passively or actively loaded with RNA, and targeted with tissue- or cell type-categorical ligands, as distribution conveyances for gene therapies.

Conclusions:

MSCs are a promising cell type for allogenic transplantation cell therapies because of their ease of isolation and expansion, multipotent differentiation capacity, and regenerative and immunomodulatory properties. Yet, challenges remain afore widespread clinical application of MSC therapies can be realized. Engineering of MSCs through gene distribution approaches could avail to surmount barriers to translation of MSC therapies and endow cells with enhanced therapeutic efficacy. A primary concern in the manufacture of genetically modified MSCs is the safety of viral vectors, incentivizing the development of nonviral vectors. Recent developments in nonviral distribution methods, including nanocarrier technology and plasmid design, in amalgamation with chemical and physical priming of cells during culture ex vivo, may sanction for amended nonviral transfection efficiency, enabling scalable translation of genetically engineered MSC therapies for a variety of applications, including guided differentiation and reprogramming, transplantation survival and directed homing, and secretion of therapeutics, potentially bringing efficacious regenerative medicine to patients.