Med Cell Europe®
SWISS STEM CELL TECHNOLOGY

Overview R&D Activities for Dr.Erri Cippini

Present and future uses of the secretome of adipose derived mesenchymal stem cells on skin diseases

(Dr. Steven James Kellner - Chief Scientist)

Münchwilen, December 2014

Med Cell Europe AG, Swiss Stem Cell Technology, Murgtalstrasse 20, CH-9542 Münchwilen, Schweiz
Tel. +41 52 511 24 24, Fax. +41 52 511 24 23, www.medcelleurope.com, info@medcelleurope.com
UID: CHE-100.541.124 MWST

Med Cell Europe®
SWISS STEM CELL TECHNOLOGY

Content

1. Summary of the safety data on adipose tissue derived mesenchymal stem cells

Review of the safety of mesenchymal stem cells for clinical application (Youwwi Wang et al., Stem Cells International Vol 2012)

Introduction

It has been shown that the transplantation of mesenchymal stem cells (MSCs) could be an effective therapy for many diseases including blood disease, acute respiratory distress syndrome, spinal cord injury, liver injury, and critical limb ischemia. To date, hundreds of clinical trials using MSCs have been registered in the database (http://www.clinicaltrials.gov/) of the US national institutes of health. Furthermore, a number of nonregistered clinical studies using MSCs are being performed in many countries.

The general practice includes the isolation of MSCs from various tissues (including bone marrow, adipose tissue, placenta umbilical cord, umbilical cord blood, peripheral blood, and dental pulp) and the cell expansion under ***in vitro*** culture conditions. The complications in the utilization of MSCs as therapeutic tools ***in vivo*** arose due to the experimental artefacts introduced by inconsistent cell culture protocols. Actually, most MSCs used for clinical trials are prepared in research laboratories, lacking sufficient preclinical studies and manufacturing quality control. Moreover, laboratories around the world lack an internationally standardized practice for ***in vitro*** expansion of MSCs, resulting in heterogeneous populations of cells and inconsistent results, both in experimental studies and clinical trials.

In addition, although MSCs have been used in both autologous and allogeneic settings, most clinical applications of MSCs are in fact personalized therapies in which the patient receives administration of MSCs provided by different donor and/or different preparation. This necessitates the establishment of standardized manufacture guidelines for the isolation, expansion, preservation, and delivery of MSCs that display minimal variability in their production and assumes large-scale produced MSCs as “cell medicine” for safety evaluation and clinical applications.

Expansion and genetic stability

Primary MSCs are rare in human tissues. The frequency of MSCs is approximately $1/10^6$ nucleated cells in adult bone marrow and $1/10^4$ nucleated cells in umbilical cord.

The number of MSCs has been noted to decrease with age. When grouped by decade, a significant decrease in MSCs per nucleated bone marrow cell could be observed, with 10-fold decrease from birth to teens and another 10-fold decrease from teens to elderly.

Despite limited number, MSCs can be expanded to a high level in long-term culture system, which permits a large-scale production of MSCs for clinical application. Usually, the adult bone marrow MSCs (BMMSCs) can grow identically in culture for 6–10 passages, whereas placenta umbilical cord MSCs can undergo 30 to 40 passages. It is known that prolonged culture of human embryonic stem cells (ESCs) can lead to adaptation and acquisition of chromosomal abnormalities

The induced pluripotent stem cells (iPSCs) undergo deletions of tumor-suppressor genes during the process of reprogramming, while duplications of oncogenic genes aroused in culture. It remains unclear whether the "culture-adapted" MSCs undergo adaptive transformation during long-term passaging ***in vitro***.

Previous data obtained from high-resolution analysis show that the ***in vitro*** expanded human BMMSCs are devoid of DNA copy number aberrations

However, senescence-associated modification at specific CpG sites has been observed in MSC during culture expansion. The key evidence for transformation based on DNA fingerprinting has not been presented in the studies in which the authors claimed that MSCs underwent malignant transformation in cultivation. More data are therefore needed to evaluate the genomic stability of MSCs during prolonged culture ***in vitro***. The high resolution of genetic analysis including balanced and unbalanced genetic change is an important method to determine the possibility of transformation of MSCs. If CNV or SNP change is detected after long-term cultivation, it refers not only to the occurrence of mutation but also to the mutation providing a survival (senescence or apoptosis resistance) or growth advantage more or less. Even if there is no difference between early- and late-passage MSCs in genome, it does not imply the absence of genomic alteration during long-term cultivation. The mutated MSCs without survival or growth advantage will be diluted in the process of cultivation and become undetectable after long-term cultivation. By contrast, the mutated MSCs with growth advantage or senescence resistance are of more risk for clinical application.

Tumor formation

Stem cells possess some features of cancer cells including long lifespan, relative apoptosis resistance, and ability to replicate for extended periods of time. In addition, similar growth regulators and control mechanisms are involved in both cancer and stem cell maintenance. Therefore, stem cells may undergo malignant transformation which is often seen as a key obstacle to the safe use of stem-cell-based medicinal products.

Some previous studies have described spontaneous transformation of MSCs ***in vitro***. However, almost all of them have not provided solid evaluation of the same origin of normal MSCs and their transformed counterparts. Actually, most of the spontaneous malignant transformed MSCs are cross-contained by HT1080, HELA, or other tumour cell lines.

There is not enough evidence for tumourigenicity of MSCs expanded ***in vitro***.

To address the safety issues, we conducted several GLP-compliant ***in vivo*** toxicity studies using NOD mice, NOD/SCID mice, guinea pigs, rabbits, and monkey models. UC-MSCs from master MSCs bank (passage 2, P2) were thawed and cultured for additional five passages (P7) and eleven passages (P13). At the end of P7 or P13, an approximate number of 6×10^9 or 5×10^{12} UC-MSCs were, respectively, harvested, allotted, and cryopreserved until use. For tumorigenic study, UC-MSCs at a dose of 1×10^7/mouse were subcutaneously transplanted into both NOD mice and NOD/SCID mice. No tumour formation was observed two months after cell transplantation in these animals. The effect of transplanted UC-MSCs on tumour growth was then studied using the Nod mice which were previously injected with K562 cells to induce leukemic tumours. Two injections (two-week interval) of different doses of UC-MSCs resulted in a significant inhibition of K562 tumour growth in the mice bearing leukemic tumours. These ***in vivo*** results are consistent with our ***in vitro*** results showing a potent inhibitory effect of UC-MSCs on the proliferation of K562 and HL-60 cells without inducing apoptosis.

In an effort to evaluate the overall toxicology of UC-MSCs, we have performed an ***in vivo*** study in cynomolgus monkeys receiving repeated administrations of UC-MSC. The administration of UC-MSC was done by intravenous injection once every two weeks for six weeks, with a dose of 2×10^6 or 1×10^7 cells/kg body weight. All animals survived until scheduled euthanasia. No significant MSCs-related changes were found in body weights, clinical signs, haematological/biochemical values, organ weights, or histopathological findings. The results of this toxicity study indicated that the transplantation of UC-MSC did not affect the general health of cynomolgus monkeys.

Moreover, the vast majority of clinical trials conducted with MSCs in regenerative medicine applications have not reported major health concerns. Centeno et al. report that two groups of patients (group 1: ***n*** = 50; group 2: ***n*** = 290) between 2006 and 2010 were treated for

various orthopaedic conditions with culture-expanded, autologous BMMSCs. Cells were cultured in monolayer culture flasks using an autologous platelet lysate technique andreinjected into peripheral joints or into intervertebral discs with use of c-arm fluoroscopy. Using both intensive high-field MRI tracking and complications surveillance in 339 patients, no neoplastic complications were detected at any stem cell reimplantation site. MSCs-based therapy has also already been used in other human disease settings, such as graft-versus-host and cardiac disease, with initial reports indicating a good safety profile. These findings indicate the lack of solid evidence for malignant transformation ***in vivo*** following implantation of MSCs for clinical use. Further studies will be required to determine if MSCs can help tumour formation and related mechanisms. Although MSCs with chromosomal alterations did not show any sign of malignant transformation either ***in vitro*** or ***in vivo***, it remains uncertain that acquired mutations will induce cellular transformation during the prolonged culture. There exists the possibility that MSCs gain copy number variation during prolonged expansion. Thus, it is necessary to conduct aCGH or SNP array to evaluate the genomic integrity of MSCs before clinical application.

Cryopreservation and Banking

To isolate and produce in large scale the MSCs for clinical use, standardized preparation processes and long-term storage of MSCs isolated from different sources are needed for future clinical applications.

Since HLA-matched adult organ donors are not always available, stem cells derived from several birth-associated perinatal tissues—including cord blood, placenta, and umbilical cord—can be banked as a safeguard against future life-threatening conditions. The clinical grade production and preservation of perinatal MSCs necessitates adhering to cGMP (current Good Manufacture Practices) to insure the delivery of a “cell drug” that is not only safe but also reproducible and efficient. Cryopreservation of cells permits the transportation of cells between sites, as well as completion of safety and quality control testing. Taken together, banking of perinatal MSCs includes the donor determination and sample collection, primary cell isolation and microbiological testing, the master stem cell selection, cell expansion, cryopreservation and banking, and the large-scale cell expansion and preparation of final stem cell products. Strict standards and management are vital to make stem cell bank work well. Validated SOPs (Standard Operation Procedures) with quality assurance programs are the key factor of a well-designed bank of MSCs. Maintenance of viability, biological characteristics, and sterility makes the banked MSCs safety and “ready to use.”

It has been demonstrated that cryopreservation does not change the biological behaviour of MSCs such as differentiation, growth, and surface marker. Serum and dimethyl sulfoxide

(DMSO) are used in research laboratory as cryoprotectant. The major challenge of freezing MSCs is the toxicity of cryoprotectant in clinical use. The toxicity of DMSO is overestimated as it could be weakened by diluting cryopreserved MSCs before clinical use. FBS (fetal bovineserum), BSA (bovine serum albumin), or HSA (human serum albumin) should not be an alternative cryoprotectant for DMSO because of a risk of contamination with human or animal viruses.

Serum-containing and serum-free cultivations

In vitro expansion of MSCs is conventionally achieved in medium containing FBS and is increased by addition of growth factors. However, for widespread clinical applications, contact of MSCs with serum must be minimized since it is a putative source of prion or virus transmission. Serum is the most uncertain factor in the expansion of clinical grade MSCs. Considering the batch-to-batch variability and possibility of viral contamination, some replacement of FBS such as human AB serum or platelet lysates cannot be considered as better choice for producing MSCs for clinical use.

Chemical-defined, xeno-free, serum-free medium (SFM) may conquer all the problems of FBS. Furthermore, comparing serum-contained medium, some evidence supported that SFM provided an adjuvant for maintenance of chromosomal stability in BMMSCs and adipose-derived MSCs. Another study focus on mouse embryo fibroblasts showed that predominantly diploid karyotype was maintained in serum-free culture even at PD60. Aneuploid karyotype was induced by the addition of serum. Probably the uncontrolled mitogenic stimulation in serum can lead to strong genetic instability. However, expansion of MSCs in SFM remains an unsolved question. SFM has its own shortcoming in the preparation of clinical grade MSCs. The attachment of MSCs in SFM needs the coating of fibronectin or other substrates which contained components of human origin and batch-to-batch variability and cannot be well defined chemically.

In fact, SFM is not as well as that some commercial companies claimed. Based on our own data, UC-MSCs proliferate more slowly in SFM than in FBS-contained media. Sometimes, UC-MSCs cannot be expanded in SFM. A number of studies have revealed that MSCs can be expanded in FBS contained medium without transformation. If MSCs are expanded in SFM, the similar safety studies are needed to determine if the serum-free system affects the genetic stability of MSCs and cause tumour formation.

In summary, the safety remains one of the main concerns in cell therapy. The production of safe cell products requires an entire process supervising to make sure the cells maintain overall phenotype, functional potential, and to ensure the cultured cells remain untransformed and no microbiological contaminations. Therefore, MSCs banking and cell products manufacturing and corresponding quality control system procedures must be applied for assuring the safety and efficiency of the final cell products. Moreover, we cannot only rely on biologists to produce MSCs which fulfils all of the requirements for clinical application. Cell engineering technologies are needed in the translation from expansion of MSCs in laboratory to large-scale manufacture in cell factory.

Stem Cells Dev. 2011 Aug; 20(8):1297-308. doi: 10.1089/scd.2010.0466. Epub 2011 Mar 17.

Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans.

Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E.

Source

Stem Cell Research Center, RNL Bio Co, Ltd, Seoul, Republic of Korea. jcra@rnl.co.kr

Abstract

Adipose tissue-derived mesenchymal stem cells (AdMSCs) represent an attractive and ethical cell source for stem cell therapy. With the recent demonstration of MSC homing properties, intravenous applications of MSCs to cell-damaged diseases have increased. In the present study, the toxicity and tumourigenicity of human AdMSCs (hAdMSCs) were investigated for clinical application. Culture-expanded hAdMSCs showed the typical appearance, immunophenotype, and differentiation capacity of MSCs, and were genetically stable at least 12 passages in culture. Cells suspended in physiological saline maintained their MSC properties in a cold storage condition for at least 3 days. To test the toxicity of hAdMSCs, different doses of hAdMSCs were injected intravenously into immunodeficient mice, and the mice were observed for 13 weeks. Even at the highest cell dose (2.5×10(8) cells/kg body weight), the SCID mice were viable and had no side effects. A tumourigenicity test was performed in Balb/c-nu nude mice for 26 weeks. Even at the highest cell dose (2×10(8) MSCs/kg), no evidence of tumour development was found. In a human clinical trial, 8 male patients who had suffered a spinal cord injury >12 months previous were intravenously administered autologous hAdMSCs (4×10(8) cells) one time. None of the patients developed

any serious adverse events related to hAdMSC transplantation during the 3-month follow-up. In conclusion, the systemic transplantation of hAdMSCs appears to be safe and does not induce tumour development.

References:

Wang Y1, Han ZB, Song YP, Han ZC.Safety of mesenchymal stem cells for clinical application.Stem Cells Int. 2012; 2012:652034

Ra J.C et al:Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans.Stem Cells Dev. 2011 Aug; 20(8):1297-308

2. The secretome: cytokines and extracellular vesicles

The secretome of adipose derived MSC consists of various proteins, RNA and extracellular vesicles, such as, but not limited to, exosomes and microvesicles.

2.1. Introduction to exosomes and microvesicles

Cells communicate and exchange information by different ways, including the secretion of soluble factors, the cell-to-cell adhesion contact, and the intercellular exchange of organelles through nanotubular structures [1]. Recent studies have proposed that cell-derived small circular membrane vesicles, called exosomes and microvesicles, represent an additional mechanism of cell-to-cell communication by transfer of membrane components as well as the cytoplasmic content [2,3]. [2,5,6]. Two distinct populations of vesicles with peculiar membrane structure, mechanism of production, pathophysiological relevance, and different size have been described. Membrane fragments of 30–90nm diameter derived from the endosomal membrane compartment after fusion of secretory granules with the plasma membrane are defined as exosomes. Once released, exosomes bind the recipient cells through receptor–ligand interactions or fuse with the target cell membrane transferring membrane components, including cell receptors [2], and discharging the portion of cytosol segregated within their lumen into the cytoplasm of recipient cells [7]. The molecular cargo content of exosomes derives from active packaging of certain nucleic acid species leading to the presence of mRNAs in exosomes that are not found in donor cells [8]. Furthermore, relatively large microvesicles (100 nm–1 mm diameter) are formed from the surface membrane of activated cells in a calcium and calpain-dependent manner following a disordered function of phospholipid transporters that results in the budding of altered membrane that exposes phosphatidylserine in the outer leaflet. Microvesicles and exosomes contain biomolecules, including mRNA and microRNA, packaged in a random process and their release is considered an expression of a pathological process in place. Molecular transfer from microvesicles and exosomes contributes to changes in the maturation and differentiation of target cells as for example microvesicles and exosomes released by endothelial progenitor cells trigger neo-angiogenesis in endothelial cells [9].

References

1. Rustom A, R Saffrich, I Markovic, P Walther and HH Gerdes. (2004). Nanotubular highways for intercellular organelle transport. Science 303:1007–1010.

2. Ratajczak J, M Wysoczynski, F Hayek, AJanowska-Wieczorek and MZ Ratajczak. (2006). Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia 20:1487–1495.

3. Camussi G, MC Deregibus, S Bruno, V Cantaluppi and L Biancone. (2010). Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 78:838–848.

4. McDaniel LD, E Young, J Delaney, F Ruhnau, KB Ritchie and JH Paul. (2010). High frequency of horizontal gene transfer in the oceans. Science 330:50.

5. Denzer K, MJ Kleijmeer, HF Heijnen, W Stoorvogel and HJ Geuze. (2000). Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. J Cell Sci 113 Pt 19:3365–3374.

6. Fevrier B and G Raposo. (2004). Exosomes: endosomal-derived vesicles shipping extracellular messages. CurrOpin Cell Biol 16:415–421.

7. Cocucci E, G Racchetti and J Meldolesi. (2009). Shedding microvesicles: artefacts no more. Trends Cell Biol 19:43–51.

8. Valadi H, K Ekstrom, ABossios, M Sjostrand, JJ Lee and JO Lotvall. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654–659.

9. Deregibus MC, V Cantaluppi, R Calogero, M Lo Iacono, C Tetta, L Biancone, S Bruno, B Bussolati and G Camussi. (2007). Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110:2440–2448.

2.2. Introduction to paracrine substances

MSCs secrete numerous growth factors and cytokines. A typical MSC secretion profile comprises growth factors, cytokines, ECM proteases, hormones, and lipid mediators. Paracrine signaling is a form of cell-cell communication in which a cell produces a signal to induce changes in nearby cells, altering the behavior or differentiation of those cells. Signaling molecules known as paracrine factors diffuse over a relatively short distance (local action), as opposed to endocrine factors (hormones which travel considerably longer distances via the circulatory system), juxtacrine interactions, and autocrine signaling. Cells that produce paracrine factors secrete them into the immediate extracellular environment. Factors then travel to nearby cells in which the gradient of factor received determines the outcome. However, the exact distance that paracrine factors can travel is not certain. Although paracrine signaling elicits a diverse array of responses in the induced cells, most paracrine factors utilize a relatively streamlined set of receptors and pathways. In fact, different organs in the body -even between different species - are known to utilize a similar sets of paracrine factors in differential development. The highly conserved receptors and pathways can be organized into four major families based on similar structures: Fibroblast growth factor (FGF) family, Hedgehog family, Wnt family, and TGF-β superfamily. Binding of a paracrine factor to its respective receptor initiates signal transduction cascades, eliciting different responses.

3. Patented* innovation regarding the production of clinical grade secretome

Background

Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts, chondrocytes, neurons, muscle cells and adipocytes. This phenomenon has been documented in specific cells and tissues in vivo and in vitro. MSCs are distributed all over the body and are responsible for regeneration. Commonly used tissues for the isolation of MSC are bone marrow, umbilical cord, cord lining and, increasingly, adipose tissue, which has a superior amount of MSCs.

All cells communicate and exchange information by different ways, including the secretion of soluble factors, the cell-to-cell adhesion contact, and the intercellular exchange of organelles. The secretome of mesenchymal stem cells includes paracrine substances, exosomes and microvesicles. Over 150 paracrine substances, also called cytokines and chemokines, can be released by mesenchymal stem cells. Two distinct populations of vesicles with peculiar membrane structure, mechanism of production, pathophysiological relevance, and different size have been described: exosomes and microvesicles. Microvesicles and exosomes contain biomolecules, including messenger RNA and micro RNA. Exosomes and the whole secretome have been used for therapies in regenerative medicine to treat various illnesses such as osteoarthritis, cardiovascular diseases, neurological diseases, pulmonary diseases, diabetes and many more.

Success rate and duration has been very variable, mainly due to the amount of proteins and RNA used in the studies. We are here presenting our ExoRAP and ExoPAN technology which increases the yield of the secretome by up to 30 times.

* Med Cell Europe AG has submitted a patent to the European Patent Office on November 27th, 2014; PCT Application Number: PCT/EP2014/075875, Submission Number: 315327

* Trademark Registrations: “EXOPAN”, “EXORAP”, “EXOSWISS”

Technology

Previous studies suggest that a hypoxic condition promotes self-renewal of undifferentiated mesenchymal stem cells and enhances their therapeutic potential.

Hypoxic exposure activates several signal transduction pathways including hypoxia inducible factor (HIF), a master transcription factor that regulates the expression of hundreds of genes to promote cellular adaptation to the hypoxic condition.

Our ExoRAP and ExoPAN technology uses a protocol of pre-treatment of the cultured MSC in special media and various anoxia conditions instead of hypoxia. With this protocol a 30 fold increase in RNA content per cell can be achieved.

The technology is successfully being used in regenerative treatments in human ExoRAP and ExoPAN technology and animal patients.

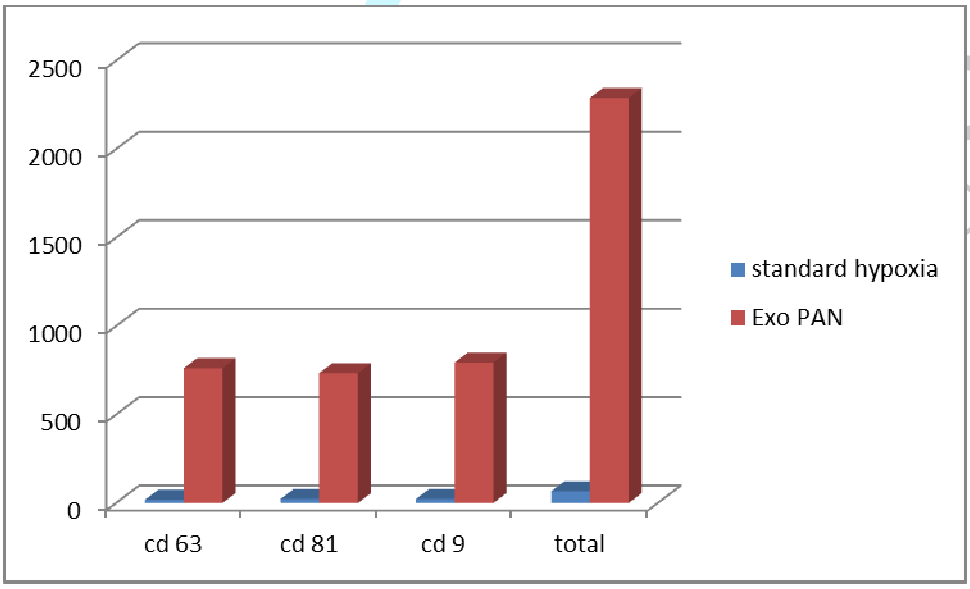

Figure 1: 300 fold increase of exosomes per 1 mio cells as measured semiquantitatively via surface markers cd 63, 81 and 9.

Med Cell Europe AG, Swiss Stem Cell Technology, Murgtalstrasse 20, CH-9542 Münchwilen, Schweiz
Tel. +41 52 511 24 24, Fax. +41 52 511 24 23, www.medcelleurope.com, info@medcelleurope.com
UID: CHE-100.541.124 MWST

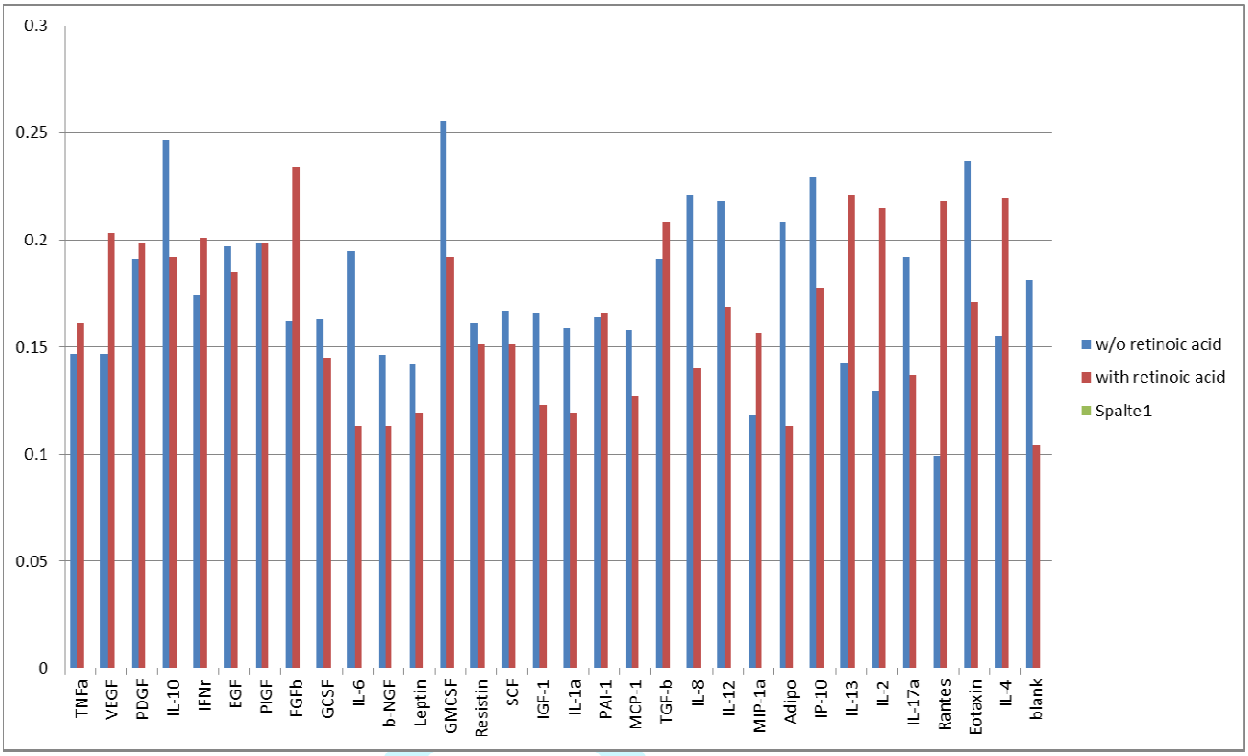

Figure 2: Comparison auf 31 Cytokines produced by hMSC-ad with and without pre-treatment with retinoic acid

Competitive Advantages

- The ExoRAP and ExoPAN technology is easy to use and achieves a much greater amount of RNA. This will be beneficial for all regenerative treatments but especially for diseases where the success rate has not been huge so far, such as diabetes, multiple sclerosis and other neurodegenerative diseases, cardiovascular diseases and so forth.
- The secretome is devoid of all cells which will decrease to risk of unwanted reactions in allogeneic use.
- The secretome has been tested in an autologous setting in hundreds of patients with no side effects so far.
- The secretome can be stored at -80^{0}C with minimal loss of activity.
- Therapeutic doses can be achieved with only 1 mio MSCs.

Applications

- Therapies for regenerative medicine for a host of diseases such as osteoarthritis, cardiovascular diseases, lung diseases, liver diseases, neurodegenerative diseases including multiple sclerosis, Parkinson, Alzheimer; GVHD, Crohn's disease and many more.
- Increase the RNA yield in a research setting, i.e. studies on novel therapies
- Content of specific RNA will enable to focus therapies, such as miR-22 for cardiovascular diseases, miR-133b for neurological diseases, miR-146 for wound healing, miR-204 for pulmonary diseases etc.

Literature

The Role of MicroRNA-146a in the Pathogenesis of the Diabetic Wound-Healing Impairment.Junwang Xu et al., Diabetes 61:2906–2912, 2012

Hypoxic Conditioned Medium from Human Amniotic Fluid-Derived Mesenchymal Stem Cells Accelerates Skin Wound Healing through TGF-β/SMAD2 and PI3K/Akt Pathways. EunKyoung Jun et al., Int. J. Mol. Sci. 2014, 15, 605-628

Emerging role of neuronal exosomes in the central nervous system.MathildeChivet et al., frontiers in physiology, May2012|Volume3|Article145| 3

ExosomalSignaling during Hypoxia Mediates Microvascular Endothelial Cell Migration and Vasculogenesis. Carlos Salomon et al., www.plosone.org 1 July 2013 | Volume 8 | Issue 7 | e68451

Exosome-Mediated Transfer of miR-133b from Multipotent Mesenchymal Stromal Cells to Neural Cells Contributes to Neurite Outgrowth. Hongqi Xin et al., Stem Cells. 2012 July ; 30(7): 1556–1564

Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy Yi Leeet al., Human Molecular Genetics, 2012, Vol. 21, Review Issue 1

Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. Takeshi Katsuda et al., SCIENTIFIC REPORTS | 3 : 1197

Exosomes Mediate the Cytoprotective Action of Mesenchymal Stromal Cells on Hypoxia-Induced Pulmonary Hypertension. Changjin Lee et al., Circulation. 2012 November 27; 126(22): 2601–2611

Ischemic Preconditioning Potentiates the Protective Effect of Stem Cells through Secretion of Exosomes by Targeting Mecp2 via miR-22. Yuliang Feng et al., PLOSONE February 2014 | Volume 9 | Issue 2 | e88685

Transfer of Growth Factor Receptor mRNA Via Exosomes Unravels the Regenerative Effect of Mesenchymal Stem Cells. Susanna Tomasoni et al., STEM CELLS AND DEVELOPMENT, Volume 22, Number 5, 2013

New Insight Into Early Events in Type 1 Diabetes: Role for Islet Stem Cell Exosomes. Miodrag L. Lukic et al., Diabetes Volume 63, March 2014

Med Cell Europe AG, Swiss Stem Cell Technology, Murgtalstrasse 20, CH-9542 Münchwilen, Schweiz
Tel. +41 52 511 24 24, Fax. +41 52 511 24 23, www.medcelleurope.com, info@medcelleurope.com
UID: CHE-100.541.124 MWST

4. Preclinical studies regarding skin diseases

4.1. Diabetic wound healing, intradermal injections, allogenic BM MSCs, rats

The Role of MicroRNA-146a in the Pathogenesis of the Diabetic Wound-Healing Impairment Correction With Mesenchymal Stem Cell Treatment

Junwang Xu, Wenjie Wu, Liping Zhang, Wanda Dorset-Martin, Michael W. Morris, Marc E. Mitchell, and Kenneth W. Liechty

Diabetes 61:2906–2912, 2012

The impairment in diabetic wound healing represents a significant clinical problem. Chronic inflammation is thought to play a central role in the pathogenesis of this impairment. We have previously shown that treatment of diabetic murine wounds with mesenchymal stem cells (MSCs) can improve healing, but the mechanisms are not completely defined. MicroRNA-146a (miR-146a) has been implicated in regulation of the immune and inflammatory responses. We hypothesized that abnormal miRNA-146a expression may contribute to the chronic inflammation. To test this hypothesis, we examined the expression of miRNA-146a and its target genes in diabetic and nondiabetic mice at baseline and after injury. MiR-146a expression was significantly downregulated in diabetic mouse wounds. Decreased miR-146a levels also closely correlated with increased gene expression of its proinflammatory target genes. Furthermore, the correction of the diabetic woundhealing impairment with MSC treatment was associated with a significant increase in the miR-146a expression level and decreased gene expression of its proinflammatory target genes. These results provide the first evidence that decreased expression of miR-146a in diabetic wounds in response to injury may, in part, be responsible for the abnormal inflammatory response seen in diabetic wounds and may contribute to wound-healing impairment.

4.2. Influencing migration and proliferation of fibroblasts with the secretom in vitro

The Effects of Cytokines in Adipose Stem Cell-Conditioned Medium on the Migration and Proliferation of Skin Fibroblasts In Vitro

Jiajia Zhao, Li Hu, Jiarong Liu, Niya Gong, and Lili Chen

BioMed Research International, Volume 2013, Article ID 578479

Although adipose stem cell-conditioned medium (ASC-CM) has demonstrated the effect of promoting the cutaneous wound healing, the mechanism for this response on the effector cells (e.g., dermal fibroblasts) during the process remains to be determined. In this study, we aim to investigate the types and contents of cytokines in ASC-CM and the effects of some kinds of common cytokines in ASC-CM, such as EGF, PDGF-AA, VEGF, and bFGF, on dermal fibroblasts proliferation and migration in wound healing process. Results showed that these four cytokines had high concentrations in ASC-CM.The migration of skin fibroblasts could be significantly stimulated by VEGF, bFGF, and PDGF-AA, and the proliferation could be significantly stimulated by bFGF and EGF in ASC-CM. Additionally, ASC-CM had more obvious promoting effect on fibroblasts proliferation and migration than single cytokine. These observations suggested that ASC-CM played an important role in the cutaneous injury partly by the synergistic actions of several cytokines in promoting dermal fibroblasts proliferation and migration, and ASC-CM was more adaptive than each single cytokine to be applied in promoting the wound healing.

4.3. Wound healing influenced by paracrine factors, human adipose derived in rats

Tumor Necrosis Factor-a-Activated Human Adipose Tissue–Derived Mesenchymal Stem Cells Accelerate Cutaneous Wound Healing through Paracrine Mechanisms

Soon ChulHeo, Eun Su Jeon, Il Hwan Lee, Hoon Soo Kim, Moon Bum Kim and Jae Ho Kim

Journal of Investigative Dermatology (2011) 131, 1559–1567

Human adipose tissue–derived mesenchymal stem cells (ASCs) stimulate regeneration of injured tissues by secretion of various cytokines and chemokines. Wound healing is mediated by multiple steps including inflammation, epithelialization, neoangiogenesis, and proliferation. To explore the paracrine functions of ASCs on regeneration of injured tissues, cells were treated with tumor necrosis factor-a (TNF-a), a key inflammatory cytokine, and the effects of TNF-a-conditioned medium (CM) on tissue regeneration were determined using a rat excisional wound model. We demonstrated that TNF-a CM accelerated wound closure, angiogenesis, proliferation, and infiltration of immune cells into the cutaneous wound in vivo. To assess the role of proinflammatory cytokines IL-6 and IL-8, which are included in TNF-a CM, IL-6 and IL-8 were depleted from TNF-a CM using immunoprecipitation. Depletion of IL-6 or IL-8 largely attenuated TNF-a CM-stimulated wound closure, angiogenesis, proliferation, and infiltration of immune cells. These results suggest that TNF-a-activated ASCs accelerate cutaneous wound healing through paracrine mechanisms involving IL-6 and IL-8.

4.4. Wound healing improved via MMP-8 und VEGF with MSC, in vivo rat model

Mesenchymal Stem Cells Improve Wound Healing In Vivo via Early Activation of Matrix Metalloproteinase-9 and Vascular Endothelial Growth Factor

Chul Han Kim, Jang Hyun Lee, Jong Ho Won and Moon Kyun Cho

J Korean Med Sci 2011; 26: 726-733

We investigated the effects of mesenchymal stem cells (MSCs) on wound healing using a three-dimensional (3D) collagen gel scaffold. Three circular full-thickness skin defects were created on the back of Sprague-Dawley rats. One site was covered with a 3D collagen gel containing 2 × 106 MSCs (MSCs+/3D collagen+). Another site was replaced with a 3D collagen gel without MSCs and the third site was left empty. The wound size was significantly reduced in the MSCs+/3D collagen+ sites. MSCs+/3D collagen+ sites exhibited the most neovascularization. FISH showed that Y-chromosome possessing cells were found within the dermis of MSCs+/3D collagen+ sites. Gelatinzymography revealed that the most intense expression of MMP-9 was detected early in the MSCs+/3D collagen+ sites. Our results indicate that MSCs upregulate the early expression of MMP-9 which induces the early mobilization of VEGF. Thus, MSCs appear to accelerate significantly wound healing via early activation of MMP-9 and VEGF.

4.5.Wound healing and hair growth

Human Wharton's Jelly Mesenchymal Stem Cells Plasticity Augments Scar-Free Skin Wound Healing with Hair Growth

VikramSabapathy, BalasubramanianSundaram, Sreelakshmi VM, PratheeshMankuzhy, Sanjay Kumar

PLoS ONE 9(4): e93726

Human mesenchymal stem cells (MSCs) are a promising candidate for cell-based transplantation and regenerative medicine therapies. Thus in the present study Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) have been derived from extra embryonic umbilical cord matrix following removal of both arteries and vein. Also, to overcome the clinical limitations posed by fetal bovine serum (FBS) supplementation because of xenogeneic origin of FBS, usual FBS cell culture supplement has been replaced with human platelet lysate (HPL). Apart from general characteristic features of bone marrow-derived MSCs, wharton jelly-derived MSCs have the ability to maintain phenotypic attributes, cell growth kinetics, cell

cycle pattern, in vitro multilineage differentiation plasticity, apoptotic pattern, normal karyotype-like intrinsic mesenchymal stem cell properties in long-term in vitro cultures. Moreover, the WJ-MSCs exhibited the in vitro multilineage differentiation capacity by giving rise to differentiated cells of not only mesodermal lineage but also to the cells of ectodermal and endodermal lineage. Also, WJ-MSC did not present any aberrant cell state upon in vivo transplantation in SCID mice and in vitro soft agar assays. The immunomodulatory potential assessed by gene expression levels of immunomodulatory factors upon exposure to inflammatory cytokines in the fetal WJ-MSCs was relatively higher compared to adult bone marrow-derived MSCs. WJMSCs seeded on decellularized amniotic membrane scaffold transplantation on the skin injury of SCID mice model demonstrates that combination of WJ-MSCs and decellularized amniotic membrane scaffold exhibited significantly better wound-healing capabilities, having reduced scar formation with hair growth and improved biomechanical properties of regenerated skin compared to WJ-MSCs alone. Further, our experimental data indicate that indocyanin green (ICG) at optimal concentration can be resourcefully used for labeling of stem cells and in vivo tracking by near infrared fluorescence non-invasive live cell imaging of labelled transplanted cells, thus proving its utility for therapeutic applications.

4.6. Wound healing, canine model

The effects of topical mesenchymal stem cell transplantation in canine experimental cutaneous wounds

Ju-Won Kim, Jong-Hwan Lee, Young S. Lyoo, Dong-In Jung and Hee-Myung Park

Vet Dermatol 2013; 24: 242–e53

Adult stem cells have been widely investigated in bioengineering approaches for tissue repair therapy. We evaluated the clinical value and safety of the application of cultured bone marrow-derived allogenic mesenchymal stem cells (MSCs) for treating skin wounds in a canine model. Adult healthy beagle dogs (n = 10; 3–6 years old; 7.2–13.1 kg) were studied. Full-thickness skin wounds were created on the dorsum of healthy beagles, and allogenic MSCs were injected intradermally. The rate of wound closure and the degree of collagen production were analysed histologically using haematoxylin and eosin staining and trichrome staining. The degree of cellular proliferation and angiogenesis was evaluated by immunocytochemistry using proliferating cell nuclear antigen-, vimentin- and a-smooth muscle actin-specific antibodies. Local mRNA expression levels of interleukin-2, interferon-c, basic fibroblast growth factor and matrix metalloproteinase-2 were evaluated by RT-PCR. Compared with the vehicle-treated wounds, MSC-treated wounds showed more rapid wound

closure and increased collagen synthesis, cellular proliferation and angiogenesis. Moreover, MSC-treated wounds showed decreased expression of pro-inflammatory cytokines (interleukin-2 and interferon-c) and wound healingrelated factors (basic fibroblast growth factor and matrix metalloproteinase-2). Conclusion and clinical importance – Topical transplantation of MSCs results in paracrine effects on cellular proliferation and angiogenesis, as well as modulation of local mRNA expression of several factors related to cutaneous wound healing.

4.7. Cell free material for wound healing

Cell-free derivatives from mesenchymal stem cells are effective in wound therapy

Pravin J Mishra, Prasun J Mishra, Debabrata Banerjee

World J Stem Cells 2012 May 26; 4(5): 35-43

Abstract

AIM: To compare the efficacy of cell-free derivatives from Bone marrow derived human mesenchymal stem cells (hMSCs) in wound therapy.

METHODS: hMSCs have been shown to play an important role in wound therapy. The present study sought to compare efficacy of hMSCs and cell-free derivatives of hMSCs, which may be clinically more relevant as they are easier to prepare, formulate and transport. hMSCs were isolated from human bone marrow and cultured. Multi lineage differentiation of hMSCs was performed to confirm their identity. The ability of hMSCs to migrate was evaluated using in vitro and in vivo migration assays. Cell lysates and conditioned medium concentrate was prepared from hMSCs (see Methods for details). Wounds were induced in mice and wound areas were measure before and after cell and cell-free derivative treatment. RNA and proteins were extracted from the skin and cytokine levels were measured.

RESULTS: Co-culture of hMSCs with keratinocytes resulted in increased expression of CXCL-12 (SDF1) and ENA78 (CXCL-5) in the conditioned media indicating that the hMSCs can respond to signals from keratinocytes. Accelerated wound closure was observed when hMSCs were injected near the site of excisional wounds in athymic as well as NOD/SCID mice. Interestingly, cell-free lysates prepared from hMSCs were also effective in inducing accelerated wound closure and increased expression of SDF1 and CXCL-5 at the wound bed. Additionally, concentrated media from hMSCs as well as an emulsion containing lysates prepared from hMSCs was also found to be more effective in rapid re-epithelialization than fibroblasts or vehicle-alone control. Use of cell-free derivatives may help replace expensive

wound care approaches including use of growth factors, epidermal/dermal substitutes, synthetic membranes, cytokines, and matrix components, and most importantly avoid transmission of pathogens from human and animal products.

CONCLUSION: These results encourage development of derivatives of hMSCs for wound care and re-epithelialization applications.

5. Clinical studies on skin diseases

5.1. Chronic wound healing

Activity of mesenchymal stem cells in therapies for chronic skin wound healing

Austin Nuschke

Organogenesis 10:1, 29–37; January/February/March 2014

Chronic or non-healing skin wounds present an ongoing challenge in advanced wound care, particularly as the number of patients increases while technology aimed at stimulating wound healing in these cases remains inefficient. Mesenchymal stem cells (MSCs) have proved to be an attractive cell type for various cell therapies due to their ability to differentiate into various cell lineages, multiple donor tissue types, and relative resilience in ex-vivo expansion, as well as immunomodulatory effects during transplants. More recently, these cells have been targeted for use in strategies to improve chronic wound healing in patients with diabetic ulcers or other stasis wounds. Here, we outline several mechanisms by which MSCs can improve healing outcomes in these cases, including reducing tissue inflammation, inducing angiogenesis in the wound bed, and reducing scarring following the repair process. Approaches to extend MSC life span in implant sites are also examined.

5.2. Skin engineering

Transdifferentiation of Adipose-Derived Stem Cells into Keratinocyte-Like Cells: Engineering a Stratified Epidermis

Claudia Chavez-Munoz, Khang T. Nguyen, Wei Xu, Seok-Jong Hong, Thomas A. Mustoe, Robert D. Galiano

PLoS ONE 8(12): e80587

Skin regeneration is an important area of research in the field of tissue-engineering, especially for cases involving loss of massive areas of skin, where current treatments are not capable of inducing permanent satisfying replacements. Human adipose-derived stem cells (ASC) have been shown to differentiate in-vitro into both mesenchymal lineages and nonmesenchymal lineages, confirming their transdifferentiation ability. This versatile differentiation potential, coupled with their ease of harvest, places ASC at the advancing front of stem cell-based therapies. In this study, we hypothesized that ASC also have the

capacity to transdifferentiate into keratinocyte-like cells and furthermore are able to engineer a stratified epidermis. ASC were successfully isolated from lipoaspirates and cell sorted (FACS). After sorting, ASC were either cocultured with human keratinocytes or with keratinocyte conditioned media. After a 14-day incubation period, ASC developed a polygonal cobblestone shape characteristic of human keratinocytes. Western blot and q-PCR analysis showed the presence of specific keratinocyte markers including cytokeratin-5, involucrin, filaggrin and stratifin in these keratinocytelike cells (KLC); these markers were absent in ASC. To further evaluate if KLC were capable of stratification akin to human keratinocytes, ASC were seeded on top of human decellularized dermis and cultured in the presence or absence of EGF and high Ca2+ concentrations. Histological analysis demonstrated a stratified structure similar to that observed in normal skin when cultured in the presence of EGF and high Ca2+. Furthermore, immunohistochemical analysis revealed the presence of keratinocyte markers such as involucrin, cytokeratin-5 and cytokeratin-10. In conclusion this study demonstrates for the first time that ASC have the capacity to transdifferentiate into KLC and engineer a stratified epidermis. This study suggests that adipose tissue is potentially a readily available and accessible source of keratinocytes, particularly for severe wounds encompassing large surface areas of the body and requiring prompt epithelialization.

5.3. Acute irradiation

Severe acute radiation syndrome: treatment of a lethally 60Co-source irradiated accident victim in China with HLA-mismatched peripheral blood stem cell transplantation and mesenchymal stem cells

Mei GUO et al.

Journal of Radiation Research, 2014, 55, 205–209

This is a case report of a 32-year-old man exposed to a total body dose of 14.5 Gyγ-radiation in a lethal 60Co-source irradiation accident in 2008 in China. Frequent nausea, vomiting and marked neutropenia and lymphopenia were observed from 30 min to 45 h after exposure. HLA-mismatched peripheral blood stem cell transplantation combined with infusion of mesenchymal stem cells was used at Day 7. Rapid hematopoietic recovery, stable donor engraftment and healing of radioactive skin ulceration were achieved during Days 18–36. The patient finally developed intestinal obstruction and died of multi-organ failure on Day 62, although intestinal obstruction was successfully released by emergency bowel resection.

5.4. Anti-scarring mechanisms

Mesenchymal stem cell therapy for attenuation of scar formation during wound healing

Wesley M Jackson, Leon J Nesti and Rocky S Tuan

Stem Cell Research & Therapy 2012, 3:20

Scars are a consequence of cutaneous wound healing that can be both unsightly and detrimental to the function of the tissue. Scar tissue is generated by excessive deposition of extracellular matrix tissue by wound healing fibroblasts and myofibroblasts, and although it is inferior to the uninjured skin, it is able to restore integrity to the boundary between the body and its environment. Scarring is not a necessary process to repair the dermal tissues. Rather, scar tissue forms due to specific mechanisms that occur during the adult wound healing process and are modulated primarily by the inflammatory response at the site of injury. Adult tissue-derived mesenchymal stem cells, which participate in normal wound healing, are trophic mediators of tissue repair. These cells participate in attenuating inflammation in the wound and reprogramming the resident immune and wound healing cells to favor tissue regeneration and inhibit fibrotic tissue formation. As a result, these cells have been considered and tested as a likely candidate for a cellular therapy to promote scar-less wound healing. This review identifies specific mechanisms by which mesenchymal stem cells can limit tissue fibrosis and summarizes recent in vivo studies where these cells have been used successfully to limit scar formation.

5.5. Review article on wound healing

Concise Review: Clinical Translation of Wound Healing Therapies Based on Mesenchymal Stem Cells

WESLEY M. JACKSON, LEON J. NESTI, ROCKY S. TUAN

STEM CELLS TRANSLATIONALMEDICINE 2012;1:44–50

There is enormous worldwide demand for therapies to promote the efficient resolution of hard-to heal wounds with minimal appearance of scarring. Recent in vitro studies with mesenchymal stem cells (MSCs) have identified numerous mechanisms by which these cells can promote the process of wound healing, and there is significant interest in the clinical translation of an MSC-based therapy to promote dermal regeneration. This review provides a systematic analysis of recent preclinical and clinical research to evaluate the use of MSCs in wound healing applications. These in vivo studies provide overwhelming evidence that MSCs

can accelerate wound closure by modulating the inflammatory environment, promoting the formation of a well-vascularized granulation matrix, encouraging the migration of keratinocytes, and inhibiting apoptosis of wound healing cells. The trophic effects of MSC therapy also appear to augment wound healing in diabetic tissues, thereby preventing the formation of non healing ulcers. Finally, a number of delivery systems have been evaluated and indicate that MSCs could be the basis of a versatile therapy to fulfill the clinical needs for dermal regeneration. However, despite the apparent advantages of MSC-based therapies, there have been only limited clinical investigations of this type of therapy in humans. Thus, our review concludes with a discussion of the translational barriers that are limiting the widespread clinical use of MSCs to enhance wound healing.

5.6. Skin engineering

Full-thickness tissue engineered skin constructed with autogenic bone marrow mesenchymal stem cells.

He L, Nan X, Wang Y, Guan L, Bai C, Shi S, Yuan H, Chen L, Liu D, Pei X.

Sci China C Life Sci. 2007 Aug;50(4):429-37

To explore the feasibility of repairing clinical cutaneous deficiency, autogenic bone marrow mesenchymal stem cells (BMSCs) were isolated and differentiated into epidermal cells and fibroblasts in vitro supplemented with different inducing factors and biomaterials to construct functional tissue engineered skin. The results showed that after 72 h induction, BMSCs displayed morphologic changes such as typical epidermal cell arrangement, from spindle shape to round or oval; tonofibrils, melanosomes and keratohyaline granules were observed under a transmission electronic microscope. The differentiated cells expressed epidermal stem cell surface marker CK19 (59.66% +/- 4.2%) and epidermal cells differentiation marker CK10. In addition, the induced epidermal cells acquired the anti-radiation capacity featured by lowered apoptosis following exposure to UVB. On the other hand, the collagen microfibrils deposition was noticed under a transmission electronic microscope after differentiating into dermis fibroblasts; RT-PCR identified collagen type I mRNA expression in differentiated cells; radioimmunoassay detected the secretion of interleukin-6 (IL-6) and interleukin-8 (IL-8) (up to 115.06 pg/mL and 0.84 ng/mL, respectively). Further in vivo implanting BMSCs with scaffold material shortened skin wound repair significantly. In one word, autogenic BMSCs have the potential to differentiate into epidermal cells and fibroblasts in vitro, and show clinical feasibility acting as epidermis-like and dermis-like seed cells in skin engineering.

6. Studies on hair

6.1. hMSCs possess the potential to differentiate into DP cells in vivo and in vitro

Minjuan Wu, Qing Sun, XiaocanGuo and Houqi Liu

Cell Biol. Int. Rep. 19(2)

DP (dermal papilla) is a mesenchyme-derived structure situated at the base of the HF (hair follicle) that plays an important role in embryonic hair morphogenesis and maintenance of the hair growth cycle. hMSCs (human mesenchymal stem cells) have gained widespread attention in the field of tissue engineering, but not much is known about the differentiation of hMSCs into DP cells. hMSCs involved in HF formation were examined in our previous study. Here, we have explored the differentiation potential of hMSCs into DP cells by co-culturing hMSCs with DP cells, which proved to be the case. During the differentiation process, the expression of versican, CD133, SCF (stem cell factor), ET-1 (endothelin-1) and bFGF (basic fibroblast growth factor) increased. Compared with hMSCs alone, the aggregate number clearly increased when cocultured with DP cells. The expression in vivo of HLA-I (human leucocyte antigen class I) was confined to DP of the newly formed HF. The data suggest that hMSCs possess the potential to differentiate into DP cells in vivo and in vitro.

6.2 miR-24 affects hair follicle morphogenesis targeting Tcf-3

I Amelio, AM Lena, E Bonanno, G Melino and E Candi

Cell Death and Disease (2013) 4, e922

During embryonic development, hair follicles (HFs) develop from an epidermal–mesenchymal cross talk between the ectoderm progenitor layer and the underlying dermis. Epidermal stem cell activation represents a crucial point both for HF morphogenesis and for hair regeneration. miR-24 is an anti-proliferative microRNA (miRNA), which is induced during differentiation of several cellular systems including the epidermis. Here, we show that miR-24 is expressed in the HF and has a role in hair morphogenesis. We generated transgenic mice ectopically expressing miR-24 under the K5 promoter. The K5::miR-24 animals display a marked defect in HF morphogenesis, with thinning of hair coat and altered HF structure. Expression of miR-24 alters the normal process of hair keratinocyte differentiation, leading to altered expression of differentiation markers. MiR-24 directly represses the hair keratinocyte stemness regulator Tcf-3. These results support the notion that microRNAs, and among them miR-24, have an important role in postnatal epidermal homeostasis.

6.3 Human Wharton's Jelly Mesenchymal Stem Cells Plasticity Augments Scar-Free Skin Wound Healing with Hair Growth

VikramSabapathy, BalasubramanianSundaram, Sreelakshmi VM, PratheeshMankuzhy, Sanjay Kumar

Human mesenchymal stem cells (MSCs) are a promising candidate for cell-based transplantation and regenerative medicine therapies. Thus in the present study Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) have been derived from extra embryonic umbilical cord matrix following removal of both arteries and vein. Also, to overcome the clinical limitations posed by fetal bovine serum (FBS) supplementation because of xenogeneic origin of FBS, usual FBS cell culture supplement has been replaced with human platelet lysate (HPL). Apart from general characteristic features of bone marrow-derived MSCs, wharton jelly-derived MSCs have the ability to maintain phenotypic attributes, cell growth kinetics, cell cycle pattern, in vitro multilineage differentiation plasticity, apoptotic pattern, normal karyotype-like intrinsic mesenchymal stem cell properties in long-term in vitro cultures. Moreover, the WJ-MSCs exhibited the in vitro multilineage differentiation capacity by giving rise to differentiated cells of not only mesodermal lineage but also to the cells of ectodermal and endodermal lineage. Also, WJ-MSC did not present any aberrant cell state upon in vivo transplantation in SCID mice and in vitro soft agar assays. The immunomodulatory potential assessed by gene expression levels of immunomodulatory factors upon exposure to inflammatory cytokines in the fetal WJ-MSCs was relatively higher compared to adult bone marrow-derived MSCs. WJMSCs seeded on decellularized amniotic membrane scaffold transplantation on the skin injury of SCID mice model demonstrates that combination of WJ-MSCs and decellularized amniotic membrane scaffold exhibited significantly better wound-healing capabilities, having reduced scar formation with hair growth and improved biomechanical properties of regenerated skin compared to WJ-MSCs alone. Further, our experimental data indicate that indocyanin green (ICG) at optimal concentration can be resourcefully used for labeling of stem cells and in vivo tracking by near infrared fluorescence non-invasive live cell imaging of labelled transplanted cells, thus proving its utility for therapeutic applications.

6.4. Signaling Involved in Hair Follicle Morphogenesis and Development

PisalRishikaysh, Kapil Dev, Daniel Diaz, WasayMohiuddin Shaikh Qureshi, Stanislav Filip and Jaroslav Mokry

Int. J. Mol. Sci. 2014, 15, 1647-1670

Hair follicle morphogenesis depends on Wnt, Shh, Notch, BMP and other signaling pathways interplay between epithelial and mesenchymal cells. The Wnt pathway plays an essential role during hair follicle induction, Shh is involved in morphogenesis and late stage differentiation, Notchsignaling determines stem cell fate while BMP is involved in cellular differentiation. The Wnt pathway is considered to be the master regulator during hair follicle morphogenesis. Wntsignaling proceeds through EDA/EDAR/NF-κBsignaling. NF-κB regulates the Wnt pathway and acts as a signal mediator by upregulating the expression of Shh ligand. Signal crosstalk between epithelial and mesenchymal cells takes place mainly through primary cilia. Primary cilia formation is initiated with epithelial laminin-511 interaction with dermal β-1 integrin, which also upregulates expression of downstream effectors of Shh pathway in dermal lineage. PDGF signal transduction essential for crosstalk is mediated through epithelial PDGF-A and PDGFRα expressed on the primary cilia. Dermal Shh and PDGF signaling up-regulates dermal noggin expression; noggin is a potent inhibitor of BMP signaling which helps in counteracting BMP mediated β-catenin inhibition. This interplay of signaling between the epithelial and dermal lineage helps in epithelial Shh signal amplification. The dermal Wnt pathway helps in upregulation of epithelial Notch expression. Dysregulation of these pathways leads to certain abnormalities and in some cases even tumor outgrowth.

6.5 Adipose Derived Stem Cells and Growth Factors Applied on Hair Transplantation. Follow-Up of Clinical Outcome

Federica Zanzottera, Emilio Lavezzari, LetiziaTrovato, Alessandro Icardi, Antonio Graziano

Journal of Cosmetics, Dermatological Sciences and Applications, 2014, 4, 268-274

Different studies show the need of immature adipose cell to induce the proliferation of bulge stem cells in order to kick off the anagen phase of hair cycle. Furthermore, the adipose derived stem cell, adipose progenitors, and growth factors secreted by mature adipocytes can help the wound healing and the vascular neogenesis. Nowadays, it is not known any protocol of tissue regeneration applied to hair transplantation, especially if aimed to the reconstruction of the main vascular network for the engraftment of transplanted hair and the healing process. The aim of the work is to investigate how the application of autologous cellular suspension obtained by Rigenera system, mechanical fragmentation procedure which

allows to obtain a physiological saline solution consisting of a heterogeneous pool of cells rich in adipose derived mesenchymal stem cells and growth factors, helps the wound healing and engraftment of the transplanted hair. During hair restoration surgery, the adipose tissue recovered from the discard of follicular slicing, was processed using the Rigenera system. The obtained cell suspension was applied in the area of hair transplantation, increasing the natural background of adipocyte lineage and raising the amount of growth factors. In addition, the cellular suspension was applied to the suture on the occipital region. The cell population was characterized by FACS. The monthly evaluation of hair transplantation follow-up with photos and the patient's impressions demonstrates that there is a faster healing of the micro-wound and a continuous growth of the transplanted hair even two months after the procedure, with a shortening of the dormant phase. In conclusion, this new approach aims to integrate regenerative medicine and hair restoration surgery in order to improve the outcome for the patient. It would be wonderful to continue this research to elaborate on the molecular cause behind this satisfying clinical.

6.6 Therapeutic strategy for hair regeneration: Hair cycle activation, niche environment modulation, wound-induced follicle neogenesis and stem cell engineering

Shan-Chang Chueh, Sung-Jan Lin, Chih-Chiang Chen, Mingxing Lei, Ling MeiWang, Randall B. Widelitz, Michael W. Hughes, Ting-Xing Jiang, and Cheng Ming Chuong

Expert OpinBiolTher. 2013 March ; 13(3): 377–391

Hair regeneration is discussed in four different categories. (1) Intra-follicle regeneration (or renewal) is the basic production of hair fibers from hair stem cells and dermal papillae in existing follicles. (2) Chimeric follicles via epithelialmesenchymal recombination to identify stem cells and signalingcenters. (3) Extra-follicular factors including local dermal and systemic factors can modulate the regenerative behavior of hair follicles, and may be relatively easy therapeutic targets. (4) Follicular neogenesis means the de novo formation of new follicles. In addition, scientists are working to engineer hair follicles, which require hair forming competent epidermal cells and hair inducing dermal cells.

6.7. β-catenin activation regulates tissue growth non-cell autonomously in the hair stem cell niche

Elizabeth R. Deschene, Peggy Myung, PanteleimonRompolas, Giovanni Zito, Thomas Yang Sun, Makoto M. Taketo, IchikoSaotome and Valentina Greco

Science. 2014 March 21; 343(6177): 1353–1356

Wnt/β-catenin signaling is critical for tissue regeneration. However, it is unclear how β-catenin controls stem cell behaviors to coordinate organized growth. Using live imaging, we show that activation of β-catenin specifically within mouse hair follicle stem cells generates new hair growth through oriented cell divisions and cellular displacement. β-catenin activation is sufficient to induce hair growth independently of mesenchymal dermal papilla-niche signals normally required for hair regeneration. Remarkably, wild-type cells are co-opted into new hair growths by β-catenin mutant cells, which non-cell autonomously activate Wntsignaling within the wild-type cells via Wnt ligands. This study demonstrates a mechanism by which Wnt/β-catenin signaling controls stem cell-dependent tissue growth non–cell autonomously and advances our understanding of the mechanisms that drive coordinated regeneration.

6.8 Bioengineering the Hair Follicle

K. Stenn, S. Parimoo Y. Zheng, T. Barrows, M. Boucher, K. Washenik

Organogenesis 3:1, -13, January/February/March 2007

The hair follicle develops from the primitive embryonic epidermis as a result of complex epithelial -mesenchymal interactions. The full follicle, consisting of epithelial cylinders under control of a proximal lying mesenchymal papilla, grows in cycles giving rise to a new hair shaft during each cycle. The ability to cycle endows the follicle with regenerative properties. The evolution of hair follicle engineering began with the recognition in the early 1960's that hair follicles could be transplanted clinically into a foreign site and still grow a shaft typical of the donor site. Since that time, it has been found that the follicular papilla has hair follicle inducing properties and that the hair follicle houses within it epithelial stem cells that can respond to hair inductive signals. These findings have laid the foundation for isolating hair - forming cells, for expanding the cells in culture, and for forming new follicles in vivo.

6.9 Treatment of MSCs with Wnt1a-conditioned medium activates DP cells and promotes hair follicle regrowth

Liang Dong, HaojieHao, Lei Xia, Jiejie Liu, DongdongTi, Chuan Tong, Qian Hou1, Qingwang Han, Yali Zhao, Huiling Liu, Xiaobing Fu &Weidong Han

SCIENTIFIC REPORTS | 4 : 5432

Hair loss (alopecia) is a common problem for people. The dermal papilla is the key signalingcenter that regulates hair growth and it engage in crosstalk with the microenvironment, including Wntsignaling and stem cells. In this study, we explored the effects of bone marrow mesenchymal stem cell overexpression of Wnt1a on mouse hair follicle regeneration. Wnt-CM accelerated hair follicle progression from telogen to anagen and enhanced the ALP expression in the DP area. Moreover, the hair induction-related genes were upregulated, as demonstrated by qRT-PCR. Wnt-CM treatment restored and increased DP cell expression of genes downregulated by dihydrotestosterone treatment, as demonstrated by qRT-PCR assays. Our study reveals that BM-MSC-generated Wnt1a promotes the DP's ability to induce hair cycling and regeneration.