MESENCELL BIOTECH

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Anti-morbidity and Anti-ageing: present and future uses of adipose derived mesenchymal stem cells and their secretome in improving health span

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Content

1. Executive summary

Ageing, Anti-ageing and Anti-morbidity

With continuously increasing life expectancy, the impact of chronic diseases and dependencies, for which no specific cures are available, also increases. This leads to an enormous burden to individuals and societies alike, which threatens to overwhelm conventional curative and care models in medicine. Unfortunately healthy life expectancy, the number of years spent free of disabilities, has grown slower than life expectancy in a number of countries. Within the European Union, for instance, life expectancy at age 50 has grown by 1.2 years between 2005 and 2010, but healthy life expectancy has only grown slightly (0.5 years in men, 0.4 years in women) in the same time frame (Fouweather et al., 2015). Ageing is a process of progressive decline in the physiological capacity of an organism, manifested by accumulated alteration and destabilization at the whole system level. Generally, oxidative stress is a cause of aging in lipid peroxidation, protein carbonyl content increase, and DNA damage. Aging is also associated with a differential gene expression pattern indicative of a marked or lower stress response of metabolic and biosynthetic genes. Additionally, cellular senescence, stem cell exhaustion and chronic low-grade inflammation known as “inflammaging” have been proposed as limitations to age span and health span.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) have been proposed as an optimal regenerative cellular therapeutic for degenerative musculoskeletal conditions like OA. These cells are found in a variety of tissues and have the ability to rapidly proliferate and differentiate to musculoskeletal lineages including bone and cartilage. A significant body of research has also demonstrated that these cells orchestrate important immunologic functions through modulation of the local inflammatory response. Taken together, these factors support the theoretical ability of MSCs to deter degenerative joint disease.

Safety

Adipose tissue-derived mesenchymal stem cells (Ad MScs) 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. The toxicity and tumourigenicity of human Ad MScs 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.

Secretome

The use of MSCs for tissue repair was initially based on the hypothesis that these cells home to and differentiate within the injured tissue into specialized cells. However, it now appears that only a small proportion of transplanted MSCs actually integrate and survive in host tissues. Thus, the predominant mechanism by which MSCs participate in tissue repair seems to be related to their paracrine activity. Indeed, MSCs provide the microenvironment with a multitude of trophic and survival signals including growth factors and cytokines. Recent discoveries suggest that lipid microvesicles released by MSCs may also be important in the physiological function of these cells. Over the past few years the biological relevance of micro- and nano-vesicles released by cells in intercellular communication has been established. Alongside the conventional mediators of cell secretome, these sophisticated nanovesicles transfer proteins, lipids and, most importantly, various forms of RNAs to neighbouring cells, thereby mediating a variety of biological responses.

Patented Production Method

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

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Preclinical studies regarding ageing and anti-ageing

The anti-inflammatory and anti-apoptic (limiting the programmed cell death of cells) effect of mesenchymal stem cells and their secretome are well documented in literature. Our own studies into the effects of the stem cell secretome on keratinocytes, cardiomyocytes, chondrocytes, cartilage explants and aged mesenchymal stem cells have shown impressive reduction of known factors of inflammation and cell death.

Clinical studies on ageing, anti-ageing and anti-morbidity

We are currently finalizing a multi-centre observational study on the effects of intravenous administration of mesenchymal stem cells and two production versions of their secretome on a selected panel of 10 biomarkers of ageing and morbidity.

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2. Ageing and Anti-ageing

With continuously increasing life expectancy, the impact of chronic diseases and dependencies, for which no specific cures are available, also increases. This leads to an enormous burden to individuals and societies alike, which threatens to overwhelm conventional curative and care models in medicine. Unfortunately healthy life expectancy, the number of years spent free of disabilities, has grown slower than life expectancy in a number of countries. Within the European Union, for instance, life expectancy at age 50 has grown by 1.2 years between 2005 and 2010, but healthy life expectancy has only grown slightly (0.5 years in men, 0.4 years in women) in the same time frame (Fouweather et al., 2015).

Understanding the factors determining extreme longevity and compression of morbidity might help to achieve extended healthy life span. Such understanding cannot simply be extrapolated from results obtained in younger populations because the power of biomarkers to predict the impact of various pathophysiological mechanisms on successful ageing is frequently dependent on age itself (Martin-Ruiz and von Zglinicki, 2014).

Aging is a process of progressive decline in the physiological capacity of an organism, manifested by accumulated alteration and destabilization at the whole system level. Generally, oxidative stress is a cause of aging in lipid peroxidation, protein carbonyl content increase, and DNA damage. Aging is also associated with a differential gene expression pattern indicative of a marked or lower stress response of metabolic and biosynthetic genes. Additionally, cellular senescence, stem cell exhaustion and chronic low-grade inflammation known as “inflammaging” have been proposed as limitations to age span and health span. Cellular senescence describes how cells can induce an irreversible proliferation disability, which is resistant to growth or proliferation factors. Exhaustion of adult stem cells is proposed as reason for the decline in self-healing and regeneration of the aged organism. Inflammation is mediated by cytokines and micro RNA. So called Inflamma-miRs, micro RNA responsible for the activation of inflammation, have been implicated in the regulation of immune and inflammatory responses. Their abnormal expression contributes to the chronic pro-inflammatory status documented in normal aging and major age-related diseases (ARDs), “inflammaging” being a significant mortality risk factor in both cases.

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. The secretome of adipose derived stem cells has a predominantly anti-inflammatory and anti-apoptic effect and target tissues.

An epigenetic profile defining the DNA methylation age (DNAm age) of an individual has been suggested to be a biomarker of aging, and thus possibly providing a tool for assessment of health and mortality. Other researchers have found a mechanistic link between carbohydrate derived metabolites and cancer which may provide a biological consequence of established factors of cancer disparity. Glycation is the non-enzymatic glycosylation of carbohydrates to macromolecules which produces reactive metabolites called advanced glycation end products (AGEs). In a study with more than 10 000 male patients, researchers found that elevated hsCRP (C reactive protein) levels were associated with a greater risk of total stroke, even after adjustment for potential confounders and cardiovascular risk factors. Risk of total stroke was significantly higher among hypertensive men with elevated hsCRP compared with normotensive men with low hsCRP. In a smaller group of female patients, oxidative stress, analysed through oxidized LDL, was increased in old healthy women, as well as the inflammatory biomarkers sVCAM and sE-selectin.

The be able to prove or refute an anti-ageing property of adipose derived mesenchymal stem cells, we have chosen biomarkers responsible for documenting inflammation, oxidative stress, DNA damage, mediators of apoptosis (programmed cell death) and advanced glycation end products and their respective receptors: 08-OHdG DNA damage (8-hydroxy-2'deoxy-Guanosine), esRAGE (endogenous secretory receptor of AGE), AGE (advanced glycation end product), DNA methylation and hydroxymethylation, m6A RNA methylation, sVCAM1 (serum vascular cell adhesion molecule), sE-selectine, oxydated LDL cholesterol, NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells), IL-6 (interleukin 6), TNF alpha (Tumor necrosis factor) and C-reactive protein.

8-OHdG Quantitation

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress. 8-OHdG, one of the oxidative DNA damage byproducts, is physiologically formed and enhanced by chemical carcinogens. During the repair of damaged DNA in vivo by exonucleases, the resulting 8-OH-dG is excreted without further metabolism into urine.

esRAGE

In multicellular organisms, most cellular and plasma proteins undergo a nonenzymatic glycation by the covalent binding of reduced sugars to the amino groups on proteins. A balance exists between early glycation products and non-glycated proteins during homeostasis. Under the condition of prolonged hyperglycemia as in diabetes, these early stage glycation products undergo additional chemical rearrangements to form irreversibly bound advanced glycation end products (AGE). AGE has been shown to be a major factor in vascular cell derangement in diabetic patients. AGE has also been implicated in Alzheimer's Disease by the glycation of amyloid-β peptide and microtubule-associated protein tau. The receptor for AGE (RAGE), a 45kDa protein and a member of the immunoglobulin superfamily of cell surface molecules, is found in mononuclear phagocytes, endothelium, vascular smooth muscle, and the central nervous system. RAGE is a multi-ligand receptor. The most pathological consequence of the AGE-RAGE engagement is induction of oxidative stress, activation of nuclear factor kB (NF-kB), resulting in disruption of homoeostatic functions in the vasculature. AGE has also been shown to up-regulate the RAGE expression in endothelial cells. Translation of RAGE mRNA isolated from human endothelial cells revealed a novel splice variant named endogenous secretory receptor (esRAGE) which can capture AGE and neutralize the effects of AGE on cells. Endogenous secretory RAGE has been found circulating in blood and in extracellular fluids of our bodies.

AGE

The non-enzymatic reaction of reducing carbohydrates with lysine side chains and N-terminal amino groups of macromolecules (proteins, phospholipids and nucleic acids) is called the

Maillard reaction or glycation. The products of this process, termed advanced glycation end products (AGEs), adversely affect the functional properties of proteins, lipids and DNA. For example, N-ε-(Carboxymethyl) lysine (CML), one of the prevalent AGEs, has been implicated in oxidative stress and vascular damage. Tissue levels of AGE increase with age and the formation of AGEs is predominantly endogenous, though these products can also be derived from exogenous sources such as food and tobacco smoke. AGE modification of proteins can contribute to the pathophysiology of aging and long-term complications of diabetes, atherosclerosis and renal failure. AGEs also interact with a variety of cell-surface AGE-binding receptors (RAGE), leading either to their endocytosis and degradation or to cellular activation and pro-oxidant or pro-inflammatory events.

DNA methylation and hydroxymethylation

DNA methylation is an epigenetic mechanism that occurs by the addition of a methyl (CH3) group to DNA, thereby often modifying the function of the genes. The most widely characterized DNA methylation process is the covalent addition of the methyl group at the 5-carbon of the cytosine ring resulting in 5-methylcytosine (5-mC), also informally known as the "fifth base" of DNA. These methyl groups project into the major groove of DNA and inhibit transcription. In human DNA, 5-methylcytosine is found in approximately 1.5% of genomic DNA. In somatic cells, 5-mC occurs almost exclusively in the context of paired symmetrical methylation of a CpG site, in which a cytosine nucleotide is located next to a guanidine nucleotide. An exception to this is seen in embryonic stem (ES) cells, where a substantial amount of 5-mC is also observed in non-CpG contexts. In the bulk of genomic DNA, most CpG sites are heavily methylated while CpG islands (sites of CpG clusters) in germ-line tissues and located near promoters of normal somatic cells, remain unmethylated, thus allowing gene expression to occur. When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed (it is turned off). The addition of methyl groups is controlled at several different levels in cells and is carried out by a family of enzymes called DNA methyltransferases (DNMTs). Three DNMTs (DNMT1, DNMT3a and DNMT3b) are required for establishment and maintenance of DNA methylation patterns. Two additional enzymes (DNMT2 and DNMT3L) may also have more specialized but related functions. DNMT1 appears to be responsible for the maintenance of established patterns of DNA methylation, while DNMT3a and 3b seem to mediate establishment of new or de novo DNA methylation patterns. Diseased cells such as cancer cells may be different in that DNMT1 alone is not responsible for maintaining normal gene hypermethylation (an increase in global DNA methylation) and both DNMTs 1 and 3b may cooperate for this function.

DNA Methylation vs DNA Hydroxymethylation

The recent discovery of 5-hydroxymethylcytosine (5-hmC) in mammalian DNA has complicated methylation analyses and stirred up the epigenetic community. The function of 5-hmC in epigenetics may be different from its forerunner, 5-methylcytosine (5-mC), as 5-hmC seems to prompt DNA demethylation. However, existing methylated DNA analysis methods including restriction enzyme digestion and bisulfite or MeDIP-mediated MS-PCR and sequencing may have been incorrectly detecting 5-hmC as 5-mC, as 5-hmC and 5-mC are virtually indistinguishable between each other with these methods. It is necessary to re-evaluate the enormous amounts of existing DNA methylation data by determining if 5-hmC also exists in the analyzed samples. It is also necessary to determine the contents of 5-mC and 5-hmC and their ratios in different cell types and in different compartments of the genome of mammalians. And it is particularly important to identify the true status of epigenetic changes at the DNA level in diseased human cells and tissues: is it methylation or hydroxymethylation?

m6A RNA methylation

N6-methyl-adenosine (m6A) is the most common and abundant modification on RNA molecules present in eukaryotes. The m6A modification is catalyzed by a methyltransferase complex METTL3 and removed by the recently discovered m6A RNA demethylases FTO and ALKBH5, which catalyze m6A demethylation in an α-ketoglutarate (α-KG)- and Fe2+-dependent manner. It was shown that METTL3, FTO and ALKBH5 play important roles in many biological processes, ranging from development and metabolism to fertility. m6A accounts for more than 80% of all RNA base methylations and exists in various species. m6A is mainly distributed in mRNA and also occurs in non-coding RNA such as tRNA, rRNA and snRNA. The relative abundance of m6A in mRNA transcripts has been shown to affect RNA metabolism processes such as splicing, nuclear export, translation ability and stability and RNA transcription. Abnormal m6A methylation levels induced by defects in m6A RNA methylase and demethylase could lead to dysfunction of RNA and cause disease. For example, abnormally low levels of m6A in target mRNAs due to increased FTO activity in patients with FTO mutations, through an as-yet undefined pathway, contributes to the onset of obesity and related diseases. The dynamic and reversible chemical m6A modification on RNA may also serve as a novel epigenetic marker of profound biological significance. Therefore, more useful information for better understanding of m6A RNA methylation levels and distribution on RNA transcripts could benefit diagnostics and therapeutics of disease.

sVCAM1

Vascular cell adhesion molecule-1 (VCAM-1) is part of the immunoglobulin superfamily. They are important in inflammation, immune responses and in intracellular signalling events. They are known to bind to leucocyte integrins CD11/CD18 during inflammation and in immune responses. VCAM-1 was first described as a cytokine-inducible endothelial adhesion molecule. It can bind to leucocyte integrin VL-4 (very late antigen-4) to recruit leucocytes to sites of inflammation. The predominant form of VCAM-1 in vivo has an N-terminal extracellular region comprising seven Ig-like domains

sE-selectine

Members of the selectin superfamily have the same domain structure: an N-terminal lectin domain followed by an EGF repeat; a variable number (between 2 and 9) of complement regulatory elements; a single trans- membrane region; and a short cytoplasmic anchor. Some studies have found distinct carbohydrate structures on leukocytes that are adhered to by selectins, suggesting that selectins are involved in the selective trafficking of blood-borne components of the immune system. CD62E mediates leukocyte rolling on activated endothelium at inflammatory sites and may also support tumor cell adhesion during hematogenous metastasis, and play a role in angiogenesis.

Oxydated LDL cholesterol

Lipoproteins are submicroscopic particles composed of lipid and protein held together by noncovalent forces. Their general structure is that of a putative spheroidal microemulsion formed from an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesteryl esters and triacylglycerols (TAG). Low density lipoprotein (LDL) is the major transport protein for cholesterol in human plasma. LDL is a spherical particle with a diameter of 20-25 nm. Each LDL particle contains cholesteryl esters in its core which are surrounded by a hydrophilic coat composed of phospholipids, cholesterol, and one molecule of a hydrophobic protein known as apolipoprotein B-100. LDL cholesterol, sometimes referred to as “bad” cholesterol, is even more dangerous when it becomes oxidized. Oxidized LDL (OxLDL) is more reactive with surrounding tissues and can collect within the inner-lining of arteries. Macrophages, cholesterol, and other lipids can accumulate at the site (atherosclerosis), ultimately forming a plaque that can lead to heart attack, stroke or death. LDL oxidation affects both the lipid and protein components of LDL. Reactive aldehyde products formed during the oxidation of polyunsaturated fatty acids, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are capable of attaching covalently to the ε-amino groups of lysine residues of of ApoB-100 to form MDA-Lys and HNE-Lys adducts (MDA-LDL and HNE-LDL). Advanced glycosylation, such as the formation of CML-LDL and CEL-LDL, are also involved in LDL oxidation.

NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells)

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family.

IL-6

Interleukin 6 (IL-6) is a cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response and plays an essential role in the final differentiation of B-cells into Ig-secreting cells. IL-6 is involved in lymphocyte and monocyte differentiation and IL-6 induces myeloma and plasmacytoma growth as well as nerve cells differentiation. B-cells, T-cells, hepatocytes, hematopoeitic progenitor cells and cells of the CNS are all responsive to IL-6. IL-6 is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance.

TNF alpha

TNF-alpha, also known as cachectin or TNFSF1A, is the prototypic ligand of the TNF superfamily which plays a central role in inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis and morphogenesis. It is expressed on macrophages, endothelial, epithelial and tumor cells as a 26kDa transmembrane protein. TNF-alpha is cleaved by proteolytic processing into six chains: (1) TNF membrane form, (2) Intracellular domain 1, (3) Intracellular domain 2, (4) C-domain 1, (5) C-domain 2 and (6) TNF soluble form. Signaling from TNF-alpha differs depending on the type of ligand initiating the signaling event (intracellular, membrane or soluble). As an example, the membrane form of TNF-alpha appears to mediate anti-tumorigenic therapeutic responses whereas the soluble ligand is linked to inflammation and proliferation.

C-reactive protein

C-Reactive Protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. CRP belongs to the pentraxin family of calcium dependent ligand-binding plasma proteins. Human CRP binds with highest affinity to phosphocholine residues, but it also binds to a variety of other autologous and extrinsic ligands, and it aggregates or precipitates the cellular, particulate, or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles, and apoptotic cells. Extrinsic ligands include many glycan, phospholipid, and other constituents of microorganisms, such as capsular and somatic components of bacteria, fungi, and parasites, as well as plant products. CRP has been associated with cardiovascular disease, atherosclerosis and inflammation, and other human diseases.

Telomere shortening as biomarker was considered, however the results from a Japanese survey with 1554 individuals aged 85 and above showed that inflammation, but not Telomere length predicted successful ageing (at extreme old age).

3. 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 and reinjected 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.

Mesenchymal stem cells do not form but inhibit tumours

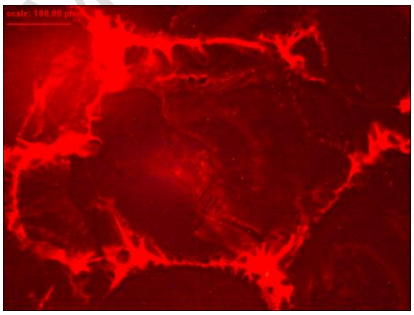
For decades we have struggled through drug regulatory panels and medicine's agencies who's scientists were implying that mesenchymal stem cells were capable of degenerating into tumours. This was based on a publication of Spanish scientists claiming to have seen tumour cell formation in vitro (the publication being withdrawn swiftly later, but this was less published then the original text) and on the lack of understanding about the real active principles behind the stem cells. As we have proven and validated over the last decade, the active principle behind stem cells are the active substances they secrete (thus our name "secretome" for this), including microvesicles, exosomes, cytokines, messenger and micro RNA to just mention some.

Over the years we and other scientists have proven that MSCs to not form cancer and indeed the secretome of MSCs inhibits growths of tumours and carcinomas, as well as inhibiting drug resistance genes, making it interesting as therapeutic co-medication to radiotherapy and chemotherapy.

Absence of Tumourgenicity of MSC and MSC derived cells and tissues

Mesencell Biotech has intensively worked on the question of tumour formation of MSCs and also of Insulin producing cells (IPCs) and Dopamine producing cells (DPCs), the new biological treatment for Diabetes type 1 and 2 and Parkinson Disease, replacing beta cells of the pancreas and substantia nigra cells of the brain respectively with autologous cells derived from MSCs.

- Our studies, performed to the current in vitro state of the art method, i.e. Soft Agar Colony Forming Assay, revealed that MSCs, IPCs and DPCs cultured and differentiated under the Mesencell Biotech system, do not show any signs of tumour formation (Kellner & Rey, in house data).



Insulin producing cells during Soft Agar Colony Forming Assay

Secretome

- Exosomes from MSCs enhance radiotherapy-induced cell death in tumour and metastatic tumour foci, making them a viable option for concurrent cancer therapies (De Araujo et al., 2018)
- MSCs have an anticancer effect with dynamic DNA methylation alteration (Wu et al., 2019)
- MSCs influence global patterns of hydroxymethylome and methylome, i.e. epigenetic modification of DNA (Kellner & Ferchichi, in house data)
- MSCs promote carcinoma cell apoptosis through the PI3K/AKT/FOXO pathway via regulating enhancer 5-hmC and 5-mC levels. (phosphoinositide 3-kinase/serine/threonine kinase/Forkhead box protein O)
- MSCs downregulate drug resistance genes via epigenetic regulation
- MSCs upregulate cancer and tumour suppressors via epigenetic mechanisms (Wu et al., 2019)

Epigenetic mechanisms function as an interface between environmental factors and the genome (see Anti-ageing project Mesencell). Cancer cells not only reshape their epigenomes in response to an altered milieu but also reprogram the tumour microenvironment toward immunosuppression. MSCs inhibit cancer cells via secretion of diverse components into the microenvironment. MSCs not only secrete anti-cancer factors to directly inhibit oncogenic pathways, but also regulate the epigenetic mechanisms of cancer cells to suppress downstream biological functions.

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 bovine serum), 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.

List of relevant publications regarding the safety of stem cells

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.

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

Source

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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 tumorigenicity 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 tumorigenicity 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 tumor 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 tumor development.

Evaluation of the use of human Mesenchymal Stem Cells for acute toxicity tests.

Scanu M, Mancuso L, Cao G.

Toxicol In Vitro. 2011 Dec;25(8):1989-95. doi: 10.1016/j.tiv.2011.07.006. Epub 2011 Jul 21.

Source

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Abstract

In vitro cytotoxicity tests are typically carried out with transformed, immortalized cell lines or primary cells. Immortalized cells are readily available and easily maintained, although they usually show anomalous behavior and phenotypes, which do not reflect the mechanisms observed in their normal homologous cells. Primary cells are indeed considered a better option as model systems for predicting toxicological behavior, although they are limited in

quantity and suffer from batch-to-batch variation due to the need to isolate them freshly for each study. In particular, human Mesenchymal Stem Cells (hMSCs) have never been adopted in order to develop in vitro model systems for acute toxicity tests of chemicals. Therefore, the aim of this study was to verify the possibility of using hMSCs as an alternative method to estimate in vivo starting dose for acute toxicity. As suggested by ICCVAM, 12 reference chemicals were assessed in the present study and a Neutral Red Uptake assay was performed. It is shown for the first time that MSCs isolated from human bone marrow can be confidently used in this area of toxicology. MSCs represent a good promise for the development of in vitro human assays.

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.

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

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 tumorigenicity 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 tumorigenicity 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 tumor 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 tumor development.

Multipotent mesenchymal stromal cell therapy and risk of malignancies.

Casiraghi F, Remuzzi G, Abbate M, Perico N.

Stem Cell Rev. 2013 Feb;9(1):65-79. doi: 10.1007/s12015-011-9345-4.

Source

Transplant Research Center Chiara Cucchi de Alessandri e Gilberto Crespi, Department of Immunology and Transplantation, Ospedali Riuniti - Mario Negri Institute for Pharmacological Research, Bergamo, Italy. federica.casiraghi@marionegri.it

Abstract

Cell therapy with Multipotent Mesenchymal Stromal Cells (MSC) holds enormous promise for the treatment of a large number of degenerative and immune/inflammatory diseases. Their multilineage differentiation potential, immunoprivilege and capacity of promoting recovery of damaged tissues coupled with anti-inflammatory and immunosuppressive properties are the focus of a multitude of clinical studies currently underway. The recognized clinical potential of MSC repairing/immunomodulatory effects now encompasses graft-versus-host disease, hematologic malignancies, cardiovascular diseases, neurologic and inherited diseases, autoimmune diseases, organ transplantation, refractory wounds, and bone/cartilage defects among others. However, it has been suggested that both the need of extensive ex vivo culture for MSC clinical use, and their proangiogenic, anti-apoptotic and immunomodulatory properties may act together as tumor promoters, raising significant safety concerns. This paper will review the available data on in vitro MSC maldifferentiation and the ability of MSC to sustain tumor growth in vivo, with the aim to clarify whether MSC-based therapeutic approaches may carry actual risk of malignancies.

Banking human umbilical cord-derived mesenchymal stromal cells for clinical use.

Gong W, Han Z, Zhao H, Wang Y, Wang J, Zhong J, Wang B, Wang S, Wang Y, Sun L, Han Z.

Cell Transplant. 2012;21(1):207-16. doi: 10.3727/096368911X586756. Epub 2011 Sep 16.

Source

National Engineering Research Center of Cell Products, AmCellGene Co. Ltd, TEDA, Tianjin, China.

Abstract

A great deal of interest has arisen recently with respect to human mesenchymal stem cells (MSCs), due to their broad therapeutic potential. However, the safety and efficacy of MSCs expanded ex vivo for clinical applications remain a concern. In this article, we establish a standardized process for manufacture of human umbilical cord-derived MSCs (UC-MSCs), which encompasses donor screening and testing, recovery, two-stage expansion, and administration. The biological properties and safety of UC-MSCs were then characterized and tested. The safety data from use in human patients have also been reported. After clinical-scale expansion, a yield of 1.03-3.78 × 10(8) MSCs was achieved in 10 batch manufacturing runs. The biological properties, such as plastic adherence, morphology, specific surface antigen (CD105, CD73, CD90, positive ≥ 95%; CD45, CD34, CD31, CD11b, CD19, HLA-DR, negative ≤2%), and multipotent differentiation potential (osteogenesis and adipogenesis) were retained. Bacterial and mycoplasma tests were negative and endotoxin levels were lower than 2 EU/ml. No adverse events were noted in two patients treated with intravenously and/or intrathecally administered MSCs. The data obtained indicate that banking UC-MSCs for clinical use is feasible.

Wound microenvironment sequesters adipose-derived stem cells in a murine model of reconstructive surgery in the setting of concurrent distant malignancy.

Altman AM, Prantl L, Muehlberg FL, Song YH, Seidensticker M, Butler CE, Alt EU.

Plast Reconstr Surg. 2011 Apr;127(4):1467-77. doi: 10.1097/PRS.0b013e31820a6400.

Source

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Abstract

BACKGROUND:

It is unclear whether mesenchymal stem cells that are applied to regenerate wound tissues can migrate to existing tumors and enhance their growth. The authors investigated whether

adipose-derived stem cells had any effect on the growth and progression of distant tumors when applied to a skin wound.

METHODS:

The authors subcutaneously injected murine 4T1 breast cancer cells into all BALB/c-nu/nu mice. After tumor injection, mice were randomized to five groups (five mice per group) based on the means of co-introduction of green fluorescent protein-labeled adipose-derived stem cells, if any. In group 1, adipose-derived stem cells were combined and co-injected subcutaneously. In group 2, they were injected subcutaneously at a distant anatomical site. In group 3, they were injected intravenously. In group 4, they were delivered via a human acellular dermal matrix to a distant skin wound. In group 5, no adipose-derived stem cells were introduced.

RESULTS:

After 2 weeks, tumor volume increased in group 1 (356.5 ± 44.4 mm(3)), followed by group 3 (256.6 ± 47.1 mm(3)) and then group 2 (201.6 ± 28.6 mm(3)). In group 4, in which adipose-derived stem cells carried on acellular dermal matrix were applied to a wound distant to the primary tumor, the tumor volume was 143.8 ± 50.9 mm(3), which was similar to that observed in the control group (group 5; 167.8 ± 29.9 mm(3)).

CONCLUSIONS:

The authors' findings suggest that the wound microenvironment can retain adipose-derived stem cells, preventing their homing and stromal contribution to a distant neoplastic focus. These findings are an important first step in establishing the feasibility and safety of utilizing adipose-derived stem cell therapy for reconstructive surgery in the setting of malignancy.

Adult human adipose tissue contains several types of multipotent cells.

Tallone T, Realini C, Böhmler A, Kornfeld C, Vassalli G, Moccetti T, Bardelli S, Soldati G.

J Cardiovasc Transl Res. 2011 Apr;4(2):200-10. doi: 10.1007/s12265-011-9257-3. Epub 2011 Feb 15.

Source

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Abstract

Multipotent mesenchymal stromal cells (MSCs) are a type of adult stem cells that can be easily isolated from various tissues and expanded in vitro. Many reports on their pluripotency and possible clinical applications have raised hopes and interest in MSCs. In an attempt to unify the terminology and the criteria to label a cell as MSC, in 2006 the International Society for Cellular Therapy (ISCT) proposed a standard set of rules to define the identity of these cells. However, MSCs are still extracted from different tissues, by diverse isolation protocols, are cultured and expanded in different media and conditions. All these variables may have profound effects on the selection of cell types and the composition of heterogeneous subpopulations, on the selective expansion of specific cell populations with totally different potentials and ergo, on the long-term fate of the cells upon in vitro culture. Therefore, specific molecular and cellular markers that identify MSCs subsets as well as standardization of expansion protocols for these cells are urgently needed. Here, we briefly discuss new useful markers and recent data supporting the rapidly emerging concept that many different types of progenitor cells are found in close association with blood vessels. This knowledge may promote the necessary technical improvements required to reduce variability and promote higher efficacy and safety when isolating and expanding these cells for therapeutic use. In the light of the discussed data, particularly the identification of new markers, and advances in the understanding of fundamental MSC biology, we also suggest a revision of the 2006 ISCT criteria.

Histocompatibility testing of cultivated human bone marrow stromal cells - a promising step towards pre-clinical screening for allogeneic stem cell therapy.

Koppula PR, Chelluri LK, Polisetti N, Vemuganti GK.

Cell Immunol. 2009;259(1):61-5. doi: 10.1016/j.cellimm.2009.05.014. Epub 2009 Jun 6.

Source

C-TRACER, Hyderabad Eye Research Foundation, L.V. Prasad Eye Institute, Hyderabad, India.

Abstract

Mesenchymal stem cells (MSCs) lack major histocompatibility complex (MHC)-II and only show minimal MHC-I expression. Despite MSCs demonstrating T-cell anergy, there are no established methods to evaluate their suitability. It is crucial to evaluate the complete mismatch of MHC compatibility in view of the hypo-immunogenic nature and immunomodulatory properties of MSCs with respect to their proliferation potential (PP) and utility in terms of passage number. With bone marrow (BM) being the major source of MSCs, the use of these cells becomes even more complicated, due to many other receptors coming

to fore and triggering alternative pathways. This prospective study included five BM aspirates for MSC cultures and five allogeneic peripheral blood mono nuclear cells (PBMNCs) from healthy volunteers. MHC compatibility was assessed by polymerase chain reaction-sequence specific primer (PCR-SSP). The PP and a T-cell response to MSCs was addressed in mixed cultures and evaluated on the basis of their stimulation index (SI). Allogeneic circulatory antibodies against the donor MSCs was performed by cytotoxicity assay. The PP of MSCs during interactions with PBMNCs (T-cells) demonstrated T-cell anergy and the response to circulatory antibodies was minimal, in consonance with other published reports. Although, the results are encouraging for potential clinical application of MSC transplantation, autologous is always preferable to allogeneic, at least until the long-term safety of these cells is established in clinical trials.

Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique.

Centeno CJ, Schultz JR, Cheever M, Robinson B, Freeman M, Marasco W.

Curr Stem Cell Res Ther. 2010 Mar;5(1):81-93.

Source

Centeno-Schultz Clinic, Broomfield, Colorado, USA. centenooffice@centenoclinic.com.

Abstract

ABSTRUCT:

Mesenchymal stem cells (MSCs) hold great promise as therapeutic agents in regenerative medicine. Numerous animal studies have documented the multipotency of MSCs, showing their capabilities for differentiating into orthopedic tissues such as muscle, bone, cartilage, and tendon. However, the complication rate for autologous MSC therapy is only now beginning to be reported.

METHODS:

Between 2005 and 2009, two groups of patients were treated for various orthopedic conditions with culture-expanded, autologous, bone marrow-derived MSCs (group 1: n=45; group 2: n=182). Cells were cultured in monolayer culture flasks using an autologous platelet lysate technique and re-injected into peripheral joints (n=213) or into intervertebral discs (n=13) with use of c-arm fluoroscopy. While both groups had prospective surveillance for complications, Group 1 additionally underwent 3.0T MRI tracking of the re-implant sites.

RESULTS:

Mean follow-up from the time of the re-implant procedure was 10.6 +/- 7.3 months. Serial MRI's at 3 months, 6 months, 1 year and 2 years failed to demonstrate any tumor formation at the re-implant sites. Formal disease surveillance for adverse events based on HHS criteria documented 7 cases of probable procedure-related complications (thought to be associated with the re-implant procedure itself) and three cases of possible stem cell complications, all of which were either self-limited or were remedied with simple therapeutic measures. One patient was diagnosed with cancer; however, this was almost certainly unrelated to the MSC therapy.

CONCLUSIONS:

Using both high field MRI tracking and general surveillance in 227 patients, no neoplastic complications were detected at any stem cell re-implantation site. These findings are consistent with other reports that also show no evidence of malignant transformation in vivo, following implantation of MSCs that were expanded in vitro for limited periods.

Intranasal delivery of cells to the brain.

Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, Burkhardt U, Proksch B, Verleysdonk S, Ayturan M, Buniatian GH, Gleiter CH, Frey WH 2nd.

Source

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Abstract

The safety and efficacy of cell-based therapies for neurodegenerative diseases depends on the mode of cell administration. We hypothesized that intranasally administered cells could bypass the blood-brain barrier by migrating from the nasal mucosa through the cribriform plate along the olfactory neural pathway into the brain and cerebrospinal fluid (CSF). This would minimize or eliminate the distribution of cellular grafts to peripheral organs and will help to dispense with neurosurgical cell implantation. Here we demonstrate transnasal delivery of cells to the brain following intranasal application of fluorescently labeled rat mesenchymal stem cells (MSC) or human glioma cells to naive mice and rats. After cells crossed the cribriform plate, two migration routes were identified: (1) migration into the olfactory bulb and to other parts of the brain; (2) entry into the CSF with movement along the surface of the cortex followed by entrance into the brain parenchyma. The delivery of

cells was enhanced by hyaluronidase treatment applied intranasally 30 min prior to the application of cells. Intranasal delivery provides a new non-invasive method for cell delivery to the CNS.

Biological characterization of long-term cultured human mesenchymal stem cells.

Kim J, Kang JW, Park JH, Choi Y, Choi KS, Park KD, Baek DH, Seong SK, Min HK, Kim HS.

Arch Pharm Res. 2009 Jan;32(1):117-26. doi: 10.1007/s12272-009-1125-1. Epub 2009 Jan 29.

Source

Pharmacological Research Department, National Institute of Toxicological Research, Korea Food & Drug Administration, Seoul, 122-704, Korea.

Abstract

Human mesenchymal stem cells (hMSCs) have generated a great deal of interest in clinical applications. The reason is that they may have the plasticity needed to differentiate into multiple lineages and the ability to expand ex vivo. For the therapeutic applications of hMSCs to be of practical use, it is crucial to assess the efficacy and safety of hMSCs in long-term ex vivo expansion. In this study, we cultured hMSCs by population doubling (PD) 60, and investigated their growth, osteogenic and adipogenic differential abilities, change of surface markers, telomerase activity, telomere length, and gene expression related to tumorigenesis. An in vivo tumorigenesis assay was also carried out. In long-term expanded hMSCs, the cells became aged above PD 30 and their adipogenic and osteogenic differentiation potential decreased. Telomerase activity unchanged whereas telomere length decreased and karyotypes were not changed. Gene expressions related to tumorigenesis decreased in proportion as the PD of hMSCs increased. In vivo transplantation of long-term cultured hMSCs to nude mice did not result in tumor formation. These findings suggest that diverse tests for cellular therapy should be considered during the ex vivo culture of hMSCs, particularly when a prolonged and extended propagation period is required.

Ex vivo expansion and in vivo infusion of bone marrow-derived Flk-1+CD31-CD34- mesenchymal stem cells: feasibility and safety from monkey to human.

Liu L, Sun Z, Chen B, Han Q, Liao L, Jia M, Cao Y, Ma J, Sun Q, Guo M, Liu Z, Ai H, Zhao RC.

Stem Cells Dev. 2006 Jun;15(3):349-57.

Source

Department of Hematology, Affiliated Hospital of Academy of Military Medicine Science, Beijing, PR China.

Abstract

Flk-1(+)CD31(-)CD34(-) mesenchymal stem cells (MSCs) are multipotent cells with extensive proliferation ability and immunomodulatory function. On the basis of our previous work showing their potential application in tissue engineering and immune diseases, we designed a preclinical and Phase I trial to evaluate the feasibility and safety of intravenous infusion of these cells after ex vivo expansion. Rhesus monkey and human Flk-1(+)CD31(-)CD34(-) MSCs were isolated from bone marrow and passaged a maximum of six passages. Karyotype analysis showed Flk-1(+)CD31(-)CD34(-) MSCs remained normally diploid within six passages of expansion. Expanded cells were transplanted into rhesus monkeys and human volunteers, respectively. During the infusion of these cells, the life signs of the recipients were normal. Laboratory tests for blood, bone marrow, kidney, and liver function were conducted and no significant changes were observed before and after cell infusion. Identification of the sry sequence from the male donor in female recipients showed that allogeneic rhesus Flk-1(+)CD31(-)CD34(-) MSCs were capable of homing to and establishing residence within the recipients' bone marrow. No significant changes were observed in lymphocyte cell subpopulation before and after infusion. These result showed that Flk-1(+)CD31(-)CD34(-) MSCs have no obvious side effects after intravenous administration, encouraging investigators to perform further studies in stem cell therapy.

Development and introduction of production standards for cell products of mesenchymal origin.

Burunova VV, Suzdaltseva YG, Voronov AV, Cheglakov IB, Vakhrushev IV, Yarygin KN, Yarygin VN.

Bull Exp Biol Med. 2008 Apr;145(4):526-30.

Source

Russian State Medical University, Russia.

Abstract

The results of the development and introduction of universal production standards for cell products of mesenchymal origin are presented: technology for obtaining and culturing of primary cell cultures from human postnatal organs and tissues, cell product quality and safety control procedures, methods for cell product storage and transportation, and the necessary files.

Evaluation of mesenchymal stem cells following implantation in alveolar sockets: a canine safety study.

De Kok IJ, Drapeau SJ, Young R, Cooper LF.

Int J Oral Maxillofac Implants. 2005 Jul-Aug;20(4):511-8.

Source

Department of Prosthodontics, University of North Carolina, Chapel Hill, North Carolina, USA.

Abstract

PURPOSE:

The overall goal of this project was to evaluate culture-expanded bone-marrow-derived mesenchymal stem cells (MSCs) for alveolar bone repair in terms of safety and potential efficacy.

MATERIALS AND METHODS:

MSCs isolated from bone marrow aspirations were culture-expanded and cryopreserved. Thawed cells were incubated with 3.2 x 5-mm hydroxyapatite/tricalcium phosphate (HA/TCP) cylinders in a closed system containing 5 x 10(7) cells/mL. Cells alone, cell-free constructs, or cell-loaded constructs were rinsed in saline and implanted in extraction sockets in the mandibular second and fourth premolar sites of 14 beagle dogs. Acute reactions were evaluated histologically after 7 or 21 days, and bone formation was examined after 49 days.

RESULTS:

Neither implanted MSC-related inflammation nor ectopic osteogenesis was observed. At 7 and 21 days, dil-labeled canine MSCs were found in more than 80% of the implant sites. Few canine MSCs were found in neighboring tissue. Mild inflammation present at 7 days diminished by 21 days. After 49 days, measured bone formation was 34%, 25%, and 35% for cell-loaded, cell-free, and untreated sockets, respectively ($P < .05$). At 21 days, bone formation was evident in all sites. Wound dehiscence was a complication associated with cell exclusionary membranes and resulted in local inflammation.

DISCUSSION:

The extraction model indicates the safety of MSCs implanted adherent to HA/TCP. Local bone repair occurred in the absence of nonspecific differentiation or migration with distant osteogenesis.

CONCLUSIONS:

An alveolar socket model may be an appropriate model for initial clinical investigation of MSC-mediated bone repair.

Biodistribution, long-term survival, and safety of human adipose tissue-derived mesenchymal stem cells transplanted in nude mice by high sensitivity non-invasive bioluminescence imaging.

Vilalta M, Dégano IR, Bagó J, Gould D, Santos M, García-Arranz M, Ayats R, Fuster C, Chernajovsky Y, García-Olmo D, Rubio N, Blanco J.

Stem Cells Dev. 2008 Oct;17(5):993-1003. doi: 10.1089/scd.2007.0201.

Source

Centre d'investigació Cardiovascular (CSIC-ICCC), CIBER de Bioingenieria, Biomateriales y Nanomedicine (CIBER-BBN), Zaragoza, Spain.

Abstract

Cultivated murine bone marrow mesenchymal stem cells (MSCs) frequently accumulate chromosome abnormalities, become oncogenically transformed, and generate sarcomas when transplanted in mice. Although human MSCs appear to be more resistant, oncogenic transformation has also been observed in MSCs cultivated past the senescence phase. Cell therapy for tissue regeneration using human autologous MSCs requires transplantation of cells previously expanded in vitro. Thus, an important concern is to determine if oncogenic transformation is a necessary outcome of the expansion procedures. We have analyzed the proliferation capacity, organ colonization, and oncogenicity of enhanced green fluorescent protein and luciferase-labeled human adipose tissue-derived mesenchymal stem cells (hAMSCs), implanted in immunocompromised mice during a prolonged time period (8 months) using a non-invasive bioluminescence imaging procedure. Our data indicates that the liver was the preferred target organ for colonization by intramuscular or intravenous implantation of hAMSCs. The implanted cells tended to maintain a steady state, population did not proliferate rapidly after implantation, and no detectable chromosomal abnormalities nor tumors formed during the 8 months of residence in the host's tissues. It would appear that hAMSCs, contrary to their murine correlatives, could be safe candidates for autologous cell therapy procedures since in our experiments they show undetectable predisposition to oncogenic transformation after cultivation in vitro and implantation in mice.

Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells.

Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J.

Stem Cells Dev. 2012 Sep 20;21(14):2724-52. doi: 10.1089/scd.2011.0722. Epub 2012 May 9.

Source

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Abstract

Mesenchymal stem/stromal cells (MSCs) comprise a heterogeneous population of cells with multilineage differentiation potential, the ability to modulate oxidative stress, and secrete various cytokines and growth factors that can have immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic effects. Recent data indicate that these paracrine factors may play a key role in MSC-mediated effects in modulating various acute and chronic pathological conditions. MSCs are found in virtually all organs of the body. Bone marrow-derived MSCs (BM-MSCs) were discovered first, and the bone marrow was considered the main source of MSCs for clinical application. Subsequently, MSCs have been isolated from various other sources with the adipose tissue, serving as one of the alternatives to bone marrow. Adipose tissue-derived MSCs (ASCs) can be more easily isolated; this approach is safer, and also, considerably larger amounts of ASCs can be obtained compared with the bone marrow. ASCs and BM-MSCs share many biological characteristics; however, there are some differences in their immunophenotype, differentiation potential, transcriptome, proteome, and immunomodulatory activity. Some of these differences may represent specific features of BM-MSCs and ASCs, while others are suggestive of the inherent heterogeneity of both BM-MSC and ASC populations. Still other differences may simply be related to different isolation and culture protocols. Most importantly, despite the minor differences between these MSC populations, ASCs seem to be as effective as BM-MSCs in clinical application, and, in some cases, may be better suited than BM-MSCs. In this review, we will examine in detail the ontology, biology, preclinical, and clinical application of BM-MSCs versus ASCs.

A systematic Review and Meta-Analysis of Clinical Trials regarding Safety of Cell Therapy with Mesenchymal Stromal Cells

Manoj M Lalu et al.

Safety of Cell Therapy; Plos one, October 2012, Vol 7, Issue 10

Methods and findings

MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (to June 2011), were searched. Prospective clinical trials that used intravascular delivery of MSCs (intravenously or intra-arterially) in adult populations or mixed adult and pediatric populations were identified. Studies using differentiated MSCs or additional cell types were excluded. The primary outcome adverse events were grouped according to immediate events (acute infusional toxicity, fever), organ system complications, infection, and longer term adverse events (death, malignancy). 2347 citations were reviewed and 36 studies met inclusion criteria. A total of 1012 participants with clinical conditions of ischemic stroke, Crohn's disease, cardiomyopathy, myocardial infarction, graft versus host disease, and healthy volunteers were included. Eight studies were randomized control trials (RCTs) and enrolled 321 participants. Meta-analysis of the RCTs did not detect an association between acute infusional toxicity, organ system complications, infection, death or malignancy. There was a significant association between MSCs and transient fever.

Introduction

Mesenchymal stromal cells (mesenchymal stem cells; MSCs) are a heterogeneous group of cells that can be isolated from many adult tissues (e.g. bone marrow, adipose tissue). First described in 1974 they have recently received attention in a number of different clinical fields for their potential therapeutic effects.

Although often described as ‘adult stem cells’, MSC's have limited cellular differentiation ability. Instead, pre-clinical evidence suggests that MSCs exert their beneficial effects largely through immunomodulatory and paracrine mechanisms. MSCs home to sites of inflammation and secrete bioactive molecules, and thus may be especially effective in proinflammatory diseases.

There is a growing body of literature demonstrating the efficacy of MSC therapy in a variety of pre-clinical models, including acute lung injury,septic shock, and acute myocardial infarction.

Several small clinical trials have investigated the efficacy and safety of MSCs in diseases including chronic heart failure, acute myocardial infarction, hematological malignancies and graft versus host disease.

There is interest in applying MSCs to pulmonary diseases (e.g. chronic obstructive pulmonary disease) and critical illness (e.g. acute respiratory distress syndrome); however, safety concerns represent a significant barrier to the successful translation of MSCs into an acceptable clinical therapeutic. These include neoplastic potential due to MSC's proliferative

capacity, susceptibility to infection given their immunomodulatory effects, embolism of the cells, zoonoses associated with cell culture reagents, and acute or chronic immunogenicity of the cells themselves.

Therefore, we conducted a systematic review of the literature to evaluate the safety of MSC-based therapy in all clinical trials.

Eligibility Criteria

We included randomized and non-randomized controlled trials as well as uncontrolled clinical trials (Phase I/II trials with more than two participants) that examined the safety of intravascularly delivered MSCs in adult (at least 18 years old) or mixed adult and pediatric participants. All clinical settings were included. We excluded studies that exclusively used non-intravascular routes of administration, ex vivo differentiated MSCs, or co-administered MSCs with other experimental cells or treatments.

Search Strategy

We conducted electronic searches without language restriction of Ovid MEDLINE (1950 to June 2011), EMBASE (1980 to Week 21, 2011), Cochrane Central Register of Controlled Trials (2nd Quarter 2011), and the Cochrane Database of Systematic Reviews (2nd Quarter 2011). Given the non-standard terminology associated with MSCs a number of terms were used (Appendix S1, search strategy). ClinicalTrials.gov was searched for ongoing or recently completed trials. Abstracts and proceedings from clinical conferences were identified and searched using Web of Science (September 2010). Bibliographies of retrieved articles and relevant reviews were searched.

Assessment of Risk of Bias

RCTs that met inclusion criteria were assessed for risk of bias according to the Cochrane Collaboration methods.

Study Selection, Data Collection and Analysis

All study selection and data extraction was performed independently by two reviewers (M.M.L., C.P.) using standardized forms. Discrepancies were resolved by discussion with a third author (L.L.M.).

Main Outcome Measure: Adverse Events

Adverse events were grouped according to the immediacy of the event (acute infusional toxicity, fever), the occurrence of organ system complications [neurological, pulmonary, cardiovascular (arrhythmias and other cardiac events), gastrointestinal and renal, and

hematologic systems], infection, and the occurrence of longer term adverse effects (death, malignancy).

Completeness of adverse events reporting was assessed using the CONSORT approach to harm reporting.

Specifically, we examined whether expected adverse events were listed and defined in the methods section, whether events were described as serious versus non serious (e.g. as per Good Clinical Practice Guidelines), and if frequency and duration of follow up of adverse events was provided.

Statistical Analysis

Meta-analyses of adverse events was performed using Comprehensive Meta-analysis (Version 2, Biostat). Data was analyzed by Peto's method with correction of zero-count cells. Pooled events were described using odds ratios (OR) and 95% confidence intervals (95% CI). An odds ratio less than 1 favoured MSC treatment. Heterogeneity between trials was evaluated as well as the $\chi 2$ test. Sensitivity analyses were planned according to the patient population, MSC type (autologous versus allogeneic; fresh versus cryopreserved), and culture media (fetal bovine serum versus human).

Adverse events for non-randomized controlled trials with control groups that did not receive any dose of MSCs were pooled and reported according to numbers and proportions.

Discussion

This is the first systematic review and meta-analysis to comprehensively summarize the safety of systemic MSC administration. Our analysis was unable to detect associations between MSC treatment and the development of acute infusional toxicity, organ system complications, infection, death, or malignancy. There was, however, a significant association between MSC administration and transient fever. Our systematic review of non-RCTs supported these results. Six of seven RCTs and all non-RCTs described equal or fewer deaths with MSC treatment compared to control treatment. The completeness of adverse event reporting in the included studies was variable. However, aside from fever, the published current clinical trials suggest that the administration of MSCs is safe.

Although malignant transformation is a theoretical risk, our pooled analysis found no association between MSCs and tumour formation. Concerns related to tumourgenicity of MSCs were raised by preclinical studies demonstrating increased tumour burden in vivo. Although recent position papers have suggested low probability of malignant transformation and tumour formation with MSCs, our review is the first systematic analysis of the issue. Malignancy occurred only in studies involving participants with ongoing or previous malignancies; no de novo malignancies were observed.

We found no evidence of increased susceptibility to infection with MSC administration. Although MSC immunomodulatory effects may be beneficial in pro-inflammatory diseases, these same effects may leave a patient susceptible to infection.

In our review, infections were common in already immunosuppressed patients (e.g. following hematopoietic stem cell transplant), however the infection rates were similar to those previously published for similar populations.

In RCTs of participants without haematological malignancies, there were no differences between MSC and control participants.

There was a significant association between MSC administration and the development of fever. Fever was transient and not associated with long term sequelae. The mechanisms for fever are not clear but could be related to acute inflammatory reactions by a subset of patients to particular preparations of MSCs, not unlike similar reactions occasionally observed with red blood cell administration.

Our review also addresses several issues and theoretical concerns with the cell product used in studies. First, concerns for immunogenicity may be unfounded as 13 studies used unmatched allogeneic MSCs with no reports of acute infusional toxicity. This supports the idea that MSCs are 'immune-privileged', a characteristic that may be explained by their low expression of MHC proteins and T-cell co-stimulatory molecules.

Second, the use of fetal bovine serum for culturing MSCs has been criticized for potentially introducing zoonotic contamination to the cell product (e.g. prion disease), and also potentially increasing the immunogenicity of the cells. Although the majority of included studies used fetal bovine serum, only one study specifically monitored for potential adverse events associated with its use. Concerns over fetal bovine serum will likely decrease in the future as expansion of MSCs in human blood products becomes more commonplace. The use of dimethylsulfoxide as a cryopreservative has been another potential concern with MSC therapy as this chemical is known to have toxic side effects and can cause hypersensitivity reactions. In our review only one study documented the occurrence of acute infusional toxicity and attributed it to dimethylsulfoxide.

A final concern is the viability of cells administered, as the administration of necrotic cells or cellular by-products may increase immunogenicity. Less than half of the studies included assessed and reported on viability of MSCs prior to infusion.

Thus, greater vigilance may be needed in future studies for reporting cellular viability and monitoring for potential dimethylsulfoxide related adverse events.

No significant relationship between MSC administration and acute infusional toxicity was observed. The only RCT which described acute adverse reactions during infusion (acute and transient pulmonary edema in three participants) delivered MSCs to participants with chronic ischemic heart failure.

MSCs initially distribute to the lungs after intravascular administration; thus, in susceptible patients this could cause a transient increase in pulmonary pressures and lead to pulmonary edema.

The reporting of adverse events was highly variable among the included studies. This may be related to editorial constraints of journals. Since use of MSCs may be associated with neoplastic growth long term, it is difficult to understand why approximately 50% of studies did not report follow up duration for adverse events. For highly experimental interventions with unestablished safety profiles, we contend that it is important to summarize the adverse reporting plan in the methods section of manuscripts and report short term and longer term events.

Our systematic review has several limitations. First, despite our comprehensive search strategy, there are a number of completed but unpublished industry sponsored studies and studies published in abstract form only that may alter the safety profile of MSCs. Second, we pooled adverse events across heterogeneous disease states. Given the limited number of clinical MSC studies, and the small sample sizes of each, it was important to pool data across trials to determine if any potential signals of harm existed. Previously, we have advocated this approach when individual trials are not adequately powered to detect potential harm.

However, we acknowledge that the occurrence, type, and severity of adverse events may vary significantly between different populations and according to different MSC characteristics (e.g. dose, type). The limited number of included RCTs precluded the conduct of these sensitivity analyses. Third, the majority of RCTs included in our analysis would be considered a high risk of bias. Although double blinding an MSC trial may be considered ethically unacceptable, it is difficult to justify the lack of concealment of the allocation of patients in many studies.

Conclusions

Our study provides a systematic examination for adverse events related to the use of MSCs. We did not identify any significant safety signals other than transient fever. Results from our

systematic review should provide some assurance to investigators and health regulators that, with the present evidence, this innovative therapy appears safe.

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

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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.

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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

4. 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.

4.1. 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].

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4.2. 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.

5. 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.

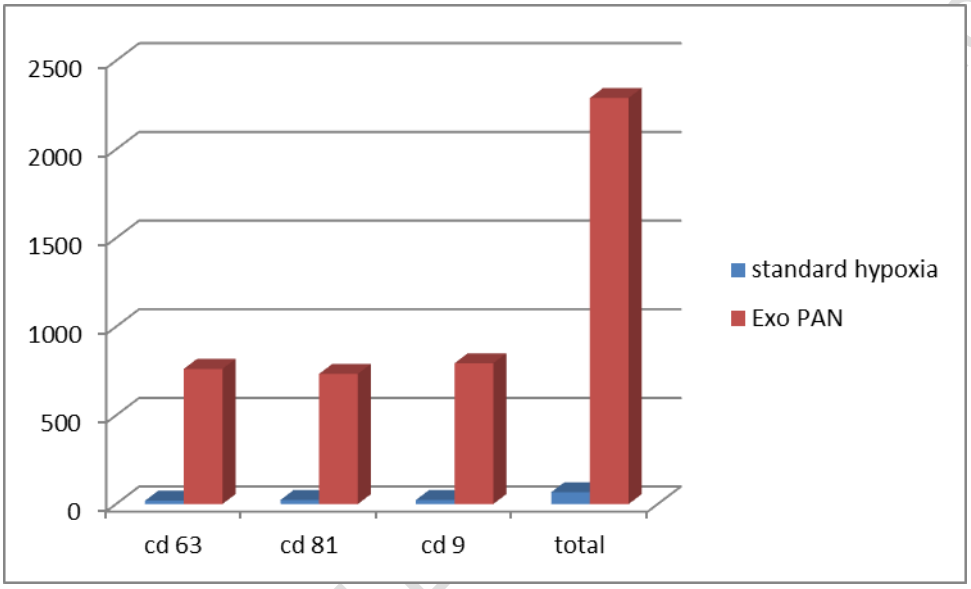

Figure 1: 300 fold increase of exosomes per 1 mio cells as measured semiquantitatively via surface markers cd 63, 81 and 9.

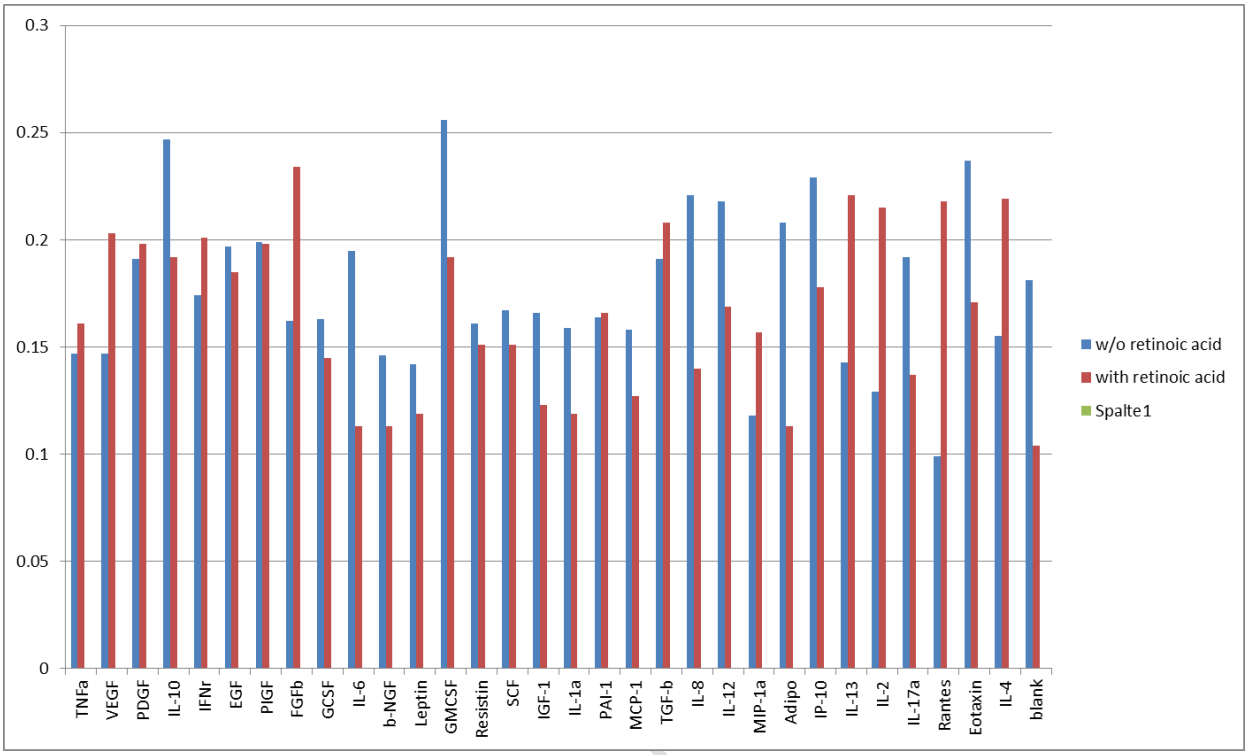

Figure 2: Comparison auf 31 Cytokines produced by hMSC-ad with and without pre-treatment with retinoic acid

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.

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6. Preclinical studies regarding anti-ageing

6.1. Own in vitro studies

Preclinical In vitro investigation of the influence of the ad-MSC secretome on the apoptosis of isolated cardiomyocytes after Glucose and Oxygen Deprivation

Several studies have reported that micro RNA is involved in the pathogenesis and progression of ischaemic diseases and that miR-22 may inhibit the inflammatory response and cell apoptosis. In our model, we are using primary cardiomyocytes which are subjected to glucose and oxygen deprivation with or without the addition of the ad-MSC secretome containing mRNA, miRNA and cytokines.

The effect was measured by the Lonza Toxilight test and the apoptosis ELISA assays on TNF-alpha, Caspase 9, Blc-2 and NF-KappaB p65.

ToxiLight™ BioAssay Kit

The ToxiLight™ BioAssay Kit is a bioluminescent, non-destructive cytolysis assay kit designed to measure the release of the enzyme, adenylate kinase (AK), from damaged cells. AK is a robust protein present in all eukaryotic cells, which is released into the culture medium when cells die. The enzyme actively phosphorylates ADP to form ATP and the resultant ATP is then measured using the bioluminescent firefly luciferase reaction.

TNF alpha

TNF-alpha is the prototypic ligand of the TNF superfamily which plays a central role in inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis and morphogenesis. The proinflammatory effect of TNF-alpha is mediated through NF-kappa B induction of IL-6, IL-8, chemokines, iNOS, cyclooxygenase 2 and lipoxygenase. TNF-alpha induced inflammation has been shown associated with neurologic diseases (depression, bipolar disorder and epilepsy), heart failure, asthma, chronic obstructive pulmonary disease, diabetes, obesity, insulin resistance and autoimmune diseases (multiple sclerosis, systemic lupus, arthritis, psoriasis and Crohn disease).

Caspase 9

Sustained TNF signalling results in activation of the intrinsic cell death pathway leading to increased cytosolic levels of cytochrome c and others and activation of caspases 3 and 9.

Caspases are the executioners of apoptosis and belong to the cysteine protease family. Caspases are synthesized as inactive proenzymes comprising an N-terminal peptide together with one large and one small subunit. Activation of caspases during apoptosis results in cleavage of critical cellular substrates thus precipitating morphological changes of apoptosis. Activated caspase 9 is the most upstream member of the apoptotic protease cascade and is localized in mitochondria. The disruption of the outer mitochondrial membrane occurring early during apoptosis is critical for its sub-cellular redistribution and activation.

Bcl-2

Bcl-2 initiates a new gene family involved in the regulation of cell death and survival without affecting cell proliferation. This precise regulation and maintenance of balance between cell proliferation and cell death in multicellular organisms is critical for tissue homeostasis. Bcl-2, encoded by a proto-oncogene, is an intracellular membrane associated protein that functions to block programmed cell death. The Bcl-2 gene has a unique function among mammalian oncogenes as a negative regulator of apoptosis. It was first discovered because of its involvement in chromosomal translocations commonly found in lymphomas. Bcl-2 is furthermore associated with stem cells committed to differentiation and morphogenesis. A decrease in Bcl-2 levels leads to cell death by apoptosis.

Overexpression of Bcl-2 on the other hand protects cells from death, but it is neither able to immortalize normal cells, nor to cause tumorgenic transformation of immortalized cells.

Nuclear factor κB transcription factor

The nuclear factor κ B transcription factor family plays a pivotal role in inflammatory and immune responses. There are five family known members, namely p65 (RelA), c - Rel, RelB, NF - κ B1 (p105/p50) and NF - κ B2 (p100/p52). The p50 and p52 products form dimeric complexes with Rel proteins, which bind DNA and regulate transcription. The most abundant form in many cell types is the p65/p50 complex.

Results

Table 1. Toxilight Test, values in RLU, in percentage to negative control

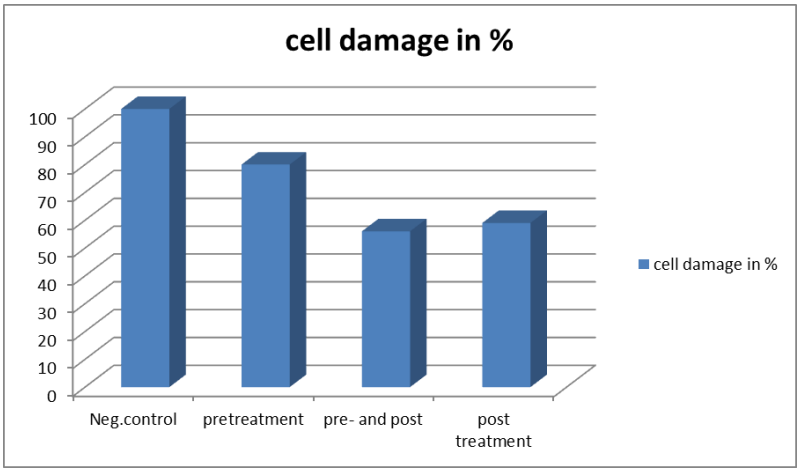
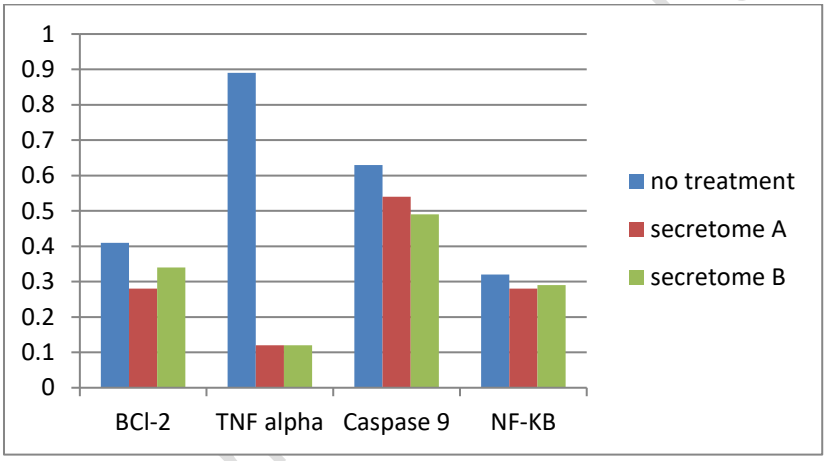


Table 2. Apoptosis/inflammation markers from cardiomyocytes with/without secretome, values OD



6.2. Literature

RNA methyltransferase NSUN2 promotes stress-induced HUVEC senescence

Xiaoyu Cai et al.

Oncotarget, Advance Publications 2016

The tRNA methyltransferase NSUN2 delays replicative senescence by regulating the translation of CDK1 and CDKN1B mRNAs. However, whether NSUN2 influences premature cellular senescence remains untested. Here we show that NSUN2 methylates SHC mRNA in vitro and in cells, thereby enhancing the translation of the three SHC proteins, p66SHC, p52SHC, and p46SHC. Our results further show that the elevation of SHC expression by NSUN2-mediated mRNA methylation increased the levels of ROS, activated p38MAPK, thereby accelerating oxidative stress- and high-glucose-induced senescence of human vascular endothelial cells (HUVEC). Our findings highlight the critical impact of NSUN2-mediated mRNA methylation in promoting premature senescence.

Metformin-mediated increase in DICER1 regulates microRNA expression and cellular senescence

Nicole Noren Hooten et al.

Aging Cell (2016) pp1–10

Metformin, an oral hypoglycemic agent, has been used for decades to treat type 2 diabetes mellitus. Recent studies indicate that mice treated with metformin live longer and have fewer manifestations of age-related chronic disease. However, the molecular mechanisms underlying this phenotype are unknown. Here, we show that metformin treatment increases the levels of the microRNA-processing protein DICER1 in mice and in humans with diabetes mellitus. Our results indicate that metformin upregulates DICER1 through a post-transcriptional mechanism involving the RNA-binding protein AUF1. Treatment with metformin altered the subcellular localization of AUF1, disrupting its interaction with DICER1 mRNA and rendering DICER1 mRNA stable, allowing DICER1 to accumulate. Consistent with the role of DICER1 in the biogenesis of microRNAs, we found differential patterns of microRNA expression in mice treated with metformin or caloric restriction, two proven life-extending interventions. Interestingly, several microRNAs previously associated with senescence and aging, including miR-20a, miR-34a, miR-130a, miR-106b, miR-125, and let-7c, were found elevated. In agreement with these findings, treatment with metformin decreased cellular senescence in several senescence models in a DICER1-dependent manner. Metformin lowered p16 and p21 protein levels and the abundance of inflammatory cytokines and oncogenes that are hallmarks of the senescence-associated secretory

phenotype (SASP). These data lead us to hypothesize that changes in DICER1 levels may be important for organismal aging and to propose that interventions that upregulate DICER1 expression (e.g., metformin) may offer new pharmacotherapeutic approaches for age-related disease.

Cyclic nucleotide phosphodiesterase 1 and vascular aging

Chen Yan

Clinical Science (2015) 129, 1077–1081

VSMCs (vascular smooth muscle cells) play critical roles in arterial remodelling with aging, hypertension and atherosclerosis. VSMCs exist in diverse phenotypes and exhibit phenotypic plasticity, e.g. changing from a quiescent/contractile phenotype to an active myofibroblast-like, often called 'synthetic', phenotype. Synthetic VSMCs are able to proliferate, migrate and secrete ECM (extracellular matrix) proteinases and ECM proteins. In addition, they produce pro-inflammatory molecules, providing an inflammatory microenvironment for leucocyte penetration, accumulation and activation. The aging VSMCs have also shown changes in cellular phenotype, responsiveness to contracting and relaxing mediators, replicating potential, matrix synthesis, inflammatory mediators and intracellular signalling. VSMC dysfunction plays a key role in age-associated vascular remodelling. Cyclic nucleotide PDEs (phosphodiesterases), by catalysing cyclic nucleotide hydrolysis, play a critical role in regulating the amplitude, duration and compartmentalization of cyclic nucleotide signalling. Abnormal alterations of PDEs and subsequent changes in cyclic nucleotide homoeostasis have been implicated in a number of different diseases. In the study published in the latest issue of Clinical Science, Bautista Nino and colleagues have shown that, in cultured senescent human VSMCs, PDE1A and PDE1C mRNA levels are significantly up-regulated and inhibition of PDE1 activity with vinpocetine reduced cellular senescent makers in senescent VSMCs. Moreover, in the premature aging mice with genomic instability (Ercc1d/−), impaired aortic ring relaxation in response to SNP (sodium nitroprusside), an NO (nitric oxide) donor, was also largely improved by vinpocetine. More interestingly, using data from human GWAS (genome-wide association studies), it has been found that PDE1A single nucleotide polymorphisms is significantly associated with diastolic blood pressure and carotid intima–media thickening, two hallmarks of human vascular dysfunction in aging. These findings establish a strong relationship between PDE1 expression regulation and vascular abnormalities in aging.

Parainflammation, chronic inflammation and age-related macular degeneration

Mei Chen and Heping Xu

J Leukoc Biol. 2015 November; 98(5): 713–725

Inflammation is an adaptive response of the immune system to noxious insults to maintain homeostasis and restore functionality. The retina is considered an immune privileged tissue due to its unique anatomical and physiological properties. During aging, the retina suffers from a low-grade chronic oxidative insult, which sustains for decades and increases in level with advancing age. As a result, the retinal innate immune system, particularly microglia and the complement system, undergo low levels of activation (para-inflammation). In many cases, this para-inflammatory response can maintain homeostasis in the healthy aging eye. However, in patients with age-related macular degeneration (AMD), this para-inflammatory response becomes dysregulated and contributes to macular damage. Factors contributing to the dysregulation of age-related retinal para-inflammation include genetic predisposition, environmental risk factors and old age. Dysregulated para-inflammation (chronic inflammation) in AMD damages the blood retina barrier (BRB), resulting in the breach of retinal immune privilege leading to the development of retinal lesions. This review discusses the basic principles of retinal innate immune responses to endogenous chronic insults in normal aging and in AMD, and explores the difference between beneficial para-inflammation and the detrimental chronic inflammation in the context of AMD.

Combined effects of aging and inflammation on renin-angiotensin system mediate mitochondrial dysfunction and phenotypic changes in cardiomyopathies

Tyesha N. Burks et al.

Oncotarget, Vol. 6, No. 14

Although the effects of aging and inflammation on the health of the cardiac muscle are well documented, the combined effects of aging and chronic inflammation on cardiac muscle are largely unknown. The renin-angiotensin system (RAS) has been linked independently to both aging and inflammation, but is understudied in the context of their collective effect. Thus, we investigated localized cardiac angiotensin II type I and type II receptors (AT_1R, AT_2R), downstream effectors, and phenotypic outcomes using mouse models of the combination of aging and inflammation and compared it to a model of aging and a model of inflammation. We show molecular distinction in the combined effect of aging and inflammation as compared to each independently. The combination maintained an increased AT_1R:AT_2R and expression of Nox2 and exhibited the lowest activity of antioxidants. Despite signaling pathway differences, the combined effect shared phenotypic similarities with aging including

oxidative damage, fibrosis, and hypertrophy. These phenotypic similarities have dubbed inflammatory conditions as premature aging, but they are, in fact, molecularly distinct. Moreover, treatment with an AT_1R blocker, losartan, selectively reversed the signaling changes and ameliorated adverse phenotypic effects in the combination of aging and inflammation as well as each independently.

7. Clinical studies on ageing and anti-ageing

Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic

Alexey Moskalev et al.

Aging Cell (2016) pp1–9

In the coming decades, a massive shift in the aging segment of the population will have major social and economic consequences around the world. One way to offset this increase is to expedite the development of geroprotectors, substances that slow aging, repair age-associated damage and extend healthy lifespan, or healthspan. While over 200 geroprotectors are now reported in model organisms and some are in human use for specific disease indications, the path toward determining whether they affect aging in humans remains obscure. Translation to the clinic is hampered by multiple issues including absence of a common set of criteria to define, select, and classify these substances, given the complexity of the aging process and their enormous diversity in mechanism of action. Translational research efforts would benefit from the formation of a scientific consensus on the following: the definition of 'geroprotector', the selection criteria for geroprotectors, a comprehensive classification system, and an analytical model. Here, we review current approaches to selection and put forth our own suggested selection criteria. Standardizing selection of geroprotectors will streamline discovery and analysis of new candidates, saving time and cost involved in translation to clinic.

A proposed panel of biomarkers of healthy ageing

Jose Lara, Rachel Cooper, Jack Nissan, Annie T Ginty, Kay-Tee Khaw, Ian J Deary, Janet M Lord, Diana Kuh2and John C Mathers

BMC Medicine (2015) 13:222

Abstract

Background: There is no criterion reference for assessing healthy ageing and this creates difficulties when conducting and comparing research on ageing across studies. A cardinal feature of ageing is loss of function which translates into wide-ranging consequences for the individual and for family, carers and society. We undertook comprehensive reviews of the literature searching for biomarkers of ageing on five ageing-related domains including physical capability and cognitive, physiological and musculoskeletal, endocrine and immune

functions. Where available, we used existing systematic reviews, meta-analyses and other authoritative reports such as the recently launched NIH Toolbox for assessment of neurological and behavioural function, which includes test batteries for cognitive and motor function (the latter described here as physical capability). We invited international experts to comment on our draft recommendations. In addition, we hosted an experts workshop in Newcastle, UK, on 22–23 October 2012, aiming to help capture the state-of-the-art in this complex area and to provide an opportunity for the wider ageing research community to critique the proposed panel of biomarkers.

Discussion: Here we have identified important biomarkers of healthy ageing classified as subdomains of the main areas proposed. Cardiovascular and lung function, glucose metabolism and musculoskeletal function are key subdomains of physiological function. Strength, locomotion, balance and dexterity are key physical capability subdomains. Memory, processing speed and executive function emerged as key subdomains of cognitive function. Markers of the HPA-axis, sex hormones and growth hormones were important biomarkers of endocrine function. Finally, inflammatory factors were identified as important biomarkers of immune function. Summary: We present recommendations for a panel of biomarkers that address these major areas of function which decline during ageing. This biomarker panel may have utility in epidemiological studies of human ageing, in health surveys of older people and as outcomes in intervention studies that aim to promote healthy ageing. Further, the inclusion of the same common panel of measures of healthy ageing in diverse study designs and populations may enhance the value of those studies by allowing the harmonisation of surrogate endpoints or outcome measures, thus facilitating less equivocal comparisons between studies and the pooling of data across studies.

Keywords: Biomarkers, Ageing, Physical capability, Cognitive function, Physiological function, Musculoskeletal function, Endocrine function, Immune function

Rationale and design of the allogeneiC human mesenchymal stem cells (hMSC) in patients with aging fRAilTy via intravenous delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty

Samuel Golpanian et al.

Oncotarget, Vol. 7, No. 11

Frailty is a syndrome associated with reduced physiological reserves that increases an individual's vulnerability for developing increased morbidity and/or mortality. While most clinical trials have focused on exercise, nutrition, pharmacologic agents, or a multifactorial approach for the prevention and attenuation of frailty, none have studied the use of cell-

based therapies. We hypothesize that the application of allogeneic human mesenchymal stem cells (allo-hMSCs) as a therapeutic agent for individuals with frailty is safe and efficacious. The CRATUS trial comprises an initial non-blinded phase I study, followed by a blinded, randomized phase I/II study (with an optional follow-up phase) that will address the safety and pre-specified beneficial effects in patients with the aging frailty syndrome. In the initial phase I protocol, allo-hMSCs will be administered in escalating doses via peripheral intravenous infusion (n=15) to patients allocated to three treatment groups: Group 1 (n=5, 20 million allo-hMSCs), Group 2 (n=5, 100 million allo-hMSCs), and Group 3 (n=5, 200 million allo-hMSCs). Subsequently, in the randomized phase, allo-hMSCs or matched placebo will be administered to patients (n=30) randomly allocated in a 1:1:1 ratio to one of two doses of MSCs versus placebo: Group A (n=10, 100 million allo-hMSCs), Group B (n=10, 200 million allo-hMSCs), and Group C (n=10, placebo). Primary and secondary objectives are, respectively, to demonstrate the safety and efficacy of allohMSCs administered in frail older individuals. This study will determine the safety of intravenous infusion of stem cells and compare phenotypic outcomes in patients with aging frailty.

Influence of aging on the quantity and quality of human cardiac stem cells

Tamami Nakamura et al.

Scientific Reports | 6:22781

Advanced age affects various tissue-specific stem cells and decreases their regenerative ability. We therefore examined whether aging affected the quantity and quality of cardiac stem cells using cells obtained from 26 patients of various ages (from 2 to 83 years old). We collected fresh right atria and cultured cardiosphere-derived cells (CDCs), which are a type of cardiac stem cell. Then we investigated growth rate, senescence, DNA damage, and the growth factor production of CDCs. All samples yielded a sufficient number of CDCs for experiments and the cellular growth rate was not obviously associated with age. The expression of senescence-associated b-galactosidase and the DNA damage marker, gH2AX, showed a slightly higher trend in CDCs from older patients (≥65 years). The expression of VEGF, HGF, IGF-1, SDF-1, and TGF-b varied among samples, and the expression of these beneficial factors did not decrease with age. An in vitro angiogenesis assay also showed that the angiogenic potency of CDCs was not impaired, even in those from older patients. Our data suggest that the impact of age on the quantity and quality of CDCs is quite limited. These findings have important clinical implications for autologous stem cell transplantation in elderly patients.

MicroRNAs in Salivary Exosome as Potential Biomarkers of Aging

Tatsuya Machida et al.

Int. J. Mol. Sci. 2015, 16, 21294-21309

The aim of this study was to examine whether salivary exosomal miRNAs could be identified as aging biomarkers. Fifteen young healthy volunteers (median age, 21.0 years) and 13 old individuals (median age, 66.0 years) were recruited. Unstimulated whole saliva was collected, salivary exosomes were isolated, and total RNA was extracted. In a microarray, 242 miRNAs were commonly detected in these two mixed samples. Based on the cut-off values of 2- or 0.5-fold changes (FC) and regulatory power for aging process, six candidate miRNAs (miR-24-3p, miR-371a-5p, miR-3175, miR-3162-5p, miR-671-5p, and miR-4667-5p) were selected. After comparing each total RNA obtained by the 15 young and 13 old individuals to validate the FC values using quantitative real-time PCR, miR-24-3p was identified as a novel candidate aging biomarker. This pilot study suggested that salivary exosomal miRNAs could be identified as candidate aging biomarkers. To confirm whether miR-24-3p in salivary exosomes are suitable biomarkers of aging, further validation research is required.

Biomarkers of oxidative stress in erythrocytes as a function of human age

Pawan Kumar Maurya, Prabhanshu Kumar, Pranjal Chandra

World J Methodol 2015 December 26; 5(4): 216-222

Despite more than 300 theories to explain the aging process, oxidative stress theory offers the best mechanism to explain aging and age related disorders. Several studies have shown the importance of oxidative stress during aging. PubMed, Science Direct and Springer online data bases are taken into consideration to write this mini-review. Human erythrocytes are most abundant and specialized cells in the body. Erythrocytes were extensively studied due to their metabolism and gas transport functions. Recent studies on erythrocytes have provided us detailed information of cell membrane and its structural organization that may help in studying the aging and age associated changes. The susceptibility of an organism is associated with the antioxidant potential of the body. Erythrocytes have potent antioxidant protection consisting of enzymatic and non enzymatic pathways that counteract with reactive oxygen species, thus maintaining the redox regulation in the body. The non-enzymatic and enzymatic antioxidants and other biomarkers associated with erythrocyte membrane transport functions are the main content of this review. Biomarkers of oxidative stress in erythrocytes and its membrane were taken into the consideration during human aging that will be the main subject of this mini review.

Inflamma-miRs in Ageing and Breast Cancer: are they reliable players?

Cristina Sorina Catana et al.

Frontiers in Medicine, Dec 15, Vol2, article 85

Human aging is characterized by chronic low-grade inflammation known as “inflammaging.” Persistent low-level inflammation also plays a key role in all stages of breast cancer since “inflammaging” is the potential link between cancer and aging through NF-kB pathways highly influenced by specific miRs. Micro-RNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at a posttranscriptional level. InflammamiRs have been implicated in the regulation of immune and inflammatory responses. Their abnormal expression contributes to the chronic pro-inflammatory status documented in normal aging and major age-related diseases (ARDs), inflammaging being a significant mortality risk factor in both cases. Nevertheless, the correct diagnosis of inflammaging is difficult to make and its hidden contribution to negative health outcomes remains unknown. This methodological work flow was aimed at defining crucial unanswered questions about inflammaging that can be used to clarify aging-related miRNAs in serum and cell lines as well as their targets, thus confirming their role in aging and breast cancer tumorigenesis. Moreover, we aim to highlight the links between the pro-inflammatory mechanism underlying the cancer and aging processes and the precise function of certain miRNAs in cellular senescence (CS). In addition, miRNAs and cancer genes represent the basis for new therapeutic findings indicating that both cancer and ARDs genes are possible candidates involved in CS and vice versa. Our goal is to obtain a focused review that could facilitate future approaches in the investigation of the mechanisms by which miRNAs control the aging process by acting as efficient ARDs inflammatory biomarkers. An understanding of the sources and modulation of inflamma-miRs along with the identification of their specific target genes could enhance their therapeutic potential.

Inflammation, But Not Telomere Length, Predicts Successful Ageing at Extreme Old Age: A Longitudinal Study of Semi-supercentenarians

Yasumichi Arai et al.

EBioMedicine 2 (2015) 1549–1558

To determine the most important drivers of successful ageing at extreme old age, we combined community based prospective cohorts: Tokyo Oldest Old Survey on Total Health (TOOTH), Tokyo Centenarians Study (TCS) and Japanese Semi-Supercentenarians Study (JSS) comprising 1554 individuals including 684 centenarians and (semi-)supercentenarians, 167 pairs of centenarian offspring and spouses, and 536 community-living very old (85 to 99 years). We combined z scores from multiple biomarkers to describe haematopoiesis, inflammation, lipid and glucose metabolism, liver function, renal function, and cellular senescence domains. In Cox proportional hazard models, inflammation predicted all-cause mortality with hazard ratios (95% CI) 1.89 (1.21 to 2.95) and 1.36 (1.05 to 1.78) in the very old and (semi-)supercentenarians, respectively. In linear forward stepwise models, inflammation predicted capability (10.8% variance explained) and cognition (8.6% variance explained) in (semi-)supercentenarians better than chronologic age or gender. The inflammation score was also lower in centenarian offspring compared to age-matched controls with Δ (95% CI) = −0.795 (−1.436 to −0.154). Centenarians and their offspring were able to maintain long telomeres, but telomere length was not a predictor of successful ageing in centenarians and semi-supercentenarians. We conclude that inflammation is an important malleable driver of ageing up to extreme old age in humans.

DNA methylation age is associated with mortality in a longitudinal Danish twin study

Lene Christiansen et al.

Aging Cell (2016) 15, pp149–154

An epigenetic profile defining the DNA methylation age (DNAm age) of an individual has been suggested to be a biomarker of aging, and thus possibly providing a tool for assessment of health and mortality. In this study, we estimated the DNAm age of 378 Danish twins, age 30–82 years, and furthermore included a 10-year longitudinal study of the 86 oldest-old twins (mean age of 86.1 at follow-up), which subsequently were followed for mortality for

8 years. We found that the DNAm age is highly correlated with chronological age across all age groups (r = 0.97), but that the rate of change ofDNAmage decreases with age. The results may in part be explained by selective mortality of those with a highDNAmage. This hypothesis was supported by a classical survival analysis showing a 35% (4–77%) increased mortality risk for each 5-year increase in the DNAm age vs. chronological age. Furthermore,

the intrapair twin analysis revealed a more-than-double mortality risk for the DNAm oldest twin compared to the co-twin and a 'dose–response pattern' with the odds of dying first increasing 3.2 (1.05–10.1) times per 5-year DNAm age difference within twin pairs, thus showing a stronger association of DNAm age with mortality in the oldest-old when controlling for familial factors. In conclusion, our results support that DNAm age qualifies as a biomarker of aging.

DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative

Morgan E. Levine et al.

AGING, September 2015, Vol 7 N 9

Lung cancer is considered an age‐associated disease, whose progression is in part due to accumulation of genomic instability as well as age‐related decline in system integrity and function. Thus even among individuals exposed to high levels of genotoxic carcinogens, such as those found in cigarette smoke, lung cancer susceptibility may vary as a function of individual differences in the rate of biological aging. We recently developed a highly accurate candidate biomarker of aging based on DNA methylation (DNAm) levels, which may prove useful in assessing risk of aging‐related diseases, such as lung cancer. Using data on 2,029 females from the Women's Health Initiative, we examined whether baseline measures of "intrinsic epigenetic age acceleration" (IEAA) predicted subsequent lung cancer incidence. We observed 43 lung cancer cases over the nearly twenty years of follow‐up. Results showed that standardized measures of IEAA were significantly associated with lung cancer incidence (HR: 1.50, P=3.4x10‐3). Furthermore, stratified Cox proportional hazard models suggested that the association may be even stronger among older individuals (70 years or above) or those who are current smokers. Overall, our results suggest that IEAA may be a useful biomarker for evaluating lung cancer susceptibility from a biological aging perspective.

AGE metabolites: A biomarker linked to cancer disparity?

Dion Foster et al.

Cancer Epidemiol Biomarkers Prev. 2014 October ; 23(10): 2186–2191

Socioeconomic and environmental influences are established factors promoting cancer disparity but the contribution of biological factors is not clear. We report a mechanistic link between carbohydrate derived metabolites and cancer which may provide a biological

consequence of established factors of cancer disparity. Glycation is the non-enzymatic glycosylation of carbohydrates to macromolecules which produces reactive metabolites called advanced glycation end products (AGEs). A sedentary lifestyle and poor diet all promote disease and the AGE accumulation pool in our bodies and also increase cancer risk. We examined AGE metabolites in clinical specimens of African American and European American prostate cancer patients and found a higher AGE concentration in these specimens among African American patients when compared to European American patients. Elevated AGE levels corresponded with expression of the receptor for AGE (RAGE or AGER). We show that AGE mediated increases in cancer associated processes is dependent upon RAGE. Aberrant AGE accumulation may represent a metabolic susceptibility difference that contributes to cancer disparity.

Alpha-Synuclein Levels in Blood Plasma Decline with Healthy Aging

Niklas K. U. Koehler et al.

PLOS ONE | DOI:10.1371/journal.pone.0123444 April 6, 2015

There is unequivocal evidence that alpha-synuclein plays a pivotal pathophysiological role in neurodegenerative diseases, and in particular in synucleinopathies. These disorders present with a variable extent of cognitive impairment and alpha-synuclein is being explored as a biomarker in CSF, blood serum and plasma. Considering key events of aging that include proteostasis, alpha-synuclein may not only be useful as a marker for differential diagnosis but also for aging per se. To explore this hypothesis, we developed a highly specific ELISA to measure alpha-synuclein. In healthy males plasma alpha-synuclein levels correlated strongly with age, revealing much lower concentrations in older (avg. 58.1 years) compared to younger (avg. 27.6 years) individuals. This difference between the age groups was enhanced after acidification of the plasmas ($p<0.0001$), possibly reflecting a decrease of alpha-synuclein-antibody complexes or chaperone activity in older individuals. Our results support the concept that alpha-synuclein homeostasis may be impaired early on, possibly due to disturbance of the proteostasis network, a key component of healthy aging. Thus, alpha-synuclein may be a novel biomarker of aging, a factor that should be considered when analyzing its presence in biological specimens.

Association Between High-Sensitivity C-Reactive Protein and Total Stroke by Hypertensive Status Among Men

Monik C. Jimenez et al.

J Am Heart Assoc. 2015;4:e002073

Background-—High-sensitivity C-reactive protein (hsCRP), a marker of systemic inflammation, may promote atherosclerosis, particularly among adults with elevated blood pressure; however, data are sparse. We examined the association between hsCRP concentrations and risk of total stroke by hypertension status (normotension, prehypertension, and hypertension) among men in the Physicians' Health Study (PHS).

Methods and Results-—Blood samples were collected (1996–1997) and assayed for hsCRP among 10 456 initially healthy men from PHS I and PHS II and followed from 1997 to 2012. Self-reported hypertension status, cardiovascular risk factors, lifestyle, and alcohol consumption were obtained from the baseline questionnaire prior to randomization in PHS II. Strokes were updated approximately annually and confirmed by medical records according to the National Survey of Stroke criteria. Multivariable Cox models were used. We observed 395 incident total strokes over 115 791 person-years. In analyses adjusted for potential confounders and stroke risk factors, clinically elevated hsCRP (>3 mg/L) was associated with a 40% significantly greater hazard of total stroke compared with hsCRP <1 mg/L (hazard ratio 1.40, 95% CI 1.06 to 1.87; Ptrend=0.01). Additional adjustment for blood pressure and biomarkers associated with cardiovascular risk marginally attenuated the estimates. Results were similar by hypertension status, although not statistically significant among normotensive and prehypertensive participants due to limited events.

Conclusions-—Elevated hsCRP levels were associated with a greater risk of total stroke, even after adjustment for potential confounders and cardiovascular risk factors. Risk of total stroke was significantly higher among hypertensive men with elevated hsCRP compared with normotensive men with low hsCRP.

Improved Blood Biomarkers but No Cognitive Effects from 16 Weeks of Multivitamin Supplementation in Healthy Older Adults

Elizabeth Harris et al.

Nutrients 2015, 7, 3796-3812

Supplementation with vitamins, minerals and phytonutrients may be beneficial for cognition, especially in older adults. The aim of this study was to assess the effects of multivitamin supplementation in older adults on cognitive function and associated blood biomarkers. In a

randomised, double blind, placebo-controlled trial, healthy women (n = 68) and men (n = 48) aged 55–65 years were supplemented daily for 16 weeks with women's and men's formula multivitamin supplements. Assessments at baseline and post-supplementation included computerised cognitive tasks and blood biomarkers relevant to cognitive aging. No cognitive improvements were observed after supplementation with either formula; however, several significant improvements were observed in blood biomarkers including increased levels of vitamins B6 and B12 in women and men; reduced C-reactive protein in women; reduced homocysteine and marginally reduced oxidative stress in men; as well as improvements to the lipid profile in men. In healthy older people, multivitamin supplementation improved a number of blood biomarkers that are relevant to cognition, but these biomarker changes were not accompanied by improved cognitive function.

Relationship between biomarkers of inflammation, oxidative stress and endothelial/microcirculatory function in successful aging versus healthy youth: a transversal study

Daniel Alexandre Bottino et al.

BMC Geriatrics (2015) 15:41

Background: There is a functional decline of endothelial- dependent vasodilatation in the aging process. The aims of this study were to investigate if various microcirculatory parameters could correlate to anthropometrical variables, oxidative stress and inflammatory biomarkers in successful aging and compare the results to young healthy controls.

Methods: Healthy elderly women (HE, 74.0 ± 8.7 years, n = 11) and young controls (YC, 23.1 ± 3.6 years, n = 24) were evaluated through nailfold videocapillaroscopy (NVC), venous occlusion plethysmography (VOP) and laboratorial analysis. Functional capillary density (FCD) and diameters, maximum red blood cell velocity (RBCVmax) during the reactive hyperemia response/RBCVbaseline after 1 min arterial occlusion at the finger base, time to reach RBCVmax were determined by NVC, peak increment of forearm blood flow (FBF) during the reactive hyperemia response (%Hyper) and after 0.4 mg sublingual nitroglycerin (%Nitro) by VOP and lipidogram, fibrinogen, fasting and postload glucose, oxidized LDL-cholesterol (oxLDL), sICAM, sVCAM, sE-Selectin, interleukines 1 and 6 and TNF-α by laboratorial analysis. Correlations and linear multiple regression (LMR) between %Hyper, %Nitro, microcirculatory parameters, oxidative stress and inflammatory biomarkers were investigated.

Results: sVCAM, sE-Selectin and oxLDL were higher and RBCVmax/RBCVbaseline and %Hyper lower in HE, while %Nitro and FCD remained unchanged. Fibrinogen, LDL-cholesterol, oxLDL correlated negatively to %Hyper while sVCAM correlated negatively to %Hyper and RBCVmax/RBCVbaseline. Healthy aged women presented dilated capillaries with sustained

perfusion and endothelial dysfunction with preserved vascular smooth muscle reactivity. Fibrinogen, LDL-cholesterol, oxidized-LDL and sVCAM correlated negatively to endothelial function but not to microcirculatory parameters. Oxidized-LDL and sVCAM could determine %Hyper through LMR.

Conclusion: Oxidized-LDL and sVCAM might be used as endothelial dysfunction biomarkers for elderly with normal cardiovascular risk factors.

Antiaging therapy: a prospective hypothesis

Mohammad Rashid et al.

Drug Design, Development and Therapy 2015:9 663–667

This hypothesis proposes a new prospective approach to slow the aging process in older humans. The hypothesis could lead to developing new treatments for age-related illnesses and help humans to live longer. This hypothesis has no previous documentation in scientific media and has no protocol. Scientists have presented evidence that systemic aging is influenced by peculiar molecules in the blood. Researchers at Albert Einstein College of Medicine, New York, and Harvard University in Cambridge discovered elevated titer of aging-related molecules (ARMs) in blood, which trigger cascade of aging process in mice; they also indicated that the process can be reduced or even reversed. By inhibiting the production of ARMs, they could reduce age-related cognitive and physical declines. The present hypothesis offers a new approach to translate these findings into medical treatment: extracorporeal adjustment of ARMs would lead to slower rates of aging. A prospective “antiaging blood filtration column” (AABFC) is a nanotechnological device that would fulfill the central role in this approach. An AABFC would set a near-youth homeostatic titer of ARMs in the blood. In this regard, the AABFC immobilizes ARMs from the blood while blood passes through the column. The AABFC harbors antibodies against ARMs. ARM antibodies would be conjugated irreversibly to ARMs on contact surfaces of the reaction platforms inside the AABFC till near-youth homeostasis is attained. The treatment is performed with the aid of a blood-circulating pump. Similar to a renal dialysis machine, blood would circulate from the body to the AABFC and from there back to the body in a closed circuit until ARMs were sufficiently depleted from the blood. The optimal application criteria, such as human age for implementation, frequency of treatments, dosage, ideal homeostasis, and similar concerns, should be revealed by appropriate investigations. If AABFC technology undergoes practical evaluations and gains approval, it would hold future promises such as: 1) prolonged lifespans; 2) slowed age-related illnesses such as low bone mass, weak muscular systems, diabetes, arthritis, Alzheimer’s disease, and impaired memory in the elderly; 3) reduced health expenses; 4) reduced cosmetic surgeries performed on the elderly; 5) healthier astronauts in extended outer space journeys; 6) reduced financial burden of advanced care

for the elderly imposed upon both government and society; and 7) rejuvenating effects in healthy, non-aged individuals.

DNA methylation age of blood predicts all-cause mortality in later life

Riccardo E Marioni et al.

Genome Biology (2015) 16:25

Background: DNA methylation levels change with age. Recent studies have identified biomarkers of chronological age based on DNA methylation levels. It is not yet known whether DNA methylation age captures aspects of biological age.

Results: Here we test whether differences between people's chronological ages and estimated ages, DNA methylation age, predict all-cause mortality in later life. The difference between DNA methylation age and chronological age (Δage) was calculated in four longitudinal cohorts of older people. Meta-analysis of proportional hazards models from the four cohorts was used to determine the association between Δage and mortality. A 5-year higher Δage is associated with a 21% higher mortality risk, adjusting for age and sex. After further adjustments for childhood IQ, education, social class, hypertension, diabetes, cardiovascular disease, and APOE e4 status, there is a 16% increased mortality risk for those with a 5-year higher Δage. A pedigree-based heritability analysis of Δage was conducted in a separate cohort. The heritability of Δage was 0.43.

Conclusions: DNA methylation-derived measures of accelerated aging are heritable traits that predict mortality independently of health status, lifestyle factors, and known genetic factors.

Establishment of the MethyLight Assay for Assessing Aging, Cigarette Smoking, and Alcohol Consumption

Kosuke Endo et al.

BioMed Research International Volume 2015, Article ID 451981

The environmental factors such as aging, smoking, and alcohol consumption have been reported to influence DNA methylation (DNAm). However, the versatility of DNAm measurement by DNAm array systems is low in clinical use. Thus, we developed the MethyLight assay as a simple method to measure DNAm. In the present study, we isolated

peripheral blood DNA from 33 healthy volunteers and selected cg25809905, cg02228185, and cg17861230 as aging, cg23576855 as smoking, and cg02583484 as alcohol consumption biomarkers. The predicted age by methylation rates of cg25809905 and cg17861230 significantly correlated with chronological age. In immortalized B-cells, DNAm rates of two sites showed a younger status than the chronological age of donor. On the other hand, the predicted age of the patients with myocardial infarction (MI) was not accelerated. The methylation rate of cg23576855 was able to discriminate the groups based on the smoking status. The DNAm rate of cg02583484 was reduced in subjects with habitual alcohol consumption compared to that of subjects without habitual alcohol consumption. In conclusion, our MethyLight assay system reconfirms that aging, smoking, and alcohol consumption influenced DNAm in peripheral blood in the Japanese. This MethyLight system will facilitate DNAm measurement in epidemiological and clinical studies.

Oxidative Stress in Atopic Dermatitis

Hongxiu Ji and Xiao-Kang Li

Oxidative Medicine and Cellular Longevity Volume 2016, Article ID 2721469

Atopic dermatitis (AD) is a chronic pruritic skin disorder affecting many people especially young children. It is a disease caused by the combination of genetic predisposition, immune dysregulation, and skin barrier defect. In recent years, emerging evidence suggests oxidative stress may play an important role in many skin diseases and skin aging, possibly including AD. In this review, we give an update on scientific progress linking oxidative stress to AD and discuss future treatment strategies for better disease control and improved quality of life for AD patients.

Aging, inflammation, stem cells, and bone healing

Gibon et al.

Stem Cell Research & Therapy (2016) 7:44

Complex interactions among cells of the monocyte-macrophage-osteoclast lineage and the

mesenchymal stem cell-osteoblast lineage play a major role in the pathophysiology of bone healing. Whereas the former lineage directs inflammatory events and bone resorption, the latter represents a source of cells for bone regeneration and immune modulation. Both of these lineages are affected by increasing age, which is associated with higher baseline levels of inflammatory mediators, and a significant reduction in osteogenic capabilities. Given the above, fracture healing, osteoporosis, and other related events in the elderly present

numerous challenges, which potentially could be aided by new therapeutic approaches to modulate both inflammation and bone regeneration.

Reduced DNA methylation patterning and transcriptional connectivity define human skin aging

Felix Bormann et al.

Aging Cell (2016) pp1–9

Epigenetic changes represent an attractive mechanism for understanding the phenotypic changes associated with human aging. Age-related changes in DNA methylation at the genome scale have been termed 'epigenetic drift', but the defining features of this phenomenon remain to be established. Human epidermis represents an excellent model for understanding age-related epigenetic changes because of its substantial cell-type homogeneity and its well-known age-related phenotype. We have now generated and analyzed the currently largest set of human epidermis methylomes (N = 108) using array-based profiling of 450 000 methylation marks in various age groups. Data analysis confirmed that age-related methylation differences are locally restricted and characterized by relatively small effect sizes. Nevertheless, methylation data could be used to predict the chronological age of sample donors with high accuracy. We also identified discontinuous methylation changes as a novel feature of the aging methylome. Finally, our analysis uncovered an agerelated erosion of DNA methylation patterns that is characterized by a reduced dynamic range and increased heterogeneity of global methylation patterns. These changes in methylation variability were accompanied by a reduced connectivity of transcriptional networks. Our findings thus define the loss of epigenetic regulatory fidelity as a key feature of the aging epigenome.

Mediation analysis of relationships between chronic inflammation and quality of life in older adults

Alexandra C. H. Nowakowski et al.

Health and Quality of Life Outcomes (2016) 14:46

Background: This article summarizes exploratory analyses of relationships between chronic inflammation, its physical consequences, and quality of life (QoL). It summarizes key findings from preliminary analyses, and contextualizes these results with extant sociomedical literature to recommend directions for future research.

Methods: Cross-sectional data from the National Social Life, Health, and Aging Project (NSHAP) were used to explore these relationships. Inflammation was assessed via the biomarker C-reactive protein (CRP). We examined associations between CRP levels and two different domains of QoL: happiness with life in general and happiness with intimate relationships. We used ordinal logistic regression with companion OLS models and Sobel-Goodman tests to assess potential mediation, and also conducted a variety of sensitivity analyses.

Results: Findings suggest that mediation pathways for the overall association between chronic inflammation and QoL may differ markedly across particular outcome constructs. Specifically, it shows mediation potential for the clinical sequelae of chronic inflammation in frameworks using happiness as an outcome measure, but not in those using relationship satisfaction. Disability appears to mediate the effect of inflammation by 27 %; chronic pain appears to exert a similar mediation effect of 21 %.

Conclusions: Pain and disability linked to chronic inflammation appear to play a small but significant mediating role in the overall reduction in QoL observed among older adults with biomarker evidence of chronic inflammation. We note that these patterns are best framed as dynamic elements of a complex causal fabric, rather than powerful determinants that override other factors contributing to QoL. Hypotheses for further exploration using longitudinal data from the NSHAP are thus offered, pending availability of Wave III data in future years.

Nutrition and lifestyle in healthy aging: the telomerase challenge

Virginia Boccardi et al.

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Nutrition and lifestyle, known to modulate aging process and age‐related diseases, might also affect telomerase activity. Short and dysfunctional telomeres rather than average telomere length are associated with longevity in animal models, and their rescue by telomerase maybe sufficient to restore cell and organismal viability. Improving telomerase activation in stem cells and potentially in other cells by diet and lifestyle interventions may represent an intriguing way to promote health‐span in humans.

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