CELL THERAPY AND STEM CELL BIOLOGY

P08–01
HOW TO MEND A BROKEN HEART – BUILDING VASCULARISED HUMAN CARDIAC TISSUE FROM STEM CELLS *IN VIVO*

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We have used both mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSC) as sources of human cardiomyocytes to grow vascularised cardiac tissue in chambers *in vivo*. To grow complex human heart tissue we require high efficiency differentiation and may need to generate hundreds of millions of cardiomyocytes per construct. Using the traditional embryoid body (EB) approach from iPSC, we have enhanced the efficiency of cardiac differentiation by the judicious use of the HDAC inhibitor trichostatin-A, along with activin A and BMP4. One of the challenges in cardiogenic differentiation is to regulate the phenotype and produce more mature cardiomyocytes. Differentiated, spontaneously-contracting cells attained a more mature phenotype after trichostatin, as assessed by chronotropic and electrophysiological responses to cardioactive drugs (isoprenaline, carbamylcholine, nifedipine), as well as calcium cycling patterns (assessed by Fluo-4) *in vitro*. We are now stimulating EB patches electrically to improve further the differentiation and maturation pathways. The mature myocytes and other cells thus produced are then grown into cardiac tissue by implantation into chambers containing the femoral vessels in nude rats. The iPSC-derived cell clusters generate spontaneously beating constructs in the chambers which incorporate human cardiac cells perfused with endogenous blood vessels. Importantly, these micro-dissected human-derived cardiomyocytes survived 4 weeks after implantation with strong troponin-T striations in the constructs. However, iPSC-derived clusters also produced clearly defined teratoma, so it remains important to purify the differentiated cells from undifferentiated or precursor cells before implantation. This tissue engineering approach incorporating endogenous vascularisation provides proof-of-principle for generating myocardium-like tissue from human iPSC, and for testing the safety of constructs generated from mixed cell populations.

P08-02
USING PLURIPOTENT STEM CELL TO STUDY AND TREAT AGING-ASSOCIATED DISEASES

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Global population aging has been causing increasing crisis around the world. Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome (WS) are two human premature aging disorders with features that closely recapitulate the features of human ageing. Mutations in *LMNA* and *WRN* genes lead to aberrant splicing product progerin and protein loss in HGPS and WS, respectively. Study on how genetic alteration leads to the cellular and organismal phenotypes of premature aging will provide clues to the molecular mechanisms that underlie physiological ageing and increased our understanding of molecular pathways contributing to healthy aging. We have generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained from patients with HGPS. Further, using targeted gene correction technology, we successfully corrected the mutated *LMNA* gene in HGPS-iPSCs. Finally, by using targeted gene 'knock-in' technique, we also created WS-specific human embryonic stem cells (hESCs) with *WRN* mutation as well as Parkinson's disease (PD)-specific hESCs with *LRRK2* mutation. Upon differentiation of these 'diseased' human pluripotent stem cells into different somatic cell types, they demonstrated tissue-specific and aging-associated phenotypic defects. Together, these tools offer an unprecedented platform to study the pathogenesis of human aging and aging-related diseases.

P08–03
REJUVENATION OF TELOMERES THROUGH PLURIPOTENT STEM CELLS

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Telomeres consist of repeated DNA sequences TTAGGG at chromosome ends enclosed in T-loop structure and stabilized with telomere associated proteins called telosome/shelterin complex, and maintain chromosomal and genomic stability. Telomeres are primarily maintained by telomerase and also can be rapidly lengthened by alternative lengthening of telomeres (ALT) which relies on recombination based mechanism. Telomeres shorten with cell division and differentiation, and also with age, mostly resulting from inactivation of telomerase and chronological oxidative stress that damages telomeres, leading to cellular senescence, aging, and aging-associated degenerative diseases or cancer. Telomeres usually become shorter in differentiated cells and in cells undergoing senescence, but long in pluripotent stem cells including embryonic stem cells (ES), induced pluripotent stem cells (iPS), nuclear transfer embryonic stem cells (ntES) and parthenogenetic embryonic stem (pES) cells. Telomere maintenance is important for unlimited self-renewal, pluripotency and genomic stability of pluripotent stem cells. We are interested in how telomeres are elongated, maintained and regulated in pluripotent stem cells. Mouse ES cells generated from early embryos sporadically enter 2-cell embryo state, to acquire rapid telomere rejuvenation. Telomeres are lengthened by both telomerase-dependent and ALT mechanisms in ES, pES and iPS and likely in ntES cells too. Oocyte factors and 2-cell genes indeed improve the iPS induction and quality. Also, telomere associated protein Rif1 and TRF1 highly expressed in ES cells play critical role in the maintenance of telomeres. Moreover, epigenetic remodeling by DNA demethylation and histone modifications including histone acetylation and methylation e.g. H3K9me3 at sub-telomeres

and telomeres also regulate telomere lengths in pluripotent stem cells. Further investigation of telomere rejuvenation through pluripotent stem cells would help understand mechanisms of unlimited self-renewal and maintenance of genomic stability of pluripotent stem cells for safer potential applications in cell therapy, and could also have implications in aging and cancer and feasibility of anti-cancer and rejuvenation of youth.

P08-04
STEM CELL AND HORMONAL BASED REJUVENATION OF THE THYMUS AND BONE MARROW TO REVERSE IMMUNODEFICIENCY, TREAT AUTOIMMUNITY AND INDUCE DONOR TRANSPLANT TOLERANCE

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Age-associated thymus atrophy leads to increased susceptibility to infections, a higher incidence and burden of cancer and very poor immune recovery from chemotherapy. We have shown that sex steroid ablation (SSA) using the clinically common hormone LHRH, profoundly enhances thymus function, restoring immune capacity. LHRH also induces significant rejuvenation of bone marrow (BM) stromal niches and haemopoietic compartments, with improved self-renewal, differentiation and repopulation potential of HSCs. We are combining this approach with thymic epithelial stem cells (TESC). We previously identified MTS 24+ as putative TESC in the embryonic thymus. We have now defined an adult TESC on the basis of variable expression of MHC Class II, thymic epithelial and other generic stem cell determinants; it is a common stem /progenitor for the adult cortex and medulla. We have also developed GFP-FoxN1 human and mouse embryonic stem cell (ESC) lines. Using the GFP-FoxN1 human ESC as spin EB cultures, in combination with 'heat map' concentration gradients of putative differentiation induction factors, we have generated putative human TEPC. These stem cell based studies, together with amnion and mesenchymal stem cells provide a novel approach to reversing immunodeficiencies where thymus atrophy is severe. The ability to rejuvenate the endogenous thymus or de novo induction of TESC, in combination with donor HSCT, provides a rational basis for creating long-term low morbidity immune tolerance to the donor stem cell therapies. We have established a very low-dose conditioning regime, combined with HSCT and LHRH induced thymus and BM rejuvenation to induce tolerance to allotransplants.

P08-05
AN UPDATE ON DMD EXON SKIPPING TRIALS: MAKING MORE SENSE WITH SPLICE SWITCHING ANTISENSE OLIGONUCLEOTIDES

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Duchenne muscular dystrophy (DMD) arises from protein truncating mutations in the *DMD* gene that preclude synthesis of a functional protein. Becker muscular dystrophy, a less severe muscle wasting disease with slower progression also arises from mutations in the dystrophin gene, but these lesions are most commonly in-frame deletions that allow synthesis of dystrophin isoforms that retain some function. Antisense oligonucleotides have been designed to redirect dystrophin pre-mRNA processing so that an exon can be specifically excluded from the mature mRNA. We have developed a morpholino oligomer that excises dystrophin exon 51, and should restore functional dystrophin expression in the most common subset of DMD deletion patients. An extended placebo-controlled study was initiated in Columbus Ohio and this oligomer, now called Eteplirsen, has been administered intravenously to trial participants on a weekly basis at doses of 30 mg/kg or 50 mg/kg. After 24 weeks administration, boys receiving Eteplirsen were unequivocally synthesizing dystrophin, but no clinically significant benefits were observed compared to the placebo group (rolled over to open label after 24 weeks). However, after 36, 48 and 84 weeks treatment, statistically significant differences in the 6 min walk test were seen in the treated groups, when compared to the placebo/delayed treatment cohort. The drug is well tolerated and has not been associated with any adverse effects. The trial is ongoing and accelerated approval is being sought from the US FDA. Additional oligomers are being designed to address different dystrophin mutations, and new clinical trials should be underway in 2013/14. The promising DMD trial results have renewed enthusiasm to pursue splice intervention therapies for other disorders. An estimated 15% of human mutations induce aberrant splicing and splice switching oligomers may be used as a personalized genetic therapy, regardless of the mutated gene.

P08-06
GENERATION OF INTEGRATION-FREE INDUCED PLURIPOTENT STEM CELLS FROM HUMAN URINE-DERIVED CELLS

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Derivation of disease-specific induced pluripotent stem cells (iPSCs) provides new opportunities to model disease and study molecular mechanisms underlying the occurrence and progression of disease *in vitro*. Differentiation of disease-specific iPSCs into disease-affected cell types offers potential platform for drug screening and gene therapy. So far, viral-based transduction of four 'Yamanaka factors' is widely used as a method to reprogram

somatic cells towards iPSCs. However, random integration into host genome hampers their further application in basic and clinical research. Here we report the generation of iPSC from urine-derived cells using episomal vector based integration-free approach. The derived colonies were characterized by their endogenous expression of pluripotent markers such as OCT4, NANOG and SOX2 at transcription and translation level, teratoma formation and their differentiation ability towards pancreas, liver and neurons *in vitro*. These cells also presented a normal karyotype, and episomal vectors were silenced after several passages. We claim that iPSCs can be successfully derived from urinary cells via integration-free method. This result suggests a potential ability of using urinary cell-derived iPSCs in the study and treatment of various diseases.

P08-07
METABOLITES PROFILES ANALYSIS IN HUMAN EMBRYONIC STEM CELLS AND THEIR NEURAL STEM CELL COUNTERPARTS

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Human embryonic stem cells and derived neural stem cells have been defined and compared based on the genetic, epigenetic and proteomic assays. However, very little is known about the differences regarding metabolic properties of them. In this study, we identified the global metabolites changes in human embryonic stem cells (ESC) and their neural stem cell (NSC) derivatives. We observed significant changes in metabolite concentration for intermediates in glycolysis, the pentose phosphate pathway, glutathione (GSH) and lipid metabolism when compared NSC cells and their ESC counterparts. Specifically, hESC cells favor glycolytic metabolism for energy generation, while NSC cells may instead depend upon oxidative metabolism for cell growth. The results suggest that metabolites profiles can provide biomarkers to assess stem cell preparations for differentiation status.

P08-08
MODELING WERNER SYNDROME WITH HUMAN PLURIPOTENT STEM CELL AND TARGET GENE-EDITING TECHNOLOGY

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A hallmark of aging is accumulation of DNA damage throughout life. Increased accumulation of genetic damage also induces numerous premature aging diseases such as Werner syndrome (WS). WS is an autosomal recessive disease caused by mutation of WRN, a RecQ helicase. Study on how WRN loss leads to genetic instability and cell senescence will improve our understanding of molecular mechanisms underlying normal aging. Here, we report the generation of human embryonic stem cells carrying WRN mutations (WS-ESCs) by using targeted gene editing approach. Then, WS-ESCs were differentiated into various somatic cells types. Upon challenging with aging associated stresses, WS specific somatic cells exhibited a number of premature aging phenotypes, including cell cycle arrest, elevated cell apoptosis, cell senescence, and overall chromatin damages. Altogether, our study indicates that WS-ESC derivations provide a new tool to recapitulate WS pathology *in vitro* and shed light on the mechanistic study of physiological aging.

P08-09
TELOMERE DYSFUNCTION AND STEM CELL AGING

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Current evidence suggests that the functional decline of adult stem cells contributes to the impaired organ homeostasis and functionality during aging. The mechanisms of stem cell aging are still poorly understood. Telomere dysfunction represent one of the molecular mechanisms limiting adult stem cell function by triggering both cell intrinsic checkpoints and cell extrinsic alterations. Deletion of DNA damage checkpoints induced by telomere dysfunction can rejuvenate the aging stem cells and improve organ function in 3rd generation telomerase knockout mice ($G3Terc^{-/-}$). However, the engrafted wild-type HSCs in $G3Terc^{-/-}$ mice showed abnormal hematopoiesis, which was associated with environmental defect induced by telomere dysfunctional in an age dependent manner. Further analysis revealed that telomere dysfunction induced alteration of systemic environmental factors contribute to the impaired lymphopoiesis and the decline stem cell functionality. By using a genetically modified mouse model, we tested the hypothesis that whether HSC transplantation could slow down the telomere-driven aging in the setting of ameliorate environmental defects. We found that wild-type HSC engrafted in $G3Terc^{-/-}$ knockout mouse showed impaired hematopoiesis, whereas HSC engrafted in $Exo\text{-}1^{-/-}$, $G3Terc^{-/-}$ double knockout mouse showed normal hematopoiesis. Further analysis showed an increased survival in $Exo\text{-}1^{-/-}$, $G3Terc^{-/-}$ double knockout mouse after wild-type HSC transplantation compared to those untreated mice, indicating that transplantation of wild-type HSC could rescue the survival of telomere dysfunctional mice in the setting of ameliorated environmental defects. We also utilized iPS and ntES approaches to rejuvenate the somatic cells from aged telomere dysfunctional mice. Our data showed that ntES cells exhibited remarkable elongation of telomere length and improved mitochondrial function, associated with better self-renewal ability and developmental potential compared to iPS cells derived from $G3Terc^{-/-}$ somatic cells.

P08-10
THE EFFECT OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) ON THE DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Human aging is associated with a progressive decline in bone mass and an accumulation of marrow fat. The marrow adipocytes and osteoblasts share common progenitors, mesenchymal stem cells (MSCs), which are capable of multi-lineage differentiation. We found that osteoblast differentiation was reduced while adipocyte formation increased in bone marrow stromal cells derived from aged mice compared with young controls. The increased adipogenesis correlated with a relatively lower Sirt1 activity and a lower intracellular NAD^+ concentration. These effects were caused by age-related reduction of the nicotinamide phosphoribosyltransferase (Nampt), the enzyme catalyzing NAD resynthesis from nicotinamide (NAM). Treatment with Nampt inhibitor, FK866, increased adipocytes formation while reduced mineralization in primary cultured bone marrow stomal cells. In mouse mesenchymal cell line C3H10T1/2 cells, Nampt expression at mRNA and protein levels increased in a time dependent manner during osteoblast differentiation. Furthermore, knock-down of Nampt in C3H10T1/2 cells resulted in decreased Sirt1 activity and enhanced adipogenesis. Besides reduced Sirt1 acitivity, Nampt deficiency also resulted in both decreased intracellular NAD^+ and increased NAM. In conclusion, the present study showed that MSCs differentiation could be affected by the activity of Nampt. The functional mechanism might lie in the deprivation of NAM by Nampt which subsequently regulates Sirt1 activity. Therefore, MSCs differentiation is to a certain extent regulated by cell energy metabolism and points to a possible mechanism for the development of senile osteoporosis.

P08-11
VEGF IN THE RECRUITMENT OF MESENCHYMAL STEM CELLS (MSCS) DURING OSTEOGENESIS

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Cell-cell interaction is believed to play important roles in the bone healing process. However, it is still unclear how transplanted cells behave and interact with host cells in cell-based therapy, especially in the site of bone defect healing. The purpose of this study was to investigate the interactions between osteogenic differentiated human bone marrow stromal cells (O-BMSCs) and mesenchymal cells (MSCs) *in vitro* and *in vivo* and the underlying mechanism of vascular endothelial growth factor (VEGF) induced CXCL12/CXCR4 axis in MSC recruitment and differentiation. Our data suggested that VEGF secretion increased dramatically in BMSCs after osteogenic differentiation and that the secretion of VEGF promoted migration of MSCs via the activation of CXCL12/CXCR4 axis. For the *in vivo* study, type I collagen scaffolds carrying O-BMSCs were implanted into skull defects in SCID mice. *In situ* hybridization demonstrated that O-BMSCs recruited host MSC cells in osteogenic process at orthotopic site. H&E and immunohistochemical staining revealed that remarkable new bone formation was correlated with the strong CXCL12/CXCR4 expression in the new bone matrix and VEGF-neutralizing by VEGF antibody resulted in significant decrease of MSC recruitment and new bone formation. This study demonstrated the VEGF secreted by osteoblasts plays a vital role in the MSCs recruitment via CXCL12/CXCR4 axis during osteogenesis.