Establishing Anti-Ageing Medicines

26th February 2014
Cineworld: The O2, London, SE10 0DX, UK
www.regonline.co.uk/AgeMed2014

The last three decades have shown us how plastic the ageing process can be. It is becoming apparent that, with increased knowledge, more and more of the negative consequences of ageing can now be tackled, postponed or avoided. This meeting aims to review the most up to date science about how we can positively modulate ageing and the implementation of such results to human ageing. This event is part of the 2014 Ageing Summit - **www.AgeingSummit2014.com.** This event has **CPD accreditation.**

Abstracts for *poster presentation only* can be submitted up to two weeks before the event. **You can download the instructions for authors at www.euroscicon.com/AbstractsForOralAndPosterPresentation.pdf**

Meeting Chair: *Dr Nadège Minois,* Biomedical Sciences Research Complex, University of St Andrews, Scotland, UK

Talk times include 5 – 10 minutes for questions

9:30 – 10:15 **Registration**

10:15 - 10:30 **Introduction by the Chair**: *Dr Nadège Minois,* Biomedical Sciences Research Complex, University of St Andrews, Scotland, UK

10:30 – 11:00 **The effects and mechanisms of action of spermidine on ageing**
Dr Nadège Minois, Biomedical Sciences Research Complex, University of St Andrews, Scotland, UK
Spermidine is a natural polyamine with important functions such as DNA stability, cell survival, growth and proliferation. Its level decreases with age and we have shown that supplementing spermidine in food or water increases life span in several model organisms and human cells in culture. It also increases stress resistance and delays age-related oxidative damage and locomotor activity decline. The main mechanisms of action of spermidine are general hypoacetylation and autophagy induction. The talk will review the findings on the role of spermidine on ageing and discuss the potential outcome for human ageing of spermidine supplementation.

11:00 – 11:30 **Stem cell ageing and chemical intervention**
Dr Ilaria Bellantuono, Reader in Stem Cell and Skeletal Ageing, The University of Sheffield, UK
Stem cells are responsible for tissue repair and maintenance and evidence suggest that changes in stem cells with age contribute to the decline in tissue function. The ability to intervene and increase even modestly the number of stem cells, delaying tissue dysfunction may have great impact in areas of degenerative diseases. Indeed limited rejuvenation of stem cells has been shown to rescue tissue function. Small molecules are attractive to amplify the endogenous stem cell pool, preserve it from ageing and direct its differentiation by targeting specific signaling pathways. Here we will present data showing how mesenchymal stem cell ageing can be delayed using chemical interventions.

11:30 – 12:00 **Speakers' photo then mid-morning break and poster exhibition and trade show**

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12:00 - 12:30 **Use of Kinetic Isotope Effect to Mitigate Age-related Oxidative Stress Diseases**
Dr Mikhail Shchepinov, CSO, Retrotope, Inc, USA
Key oxidation prone positions within biomolecules can be reinforced with deuterium to make them more resistant to oxidative stress courtesy of the isotope effect. Use of this approach in mitigation of age-related diseases will be discussed.

12:30 – 13:00 **Tenocyte metabolism in ageing and estrogen deficiency**
Dr. Francesca Veronesi, Biotechnologist researcher, Laboratory of Preclinical and Surgical Studies, Rizzoli Orthopaedic Institute, Bologna, Italy
The study evaluated in vitro proliferation, metabolism and micro-wound healing of tenocytes isolated from the Achilles tendons of ovariectomised (OVX), middle-aged (OLD) and young (YOUNG) rats. OLD and OVX showed a lower proliferation, collagen I, aggrecan, elastin and nitric oxide production than YOUNG and fibronectin and elastin were lower in OVX than YOUNG and OLD. Vascular endothelial growth factor, collagen III and metalloproteinases-13 increased in OVX than in YOUNG and OLD. A lower healing rate was observed in OVX than OLD and YOUNG. Results highlighted how aging and more estrogen deficiency negatively affect tendon metabolism and healing.

13:00 - 14:00 **Lunch, poster exhibition and trade show**
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14:00 - 15:00 **Discussion Panel**
This discussion session is an informal question and answer session. This is an ideal opportunity to get advice and opinion from experts in this area. This session is not for questions about specific talks, which can be asked after the speakers session, but for discussing either general topics or specific issues.

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3. During the session you can *put your hand up* and join in

15:00 – 15:30 **Afternoon Tea, last poster session and trade show**

15:30 – 16:00 **Stress signalling pathways as targets for intervention in the premature ageing Werner Syndrome**
Professor D Kipling, Cardiff University, School of Medicine, UK
Werner syndrome (WS, or adult progeria) is a rare human genetic disease associated with accelerated ageing. Cells from WS individuals undergo premature senescence in culture, a phenomenon long suggested to underpin the clinical symptoms. We have shown that small-molecule inhibition of the p38/MK2 stress signalling pathway can prevent the accelerated senescence of WS cells in culture. We will discuss how such inhibitors, many of which are in advanced clinical trials for inflammatory disease, may provide a route to therapeutic intervention in WS. They may

also have potentially beneficial anti-ageing effects in normal individuals, via modulation of the senescence-associated secretory phenotype.

16:00 – 16:30 **Experimental in vitro study on the behaviour of osteoclast in osteoporosis**
Dr Francesca Salamanna, Biologist, Laboratory of Biocompatibility, Technological Innovations and Advanced Therapies, Rizzoli Research Innovation Technology, Rizzoli Orthopaedic Instistute, Bologna, Italy.
The aim of the study was to set up an in vitro method for determining a pathology associated with an increased local and/or systemic bone resorption such as osteoporosis. We found a significant increase in ovariectomized osteoclast (OC) viability, differentiation, bone reabsorbing activity formation, Cathepsin-K and MMP-7 cells culture both with basal medium only (no differentiating factors toward the OC phenothype) and with differentiation medium (with diffrentiating factors toward OC phenothype) demonstrating an increased spontaneous osteoclastogenesis. Thus, the study could offer a methods for diagnosing increased local and/or systemic bone resorption that would prevent and/or limit the complications associated with osteoporosis conditions.

16:30 - 17:00 **Gut microbes as drug targets to slow ageing**
Dr David Weinkove, Lecturer, School of Biological and Biomedical Sciences, Durham University, UK
We have coevolved with an amazing diversity of microbes. Evolution theories of ageing predict that gut microbes that aid nutrition might also increase ageing. Our research with the nematode Caenorhabditis elegans suggests that microbes limit lifespan through specific metabolic pathways. Targeting microbes with antibiotics disrupts microbiome diversity and increases susceptibility to harmful infection. However, we show the production of excess folate by the bacteria Escherichia coli can be inhibited without slowing the growth of either the microbe or animal. We propose that drugs that target specific pathways of microbial metabolism without killing cells are viable prospects for anti-ageing medicines.

17:00 **Chairman's summing up**

Registration Website: www.regonline.co.uk/AgeMed2014

A meeting report from this event will be published by *HONNAO* publishing

Keywords: Spermidine, Longevity, Ageing, Model Organisms, Autophagy, Health; Stem cells, small molecules, ageing, DNA damage, microbes, ageing, folate, metabolic targets, isotope effect; ROS; age-related disease; essential polyunsaturated fatty acid; deuterium, Senescence, p38MAPK, Werner Syndrome, telomere, stress, Osteoporosis , Bone Resorption, Osteoclasts, Achilles tendons, Tenocytes, Aging, Estrogen deficiency, Tendon healing

About the Chair

Nadège Minois has been studying ageing since her PhD in Experimental Gerontology. She has worked at the University of Minnesota (USA), the Max Planck Institute for Demographic Research (Germany), the Research Institute of Molecular Pathology (Austria) and the University of St Andrews. She has tackled projects such as the effects of stress on ageing using the fly Drosophila melanogaster, the shape of mortality rates in the yeast Saccharomyces cerevisiae and a large-scale genetic screen to identify genes modulating life span in Drosophila. Her latest project is to study how spermidine, a natural polyamine, is involved in ageing using Drosophila.

About the Speakers

Ilaria Bellantuonois Reader in Stem cell and Skeletal Ageing at the Mellanby Centre for Bone Research (MCBR), University of Sheffield and her research interests is in understanding what changes mesenchymal stem cells undergo with age and how these impact on bone loss. Central to her research programme is the identification of molecules which target stem cells in vivo to delay their ageing. Ilaria is Head of the Bone Analysis Laboratory at the MCBR, a facility which provides access to contemporary approaches for the analysis of bone. She heads the Shared Ageing Research Models (ShARM) funded by Wellcome Trust, which combines web-based information systems with a physical tissue bank of ageing mouse models and is the Director of training of the MRC- Arthritis Research UK Centre of Integrated research into Musculoskeletal Ageing (CIMA).

Mikhail Shchepinov: MSc (Mendeleev Institute of Chemical Technology), PhD (Shemyakin-Ovchinnikov Bioorganic Chemistry Institute, Moscow). Worked in academia and industry (Oxford, UK; San Diego, USA) from 1995.

Francesca Veronesi: She was born in Bologna (Italy) in 1982 and she graduated in Medical Biotechnology (110/110L) in 2006. From 2007 she works at the Priclinical and Surgical Studies laboratory of Rizzoli Orthopaedic Institute and she is involved in studies on bone, cartilage, tendon and ligament regeneration, working with alternative in vitro and in vivo models of pathologies (such as osteoporosis, osteoarthritis and osteochondral defects). From 2012 she is Ph.D.in Biomaterials (thesis entitled “Polymeric and ceramic biomaterials in bone regeneration”). She is the author of 12 publications in impacted journals and she has participated in National and International conferences.

David Kipling originally trained as a zoologist. After completing a DPhil in the Zoology Department at Oxford University he moved to the MRC Human Genetics Unit in Edinburgh as a post-doctoral researcher. In 1997 he took up a permanent academic position in the School of Medicine at Cardiff University. His research group studies the fundamental processes underpinning human ageing, with a particular interest in telomeres and replicative senescence. He was the academic coordinator of two BBSRC funding initiatives on the biology of ageing, and is a Trustee of the British Society for Research on Ageing.

Francesca Salamanna: She was born in Nardò (LECCE, Italy) in 1982 and she graduated in Biological Sciences in 2006. From 2007 she works at the Priclinical and Surgical Studies laboratory of Rizzoli Orthopaedic Institute and she is involved in studies on bone, cartilage, tendon and ligament regeneration, working with alternative in vitro and in vivo models of pathologies. From 2012 she is PhD in Biomaterials. She is author of publications in impacted journals and she has participated in National and International conferences.

David Weinkove is a Lecturer in the School of Biological and Biomedical Sciences at Durham University. Throughout his career, his research has always involved using model organisms to address key questions in biology, combining genetic and biochemical techniques. Having discovered that an enzyme involved in cell signalling regulated growth in the fruitfly Drosophila during his PhD, he moved to the nematode C. elegans, training in the Netherlands, USA and UK. Using C. elegans he has made a number of contributions to ageing research and now works on how the microbe:animal interaction influences ageing, applying his work to mammals through collaborations.

POSTER PRESENTATIONS

TENOCYTE METABOLISM IN AGEING AND ESTROGEN DEFICIENCY

F.Veronesi[1], P.Torricelli[1,2], S. Pagani[1,2], N. Maffulli[3], A. Frizziero[4], M. Fini[1,2]
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INTRODUCTION Tenocytes are the cells responsible for the characteristics and the repair process of the tendons due to their ability in synthesizing and remodeling tendon extracellular matrix (ECM). In literature there are few studies regarding the effect of ageing on tendons and most of them observed only their mechanical behavior. In addition it was observed a higher risk of tendinopathies in post-menopausal years, characterized by lower tendon collagen turnover and elasticity. Nowadays little is known about the *in vitro* tenocyte ECM production and metabolism and about their behavior during ageing and estrogen deficiency situation. So, the aim of this study was to *in vitro* compare proliferation and synthetic activity of tenocytes from ovariectomised (OVX), middle-aged (OLD) and young (YOUNG) rats both in standard culture conditions and in an *in vitro* model of micro-wound healing. **MATERIALS AND METHODS** Tenocytes were obtained from tendons of young (5 months old), middle-aged (13 months old) and ovariectomized (10 months at ovariectomy and euthanized 3 months later at the age of 13 months) rats, seeded onto 24-well plates at a concentration of 1×10^4 cells/ml and maintained in standard conditions for up to 14 days. At the end of experimental times (3, 7 and 14 days), proliferation (WST-1 test) and protein production of collagen I and III (COLL I and III), aggrecan (AGN), nitric oxide (NO), fibronectin (FBN), elastin, matrix metalloproteinase-13 (MMP-13), tissue inhibitor of metalloproteinases-1 (TIMP-1) and vascular and endothelial growth factor (VEGF) were evaluated. Moreover, once they reached the confluence, an artificial wound (650 µm) was done by scraping the cell layer with a sterile tip and the wound healing, Coll I, Coll III, FBN, elastin, NO, MMP-13 and VEGF production were evaluated after 1 (T1), 4 (T4) and 24 (T24) hours. **RESULTS** Tenocytes of the YOUNG rats proliferated significantly more, at 3 and 14 days, and produced significantly higher Coll I, AGN, elastin, at 3 days, in comparison to both OLD and OVX groups. Coll I/III ratio was significantly lower at 3 days in the OLD and OVX groups than in the YOUNG one and FBN of YOUNG group, at 14 days, was significantly higher when compared to the OVX group. MMP-13, at 3 days, and VEGF, at 3 and 14 days, were respectively significantly higher and lower in OVX in comparison to YOUNG and OLD cells. In the micro-wound healing model, at T1 OVX and OLD tenocytes showed a significantly lower healing rate than YOUNG ones and at T4 and T24 lower values were found for OVX tenocytes than OLD and YOUNG. At T4 and T24, proliferation was significantly lower in OVX tenocytes in comparison to both YOUNG and OLD and in the OLD in comparison to YOUNG at T24. Coll I was significantly higher in YOUNG rats when compared to OLD and OVX ones, whereas Coll III was significantly higher in OVX rats than in OLD and YOUNG ones. Col/Coll III ratio was significantly lower in OLD and OVX groups than in YOUNG as well as FBN production in the OVX group in comparison to OLD and YOUNG. Elastin concentration showed higher values in YOUNG rats than in OVX ones, NO in the YOUNG than in OLD and OVX and MMP-13 in the OVX than in YOUNG and OLD. **DISCUSSION** Proliferation and tenocyte synthetic activity are negatively affected by ageing and estrogen deficiency, even if estrogen deficiency has a greater negative effect in both standard culture conditions and micro-wound healing model. This study improves the knowledge about ageing and estrogen deficiency effects on tenocyte behavior in terms of proliferation, synthetic activity and micro-wound healing. These results might lead to a better identification of therapeutic interventions in clinic in these two situations.

EXPERIMENTAL *IN VITRO* STUDY ON THE BEHAVIOUR OF OSTEOCLAST IN OSTEOPOROSIS.

Salamanna Francesca[1]*, PhD, Pagani Stefania[1,2], BSc, Giavaresi Gianluca[1,2] MD, Maglio Melania[2], BSc, Fini Milena[1,2,] MD.
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Osteoporosis is a major global problem, because over 10 million people are currently diagnosed with osteoporosis. (Sung BP 2011) The age matched prevalence of osteoporosis is 17-20% of women over 50 years old, 26% over 65 years old and 50% over 85 years old in the United States. Osteoclasts are cells involved in bone reabsorbing and hence in postmenopausal bone loss. The aim of the study was to set up an *in vitro* method for determining a pathology associated with an increased local and/or systemic bone resorption such as osteoporosis. Circulating monocytes, obtained from peripheral blood from healthy and ovariectomized (OVX) rats, were reefed with different media, representing alternative cell culture conditions: 1) Basal medium only : monocyte from healthy and OVX rat were cultured with basal medium in the absence of differentiating factors towards the OC phenotype; 2) Differentiation medium : Monocyte from healthy and OVX rats were cultured with basal medium added with differentiating factors towards the OC phenotype (30 ng/ml of RANKL, 25 ng/ml of M-CSF and10-7M PTH). Bone cells viability, differentiation state and synthetic activity, i.e Cathepsin K, metalloproteinase (MMP)-7, MMP-9 and tartrate-resistant acid phosphatase (TRAP) staining and osteoclast pit resorption assay, were evaluated at 1, 2 and 3 weeks after seeding for each culture condition.We found a significant increase in OVX osteoclast viability, differentiation, bone reabsorbing activity formation, Cathepsin K and MMP-7 cells culture both with basal medium only and with differentiation medium. Our data demonstrated an increased spontaneous osteoclastogenesis in rats affected by postmenopausal osteoporosis: this increase may be explained by the higher production of TRAP and Cathepsin K by monocytes cultures of osteoporotic patients. Thus, the study could offer a methods for diagnosing increased local and/or systemic bone resorption that would prevent and/or limit the complications associated with osteoporosis conditions.

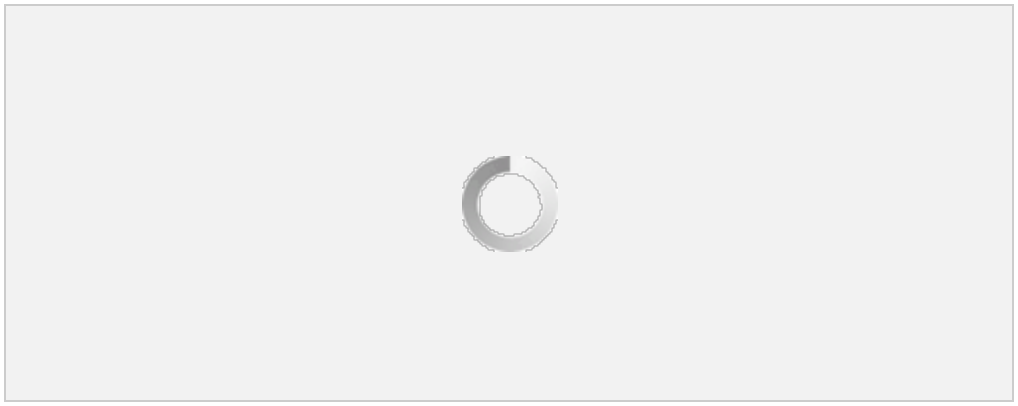
Determination of the senescent associated secretory phenotype (SASP) in normal human primary fibroblasts using the Mesoscale Discovery platform

D.Alimbetov[1,2], T.Davis[2], A.Brook[2], L.S.Cox[3], R.Faragher[4], T.Nurgozhin[1], Zh.Zhumadilov[1] and D.Kipling[2]
[1]Centre for Life Sciences, Nazarbayev University, Astana, Kazakhstan; [2]School of Medicine, Cardiff University, UK; [3]Department of Biochemistry, University of Oxford, UK; [4]School of Pharmacy and Biomolecular Sciences, Brighton University, UK.

One biological mechanism underpinning human ageing is replicative senescence. This refers to the permanent entry of primary human cells into a viable but non-dividing state, as the result of repeated cell division in culture or during life [1]. Senescence probably evolved to confer a tumour-suppressive function. However, the gradual accumulation of senescent cells with an altered phenotype during life may also contribute to age-related diseases and degeneration. In addition to cell cycle arrest, senescent cells also show changes in their pattern of secretion of a range of bio-active proteins, termed the senescence associated secretory phenotype (SASP). SASP components include cytokines (notably IL6 and IL-8), chemokines, ECM components, and ECM-metabolising proteases. Their elevated secretion by senescent cells provides one route whereby senescent cells can have deleterious effects in the context of ageing, although it has also been suggested that the SASP may contribute to clearance of

senescent cells by the immune system [2].

In this project we have investigated the use of the Mesoscale Discovery (MSD) multiplex ELISA platform for the detection of SASP components secreted by senescent primary human fibroblasts in culture. Three human dermal fibroblasts strains were grown to senescence and conditioned media collected as described previously [3]. Multiplex and singleplex MSD assays were used to detect selected SASP components using a Sector Imager SI-6000.



In parallel, key features of the senescent cell phenotype were measured, including markers of proliferation (BrdU labeling) and senescence-associated β-galactosidase. Senescent cells were defined as having a low BrdU labeling index (generally in the range 0-5%) and staining positive for SA-β-Gal. BrdU labeling gradually declined, showing an inverse relationship with the number of population doublings undergone (not shown). At the end of their proliferative lifespan most senescent cells were positive for SA-β-gal staining (closed circles in figure). One strain from a young donor demonstrated a particularly robust up-regulation of key SASP analytes, illustrated here by MCP-3a and TNFa. Note that the senescent cells (defined as low BrdU labeling and SA-b-Gal positive – closed circles) have elevated expression levels of MCP-3a and TNFa compared to the younger cultures (open circles – high BrdU labeling and negative for SA-b-Gal). A total of 12 SASP components were analysed using the MSD platform in young and senescent fibroblasts, with the majority showing increased concentrations in the senescent cultures.

This study demonstrates the utility of the MSD platform to measure the SASP, which would thus allow this medium-throughput platform to be used for SASP analysis as part of a drug discovery or biomarker detection programme.

References

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3. Coppe, J. P., C. K. Patil et al. (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* **6**: 2853-2868.

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