

Plasticity, fate control and therapeutic safety of stem cells

Orlando, Florida Wyndham Orlando Resort
January 8 h. 13.00

13.00 – 13.15

Fulvio Gandolfi

Welcome and opening remarks

13.15 – 13.45: Fausto Cremonesi and Anna Lange-Consiglio

Equine amniotic derived stem cells: progresses and perspectives

Fetal adnexa such as umbilical cord, amnion and amniotic fluid have been recently suggested as ideal sources of different stem cell lineages, due to the not invasive nature of the isolation procedure, the large tissue mass to harvest cells with high efficiency, and the differentiation potentials. Moreover, especially in human medicine, the low ethical implications make these sources of stem cells very attractive for regenerative therapies and biotechnological applications. In the complexity of stem cell origin and definition, a background of the fundamental processes of initial embryo development is illustrated, since the peculiar fitting of the fetal extraembryonic derived stem cells on the continuum of stem cell ontogeny suggests that these cells may preserve some of the characteristics of the primitive embryonic layers where they originate from. Indeed, many studies reported so far indicate that these stem cells exhibit several features of embryonic stem cells, expressing embryonic markers, sharing similar proliferation capability and displaying a negligible immunogenicity. However, their differentiation potential, either in vivo or in vitro, is intermediate between the pluripotent embryonic stem cells and the multipotent adult stem cells. Non embryonic but plastic stem cells have opened new perspectives for developmental biology and for regenerative medicine, not only in humans but also in animals. In this update, we report the state of the art of fetal adnexa-derived stem cells from domestic animals and analyze their applications and expectations in veterinary medicine.

13.45 . 14.15: Heiner Niemann

Production of Oct4/GFP transgenic pigs: a new large animal model for reprogramming

The domesticated pig has emerged as an important tool in biomedical research, including the development of novel surgical techniques, advancement of xenotransplantation, creation of important disease models and preclinical testing of novel cell therapies. However, germ line competent pluripotent porcine stem cells have not yet been derived from any origin. This is a major drawback for using the pig as a model in regenerative medicine. The transcription factor Oct4 is essential for maintenance of pluripotency and critical for reprogramming somatic cells to a pluripotent state. We have produced transgenic pigs carrying the 18 kb genomic sequence of the murine Oct4 gene fused to the enhanced green fluorescent protein (EGFP) cDNA to allow monitoring of Oct4 expression by EGFP fluorescence. Eleven viable transgenic piglets were produced by somatic cell nuclear transfer (SCNT) and germ line specific expression of the EGFP reporter construct was confirmed. EGFP fluorescence was detected in the inner cell mass and trophectoderm of blastocysts, and germ cells and testicular cells. Reprogramming approaches of fibroblasts from these animals by (i) SCNT, (ii) fusion with pluripotent murine embryonic stem cells and by (iii) viral transduction with human OCT4, SOX2, KLF4 and cMYC cDNAs resulted in Oct4-EGFP reactivation, and was compatible with monitoring the kinetics and maintenance of pluripotency in porcine cells. Results indicate that these Oct4-EGFP transgenic pigs facilitate studies on derivation and characterization of pluripotent cells in this important domesticated animal and will improve efficiency and safety of cell therapies.

14.15 – 14.45: Catharina De Schauwer and Ann Van Soom

Markers of stemness in equine mesenchymal stem cells: a plea for uniformity

Mesenchymal stem cells (MSC) represent a promising population for cell-based therapies in veterinary medicine. In spite of the advances in the knowledge of adult stem cells during the past few years, the identification of MSC still remains a difficult issue. In human medicine, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has proposed three criteria to define MSC. First, these cells must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD73, CD90 and CD105, and lack expression of CD34, CD45, CD14 or CD11b, CD79a or CD19 and MHC class II antigens. Third, MSC must differentiate into osteoblasts, adipocytes and chondroblasts in vitro. The successful culture and differentiation of equine MSC from different sources like bone marrow, fat tissue, umbilical cord blood or Wharton's Jelly has been reported

by several research groups, but a complete characterization of these equine MSC by means of immunophenotypic markers, as advocated by the ISCT, remains very difficult. The lack of a single specific marker for MSC and the present limited availability of monoclonal anti-horse antibodies, are major complicating factors for the progress of this type of research. Since commercial antibodies which recognize the equine epitopes, are at present only available for CD44 and MHC-II, different clones of antibodies need to be tested for all other markers in search for cross reactivity. Furthermore, the expression of some markers for adult stem cells may differ between species, so we propose to define a set of CD markers which can be uniformly applied for the identification of equine MSC.

14.45 – 15.15: Randall Prather and Mingtao Zhao

The multi-potentiality of skin-derived stem cells in pigs.

Multipotent skin derived progenitor (SKP) cells can generate both neural and mesodermal progeny, representing neural crest-derived progenitors during embryogenesis through adulthood. SKP cells develop into spheres in suspension and can differentiate into fibroblast-like cells (SFC) in adhesive culture with serum. Concomitantly they gradually lose the neural potential but retain certain mesodermal potential. Transcriptional characterization of porcine SKP spheres and SFC found the down-regulated genes are mostly involved in intrinsic programs like the *Dicer* pathway and asymmetric cell division; whereas up-regulated genes are likely to participate in extrinsic signaling pathways such as ErbB signaling, MAPK signaling, ECM-receptor reaction, Wnt signaling, cell communication and TGF-beta signaling pathways. We speculate that these potential signaling pathways may play an important role in regulating the cell fate transition between SKP spheres and SFC *in vitro*. Further experiments evaluating the "stemness" of neural/progenitor cells showed that the differentially expressed genes between SKP spheres and neurospheres are mainly involved in ECM-receptor interaction and the TGF- β signaling pathway, and that leukemia inhibitory factor (LIF) or MEK inhibitors result in a distinctive impact on the "stemness" and differentiation genes of SKP spheres and neurospheres. Thus the cell-intrinsic genetic program may contribute to the innate "stemness" of SKP spheres and neurospheres in a similar local microenvironment. Finally, chimera's with porcine SKP cells can produce both neural and mesodermal progeny *in vivo*. Further studies will be needed to determine if these cells can contribute to the germ line. If they can contribute to the germ line, then they might be used in a manner similar to mouse embryonic stem cells, i.e. for use in genetic modification of pigs or to test the ability of these cells to be used therapeutically.

15.15 – 15.45 coffee break

15.45 – 16.15: Carol Keefer

Mechanical Phenotyping of Embryonic Stem Cells

Elasticity and visco-elasticity are mechanical properties of cells which directly reflect cellular composition, internal structure (cytoskeleton), and external interactions (cell-cell and/or cell-surface). A variety of techniques involving probing, pulling, or deforming cells have been used to characterize these mechanical properties. With continuing advances in the technology, it may be possible to establish mechanical phenotypes that can be used to identify cells at specific points of differentiation and dedifferentiation with direct applications to therapeutics and diagnostics.

16.15 – 16.45: Vanessa Hall and Poul Hyttel

Development of porcine neural progenitor stem cells for studying and treating Alzheimer's Disease

The pig is an excellent biomedical model which can be used for studying human disease, due to its similar physiology, anatomy and size, to humans. We have recently developed porcine neural progenitor cells which display multipotent characteristics and can be cultured for an extended period in-vitro. These cells form both neurons and glia upon directed differentiation. Furthermore, we are developing in-vitro differentiation protocols that may be useful for future cell transplantation into animal models of Alzheimer's disease. We are also establishing neural progenitor cell lines from the transgenic Alzheimer pig, which may provide a useful in-vitro cell tool for studying mechanisms relating to amyloid precursor protein accumulation. The development of both wildtype and transgenic cells provides a unique opportunity for studying both disease mechanisms and for potential future treatment of the disease.

16.45 – 17.15 Matt Wheeler

Strategies for regeneration of the bone using porcine adult adipose-derived mesenchymal stem cells

Regeneration/reconstruction in the craniofacial region must satisfy the cosmetic needs as well as the functional requirements of the patient. The craniofacial structures protect vital organs, such as the brain

and the eye, and provide support to the masticatory apparatus. Mesenchymal stem cells from bone marrow is one current source of adult stem cells for craniofacial therapeutic purposes; however, the magnitude and accessibility of subcutaneous adipose tissue in humans make it an attractive alternative source for mesenchymal stem cells. Numerous in vitro studies have been conducted to determine how these cells act in vitro, but it is imperative to determine the vast abilities of these cells in vivo. The objective of this study was to evaluate in vivo migration and bone healing ability after transplanting adipose-derived stem cells (ADSC) in a swine model. The clinical implications of such results are significant for treating many diseases where inflammation or defects exist, such as cardiac disease, neurological disease or traumatic injuries to both soft and hard tissue. If the adult stem cells can be harvested from fat and encouraged to produce bone or cartilage, and then reinserted into defects, treatment protocols for trauma victims can be developed that would reduce the need for alternate harvesting techniques for bone.

17.15 – 17.45: Fulvio Gandolfi and Tiziana Brevini

Porcine cardiac progenitor cells: a promising biomedical model

Different cardiac stem/progenitor cells have been recently identified in the post-natal heart. In particular cardiac mesoangioblasts are self-renewing progenitors with high spontaneous cardiac differentiation that can be expanded in vitro to numbers suitable for systemic delivery, and, upon transplantation, regenerate the infarcted heart and new myocardium. Most of the information available is confined to the mouse, making their clinical relevance limited by the enormous diversities between the mouse and the human. Considering the potential future application of these cells for human regenerative therapy, we propose the pig as a complementary model, due to its well known morphological and functional affinity with the human. We describe the isolation, expansion and in vitro proliferation ability of pig mesoangioblasts. We performed their molecular characterization and assessed their differentiation plasticity. The results obtained indicate that cardiac mesoangioblasts can be isolated in the porcine species, stably proliferate in culture for many passages and represent a relevant animal model for cardiac regenerative medicine.

17.45 – 18.00 Concluding remarks