P268 Donor variability of ovine mesenchymal stem cell differentiation potential -clinical implications for cell therapies

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Introduction: Mesenchymal stem cells are the focus of cell-based repair strategies, but little is understood about the impact of donor variability on their clinical outcome. Due to similarities in size, weight, architecture and healing mechanism sheep animal models enable clinical translation of cell-based orthopaedic treatments. To date, ovine mesenchymal stem cells (oMSC) have not been fully characterized and their differentiation potential is not well understood. This study aims to investigate donor variability and chondrogenic potential of oMSCs *in vitro* for the translation of cell-based therapies for osteoarthritis and cartilage defects. **Methods:** Bone marrow oMSCs were isolated from 12 adult English mule ewes (age group 2-4 years) to assess the donor variation. To assess the differentiation potential of oMSCs for both adipogenesis and osteogenesis in 2D and for chondrogenesis in 3D cell organoids, cells were cultured over 20 days. Donor variation was assessed histologically using Oil-o-Red, Alizarin red and Alcian blue staining respectively and semi-quantitatively for both adipogenesis and osteogenesis, while chondrogenesis potential, was assessed by DMMB assay for GAG production. CD marker expression was studied by flow cytometry. MNP labelled STRO-4 positive oMSCs of five donors were seeded in 3D collagen I gels, cultured for 20 days and subjected to mechanical stimulation in a magnetic bioreactor (MICA) to assess the chondrogenic potential. Simultaneously, native sheep cartilage (6 donors, 8mm diameter samples, n=4) was harvested from femoral distal condyle. Native and engineered cartilage's tissue matrix composition (GAG, total Collagen, Aggrecan, COL II, DNA, total protein) were assessed by histology, and biochemical assays and mechanical testing. **Results:** Our data revealed donor variation in the tri-lineage differentiation and CD marker expression across the all donors. No clear correlation between donors was observed among the three lineages. For example, a donor that was highly responsive during osteogenic differentiation was not necessarily as responsive to chondrogenic or adipogenic differentiation. In addition, mechanical stimulation resulted in enhanced chondrogenesis compared with unstimulated controls indicated by increased GAG production (23.3-28.6 µg GAG/µg DNA) but fell short of native cartilage (73.2-119.4 µg GAG /µg DNA). Variations were observed across the donors. Similarly, there is clear variation in characterisation of native cartilage between different donors. **Conclusion:** oMSCs appear to share similarities in characteristics across individual donors, but at the same time, express differences regarding biological properties that may influence the number, phenotype and *in vitro* biological characteristics. This study investigated the use of 2D and 3D culture to assess differentiation capacity between different donors before successfully transmitted to preclinical animal studies. **Acknowledgements:** Al-Mutheffer was supported by Iraqi Ministry of Higher Education. Special thanks Dr J McLaren from the University of Nottingham for her assistance in collecting sheep bone marrow and cartilage and to Prof El Haj's group in particular Dr Markides.