Making Matrix: Early Signals in Chondrogenesis of Human Adult Stem Cells

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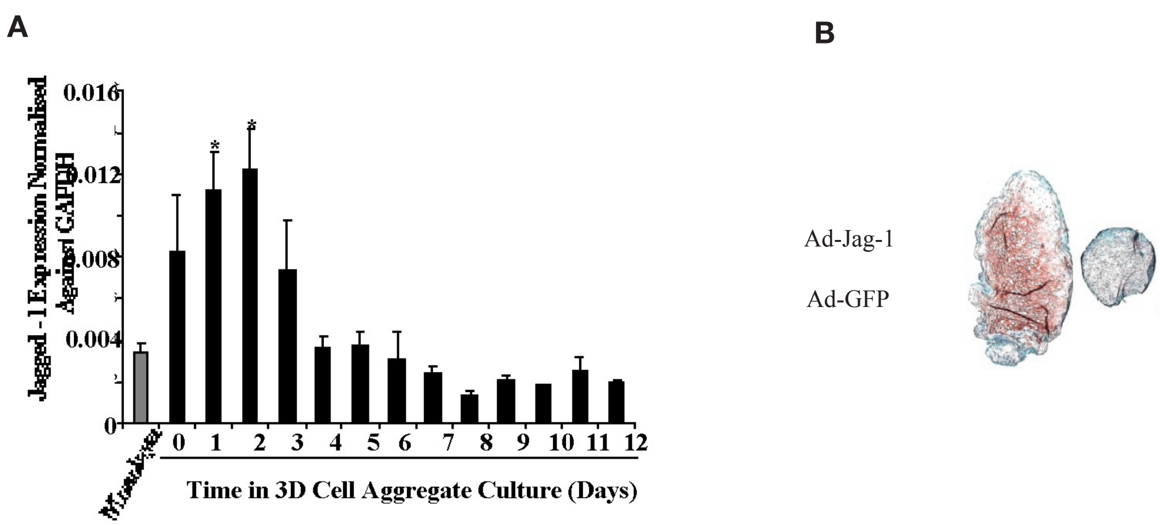
Professor Hardingham was previously Head of Biochemistry at the Kennedy Institute of Rheumatology in London and is the current Chairman of the Arthritis Research Campaign (UK) Grants Committee. Tim is also immediate past Chairman of the British Society for Matrix Biology and his honours include the Colworth Medal of The Biochemical Society (1978), co-winner of the Roussel International Award for Basic Research in Osteoarthritis (1989) and the Carol Nachman International Prize for Research in Rheumatology, (1991). Professor Hardingham is a founding Trustee of the newly formed International Society for Hyaluronan Sciences. He was elected (2001-5) to the Governing Board of Tissue Engineering Society International (TESi) and currently co-heads the Tissue Regeneration Section of the newly launched Faculty of 1000 Medicine.

Professor Hardingham's research interests are in the biology and pathology of cartilage and the degenerative processes in osteoarthritis and other joint diseases. He has long standing research interests in the physical properties and biological functions of proteoglycans and hyaluronan in the extracellular matrix. Current research is focussed on the biology of chondrocytes and adult and embryonic stem cells and the development of cartilage and other musculoskeletal tissues for tissue engineering applications, and in the broad applications of tissue engineering in medicine.

Figure 1: Chondrogenesis of human bone marrow stem cells. (A) Jagged-1 gene expression in hMSCs during cell aggregate culture. * = P< 0.05 compared to monolayer expression levels. (B) Safranin O stain of Ad-GFP and Ad-Jag-1 cell aggregates at day 14.



Introduction - Chondrogenic differentiation of human mesenchymal stem cells (hMSCs) offers great therapeutic potential for the treatment of articular cartilage defects. At present, *in vitro* chondrogenesis is driven by empirically derived protocols comprising a high-density cell aggregate culture system1. The Notch pathway is a highly conserved signalling mechanism involved in many processes determining cell fate during development. In this study we analysed the Notch signalling pathway during chondrogenic differentiation of human mesenchymal stem cells.

Materials and Methods - hMSCs from bone marrow (Cambrex) were expanded to passage 4 in the presence of FGF-2, (5ng/ml). hMSCs were placed into 3D cell aggregate culture, (500,000 cells /cell aggregate)1 for up to 14 days. Real-time qPCR using Sybr Green I was carried out to analyse the gene expression profiles of the four Notch receptors, their five ligands and downstream effectors HES-1 and HEY-1. Jagged-1 protein expression and localisation was analysed by immunohistochemistry of 3D cell aggregates. Full length Jagged-1 cDNA was generated from human placental RNA to produce recombinant Jagged-1 adenovirus (Ad-Jag-1). hMSCs were transduced with either Ad-Jag-1 or with a control adenoviral GFP (Ad-GFP). Post-transduction, hMSCs were placed into chondrogenic cell aggregate culture. Notch activation was assessed by qPCR of HEY-1 gene expression. Chondrogenesis was assessed by qPCR analysis the known chondrocyte markers collagen II and aggrecan and by histological and immunohistochemical characterization at 14 days.

Results - Initiation of chondrogenesis by 3D cell aggregate culture was accompanied by a general down-regulation of Notch receptors. Jagged-1 was the only Notch ligand expressed and it increased transiently by 3-fold to a peak at day 2 ($P<0.05$) (figure 1A), this was concurrent Jagged-1 protein was immunolocalised only within regions that subsequently showed chondrogenic differentiation. Transduction of hMSCs with Ad-Jag-1 caused a 45-fold increase in Jagged-1 message level ($P<0.05$) over Ad-GFP control hMSCs, this level of expression was maintained throughout 14 days of 3D cell aggregate culture. qPCR analysis for HEY-12 revealed that the increased expression of Jagged-1 resulted in continuous Notch signalling throughout the culture period. Over-expression of Jagged-1 ligand and the subsequent persistence of Notch signaling over 14 days resulted in complete inhibition of chondrogenesis. Ad-Jag-1 cell aggregates were smaller than the Ad-GFP controls; cells did not acquire a chondrocyte-morphology and failed to deposit a GAG-rich matrix as displayed by safranin O stain (figure 1B). Collagen II immunolabelling was also negative and collagen II and aggrecan gene expression were 1.5% and 15% that of the Ad-GFP controls at day 14 respectively ($P<0.05$).

Discussion - Notch signalling was active transiently during the early stages of chondrogenesis (days 1-4). The transient rise in Jagged-1 was also strongly suggestive of an active role for Notch signalling in chondrogenesis. The sustained expression of HEY-1 in Ad-Jag-1 transduced cells in cell aggregate culture showed sustained Notch signalling, and hence suggested that Notch signalling must be stopped for chondrogenesis to proceed. Jagged-1 expression on osteoblasts in bone marrow is has been suggested to maintain the self-renewal of haematopoietic stem cells and prevent further differentiation. Therefore, Notch signalling amongst mesenchymal stem cells may retain them undifferentiated and transient Notch in cell aggregates may prime and synchronise chondrogenesis in adult stem cells.

References: (1) Mastrogiacomo et al (2001) OA and Cart. 9: S36-S40. (2) Iso et al (2003) J Cell Physiol 194: 237-255
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