Characterisation of the epigenome of an *in vitro* model of chondrogenesis

Kathleen Cheung, Matt Barter, Louise Reynard, Carole Proctor and David Young

(Institute of Genetic Medicine, Newcastle University)

Chondrogenesis, the differentiation of mesenchymal progenitor cells from the mesoderm germ layer during embryonic development, is partly regulated by epigenetic mechanisms such as histone modifications and DNA methylation. Histone proteins possess protruding N-terminal tails which may be post-translationally modified to alter the structure of chromatin resulting in a change in the accessibility of genes to the transcription machinery. In the genome, histone modifications mark *cis*-regulatory elements such as gene promoters and enhancers while DNA methylation occurs on cytosine residues at CpG sites and typically leading to transcriptional repression.

The aim of this project was to characterise the epigenome during *in vitro* differentiation of human mesenchymal stem cells (hMSCs) into chondrocytes. Chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) was used to assess genome-wide a range of N-terminal post-transcriptional modifications (marks) to histone H3 lysines (H3K4me3, H3K4me1, H3K27ac, H3K27me3 and H3K36me3) in both hMSCs and differentiated chondrocytes. Chromatin states were characterised using the software ChromHMM and *cis*-regulatory elements were identified. Integration of DNA methylation data with chondrogenesis chromatin states revealed that enhancers marked by H3K4me1 and H3K27ac were hypomethylated during *in vitro* chondrogenesis. Similarity analysis between chondrogenesis chromatin states with epigenomes of cell types defined by the Roadmap Epigenomics project revealed that enhancers are more distinct between cell types compared to other chromatin states. SOX9 is regarded as the master transcription factor for chondrogenesis. An external mouse Sox9 ChIP-seq dataset was used to identify super enhancers in chondrocytes. Luciferase reporter assays showed that selected regions of super enhancers exhibit independent enhancer activity.

In conclusion, we observed that CpG sites within enhancers are de-methylated during hMSC differentiation into chondrocytes and propose that gene transcription during chondrogenesis is regulated by epigenetic changes at enhancers. Epigenetic changes have been implicated in cartilage diseases and greater understanding of the chondrocyte epigenome may have potential therapeutic value.