Development of a human-based platform to assess AAV vector genotoxicity

Stefany Perera, Serena Fawaz, Dr. Saqlain Suleman and Professor Michael Themis

Gene therapy is an effective way to treat genetic disorders by delivery of functional genes to tissues, such as the liver. This can be achieved by the transfer of genetic material using viral vectors such as AAV where the therapeutic genetic cargo can remain episomal or integrate into the host DNA. In recent years, AAV has been shown associated with hepatocellular carcinoma in mice, which is most likely as a result of vector effects on local gene(s) near to the site of insertion. Models to determine vector side effects have been developed, however, these use animals or cell lines that may not accurately predict the risk of genotoxicity of AAV in humans. To overcome this limitation, we chose to develop a human model using induced pluripotent cells (iPSc) and their hepatocyte-like (HLC) derivatives. iPSc and these cells differentiated to HLC were fully characterised by immunostaining for pluripotency and terminal differentiation, respectively, and then transcriptomically using RNASeq that demonstrated HLC gene expression aligns with that of primary hepatocytes. Cells were then exposed to AAV vector carrying a strong Chicken β-actin (CB7) or a weak Apo-lipoprotein (ApoE) promoter to drive EGF expression and AAV insertion sites (IS) determined via EPTS/LM-PCR. Gene expression of iPSc pre and post infection was next compared and IS were then identified in cancer genes (CG) according to their level of gene expression. By this analysis, we show *** versus *** integrations in CGs by the AAV/CB7 and AAV/ApoE vectors, respectively, before exploring AAV interactions between the vector genome and that of the host.