[6] Generation of Patient-Specific iPSCs for Hirschsprung’s Disease Modelling

Jasmine SY Yung\textsuperscript{1}, Kim HM Chow\textsuperscript{1,3}, Hung-Fat Tse\textsuperscript{2}, Paul KH Tam\textsuperscript{1}, Elly SW Ngan\textsuperscript{1}
\textsuperscript{1} Department of Surgery, \textsuperscript{2} Department of Medicine, \textsuperscript{3} Stem cell and Regenerative Medicine Consortium, Li Ka Shing Faculty of Medicine, HKU

Hirschspung’s (HSCR) disease is a congenital disorder of the colon in which certain nerve cells are absent due to incomplete colonization of bowel with enteric neural crest (NC) cells, causing chronic constipation. \textit{RET} gene encodes for a tyrosine kinase receptor and is highly implicated in the neural crest development. Mutations or genetic variants in \textit{RET} have accounted for most of the HSCR cases. In particular, a single nucleotide polymorphisms (SNP, rs2435362) residing in the intron one of \textit{RET} gene are predominantly found in HSCR, which may cause a reduced \textit{c-RET} expression in patient. In this study, a HSCR patient carrying a risk allele T in rs2435362 of \textit{RET} gene and exhibiting a short segment aganglionosis and atrial/ventricular septal defects (ASD/VSD) was selected to establish a human model for HSCR. We reprogrammed patient’s fibroblast cells into iPSCs by ectopic expression of four reprogramming factors. Three patient-specific iPSC cell lines were currently obtained. They were ES-like, expressing the pluripotency markers and with low DNA methylation levels of CpG sites in the promoter regions of \textit{NANOG} and \textit{OCT3/4}. Importantly, they could generate teratoma comprising all three germ layers when they were injected in SCID mice, further corroborating the cells had acquired pluripotency. Subsequent differentiation experiments revealed that these HSCR iPSC cells were able to differentiate into NC cells of a comparable capacity as that of the control iPSC cells (IMR90). In addition, these iPSC-derived NC cells were multipotent and could commit to both neurogenic and smooth muscle lineages under defined differentiation conditions. Nevertheless, in general, all the HSCR-iPSC cells showed a lower competency to form neurons and smooth muscle cells, suggesting that differentiation defects of NC may represent a cause of HSCR and other NC-associated disorders. Taken together, these results substantiate the potential use of our patient-specific model to study the etiology of HSCR and other NC-associated diseases.