

## 66] Neural crest derived from Hirschsprung iPS cells show a reduced neural plasticity

Kim HM CHOW<sup>1,2</sup>, Jasmine SY Yung<sup>2</sup>, and Elly SW NGAN<sup>2</sup>

<sup>1</sup>Stem cell and Regenerative Medicine Consortium, <sup>2</sup>Department of Surgery, Li Ka Shing Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong.

Hirschsprung's disease (HSCR) is a congenital disease characterized by the absence of ganglionic cells in the colon. It would be attributed to the defects in neural differentiation and/or migration of enteric neural crest (NC) cells. For a better understanding of disease pathogenesis of HSCR, our laboratory has recently established two iPS cell lines from a HSCR patient. With a gradient switch from KSR medium to a neural inductive N2 medium supplemented with various neurotropic factors, we could direct human iPS cells to differentiate towards NC cells (HNK1<sup>+</sup>/p75<sup>+</sup>). Both the HSCR and control (IMR90) iPS lines could generate NC cells of similar capacity. However, we found that HSCR iPS cells exhibited a lower efficiency to produce enteric NC cells (HNK1<sup>+</sup>/RET<sup>+</sup>). A significantly less (18.9±1.0%) number of HNK1<sup>+</sup>/RET<sup>+</sup> cells were obtained from HSCR iPS cells on day 9 in the differentiation medium. Despite the patient NC cells could differentiate further along neuronal lineage, number of neuronal precursors (TH<sup>+</sup>/Tuj1<sup>+</sup>) obtained from the patient lines was also reduced by 49.3±2.8%. In addition, they were not able to fully differentiate to mature neurons (PGP9.5<sup>+</sup>) of proper neurite outgrowth and showed a reduced neural plasticity to form enteric neurons (such as VIP<sup>+</sup> neurons).

In parallel, an *ex vivo* gut culture experiment was performed and revealed that iPS-derived NC cells were able to engraft in the muscle layers of the aganglionic gut excised from a HSCR patient. More importantly, these engrafted cells could differentiate into neuronal precursors (Tuj1<sup>+</sup>) in the diseased bowel.

In summary, we have demonstrated that HSCR-iPS cells derived NC cells may harbor the intrinsic neuronal differentiation defects, while iPS cells from healthy individual may represent a powerful tool to reconstitute/replenish absent ganglia in HSCR bowel.