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

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Hirschsprung's disease (HSCR) is a congenital disease characterized by the absence of ganglian 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 ($HNK1^+/p75^+$). 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 ($HNK1^+/RET^+$). A significantly less (18.9±1.0%) number of $HNK1^+/RET^+$ 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 ($TH^+/Tuj1^+$) 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 ($PGP9.5^+$) of proper neurite outgrowth and showed a reduced neural plasticity to form enteric neurons (such as VIP^+ 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 ($Tuj1^+$) 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.