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Migration of mouse sacral neural crest cells from the neural tube to the hindgut

X Wang[1], AK Chan[1], SY Cheung[1], TF Wan[1], AJ Burns[2], WY Chan[1]*

[1]Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, [2]University College London, Institute of Child Health, UK

Neural crest cells are a group of migratory embryonic cells which emerge from the neural tube and migrate along defined pathways to various locations throughout the embryo. Studies in avians have shown that sacral neural crest cells initially form an extramural nerve, the nerve of Remak, then migrate into the caudal hindgut and contribute a significant number of neurons and glial cells to the hindgut enteric nervous system. A similar population of sacral neural crest cells has been shown to form the extramural pelvic plexus in mammals but information on the migration of mammalian sacral neural crest cells to and within the hindgut is still scarce. To trace the migration of sacral neural crest cells from the neural tube to the mouse hindgut, we first used *in situ* labelling of pre-migratory sacral neural crest cells with wheat germ agglutinin-gold conjugates (WGA-Au) followed by whole embryo culture to determine the initial migration from E9.5 to E10.5. We then mapped immunohistochemically neural crest cell migration from E11.5 onwards with an antibody to the neurotrophin receptor $p75^{NTR}$, which is expressed in all neural crest-derived cells in the gut. The spatio-temporal distribution of labelled cells was analyzed with confocal microscopy and 3-dimensional images of the embryos were reconstructed from serial sections. Sacral neural crest cells caudal to somite 24 were found to start their migration in embryos with 27-28 somites and they traversed dorsomedially through the mesenchyme between the somites and the neural tube to reach the peri-aortic region within 24 hours. Some of these cells continued to migrate more ventrally and exhibited $p75^{NTR}$ immunoreactivity. The pioneer neural crest cells (the most ventrally located $p75^{NTR+}$ cells) were found in regions dorsolateral to the caudal hindgut at E11.5 and on the lateral sides of the hindgut at E12.5. By E13.5, neural crest cells ($p75^{NTR+}$) were found in pelvic plexi ventrolateral to the caudal hindgut but no $p75^{NTR+}$ cells were found in the caudal hindgut. However, at this stage, vagal neural crest-derived enteric cells (also $p75^{NTR+}$) had already advanced to colonise the rostral half of the hindgut. At E14.0, when the rostral two-thirds of the hindgut had been colonized by vagal neural crest-derived cells, isolated $p75^{NTR+}$ cells were apparent in the terminal hindgut. At this stage a hindgut region of about 800 μm remained devoid of any positive cells. This uncolonised region was located between the rostral (vagal neural crest-derived) and the caudal (probably sacral neural crest-derived) groups of $p75^{NTR+}$ cells. Streams of $p75^{NTR+}$ cells were also found on the ventral side of the hindgut connecting the pelvic plexi with the terminal hindgut at the sacral level of S2/S3. At E14.5, the entire length of the hindgut was fully colonized by $p75^{NTR+}$ cells. We therefore conclude that sacral neural crest cells begin to enter the terminal hindgut via pelvic plexi at E14.0 at the sacral level of S2/S3, prior to the arrival of vagal neural crest cells in the caudal hindgut.

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*Corresponding author
E-mail: wy-chan@cuhk.edu.hk