Hlx Homeobox Transcription Factor Regulation of Genes Required for Enteric Nervous System Development

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The enteric nervous system (ENS) is an independent set of neurons that control gut motility patterns. The ENS is derived from neural crest cells of ectodermal origin that migrate into the gut mesenchyme during embryonic development. This migration is mediated not only by the neurons but most importantly the intestinal mesenchyme. The goal of our research is to understand how intestinal mesenchyme regulates the migration of neural crest cells into the gut. Alteration of ENS development results in a variety of intestinal motility disorders such as Hirschsprung disease. We have previously shown that Hlx, a homeobox transcription factor that is required for normal growth and development of the intestine and liver, is also required for normal development of the ENS. Our goal was to identify factors important for neuronal migration that are regulated by Hlx. We isolated RNA from intestines dissected from $Hlx^{+/+}$ and $Hlx^{-/-}$ mouse embryos at embryonic days 10.5-12.5. We compared expression of seven candidate genes known or suspected of regulating ENS development in the presence and absence of *Hlx* using real-time PCR assays that we developed, normalizing based on expression of *GAPDH*, a housekeeping gene. We found that the expression the gene encoding semaphorin 3C, an inhibitor of neuronal migration, was significantly higher in E12.5 Hlx knockout intestine ($p < 0.01$). This result supports the idea that migration of neural crest cells into the embryonic intestine is blocked in Hlx knockouts by the presence of an inhibitory factor. For other genes studied, there were trends towards significantly different expression that warrant further evaluation. Thus, expression of the genes encoding semaphorin 3C and perhaps other regulators of ENS development are dependent upon Hlx.