Hlx regulation of smooth muscle/mesenchymal promoters

Siddharth Munsif and Michael Bates, MD, PhD

Pediatrics/Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center

Background and Significance: Intestinal peristalsis is mediated by enteric smooth muscle that is derived from primitive intestinal mesenchyme. However, very little is known about enteric smooth muscle differentiation. *Hlx* is a homeobox transcription factor gene that is expressed in embryonic mouse intestinal mesenchyme and is required for growth of the intestine. Previous work has shown that *Hlx* is required for normal smooth muscle differentiation.

Hypothesis: *Hlx* regulates expression of enteric smooth muscle in the GI tract.

Methods: Computational biology resources (MultiPipmaker, TraFAC, Genome TraFAC, BLAST) were used to identify conserved regions and regulatory sites. These highly conserved gene sequences were used to direct preparation of deletion constructs (in luciferase reporter vectors) for assay of baseline promoter activity and regulation by *Hlx*. A truncation mutant of *Hlx* was also prepared by point mutation. Promoter constructs and *Hlx* expression constructs were transfected in *Hlx* knockout cell lines in triplicate, and luciferase activity was measured.

Results: We found that the enteric smooth muscle genes are highly conserved, including elements that have been previously shown by others to be important for gene regulation. These results have been used to plan the preparation of deletion constructs to identify more precisely the regions required for *Hlx* regulation of gene expression; the preparation and testing of these constructs is in progress. Truncation of the *Hlx* coding region resulted in a loss of gene regulatory activity. It remains to be determined whether this is due to the synthesis of an unstable or an inactive protein.

Conclusion: *Hlx* regulation of enteric smooth muscle gene expression is likely mediated through conserved regions of enteric smooth muscle promoters. Understanding how *Hlx* regulates gene expression will provide insights into the mechanisms underlying enteric smooth muscle differentiation.