383 | **The N-Myc downstream-regulated gene 4 is required for enteric nervous system development and function**

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Background: We recently described that the N-Myc Downstream-Regulated Gene 4 (*NDRG4*), an established early-detection DNA-methylation marker for colorectal cancer (CRC), is specifically expressed by enteric neurons. So far, our studies focused on how enteric neuronal NDRG4 is involved in the development and progression of CRC. However, the role of NDRG4 in the development and function of the enteric nervous system (ENS) during gastrointestinal homeostasis also remains to be elucidated.

Methods: To explore the role of *ndrg4* in ENS morphogenesis and intestinal motility, we used a (i) morpholino (MO) and (ii) CRISPR-Cas9 knockdown approach in transgenic zebrafish (*Danio rerio*) expressing the fluorescent-kaede-protein in enteric precursor cells and enteric neurons (Tg(-8.3phox2b:kaede) zebrafish). We quantified the number of enteric neurons in the distal intestine and created spatiotemporal maps from video recordings to examine intestinal motility in wild-type and *ndrg4*-knockdown ($ndrg4^{-/-}$) zebrafish larvae (5 and 7 days post-fertilization (dpf), respectively).

Results: In agreement with mouse and human expression studies, we found that *ndrg4* is also expressed within the GI-tract of zebrafish larvae. In 5dpf zebrafish, loss of *ndrg4* is associated with a significant reduction in the number of enteric neurons: (i) average number 102.4 control-injected vs. 67.4 *ndrg4*-MO injected ($P < 0.001$) and (ii) average number 110.9 $ndrg4^{+/+}$ vs. 97.8 $ndrg4^{-/-}$ zebrafish ($P = 0.028$). Although the frequency, velocity and contraction interval of peristaltic waves were not affected in 7dpf $ndrg4^{-/-}$ zebrafish, their travel distance was significantly shorter in $ndrg4^{-/-}$ as compared to $ndrg4^{+/+}$zebrafish ($P = 0.040$).

Conclusion: This is the first study describing *ndrg4* expression in the zebrafish intestinal tract. Our data suggest that loss of *ndrg4* has consequences for ENS development and function.