

Purification, culture and multi-lineage differentiation of zebrafish neural crest cells

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Researchers at the Massachusetts General Hospital (MGH)/Harvard Medical School, Drs. Beste Kinikoglu and Yawei Kong, led by Dr. Eric C. Liao, cultured and characterized for the first time multipotent neural crest cells isolated from zebrafish embryos. This important study is reported in the February 2014 issue of *Experimental Biology and Medicine*.

Neural crest is a unique cell population induced at the lateral border of the neural plate during embryogenesis and vertebrate development depends on these multipotent migratory [cells](#). Defects in neural crest development result in a wide range of malformations, such as cleft lip and palate, and diseases, such as melanoma. Dr. Liao's laboratory uses zebrafish as a model vertebrate to study the genetic basis of neural crest related craniofacial malformations. Zebrafish has long been used to study early development and recently emerged as a model to study disease. "Development of in vitro culture of neural crest cells and reproducible functional assays will provide a valuable and complementary approach to the in vivo experiments in zebrafish" said Dr. Eric C. Liao, senior author of the study and an Assistant Professor of Surgery at MGH, and Principal Faculty at the Harvard Stem Cell Institute.

The team took advantage of the sox 10 reporter transgenic model to enrich and isolate the [neural crest cells](#) (NCCs), which were subsequently cultured under optimized culture conditions. Cultured NCCs were found to express major neural crest lineage markers such as sox10, sox9a, hnk1, p75, dlx2a, and pax3, and the pluripotency markers c-myc and klf4. The cells could be further differentiated into multiple neural crest lineages, contributing to neurons, glial cells, smooth muscle cells, melanocytes, and chondrocytes. Using the functional cell behavior assays that they developed, the team was able to assess the influence of retinoic acid, an endogenously synthesized, powerful, morphogenetic molecule,

on NCC behavior. This study showed that retinoic acid had a profound effect on NCC morphology and differentiation, significantly inhibited proliferation and enhanced cell migration. The data implicate NCCs as a target cell population for retinoic acid and suggest that it plays multiple critical roles in NCC development.

"We hope that our novel neural crest system will be useful to gain mechanistic understanding of NCC development and for cell-based high-throughput drug screening applications" said Dr. Beste Kinikoglu, a postdoctoral fellow in Dr. Liao's laboratory and the study's first author. Dr. Steven R. Goodman, Editor-in-Chief of *Experimental Biology and Medicine* said "Liao and colleagues have provided the first zebrafish embryo derived NCC pure population in vitro model for the study of [neural crest](#) development. I believe that this will be a valuable tool for this purpose".

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