Identifying stem cell phenotypes involved in brain repair using immuno correlative light electron microscopy methods.

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Which stem cells contribute to the reparative process following injury to the adult brain remains a fundamental question. Uncovering the identify of endogenous adult neural stem cell populations has great promise for developing future therapeutic strategies for patients with brain injury or neurodegenerative disease. While glial-scarring following lesion largely prevents neural regeneration in mammals, the adult zebrafish has become a leading model for CNS repair owing to its exquisite ability to regenerate major regions of its brain following injury using stem/progenitor cells. The heterogeniety of forebrain stem/progenitor cells in the zebrafish has been an obstacle however in elucidating the subset of these cells involved in the regenerative process given the lack of cell-specific markers available. To overcome this problem, we have developed novel correlative-light electron microscopy (CLEM) techniques that take advantage of the high degree of fluorescent labelling possible using the Tokuyasu cryo-embedding approach. By combining Tokuyasu sample preparation with scanning electron microscopy (SEM), scanning-transmission electron-microscopy (STEM), and transmission electron microscopy (TEM), we are able to seamlessly observe and quantify the morphological profiles of adult neural stem cells using both immuno-fluorescent antibody markers and ultrastructural details. Our data thus far suggests that proliferative cells of a non-glial phenotype may play a larger role in the regenerative process than initially thought. The strength of this approach will enable us to conclusively identify and compare the population and hierarchy of stem cells in the healthy and injured brain, and will serve as a leading method to reliable distinguish stem cells across organ tissues.