

Fluorescence Labeling Strategies for the Isolation and Characterization of Viable embryonic Stem-Cell Derived DA Neurons

Abstract

The concept of cell replacement therapy has been attractive for disease entities such as Parkinson's disease (PD), where a plethora of debilitating motor symptoms can be localized to the degeneration of a particular class of neurons that innervate an anatomically defined location. The ability to derive an unlimited and non-tumorigenic source of such cells, the dopaminergic (DA) neurons, is thus paramount. However, human embryonic stem cell (hESC)-derived cultures exhibit phenotypic and developmental heterogeneity, believed to be the cause of tumorigenic or ineffective grafts. We were thus interested in fluorescence activated cell sorting (FACS), which can separate cell populations based on fluorescence intensity. Here, we investigate three fluorescent labeling strategies: (1) genetic engineering of promoter-driven fluorescent proteins, (2) fluorescent antibodies against cell surface antigens, and (3) activity-dependent internalization of fluorescent dye. By immunocytochemistry, FACS analysis, purification, and transplantation using hESC-derived cells and mouse primary cultures, we assessed the efficacy of the three strategies in detecting and differentially labeling cell populations and, to varying degrees, their amenability to sorting and in vivo survival. We found that mature, hESC-derived Synapsin eGFP+ cells could be sorted, that the surface antigens NCAM and FORSE-1 could be used for positive or negative selection, and that mature, neuronal cells tended to uptake FM1-43. Thus, heterogeneous populations can be selectively labeled, and oftentimes sorted, through a variety of complementary fluorescence-labeling techniques. Although there are many remaining obstacles, these fluorescent labeling strategies could eventually allow for more targeted selection of cell populations relevant for cell-based therapies.