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Purification and Culture of Dorsal Root Ganglion Neurons

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Abstract

Dorsal root ganglion neurons (DRGs) are sensory neurons that reside in ganglions on the dorsal root of the spinal cord. Here we introduce a method for the acute, prospective purification and culture of DRGs from rodents in a serum-free, defined medium, in the absence of glial cells. This immunopanning-based method facilitates the study of DRG biology and function.

INTRODUCTION

Dorsal root ganglion neurons (DRGs) are sensory neurons that occupy both the peripheral nervous system (PNS) and central nervous system (CNS). They reside in ganglions on the dorsal root of the spinal cord and have afferent axons that transmit sensory stimuli into the CNS. Multiple subsets of DRGs exist that respond to different sensory modalities (Dodd et al. 1984; Ruit et al. 1992; Friedel et al. 1997; Lallemand and Ernfors 2012). Their role in somatosensation has been extensively studied, and they have also been used at length to study neurite outgrowth, regeneration, and degeneration (Lindsay 1988; Wang et al. 2001; Teng and Tang 2006) and PNS and CNS myelination (Salzer and Bunge 1980; Wood and Bunge 1986; Chan et al. 2004).

NONPROSPECTIVE DRG PURIFICATION STRATEGIES

To date, purification strategies for DRG neurons do not prospectively isolate DRGs away from other contaminating cells, but generally rely on several weeks of growth in the presence of antimetabolic substances (Wood 1976, 1980; Wood and Bunge 1986) or other cytotoxic treatments to target dividing cells and obtain pure cultures (Andersen et al. 2003). Typically these protocols require culturing neurons in the presence of serum, to which DRGs in the uninjured nerve are not normally exposed. Methods to separate DRGs away from other cell types based on their large size—by Percoll gradient centrifugation or filtering dissociated cells through a 10- μ m mesh to retain DRGs—result in a low yield (Goldenberg and De Boni 1983; Delree et al. 1989). An ideal purification strategy would (1) be prospective (meaning the cells are directly selected, without requiring extended culture time), (2) avoid prolonged exposure to cytotoxic agents and serum, and (3) have a high yield.

PROSPECTIVE ISOLATION OF DRGS

In Purification of Dorsal Root Ganglion Neurons from Rat by Immunopanning (Zuchero 2014), we describe how to rapidly generate pure cultures of DRG neurons. These cultures are free of Schwann cells and other glia and thus can be used to study the role of glia in the biology of DRG neurons. They do not require extended time in the presence of antimetabolic agents, nor do they require growth in the presence of serum. The protocol is based on previously described dissociation and neuronal immunopanning methods for other cell types (Huettnner and Baughman 1986; Barres et al. 1988, 1992; Meyer-Franke et al. 1995) and uses defined medium with the B27-alternative NS21 (Chen et al. 2008). Immediately following purification, these DRGs require nerve growth factor (NGF) for survival, but to select for different populations of DRGs, NGF can be replaced with other neurotrophins (in isolation or in combination) the day after purification.

To enrich for DRGs, we first deplete blood cells and endothelial cells using their tight interaction with the lectin BSL-1. We then deplete glia using a monoclonal antibody to the tetraspanin protein CD9, which will recognize developing Schwann cells and oligodendrocyte precursor cells (Tole and Patterson 1993; Terada et al. 2002). It is worth noting that CD9 has been described as being expressed in DRGs (Tole and Patterson 1993), but in our hands we do not see significant binding of DRGs to our anti-CD9 immunopanning plates, perhaps because surface levels of CD9 are not as high in dissociated DRGs as in glia.

Peripheral CD9 expression increases after birth in rats (Kaprielian et al. 1995). Therefore, it should be possible to adapt this protocol to purification of neonatal or adult DRGs by modifying the dissociation protocol (Lindsay 1988; Delree et al. 1989; Malin et al. 2007). Additionally, CD9 is expressed ubiquitously by mouse and human glia (Nakamura et al. 1996; Terada et al. 2002; Sim et al. 2011), so this technique may be suitable for purifying DRGs from a wide range of species (Scott 1977).

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