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Method Article

An efficient method for the transduction of primary pediatric glioma neurospheres

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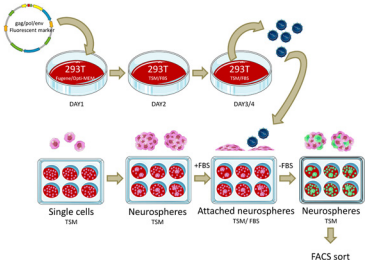
GRAPHICAL ABSTRACT



ABSTRACT

Pediatric high grade glioma (pHGG) and diffuse intrinsic pontine glioma (DIPG) are rare, but rapidly fatal malignancies of the central nervous system (CNS), and the leading cause of cancer-related death in children. Besides the scarcity of available biological material for research, the study of these diseases has been hampered by methodological problems. One of the major obstacles is the difficulty with which these cells can be genetically modified by conventional laboratory methods, such as lentiviral transduction. As a result, only very few successful stable modifications have been reported. As pHGG and DIPG cells are most often cultured as neurospheres, and therefore retain stem cell-like characteristics, we hypothesized that this culture method is also responsible for their resistance to transduction. We therefore developed a protocol in which pHGG and DIPG cells are temporarily forced to form an adherent monolayer by exposure to serum, to create a window-of-opportunity for lentiviral transduction. We here demonstrate that this protocol reliably and reproducibly introduces stable genetic modifications in primary DIPG and pHGG cells.

- Short-term serum exposure enables lentiviral transduction of primary pediatric glioma neurospheres.

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Method details

Background

Pediatric high grade gliomas (pHGG), including diffuse intrinsic pontine glioma (DIPG), are among the most lethal types of cancer occurring in children [1,2]. Major advances in our understanding of the biology of the disease have been made in the past years. These advances include the discovery that these tumors are often driven by unique epigenetic events which are not seen in their adult counterparts, most importantly caused by mutations in the gene encoding Histone 3 [3,4]. Besides being important for the development of a therapy for these diseases, the discovery of the unique epigenetic profile of pHGG and DIPG makes these tumors useful models for the study of epigenetic regulation of gene expression in general. However, research into these tumors and their epigenetic landscape has been hampered by the difficulty with which the tumor cells can be genetically modified. For unknown reasons, primary cultures of pHGG and DIPG cells are impervious to the introduction of genetic modifications by retro- or lentiviral transduction using standard laboratory techniques, which have been around since 1996 [5]. So far, only a few successful stable transductions of pHGG/DIPG cells have been reported [6–9]. As primary pHGG and DIPG cells are generally cultured as neurospheres in serum-free medium, we hypothesized that this culture methodology is, at least partially, responsible for their resistance to retro- and lentiviral transduction, possibly as a result of the stem-like phenotype these cells adopt under serum-free conditions [10–12]. Alternatively, it is possible that fetal bovine serum (FBS) contains components that render cells susceptible to viral infection via unknown mechanisms. In line with this hypothesis, we successfully introduced genes in primary pHGG and DIPG cells by exposing these cells to FBS for a short period of time during the lentiviral transduction protocol. Hereby, we report the first reliable and reproducible lentiviral transduction protocol for primary pHGG and DIPG cells, allowing researchers to study their unique biological background and epigenetic landscape in more detail than before. This protocol has already been used to transduce primary DIPG neurospheres for use in a recent study by our group [13].

Materials

Reagents

DMEM/F12 with Phenol Red without glutamine (Thermo Fisher, Waltham, MA, USA, #12634010)
Neurobasal-A medium (Thermo Fisher, Waltham, MA, USA, #10888022)
Opti-MEM reduced serum medium (Thermo Fisher, Waltham, MA, USA, #31985070)
HEPES 1M (Thermo Fisher, Waltham, MA, USA, #15630056)
MEM Non-essential amino acid solution (Thermo Fisher, Waltham, MA, USA, #11140035)
GlutaMAX Supplement (Thermo Fisher, Waltham, MA, USA, #35050038)
Sodium Pyruvate 100 mM (Thermo Fisher, Waltham, MA, USA, #11360039)
B27 Supplement without vitamin A (Thermo Fisher, Waltham, MA, USA, #12587-010)
Basic Fibroblast Growth Factor (Peprotech, London, UK, #100-18B)
Epidermal Growth Factor (Peprotech, London, UK, #AF-100-15)
Platelet-derived Growth Factor AA (Peprotech, London, UK, #100-13A)