Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Pediatric glioblastoma cells inhibit neurogenesis and promote astrogenesis, phenotypic transformation and migration of human neural progenitor cells within cocultures

Kurt Farrell[a], Gautam Mahajan[a], Parthasarathy Srinivasan[b], Moo-Yeal Lee[a], Chandrasekhar R. Kothapalli[a,*]

[a] Department of Chemical and Biomedical Engineering, Cleveland State University, Cleveland, OH 44115, USA
[b] Department of Mathematics, Cleveland State University, Cleveland, OH 44115, USA

ARTICLE INFO

Keywords:
Pediatric glioblastoma cells
Neural progenitor cells
Differentiation
Migration
GSK-3β

ABSTRACT

Neural progenitor cell (NPC) fate is influenced by a variety of biological cues elicited from the surrounding microenvironment and recent studies suggest their possible role in pediatric glioblastoma multiforme (GBM) development. Since a few GBM cells also display NPC characteristics, it is not clear whether NPCs transform to tumor cell phenotype leading to the onset of GBM formation, or NPCs migrate to developing tumor sites in response to paracrine signaling from GBM cells. Elucidating the paracrine interactions between GBM cells and NPCs *in vivo* is challenging due to the inherent complexity of the CNS. Here, we investigated the interactions between human NPCs (ReNcell) and human pediatric GBM-derived cells (SJ-GBM2) using a Transwell® coculture setup to assess the effects of GBM cells on ReNcells (cytokine and chemokine release, viability, phenotype, differentiation, migration). Standalone ReNcell or GBM cultures served as controls. Qualitative and quantitative results from ELISA®, Live/Dead® and BrdU assays, immunofluorescence labeling, western blot analysis, and scratch test suggests that although ReNcell viability remained unaffected in the presence of pediatric GBM cells, their morphology, phenotype, differentiation patterns, neurite outgrowth, migration patterns (average speed, distance, number of cells) and GSK-3β expression were significantly influenced. The cumulative distance migrated by the cells in each condition was fit to Furth's formula, derived formally from Ornstein-Uhlenbeck process. ReNcell differentiation into neural lineage was compromised and astrogenesis promoted within cocultures. Such coculture platform could be extended to identify the specific molecules contributing to the observed phenomena, to investigate whether NPCs could be transplanted to replace lesions of excised tumor sites, and to elucidate the underlying molecular pathways involved in GBM-NPC interactions within the tumor microenvironment.

1. Introduction

Glioblastoma multiforme (GBM) is a highly malignant form of cancer found within the central nervous system (CNS) which, when diagnosed, has a median patient survival time of less than a year [1]. GBM affects populations of all ages, although the pediatric form is understudied due to the histopathological diversity of the ailment. Pediatric GBM is also more challenging to treat due to intrinsic drug resistance [2]. Although the cellular origins of pediatric GBM are unclear, one theory suggests that they could arise from the transformation of proliferating NPCs during embryogenesis [3–6]. In addition to cancer stem cell markers (e.g., CD133) [7,8], GBM cells also express a variety of lineage markers including pre-neural and astrocyte, and a variety of mature neuronal markers including GABA and GalC [4].

NPCs respond to various spatio-temporal cues to determine their progeny, and their maturation is driven by a combination of intrinsic temporal factors as well as extracellular signals from the developing brain microenvironment. For example, at mid-gestation, young neurons migrate above the germinal ventricular zone (VZ) and eventually to the subventricular zone (SVZ). By postnatal stage, radial glia transform into astrocytes and the VZ disappears, but the SVZ remains into adulthood where NPCs continue to proliferate [9], and respond to a variety of growth factors mimicking responses seen during embryogenesis. When cultured *in vitro*, NPCs have been shown to expand and maintain an undifferentiated phenotype in the presence of epidermal growth factor (EGF) and/or basic fibroblast growth factor (bFGF), and could

* Corresponding author.
E-mail address: c.kothapalli@csuohio.edu (C.R. Kothapalli).

https://doi.org/10.1016/j.yexcr.2017.11.013
Received 9 June 2017; Received in revised form 7 November 2017; Accepted 8 November 2017

differentiate into both neuronal and glial lineages upon removal of these mitogens [10]. NPCs have been shown to migrate and integrate into the surrounding tumor microenvironment, and therefore offer potential to impede GBM progression by delivering relevant suppression molecules to the tumor site [11]. For example, studies have shown that GBM cells have a BMP cell-cycle exit pathway similar to that of NPCs, which could impede tumor progression if manipulated [12].

The therapeutic potential of NPCs in a cancerous microenvironment is currently limited by the potential transformation of NPCs to tumorigenic phenotype, which is thought to be triggered by a variety of molecular mechanisms [6]. Holland et al. reported that NPCs can produce similar histological characteristics as GBM in a rodent model, *via* transduction proteins such as Akt and KRas [13]. Genetic analysis suggested that Olig2 regulates both NPC and GBM lineages and is critical for cell proliferation in both populations [14]. Dai et al. found that mature mouse astrocytes transfected with the platelet-derived growth factor appeared to be more susceptible to GBM transformation *in vivo* [15]. Other studies suggest that the mechanisms driving GBM transformation are based on increased production of the glycoprotein CD133, which is also a NPC marker. Thus, the origin of GBM is likely either a derivation from CD133 expressing cells which are normally not present in the adult brain, or from CD133-positive ependymal cells in the adult brain [16]. In general, NPCs are at risk for malignant transformation based on activated pro-mitotic genes, telomerase activity, and anti-apoptotic genes, which can be triggered by a combination of less than seven mutations [17,18].

Currently, limited information exists pertaining to the influence of GBMs on NPCs and *vice versa* [6]. NPCs injected *in vivo* to mouse tumor sites have been shown to aggressively migrate into tumor sites and overexpress several cytokines and chemokines, which has the potential to trigger antitumor NPC-mediated immunity [11,19]. GBMs also appear to transmit signals to the surrounding environment that cause the co-expression of several unique neuronal markers in both themselves (autocrine) as well as surrounding stem cells (paracrine) [3,20]. Investigating the paracrine signaling between GBM cells and NPCs in a controlled microenvironment (e.g., cocultures) might help in direct quantification of the influence of each cell type on the other, in the identification of molecules which arrest GBM growth, metastasis and tumor formation suppression, and in evaluating the potential of NPC transplantation in restoring lost cell populations at the lesions site after surgical removal of CNS tumor [6]. Using a biomimetic coculture system, limitations associated with *in vivo* studies could be overcome and direct cell-cell contact could be eliminated, while effectively exposing GBM cells and NPCs to the signaling molecules released by the other.

This study is based on the hypothesis that the biochemical signals released by pediatric GBM-derived cells influence the normal NPC phenotype by altering their morphology, survival, migration, differentiation patterns, and release of various cytokines and chemokines. Given the similarities in the cellular pathways which regulate both NPC and GBM cell differentiation and proliferation, such coculture studies would also provide key insights into the effect NPCs have on GBM cells. The outcomes from such studies might help elucidate the conditions leading to the onset and progression of pediatric GBMs, identify the target molecules and pathways which might help impede GBM progression, and unlock the interactions of NPCs in tumor microenvironment [21].

2. Materials and methods

2.1. NPC and GBM cell expansion

Human NPCs (ReNcell VM Human Neural Progenitor Cell Line; SCC008) and all media components were purchased from EMD Millipore. ReNcells were maintained in an undifferentiated state by culturing on laminin-coated T-75 flasks in the presence of *non-differentiating* maintenance media (Millipore Cat. No. SCM005) containing 20 ng/mL of freshly-thawed bFGF and EGF. Media was changed every 24 h, and after 7 days, ReNcells were gently detached using Accutase (Life Technologies, Carlsbad, CA) and frozen in ReNcell freezing medium (Millipore Cat. No. SCM007). *Differentiation media* was ReNcell maintenance media without any bFGF or EGF. All cells used in this study were before passage 10.

Human pediatric glioblastoma multiforme cells (GBM) were obtained from the Children's Oncology Group (COG) Cell Culture and Xenograft Repository at Texas Tech University Health Sciences Center School of Medicine. Cells were derived from a five-year old female and labeled as the "SJ-GBM2″ cell line [22]. The SJ-GBM2 cell line was expanded in uncoated T-75 flasks. All media products were purchased from Life Technologies unless otherwise noted. GBM media was prepared using Iscove's modified Dulbecco's medium containing 20% fetal bovine serum, 4 mM L-glutamine, and ITS supplement (5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL selenous acid). Media was replaced every 3 days, cells detached using Pucks EDTA (140 nM NaCl, 5 mM KCl, 5.5 mM glucose, 4 mM $NaHCO_3$, 13 μM phenol red, 0.8 mM EDTA, and 9 mM HEPES) after 7 days, and frozen in a solution containing 50% fetal bovine serum and 7.5% DMSO in Iscove's modified Dulbecco's medium. Cells were used prior to passage 20. Henceforth, SJ-GBM2 cells will be referred to as *GBM*, and undifferentiated ReNcells as *ReNcell-bFGF*. All cultures in this study were performed on 2D tissue culture grade plastic, and ReNcells were always plated on laminin-coated dishes.

2.2. Transwell® coculture conditions

After initial expansion, test cultures were run for 5 or 10 days under these conditions, in parallel: ReNcells cultured alone in non-differentiating media (*ReNcell bFGF*), ReNcells cultured alone in differentiation media (*ReNcells alone*), GBMs cultured alone in GBM media (*GBM alone*), ReNcells cocultured with GBMs seeded in transwell insert (*ReNcell cocultures*), and GBMs cocultured with ReNcells seeded in transwell insert (*GBM cocultures*). For these cocultures, ReNcell differentiation media and GBM media were mixed in 1:1 ratio and supplemented. To ensure no direct cell-cell interactions, 1-μm PET membrane Transwell® cell culture inserts (Flacon/Corning, Durham, NC) were used. NPCs were first seeded in laminin-coated 24-well plates at a density of 4×10^4 cells/well and cultured for 3 h with 200 μL of differentiation media or non-differentiating media. For cocultures, after 3 h, Transwell® inserts were placed and 4×10^4 GBMs were seeded within the uncoated inserts. Respective media for both cell types was replaced daily. In all the cases, cells were cultured on 2D substrates and not within 3D scaffolds.

2.3. Cytokine/chemokine analysis

Cytokine and chemokine analyses were performed using Discovery Assays® (Eve Technologies, Alberta, Canada). Cell culture supernatants (150 μL) were collected from each well after 24 h culture, spun down at 3000 *g* for 5 min, and stored at −20 °C. The supernatants were then processed using multiplexing LASER bead technology and processed on a dual-laser flow-cytometry system (Bio-Plex 200). The technology works by utilizing different combinations of red and infrared fluorophore beads conjugated to specific antibodies targeted to the cytokine or chemokine of choice and were read using a flow-cytometry based system. The quantity of the specific analyte generated was based off of a series of standards set forth by the company. The following cases were tested: ReNcells alone, GBM alone, ReNcells bFGF, ReNcell cocultures and GBM cocultures. For ReNcell cocultures, ReNcells were plated in the bottom dish of the 24-well plates and GBM cells were seeded in the Transwell inserts. Similarly, for GBM cocultures, GBM cells were plated in the bottom dish of the 24-well plates and ReNcells were seeded in the Transwell inserts. The presence of following 42 cytokines and