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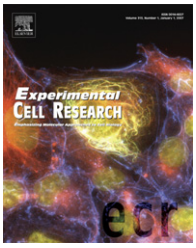

Research Article

HDAC inhibition amplifies gap junction communication in neural progenitors: Potential for cell-mediated enzyme prodrug therapy

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ABSTRACT

Enzyme prodrug therapy using neural progenitor cells (NPCs) as delivery vehicles has been applied in animal models of gliomas and relies on gap junction communication (GJC) between delivery and target cells. This study investigated the effects of histone deacetylase (HDAC) inhibitors on GJC for the purpose of facilitating transfer of therapeutic molecules from recombinant NPCs. We studied a novel immortalized midbrain cell line, NGC-407 of embryonic human origin having neural precursor characteristics, as a potential delivery vehicle. The expression of gap junction protein connexin 43 (C×43) was analyzed by western blot and immunocytochemistry. While C×43 levels were decreased in untreated differentiating NGC-407 cells, the HDAC inhibitor 4-phenylbutyrate (4-PB) increased C×43 expression along with increased membranous deposition in both proliferating and differentiating cells. Simultaneously, Ser 279/282-phosphorylated form of C×43 was declined in both culture conditions by 4-PB. The 4-PB effect in NGC-407 cells was verified by using HNSC.100 human neural progenitors and Trichostatin A. Improved functional GJC is of imperative importance for therapeutic strategies involving intercellular transport of low molecular-weight compounds. We show here an enhancement by 4-PB, of the functional GJC among NGC-407 cells, as well as between NGC-407 and human glioma cells, as indicated by increased fluorescent dye transfer.

Introduction

Malignant glioma is the most common type of brain tumor in adults and very progressive [1]. These tumors are refractory to surgery, radio- and chemotherapy with a median survival of only 9–12 months after diagnosis [2]. The resistance is related to the exceptional migratory nature of tumor cells, and their evolving heterogeneity [3]. Different gene therapeutic strategies have been developed for their treatment and some were tested in clinical trials, albeit with limited success [4]. Most

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trials were performed with retroviral vectors armed with the herpes simplex virus thymidine kinase (HSV-tk) suicide gene. However, viral vectors penetrate glioma tissue inefficiently [5]. Furthermore, packaging cell lines transplanted directly into the brain did not migrate. Thus, one major cause for the lack of success of gene therapy for malignant glioma has been an insufficient gene transfer technology [6]. A further complicating factor is the finding that several of these tumors, including the most malignant form, glioblastoma multiforme (GBM), manifest as multiple discrete masses within the brain [7].

To address these problems, a new therapeutic strategy based on neural progenitor cells (NPCs) has been developed. NPCs show extensive tropism towards GBM in *in vitro* as well as *in vivo* models. When implanted contralaterally into the brain or even intravenously, they infiltrate the tumor bed and track down migrated GBM cells [8–10]. Thus, genetically engineered NPCs can be used to deliver therapeutic molecules to the lesion site [11]. Several investigators have already demonstrated that NPCs can efficiently deliver tumor-toxic gene products, for example IL-4, IL-12, TRAIL, cytosine deaminase and HSV-tk activated prodrug to the disseminated GBM [10,12–16]. Successful exploitation of these favorable properties of NPCs depends not only on their survival, engraftment and stable gene expression, but also on their intercellular communication. Cell-mediated suicide gene therapy is based on the delivery of cytotoxic products to cancer cells, rather than recombinant gene transfer. The cell-killing effect is dependent on the so called 'bystander effect', whereby activated prodrug is transferred from delivery cells, expressing the prodrug-activating enzyme, to cancer cells exploiting gap junction communications (GJC) [16–19]. Therefore, the extent of GJC is the bottle neck for any NPC-mediated enzyme prodrug therapy which relies on bystander effect. Gap junctions are proteinaceous intercellular channels that permit small water soluble molecules to be exchanged between cells [20–22]. Gap junction protein connexin 43 (C×43) is involved in facilitating the bystander effect, as was demonstrated by using glioma, melanoma and colorectal carcinoma cell lines with differentiated expression of C×43 [23].

Histone deacetylase (HDAC) inhibitors are promising anti-cancer drugs. They induce histone hyperacetylation, altered chromatin structure and selective modulation of gene expression through epigenetic alterations, making genes available for transcription. The resulting effects include differentiation, cell cycle arrest and apoptosis in different tumor categories [24]. HDAC activity also plays a critical role during specific transitional periods of development, when cells leave the cell cycle and start to differentiate [25]. HDAC activity is necessary for embryonic stem cell differentiation [26] and its inhibitors promote self-renewal and expansion of hematopoietic stem cells [27].

In previous studies, we reported that the HDAC inhibitor 4-phenylbutyrate (4-PB) substantially increases C×43 expression and thereby the GJC in human primary GBM cultures [28] and modulates the bystander effect in a glioma cell line transfected with HSV-tk [29]. In the current paper, we (1) extend the observations made for C×43 expression to human NPCs; (2) report on the effect of HDAC inhibitors on C×43 phosphorylation; and (3) semi-quantify the relative functional GJC among NPCs, and between NPCs and human GBM cells for the purpose of exploring the opportunities for cell-mediated enzyme prodrug therapy.

Materials and methods

Cell lines and chemicals

Two NPC lines, NGC-407 and HNSC.100 and two established GBM cell lines, U87MG and U343MGa [30,31] were used in this study. The NGC-407 cell line was developed by NsGene A/S, Denmark, in collaboration with the University of Lund, Lund, Sweden. This cell line was established by immortalization of cells derived from the ventral mesencephalon of a 7-week-old human embryo, by using a retroviral vector containing the v-myc oncogene. The embryonic tissue was derived from an elective first trimester abortion, and was recovered with the permission of the Ethics committee of the University of Lund (ethical permission no. LU 549-02), and with the consent of the patient. The HNSC.100 cell line – used here as a reference cell line – obtained from human fetal diencephalon and telencephalon (gestational age 10–10.5 weeks), has already been characterized [32].

The HDAC inhibitor 4-PB (Triple Crown America Inc., Perkasie, PA, USA) was dissolved in phosphate-buffered saline (PBS), and Trichostatin A (TSA; Wako Chemicals GmbH, Germany) was dissolved in dimethyl sulfoxide for use in tissue culture experiments.

Cell culture protocols

NGC-407 cells were plated on poly-L-lysine coated (Sigma, P4832) Petri dishes, and cultured as adherent cells at 37 °C and 5% CO_2. Culture medium DMEM-F12 (1:1) with Glutamax I (Gibco, 31331-028) was supplemented with 40 ng/ml basic fibroblast growth factor (bFGF) (R&D Systems, 233-FB), 20 ng/ml epidermal growth factor (EGF) (Invitrogen, 13247-051), N2 supplement 1× (Invitrogen, 17502-048), 0.5% human serum albumin (Sigma, A1653), 6 g/L glucose (Sigma, G8769), 5 μM HEPES buffer solution (Invitrogen, 15630-56), 1× non-essential amino acids (Invitrogen, 11140-035), penicillin (50 units/ml) and streptomycin (50 μg/ml) (Gibco). HNSC.100 cells were cultured under identical conditions except that bFGF was used at 20 ng/ml, and human serum albumin was replaced by 0.5% Albumax-I (Gibco, 11020-021). Cells were differentiated by withdrawing both mitogens (bFGF and EGF) from the culture medium. Briefly, 1.5×10^6 NPCs were plated on poly-L-lysine pre-coated 100 mm Petri dishes. The following day, cells were fed differentiation medium, which was replaced every second day, leaving 30% conditioned medium. Differentiation of cells under mitogen depletion was continued for up to 120 h. U87MG and U343MGa cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), penicillin 100 units/ml and streptomycin 100 μg/ml (Gibco). U87MG or U343MGa cells were co-cultured with NGC-407 cells in either proliferation or differentiation medium for fluorescent dye transfer experiments.