EXPERIMENTAL CELL RESEARCH 317 (2011) 2086–2098

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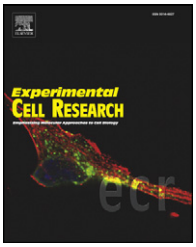

Research Article

Inhibition of glycogen synthase kinase-3 enhances the differentiation and reduces the proliferation of adult human olfactory epithelium neural precursors

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ARTICLE INFORMATION

Article Chronology:
Received 20 November 2010
Revised version received 7 June 2011
Accepted 10 June 2011
Available online 25 June 2011

Keywords:
Stem cell
GSK-3
Olfactory mucosa
TAT(47–57)
Differentiation

ABSTRACT

The olfactory epithelium (OE) contains neural precursor cells which can be easily harvested from a minimally invasive nasal biopsy, making them a valuable cell source to study human neural cell lineages in health and disease. Glycogen synthase kinase-3 (GSK-3) has been implicated in the etiology and treatment of neuropsychiatric disorders and also in the regulation of murine neural precursor cell fate in vitro and in vivo. In this study, we examined the impact of decreased GSK-3 activity on the fate of adult human OE neural precursors in vitro. GSK-3 inhibition was achieved using ATP-competitive (6-bromoindirubin-3′-oxime and CHIR99021) or substrate-competitive (TAT-eIF2B) inhibitors to eliminate potential confounding effects on cell fate due to off-target kinase inhibition. GSK-3 inhibitors decreased the number of neural precursor cells in OE cell cultures through a reduction in proliferation. Decreased proliferation was not associated with a reduction in cell survival but was accompanied by a reduction in nestin expression and a substantial increase in the expression of the neuronal differentiation markers MAP1B and neurofilament (NF-M) after 10 days in culture. Taken together, these results suggest that GSK-3 inhibition promotes the early stages of neuronal differentiation in cultures of adult human neural precursors and provide insights into the mechanisms by which alterations in GSK-3 signaling affect adult human neurogenesis, a cellular process strongly suspected to play a role in the etiology of neuropsychiatric disorders.

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doi:10.1016/j.yexcr.2011.06.004

Introduction

Neural stem cells were first identified in the adult mammalian brain by their ability to self-renew and, in the presence of appropriate cues, differentiate into the three lineages of the central nervous system: neurons, astrocytes and oligodendrocytes [1,2]. However, due to the limited access to live adult human brain tissues, alternative sources of adult human neural precursors are being sought, in particular for the development of patient-based, disease-relevant cellular models. One promising source is the use of neural precursors present in the adult human olfactory epithelium (OE). The neural precursor cells of the OE are responsible for the constant regeneration of the olfactory sensory neurons in the nose; they proliferate and differentiate throughout life and [3–6], unlike most other tissues containing human adult neural progenitors, OE can be safely biopsied. Interestingly, OE-derived neural precursors obtained from Rett syndrome [7], schizophrenia and Parkinson's disease [8] patients were found to have altered cellular functions which suggest that they can be useful for the development of cellular models of neuropsychiatric disorders. Furthermore, a recent study by Matigian et al. [8] reports changes in genes and protein expression in patient-derived OE cells that were not found in fibroblasts, arguing that OE cells may be a more representative cell model; notably, these authors report that glycogen synthase kinase-3ß (GSK3β) mRNA expression levels are significantly lower in cells derived from neural precursors obtained from the OE of schizophrenia patients, compared with healthy subjects [8]. A body of research already suggests that GSK-3 plays a role in the etiology and treatment of neurospychiatric disorders [9–15]. In particular, lower GSK-3β protein and activity levels were found in post-mortem brains of schizophrenia patients [16–18]. However, the mechanisms by which altered GSK-3 levels affect adult human neurogenesis and cell resilience and its possible role in the etiology of neuropsychiatric disorders remain elusive, due in part to the lack of appropriate cellular models.

The identification of culture conditions to expand human adult neural precursor cells in culture and/or generate neural cells of different lineages and at different stages of maturation is crucial for the development of tissue and disease-relevant cellular models of neuropsychiatric disorders. For instance, glycogen synthase kinase-3 (GSK-3) inhibitors have been used successfully in cultures of adult rodent neural precursor cells to promote neuronal differentiation [19]. However, our ability to modulate the activity of specific signaling enzymes in live cells is limited by the availability of inhibitors which tend to exhibit off-target effects that may impact on other cellular activities such as cell proliferation, differentiation and/or survival [20]. Nonetheless, the use of small molecule inhibitors remains a powerful and convenient means to modulate signaling pathways and alter cell fate decisions in culture, in various applications [21] and, when possible, is generally preferred over recombinant growth factors/ligands or genetic approaches for reasons of efficacy, simplicity and costs. In the present study, we used three different inhibitors to modulate the activity of GSK-3 in cultures of human OE cells. We tested a substrate-competitive inhibitor based on a TAT-conjugated pseudosubstrate derived from the eIF2B protein (TAT-eIF2B) [22] and two commonly used ATP-competitive GSK-3 inhibitors: (2′*Z*,3′*E*)-6-bromoindirubin-3′-oxime (BIO) and CHIR99021, the most specific commercially available GSK-3 inhibitor [23]. These ATP-competitive inhibitors have targets other than GSK-3. For example, BIO inhibits extracellular-regulated kinase (ERK)1/2 in murine embryonic stem cells (ESCs) at concentrations higher than 200 nM [24]. Both BIO and CHIR99021 display activity against cyclin D kinase 1 (CDK1) presumably because GSK-3 and CDK1 share a high level of homology between their ATP-binding sites [25,26]. Substrate-binding sites are generally less promiscuous which usually confers pseudosubstrate inhibitors a higher specificity than ATP-competitive inhibitors [27]. The substrate-competitive inhibitor TAT-eIF2B [22] is based on a peptide derived from the eIF2B protein, a GSK-3 substrate [28]. The fact that GSK-3 has an unusual preference for pre-phosphorylated substrates (SXXXS(p) recognition motif) significantly facilitates the development of highly specific substrate-competitive GSK-3 inhibitors [27,28]. Therefore, in this study we compare three different inhibitors to reveal the impact of GSK-3 inhibition on the survival, proliferation and differentiation of adult human neural precursor cells derived from OE.

Material and methods

GSK-3 inhibitors

CHIR99021 [29] was purchased from Stemgent (San Diego, CA) and BIO [30] from Sigma (Oakville, Ontario). TAT-eIF2B was custom synthesized by AnaSpec (Fremont, CA) with a fluorescein label (5-FAM), as described in our previous study [22].

Cell lines and cell propagation

All cells were cultured at 37 °C in 5% CO_2. Wild-type (WT) and GSK-3α/β double knock-out (dKO) mouse embryonic stem cells (ESCs), i.e. in which both GSK-3α and GSK-3β have been knocked out [31], were grown in gelatine-coated culture flasks in Dulbecco's Modified Eagle Medium (DMEM) high glucose, supplemented with 0.1 mM non-essential amino acids (GIBCO), 1 mM sodium pyruvate (GIBCO), 100 μM β-mercaptoethanol (Sigma, Oakville, Ontario), 15% fetal bovine serum (FBS), penicillin and streptomycin (final concentration 50 μg/ml each) and 1000 U/ml Leukemia Inhibitory Factor as described by Fok and Zandstra [32].

The three human OE cell lines used in this study were derived from nasal biopsies of three healthy subjects, under the approval of the Human Subjects Review Board of the Centre for Addiction and Mental Health and Mount Sinai Hospital, University of Toronto. The Declaration of Helsinki protocols were followed and the subjects gave their written, informed consent. One of the cell lines was derived from an individual who completed a full research diagnostic assessment to confirm the absence of current medical illness, including diabetes or hypertension, past or present history of psychiatric illness and family history of psychiatric disorders in first-degree relatives. Preparation of the biopsied tissues and initial cultures were carried out as previously described by McCurdy et al. 2006 [33], 2007 [34] and Matigian et al. 2010 [8]. OE cells were grown in T-flasks (Sarstedt) in DMEM-F12 supplemented with 10% FBS. For all experiments, OE cells were used between passage 2 and 7. The general profiles of proliferation, viability and differentiation measures from each subject cell line were similar. Cell lines 1 and 2 were used for the neurosphere assay and cell viability measurements (Figs. 3 and 4) while cell lines 2 and 3 were used to evaluate proliferation and differentiation (Figs. 5 to 8).