Exploiting Müller Glia Progenitor Cell Signaling Pathways for Retinal Regeneration in Chick Retinas Using Small Molecule Drug Cocktails

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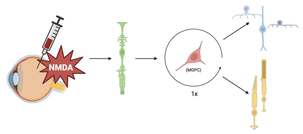
Introduction

Unlike animals such as chicks and zebrafish, humans are unable to partially or fully regenerate the retina. Over the past few decades, many potential avenues for retinal regeneration have been discovered, with Müller glia cells as the common denominator. Müller glia (MG) are support cells of the retina, performing a variety of functions. It wasn't until 2001, however, that MG were observed to possess regenerative potential in chicks [1].

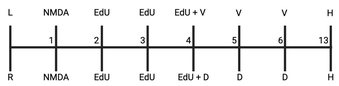


A potential also seen in zebrafish, it was discovered that in response to acute excitotoxic damage, MG have the ability to proliferate and de-differentiate into retinal progenitors called Müller glia progenitor cells (MGPCs), undergo a single round of cell division, and either stochastically differentiate into glia and neurons or remain undifferentiated [1,10]. Employing Müller glia as neurogenesis agents is an exciting prospect, and could lead to positive therapeutic outcomes for humans. Yet, neurogenesis of Müller glia is a rare event (<5%) [2]. Thus, this project investigates methods for amplifying neurogenesis levels of MGPCs in chick retinas using drug cocktails of small molecules known to induce neurogenic fate.

Methods

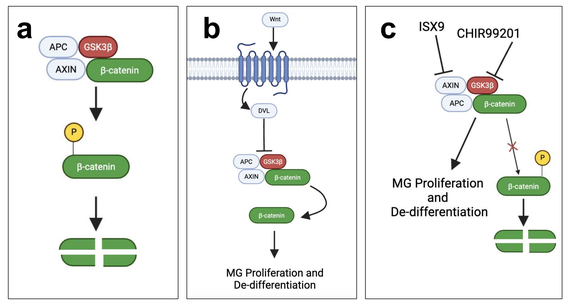
P3 chicks were intraocularly injected with N-methyl-D-aspartate (NMDA), ethynyl deoxyuridine (EdU), and multiple iterations of drug cocktails (D) or vehicles (V), all using the injection paradigm below.
Neurogenic agents:

- Adenylate cyclase activator: Forskolin (F)
- BET family bromodomain inhibitor: I-BET151 (B)
- Wnt Agonist: CHIR99201 (CHIR [C])
- Protein kinase A activator : Bucladesine
- Rho-associated, coiled-coil containing protein kinase inhibitor: Y-27632
- Wnt Agonist: ISX9 (I)



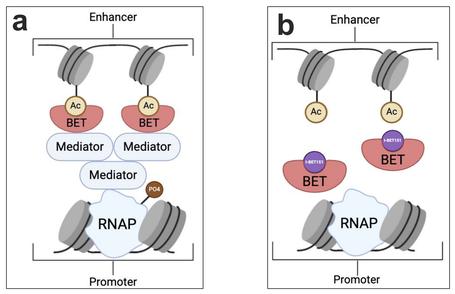
Harvested, fixed and mounted retinal sections were visualized with a HuD/EdU (both 1:1000) immunostain and MGPCs that underwent neurogenesis were quantified. Statistical analysis was performed using a two-tailed t-test.

Wnt/β-catenin Pathway in MGPC Formation



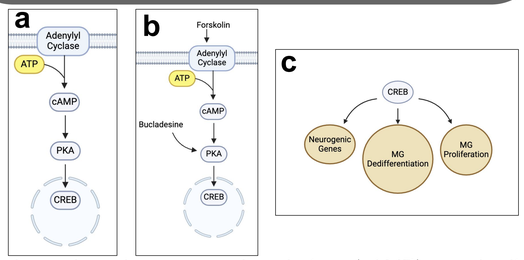
Activation of the canonical Wnt pathway in chick retinas is required for stimulation of Müller glia proliferation and de-differentiation [3]. ISX9 is also a neural differentiation inducer that increases expression of NeuroD, and might act by affecting chromatin structure. (a) Normal function of the β-catenin destruction complex. (b) The canonical Wnt pathway relevant to MGPCs. (c) Inhibition of the β-catenin destruction complex promotes MGCP formation.

Inhibition of BET Proteins in Neurogenesis



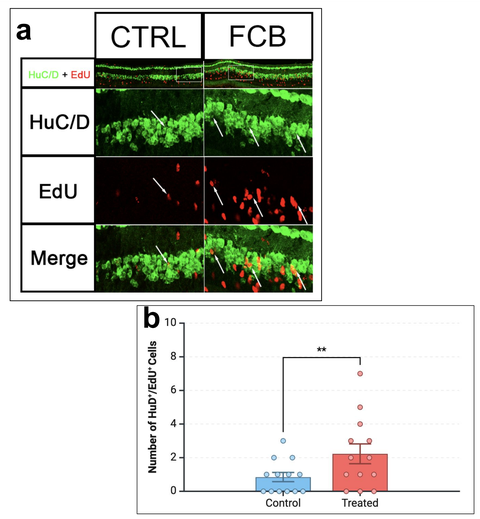
Bromo and extraterminal domain (BET) proteins are epigenetic readers that bind to acetylated lysine residues and function in a variety of epigenetic and transcriptional mechanisms [9]. Inhibition of BET proteins has been shown to promote neurogenesis, while inhibiting fibroblast-specific programs, cell-cycle progression, and gliogenesis [5,9].
(a) Typical function of BET proteins in the formation of the transcription complex. (b) Competitive inhibition of BET proteins by I-BET151.

cAMP and CREB Signalling in Neurogenesis



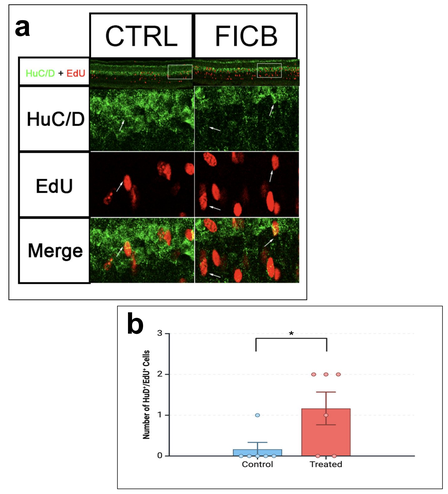
Cyclic adenosine monophosphate (cAMP) and its downstream effector cAMP-response element binding protein (CREB) have been reported to promote neural fate in cell culture [5,8]. Thus, cAMP, CREB, and other steps along this signalling pathway have the potential to be targets for a variety of small molecules, such as Forskolin or Bucladesine, which serve as activators for adenylyl cyclase or protein kinase A (PKA) respectively [5].
(a) cAMP signaling pathway. (b) cAMP/PKA activators in the cAMP signalling pathway. (c) Relevant functions of CREB signalling [8].

FCB Cocktail



(a) Retinal sections were labeled with HuD (green; pan-neuronal marker) antibodies and EdU (red; cell lineage tracer). (b) Bar graphs depict mean (±SEM) number of actively dividing neurons. Significance: **p < 0.01

FICB Cocktail



(a) Retinal sections were labeled with antibodies to HuC/D (green; pan-neuronal marker) and EdU (red; cell lineage tracer). (b) Bar graphs illustrate mean (±SEM) number of neurons differentiated from MGPCs. Significance: *p < 0.05

Conclusions

- BET proteins play a role in the differentiation of MGPCs into new neurons.
- Wnt pathway molecules are important in the dedifferentiation of Müller glia.
- CREB signalling pathways play an important role in neurogenesis from MGPCs
- MGPCs have a chemically-exploitable potential for increased neurogenesis rates *in vivo*

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