HIV induces astrocyte senescence and is reversed by β-catenin induction

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ABSTRACT

Background: Neuroinflammation and neurodegeneration are hallmarks of HIV-Associated Neurocognitive Disorders (HAND). Astrocyte senescence induces neuropathologies due to increased oxidative stress and altered cytokine secretion leading to neuroinflammation. These processes reduce the neuroprotective capacity of astrocytes leading to long-term inflammation and neurodegeneration. We evaluated the impact of HIV on astrocyte senescence and explored the role of Wnt/β-catenin, a pro-survival pathway, in astrocyte senescence in the context of HIV.

Methods: Human Fetal Astrocytes (HFAs) were infected with VSVG pseudotyped-HIV_{BAL} or transfected with a full length HIV_{BAL} plasmid ($pHIV_{BAL}$) or corresponding controls. HFAs were transfected with β-catenin siRNA, scrambled siRNA, constitutively active β-catenin plasmid (pABC), or background. Dickkopf-related protein 1 (DKK1) was used as an antagonist of the Wnt/β-catenin pathway. Six days post treatment, HFAs were fixed and stained for senescence-associated β-galactosidase (SA-βGAL); percent positive SA-βGAL cells were recorded.

Results: VSVG-HIV_{BAL} and $pHIV_{BAL}$ significantly induced SA-β-gal staining in HFAs by 5- and 2-folds, respectively. β-catenin knockdown or antagonizing Wnt/β-catenin signaling through DKK-1 treatment induced SA-β-gal expression in HFA by ≥3.5 folds. Conversely, pABC inhibited HFA senescence by ≈60%.

Conclusions: HIV induces astrocyte senescence. Given that we previously demonstrated that HIV diminishes β -catenin signaling in astrocytes and we show here that reduction in β-catenin expression promotes while induction of β-catenin protects against astrocyte senescence suggests that HIV induces astrocyte senescence through inhibition of β-catenin signaling. If so, activation of Wnt/β-catenin in astrocytes may emerge as a prominent pathway to reverse astrocyte senescence associated with aging and/or neurodegenerative diseases beyond HAND.

INTRODUCTION

Astrocytes are key regulators of CNS homeostasis and function. They are neuroprotective and alteration in their function including changes in secretory factors induces long-term inflammation and neuronal injury, which are evident in many neurological disorders including HIV-Associated Neurocognitive Disorders (HAND).

Astrocyte senescence is an irreversible process whereby cells undergo functional decline and naturally occurs with aging. Changes in secretion include increased release of inflammatory mediators including IL-6 and IL-1β and proteinases matrix metalloproteinase-1 (MMP-1)[1]. Further, astrocyte senescence decreases their neuroprotective capacity leading to neuronal aging[2]. Factors known to increase cell senescence include oxidative stress, telomere attrition and DNA damage[3]. Senescence-associated-β-galactosidase (SA-βGAL) is the most widely used senescent marker.

Wnt/β-catenin signaling (Figure 1) induces pro-survival pathways through regulation of gene transcription regulating cell structure, synaptic activity, cell proliferation and survival along with the extensive studies of β-catenin activity in neurodevelopment[4]. Dickkhopf 1 (DKK1) is a natural antagonist of Wnt/β-catenin signaling. The ability of HIV and Wnt/β-catenin signaling in astrocytes to regulate astrocyte senescence remains undefined.

The purpose of these studies was to determine the impact of HIV on astrocyte senescence and explore the role of Wnt/β-catenin signaling in astrocyte senescence in the context of HIV. To this end, we infected primary human fetal astrocytes (Lonza, HFAs) with HIV_{BAL}, VSVg pseudotyped HIV_{BAL} or full length plasmid HIV_{BAL} or manipulated Wnt/β-catenin signaling in HFAs by loss and gain of function studies and measured astrocyte cell senescence by SA-βGAL staining.

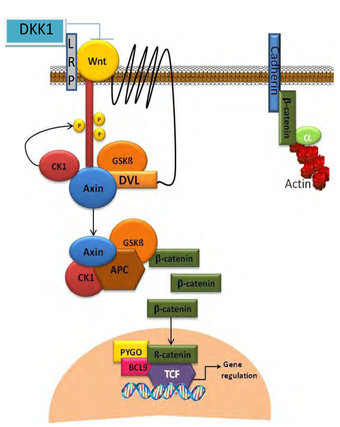


Figure 1: **Wnt/β-catenin signaling pathway.** Wnts are small secreted glycoproteins that bind to Frizzled receptors and the co-receptor low-density lipoprotein receptor related protein (LRP) 5/6 leading to destabilization of a β-catenin destruction complex (Axin, APC, CK1α, GSK3β). Hypophosphorylated β-catenin translocates to the nucleus, where it interacts with TCF/LEF to displace its co-repressors and recruit either positive or negative transcription co-factors to regulate Wnt target genes. In the absence of Wnts cytosolic β-catenin is phosphorylated by the destruction complex and undergoes βTrcp-mediated ubiquitination and proteasomal degradation. Further, Wnt signaling is inhibited when DKK1 binds to LRP.

RESULTS

HIV induces human astrocyte senescence

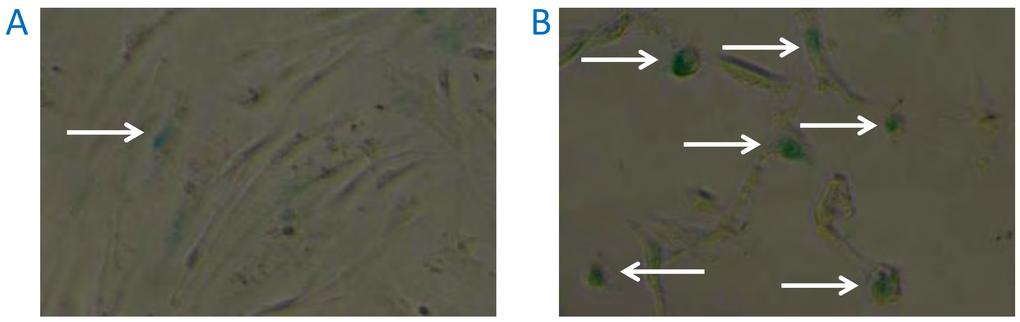


Figure 2: Representative SA-βGAL staining. HFAs were transfected with control (A) or $VSVg\text{-}HIV_{NL4/3}$. Cells were washed the day following treatment and fixed 6 days after treatment with 4% paraformaldehyde. Cells were stained for SA-βGAL and the percent of total cells positive were counted. Arrows indicate a SA-βGAL positive cell.

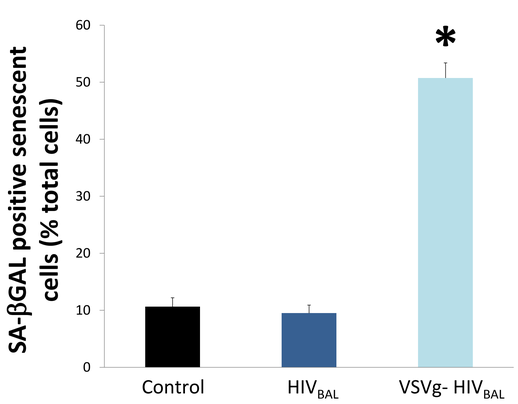


Figure 3: HFAs were infected with HIV_{BAL} (10 ng/ml) or VSVg-HIV_{BAL} overnight. Cells were washed and fixed with 4% paraformaldehyde 6 days post treatment. Cells were stained for SA-βGAL and % of total cells positive for SA-βGAL is shown. * indicates $p < 0.05$ using T-test from control treated well.

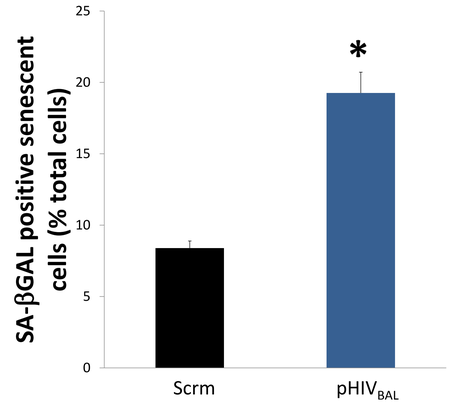


Figure 4: HFAs were transfected with scrambled (Scrm) or $pHIV_{BAL}$ using Lipofectamine 2000. The following day, cells were washed. Six days post treatment cells were fixed with 4% paraformaldehyde. Cells were stained for SA-βGAL and % of total cells positive for SA-βGAL is shown. * indicates $p < 0.05$ using T-test.

β-catenin positively regulates human astrocyte senescence

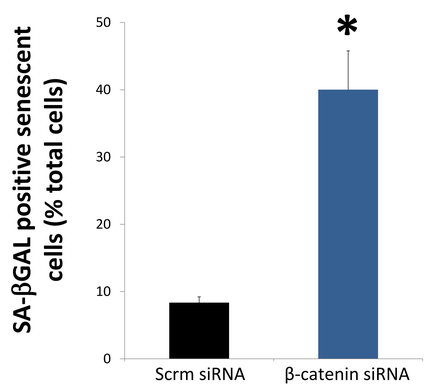


Figure 5: HFAs were transfected with scrambled (Scrm) or β-catenin siRNA using Lipofectamine siRNAmax. The following day, cells were washed. Six days post treatment, cells were fixed with 4% paraformaldehyde. Cells were stained for SA-βGAL and % of total cells positive for SA-βGAL is shown. * indicates $p < 0.05$ using T-test.

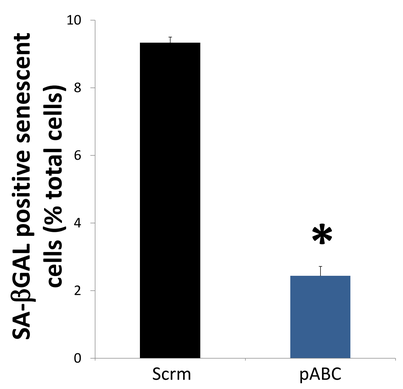


Figure 6: HFAs were transfected with scrambled (Scrm) or active β-catenin (pABC) plasmids using Lipofectamine 2000. The following day, cells were washed. Six days post treatment, cells were fixed with 4% paraformaldehyde. Cells were stained for SA-βGAL and % of total cells positive for SA-βGAL is shown. * indicates $p < 0.05$ using T-test.

DKK1 induces human astrocyte senescence

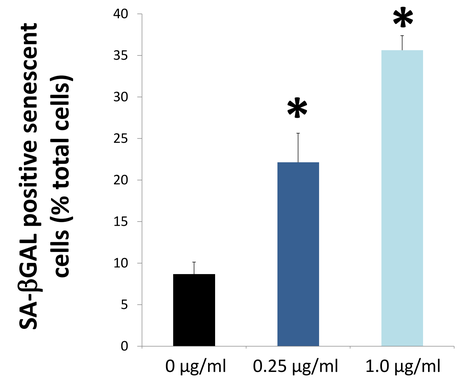


Figure 7: DKK-1 is an antagonist of Wnt/β-catenin signaling. HFAs were treated with Dickkhopf-related protein 1 (DKK1) at 0, 0.25 or 1 µg/ml. Cells were washed and fixed with 4% paraformaldehyde at day 6 post treatment. Cells were stained for SA-βGAL and % of total cells positive for SA-βGAL is shown. * indicates $p < 0.05$ using T-test from control treated well.

SUMMARY

Senescence is an irreversible ageing process resulting in a functionally deficient cell. Astrocyte senescence, while not completely understood, results in altered secretion of factors including inflammatory cytokines to directly effect the microenvironment. This results in decreased neuroprotection leading to neuroinflammation and neuropathologies.

We show here that HIV induces astrocyte senescence as does inhibition of β-catenin. Given our previous data[5-6] showing HIV diminishes β-catenin signaling in astrocytes suggests that HIV induces astrocyte senescence through inhibition of Wnt/β-catenin signaling.

Understanding the molecular mechanism by which HIV and Wnt/β-catenin regulates astrocyte senescence could emerge as a pathway to prevent senescence associated with ageing and/or neurodegenerative diseases such as HAND.

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