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Role of β -Catenin/TCF-4 Signaling in HIV Replication and Pathogenesis: Insights to Informing Novel Anti-HIV Molecular Therapeutics

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Abstract

A greater understanding of the interaction between HIV and host signaling pathways that restrict virus production may lead to new methods to purge virus from latent reservoirs and enhance survival/function of cells targeted by HIV. This review highlights the role of the Wnt/ β -catenin pathway as a host factor that represses HIV replication in multiple targets, especially those relevant to HIV in the central nervous system.

Keywords

HIV neuropathogenesis; Astrocyte; Signaling pathway

Introduction

Approximately 34 million people worldwide are currently infected with the human immunodeficiency virus (HIV), with the greatest impact on sub-Saharan Africa. Although great strides have been made in prolonging the life expectancy of HIV-infected individuals, HIV remains a chronic disease with major obstacles toward eradication. It is estimated that even with potent suppression of HIV replication with highly active antiretroviral therapy (HAART), it would take several decades to purge the latent reservoir (Rong and Perelson 2009). This mathematical modeling shatters any chance for HAART-mediated successful eradication of HIV during a patient's life time.

Initially, HIV treatment strategies concentrated on viral proteins that can be targeted for therapy. Advances in understanding the virology of HIV have informed current inhibitors of HIV that include reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, and even entry inhibitors. Understanding virus–host interaction as it impacts the replicative cycle of HIV will provide the next wave of anti-HIV therapeutics. HIV/host interaction is complex, as evidenced by a high-throughput siRNA screen that identified hundreds of host

factors that HIV requires for its propagation (Brass et al. 2008). This arsenal of potential new drugs targeting host factors is critical given the mutation rate of HIV against current drugs and the toxicities associated with them.

Signaling pathways that interface with HIV can be harnessed for anti-HIV therapeutics. The Wnt/ β -catenin signaling pathway has recently emerged as a potent repressor of HIV replication. In this review, the relevance of this pathway to CNS homeostasis and its role in regulating HIV replication within and outside of the CNS will be reviewed.

Signal transduction cascade of the Wnt/ β -catenin pathway

The Wnt family is comprised of 19 soluble secreted glycoproteins that regulate signaling pathways that control transcriptional activity of hundreds of genes involved in cell differentiation, communication, apoptosis/survival, and proliferation (Table 1). The most extensively studied Wnt pathway is the Wnt/ β -catenin or “canonical” pathway, in which signaling is initiated by binding of a Wnt ligand to a member of the Frizzled (Fz) family of seven transmembrane receptors and a co-receptor such as low-density lipoprotein receptor-related protein (LRP) 5/6. The central mediator of the canonical pathway is β -catenin, a multi-functional protein that can either associate with cadherins at the cell membrane to regulate cellular adhesion or can translocate to the nucleus where it functions as a transcriptional co-activator. Shuttling of β -catenin between the membrane, cytoplasm, and nucleus is largely controlled by its phosphorylation state, which is regulated by a wide variety of tyrosine and serine/threonine kinases. When Wnt signaling is inactive, a multi-protein destruction complex composed of Axin, adenomatous polyposis coli, casein kinase 1 (Ck1), and glycogen synthase kinase 3 (GSK3) binds to cytosolic β -catenin. Ck1-mediated phosphorylation on Ser45, followed by GSK3-mediated phosphorylation on Thr41, Ser33, and Ser37, targets β -catenin for ubiquitination by Trcp and degradation through the proteasomal pathway. Binding of a Wnt ligand initiates a cascade of events that results in destabilization of the destruction complex and accumulation of a stable, hypophosphorylated β -catenin that is able to translocate to the nucleus and associate with a member of the T cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors. Within the nucleus, β -catenin displaces negative regulatory elements from TCF/LEF, such as transducin-like enhancer protein and histone deacetylases (HDACs), and recruits co-factors such as BCL9, Pygopus, and CBP/p300 to activate transcription of Wnt target genes. Conversely, nuclear β -catenin may be inhibited by negative regulators such as inhibitor of β -catenin and TCF-4 and Chibby, which physically interact with β -catenin to prevent binding with TCF/LEF and which promote nuclear export of β -catenin. Key events in canonical β -catenin/TCF/LEF signaling are depicted in Fig. 1. In certain circumstances, such as oxidative stress, forkhead transcriptional factors (FOXO) may compete with TCF/LEF for β -catenin binding (Jin et al. 2008). Association of FOXO with β -catenin has been linked to inhibition of cell proliferation and senescence (DeCarolis et al. 2008).

In addition to the canonical (Wnt/ β -catenin) pathway, there are at least two “non-canonical” pathways that signal independently of β -catenin: the Wnt/Jun-N-terminal kinase (JNK) pathway, also called the planar cell polarity pathway, and the Wnt/ Ca^{2+} pathway. In the Wnt/JNK pathway, Wnt ligand binding to Frizzled leads to activation of JNK, resulting in cytoskeletal rearrangement. Induction of the Wnt/ Ca^{2+} pathway results in an increase in intracellular calcium leading to activation of calcium/calmodulin-dependent protein kinase II and nuclear import of nuclear factor of activated T cells. Activation of canonical vs. non-canonical signaling is dependent on a number of factors, including the member of the Wnt family that binds to Frizzled. Wnt-1 and Wnt3a are classical “canonical” Wnts, while Wnt4, Wnt5a, and Wnt11 are usually associated with non-canonical signaling. However, receptor context also plays a large part in determining the response to a Wnt stimulus. Ten Frizzled

family members have been identified so far, as well as several receptor kinases that can function as co-receptors for Fz to transduce a Wnt signal. These include Kremen, which functions as a high-affinity receptor for Dickkopf-1 (DKK1), an inhibitor of Wnt signaling that competes with Wnts for binding to LRP 5/6 but may promote activation of the canonical pathway in the absence of DKK1 (Hassler et al. 2007; Mao et al. 2002; Cselenyi and Lee 2008); the receptor tyrosine kinase-like orphan receptor 2, which transduces signals stimulated by Wnt5a to activate JNK (Angers and Moon 2009); and Ryk (related to receptor tyrosine kinase) which is required for neurite outgrowth during development and is associated with enhancement of canonical signals (Angers and Moon 2009).

Wnt signaling is typically induced by binding of Wnt glycoproteins in an autocrine/paracrine manner. However, recent evidence indicates that the tetraspanin membrane proteins CD9 and CD82 are involved in regulating Wnt/ β -catenin signaling by promoting exosomal release of β -catenin from the cell, resulting in decreased levels of β -catenin independent of GSK3 activity or proteasomal degradation (Chairoungdua et al. 2010). Additionally, pharmacological inhibitors of GSK3 activity such as synthetic small molecules and lithium chloride can induce β -catenin-dependent signaling in the absence of Wnt ligands.

Biologic properties of Wnt/ β -catenin

Extensive reviews exist on the role of the Wnt pathway, both canonical and non-canonical on many organ systems, including the CNS (Inestrosa and Arenas 2010; Staal and Luis 2010), and even aging (DeCarolis et al. 2008). We refer you to these reviews for detailed information. We will focus here on the biologic importance of the Wnt pathway in the CNS and how it impacts HIV replication.

Wnt/ β -catenin in the CNS

The Wnt/ β -catenin pathway plays a vital role in the formation of the CNS by regulating cellular proliferation, neurogenesis, and axis polarization in the developing brain (Malaterre et al. 2007). β -Catenin continues to be widely expressed in the adult brain and regulates a variety of functions that impact neural proliferation, differentiation, and survival as well as synaptic plasticity and dendrite outgrowth (Table 2). Frizzled receptors (Fz1) are concentrated at presynaptic terminals in hippocampal neurons. Induction of the canonical pathway by treatment with Wnt3a induces clustering of presynaptic proteins and facilitates activity-dependent neurotransmitter release and vesicle recycling (Varela-Nallar et al. 2009). Similarly, tetanic stimulation induces release of Wnt3a from presynaptic terminals that results in long-term potentiation in postsynaptic neurons via activation of Wnt/ β -catenin signaling (Chen et al. 2006). Depolarization also causes phosphorylation-dependent redistribution of β -catenin from dendritic shafts to spines and resultant association with cadherins that is associated with clustering of presynaptic proteins and increased synaptic strength (Murase et al. 2002). Together, these findings indicate that canonical signaling is involved in both pre- and postsynaptic function and is important in controlling the strength and duration of neural signals. Experiments with transgenic mice also indicate that β -catenin expression is required for memory consolidation in the amygdala (Maguschak and Ressler 2008). Astrocytes are a vital source for Wnt ligands, which promotes hippocampal neurogenesis, including the differentiation of dopaminergic neurons (Inestrosa and Arenas 2010). Further, Wnt ligands can rescue NG2 gliogenesis after injury (White et al. 2010).

The Wnt/ β -catenin pathway also has a neuroprotective role in the CNS. Recent evidence suggests that dysregulated β -catenin-dependent signaling may play a role in the pathogenesis of several neurodegenerative diseases including Alzheimer's disease and Parkinson's disease as well as other conditions such as bipolar disorder and schizophrenia (Inestrosa and

Arenas 2010). Induction of the Wnt/ β -catenin pathway through Wnt3a or lithium treatment protects against a variety of excitotoxic insults such as glutamate, NMDA, and β -amyloid, either through upregulation of anti-apoptotic target genes or inhibition of apoptotic signaling such as GSK3 β -mediated phosphorylation of the pro-apoptotic Bax (Chuang 2005; Gould and Manji 2002; Inestrosa et al. 2004).

Wnt/ β -catenin signaling inhibits HIV replication in multiple compartments

We and others have identified the Wnt/ β -catenin pathway as an important host factor that inhibits HIV replication in multiple target cells, including astrocytes, monocytes/macrophages, and T cells (Aljawai et al., submitted for publication; Carroll-Anzinger et al. 2007; Kumar et al. 2008; Wortman et al. 2002). An inverse relationship exists between an intact Wnt/ β -catenin signal and HIV, whereby when this signaling pathway is intact, HIV replication is low and when it is interrupted, HIV replication is increased (Aljawai et al., submitted for publication; Carroll-Anzinger et al. 2007; Kumar et al. 2008).

Establishing Wnt/ β -catenin as a repressor of HIV replication, a paradigm first developed in astrocytes

The relationship between Wnt/ β -catenin signaling and HIV was first observed in astrocytes. Astrocytes constitute 40–70% of brain cells and perform vital functions to maintain brain homeostasis. They regulate neuronal development, integrity of the blood–brain barrier, metabolism of neurotransmitters, and secretion of neurotrophic factors and contribute to immune surveillance in the brain through secretion of cytokines/chemokines. Indeed, dysregulation of astrocytes is associated with a number of neurodegenerative diseases, including HIV-associated dementia (HAD) (Borjabad et al. 2010). Therefore, any level of HIV replication in astrocytes can contribute to HIV load in the CNS as well as release of verotoxins such as Tat and gp120 that mediate further dysregulation in the CNS.

Historically, a disconnect existed between in vitro and postmortem studies in determining whether or not astrocytes are permissive to productive HIV replication. In vitro, using primary human fetal astrocytes and astrocytoma cell lines, a number of groups have demonstrated that HIV can infect astrocytes (Carroll-Anzinger and Al-Harathi 2006; Di Rienzo et al. 1998; Nath et al. 1995; Tornatore et al. 1991). The level of HIV infection of astrocytes ranges from 0.8 to 2 ng of HIV p24/ml from 1×10^6 cells, which is equivalent to up to 30 million virions/ml (Carroll-Anzinger and Al-Harathi 2006). Even though this level of infection is lower than can be achieved by infection of classical targets of HIV in the CNS (e.g., microglia/macrophages/infiltrating lymphocytes), 30 million virions/ml can easily contribute to the overall pathology associated with HIV invasion in the CNS. HIV replication in astrocytes is dramatically increased by CD4 transfection or pseudotyping HIV with envelope glycoproteins of amphotropic murine leukemia virus or vesicular stomatitis virus G (VSV-G) envelope (Canki et al. 2001; Schweighardt and Atwood 2001). Although these studies indicate that bypassing HIV entry leads to dramatic HIV replication in astrocytes, it is unclear if VSV itself or CD4 transfection may perturb signaling pathways in astrocytes that otherwise restrict HIV replication.

Pre-treating astrocytes with IFN γ induces productive HIV replication in astrocytes (Carroll-Anzinger and Al-Harathi 2006). IFN γ is a pleiotropic cytokine involved in enhancing bactericidal activity of phagocytes, antigen presentation through major histocompatibility complex class I and class II, anti-viral, and anti-tumor responses. The primary sources of IFN γ in the brain are microglia/ macrophages, infiltrating T cells, and astrocytes (Lau and Yu 2001). Elevated IFN γ expression is observed in both the brains of HIV-infected individuals and in simian immunodeficiency virus-infected macaques (Lane et al. 1996;

Shapshak et al. 2004). It is associated with HIV neuroinvasion and pathology of HIV in the brain (Orandle et al. 2002). In order for IFN γ signaling to be effective in inducing HIV replication in astrocytes, astrocytes must be exposed to IFN γ prior to HIV infection. If the cells are infected then treated with IFN γ , no productive replication ensues (Carroll-Anzinger and Al-Harathi 2006). These findings suggest that astrocytes require a priming signal to support productive HIV replication. If this signal, whether IFN γ or any other HIV inducing signal, is encountered in vivo, it could lead to productive HIV replication. If the signal is missing, however, as is the case in many in vitro model systems, astrocytes are blocked in HIV replication.

Recent data by Churchill and colleagues lend support to our in vitro model demonstrating that the environmental milieu, especially priming signals, can impact the degree of HIV productive replication in astrocytes. Using laser capture microdissection assay in combination with ultra-sensitive PCR, Churchill and colleagues demonstrated that up to 19% of GFAP+ astrocytes are indeed infected by HIV (Churchill et al. 2009). The level of astrocyte infection correlated with degree of HIV-associated cognitive impairment and proximity to microglia/macrophages (Churchill et al. 2009). The correlation between the level of astrocyte infection and their proximity to perivascular macrophages/ microglia (Churchill et al. 2009), combined with the observation that IFN γ primes astrocytes for productive replication (Carroll-Anzinger and Al-Harathi 2006), suggests that immune cells, whether macrophages or infiltrating lymphocytes, may be a significant source of IFN γ in the CNS that contributes to enhanced HIV infection of astrocytes in vivo and under inflammatory conditions. Further, 2–6% of astrocytes from brain tissue of patients without severe HAD and from regions that are not close to blood vessels were also positive for HIV DNA. This level of infection is reminiscent of the latent reservoir in HIV $\pm$ patients who are maximally suppressed, which is equivalent to 2–5% of CD4 $^{+}$ cells. These remarkable findings suggest that astrocytes can be a significant reservoir for HIV.

How HIV enters astrocytes is still not clear but is likely to involve unconventional/CD4 independent modes of entry. Astrocytes are CD4 $^{-}$ but may express alternative receptors for HIV entry including D6, a promiscuous CC chemokine receptor (Neil et al. 2005), and mannose receptors, which may support HIV entry through endocytosis and subsequent escape from endosomal vesicles (Liu et al. 2004; Permanyer et al. 2010; Vijaykumar et al. 2008). IFN γ priming induces D6 expression on astrocytes (Al-Harathi, unpublished data). Regardless of how HIV enters astrocytes, the finding by Churchill et al. (2009) demonstrates that astrocytes are indeed infected in vivo and may utilize cell-to-cell contact for transfer of HIV from highly permissive targets for HIV infection to astrocytes.

Studies to evaluate the signals in astrocytes that are required to prime them for productive HIV replication led us to the observation that intact Wnt/ β -catenin signaling restricts HIV replication in astrocytes and that its inhibition is required for productive HIV replication. Astrocytes have robust endogenous levels of Wnt/ β -catenin, as determined by high level of TCF/LEF reporter activity and high expression of active hypophosphorylated β -catenin (Carroll-Anzinger et al. 2007; Wei et al., submitted for publication). TCF-4, a downstream effector of the Wnt/ β -catenin pathway, is a repressor of HIV transcription (Wortman et al. 2002). Using an LTR reporter construct combined with TCF-4 and Tat expression vectors demonstrated that TCF-4 expression represses both basal and Tat-mediated HIV transcription in astrocytes (Wortman et al. 2002). This study also showed that there is a physical interaction between Tat and TCF-4 and that co-expression of these proteins results in their retention in the cytoplasm. Further confirmation of Tat and TCF-4 association was observed through proteomic analyses (Pumfery et al. 2003). Tat binding to TCF-4 prevents TCF-4-mediated dephosphorylation of Sp1 (Rossi et al. 2006). Although the exact mechanism by which β -catenin/TCF-4 inhibits HIV replication at the transcriptional level is

under investigation, the data suggest several mechanisms for the inverse relationship between Wnt/ β -catenin signaling and HIV replication in which (1) Tat binding to TCF-4 sequesters both proteins away from their respective binding partners, possibly resulting in both decreased transactivation of the HIV LTR and reduced β -catenin signaling, or (2) TCF-4 antagonizes HIV at the transcriptional level by inhibiting positive regulators such as Sp1 in the absence of Tat. Furthermore, Salim and Ratner (2008) showed that Vpu, a viral protein that enhances HIV particle release, functions at least in part by preventing β -catenin association with E-cadherin, indicating that β -catenin/E-cadherin interaction also inhibits virion release, albeit in macrophages. Together, these studies suggest that components of Wnt/ β -catenin signaling may act on HIV at several points in the viral life cycle and that the virus has evolved mechanisms (Vpu) to circumvent these restrictions.

In examining the mechanism by which IFN γ overcomes restricted HIV replication, chromatin immunoprecipitation assays showed that TCF-4 is docked on the HIV LTR in infected astrocytes, and this binding is associated with repression of HIV replication (Carroll-Anzinger et al. 2007). Furthermore, astrocytes transfected with a dominant negative (DN) TCF-4 that is incapable of interacting with β -catenin are productively infected by HIV (Carroll-Anzinger et al. 2007). These studies support findings from transfection experiments that TCF-4 acts at least partly at the level of viral transcription and also suggest that β -catenin may be involved in TCF-4-mediated inhibition of HIV. The requirement for β -catenin in this system is contradictory to the observation made by Wortman et al., in which they showed that TCF-4-mediated repression of HIV LTR promoter activity was β -catenin independent (Wortman et al. 2002). Four TCF-4 binding sites have been identified on the HIV LTR with 71–100% homology to the TCF-4 consensus sequence. Out of 500 HIV isolates evaluated by informatics analysis, one third contained two to four TCF-4 binding sites (Narasipura et al. 2010). This suggests that strain variation may account for these divergent findings. Collectively, these findings challenge the dogma that astrocytes are not productively infected by HIV and point to the requirement for biologic signals that inhibit Wnt/ β -catenin signaling in order to induce a permissive state for HIV infectivity, which may be lacking in an in vitro model system.

Role of Wnt/ β -catenin in regulating HIV replication in PBMCs

The relationship between HIV replication in astrocytes and Wnt/ β -catenin pathway is not restricted to astrocytes. PBMCs, typically, have a lower level of β -catenin activity. Inducing the β -catenin pathway in PBMCs by lithium chloride led to a potent reduction in HIV replication in PBMCs (Kumar et al. 2008). Lithium-mediated inhibition of HIV replication in PBMCs was dependent on its well-established ability to induce β -catenin, as inhibiting β -catenin or TCF-4 abrogated the anti-HIV effect of lithium (Kumar et al. 2008). Conversely, transfection with either a DN mutant of β -catenin or DN TCF-4 significantly enhances HIV infection in these cells (Kumar et al. 2008), suggesting that the low level of endogenous Wnt activity in PBMCs may contribute to limiting virus replication even in permissive targets (Kumar et al. 2008). Overexpression of β -catenin in PBLs also leads to robust inhibition of HIV replication, similar to levels achieved through AZT treatment (Al-Harhi, personal observation). Interestingly, an association between lithium use and lower viral load was also observed among a small cohort of HIV+ patients. A retrospective study of bipolar patients on lithium who are HIV+ but ART naïve demonstrated a reduction in HIV VL with lithium use (Kumar et al. 2008). Well-controlled prospective studies are warranted to evaluate the anti-viral effects of lithium and the mechanism by which it inhibits HIV replication in vivo.

Association between lithium chloride, HIV, and neuroprotection

The two major therapeutic targets of lithium are glycogen synthase kinase 3 (GSK-3) and signal transduction via inositol (1,4,5) trisphosphate (IP₃; Williams and Harwood 2000). Lithium inhibits the activity of both the α and β isoforms of GSK3, resulting in the activation of the Wnt signaling pathway (Hedgepeth et al. 1997; Klein and Melton 1996; Rao et al. 2005; Williams and Harwood 2000). At higher concentrations (4–5 mM), lithium inhibits inositol monophosphatase and inositol polyphosphatase, leading to a decreased IP₃ response (Sarkar et al. 2005). Although lithium side effects vary depending on its concentration, most patients are at risk of toxicity if the plasma level exceeds 2 mM. At high concentrations, lithium can cause tremors and diarrhea, increase in urine volume, and reduction of renal concentration ability (Schou 1988, 1989). In a small pilot study evaluating the therapeutic benefit of lithium in improving cognition in HIV+ patients, within 12 weeks of low-oral dose lithium therapy, the cognition score improved in all participants ($n=8$) and became unimpaired in 75% of the enrolled patients (Letendre et al. 2006). Further, lithium (600–1,200 mg/day) was well tolerated in this small clinical study with no grade 3 or 4 adverse events or withdrawal from study because of adverse effects (Letendre et al. 2006). Although this study did not assess the impact of lithium on HIV viral load (VL) because the inclusion criteria consisted of patients who were on stable HAART with VL<400 copies/ml in plasma, lithium did not increase VL. This small clinical study provides additional evidence that lithium, when monitored carefully, is well tolerated and improves HIV-associated neurocognitive impairment (Letendre et al. 2006). Several animal and human studies suggest a benefit of lithium administration in neuroprotection in HIV. Specifically, lithium prevents gp120- and Tat-induced HIV neurodegeneration in vitro (Everall et al. 2002; Maggirwar et al. 1999) and increases soluble TNF receptor, which absorbs the neurotoxic TNF cytokine (Himmerich et al. 2005). Lithium was also effective in regulating murine immunodeficiency virus, which has similarities to HIV (Dou et al. 2005; Gallicchio et al. 1993). These collective in vitro and in vivo studies indicate that lithium is neuroprotective for HIV. Lithium may also exert an additional benefit by suppressing HIV replication in PBMCs and at least in vitro it does so by activating the β -catenin pathway (Kumar et al. 2008). No study to date has specifically evaluated the role of lithium in direct HIV suppression in a well-controlled clinical study.

Lithium vs. valproic acid and association with β -catenin

Although both lithium and valproic acid activate β -catenin, they seem to have divergent effects on HIV replication (Kumar et al. 2008; Smith 2005). Lithium is a suppressor of HIV replication in a β -catenin-dependent manner (Kumar et al. 2008). However, valproic acid, a nonselective HDAC inhibitor, induces HIV replication in PBMCs and was proposed as a means in combination with HAART to purge the latent HIV reservoir (Smith 2005). Clinical studies thus far have not shown a promise for valproic acid in successfully purging the latent reservoir, at least in resting CD4+ memory T cells (Archin et al. 2008). The divergent effects of lithium and valproic acid, despite their effect on induction of β -catenin, are intriguing and may suggest effects beyond β -catenin. In particular, lithium exerts a consistent inhibition of GSK3 leading to β -catenin stabilization while valproic acid inhibition of GSK3 is cell-type-dependent and thus its effects on β -catenin levels may also be cell-type-dependent (Jonathan Ryves et al. 2005).

Role of host factors in modulating Wnt/ β -catenin activity and impacting level of HIV replication

Since the β -catenin pathway regulates HIV replication in multiple compartments, any signal, whether viral or host- derived, to modulate the endogenous level of this signaling pathway

could potentially impact HIV viral load. As mentioned previously, IFN pretreatment induces HIV replication in astrocytes by down regulating β -catenin signaling (Carroll-Anzinger et al. 2007). Studies from our lab indicate that IFN inhibits β -catenin by inducing DKK1, a β -catenin pathway antagonist (Fig. 1) in a Stat 3-dependent manner leading to enhanced HIV replication (Wei et al., submitted for publication). GM-CSF, which differentiates monocytes to macrophages and is secreted by glia under conditions of inflammation, also induces HIV replication by downregulating β -catenin (Aljawai et al., submitted for publication; Carroll-Anzinger and Al-Harhi 2006). Hormones such as estrogen are reported to interact with this pathway. In particular, cross-talk between 17 β -estradiol and Wnt/ β -catenin is well documented. The estrogen receptor (Er) is physically associated with β -catenin, as demonstrated in human colon and breast cancer cells (Kouzmano et al. 2004). Further, each protein enhances the transactivation of its cognate reporter genes and is reciprocally recruited to cognate response elements in the promoters of endogenous target genes. Treating the cells with estrogen further enhances this physical interaction between Er and β -catenin (Kouzmano et al. 2004). β -Catenin can translocate to the nucleus where it binds to TCF/LEF transcriptional factors and directly or with binding to ER regulates target gene expression. Further, compelling evidence exists in the literature pointing to a neuroprotective role of estrogen (Arnold and Beyer 2009; Correia et al. 2010). These effects may be largely mediated by the ability of estrogen to impact the Wnt/ β -catenin pathway.

Interaction between Wnt/ β -catenin and other signaling pathways: link to GSK3 β , AKT, and p38 MAP kinase

Extensive cross-talk exists between the Wnt/ β -catenin pathway and other signal transduction cascades. These include the phosphoinositide 3-kinase/Akt and the p38 mitogen-activated protein kinase (MAPK) pathways, which interact with the canonical pathway by converging on a third signaling partner: glycogen synthase kinase-3 (Fig. 2). Phosphorylation of GSK3 on Ser9 is a well-described mechanism by which Akt inhibits GSK3 activity. Additionally, P38 can phosphorylate GSK3 at Ser839 through a non-canonical MAPK pathway that is active in the brain and in developing thymocytes (Thornton et al. 2008). Similarly, some of the neuroprotective effects of growth factors such as brain-derived neurotrophic factor are derived from inactivation of GSK3 (Ji et al. 2010; Li et al. 2007). Given that inhibition of GSK3 destabilizes the destruction complex and leads to accumulation of β -catenin, stimuli that suppress GSK3 activity will likely lead to activation of β -catenin-dependent signaling.

Conversely, signals that enhance GSK3 activity or that directly inhibit β -catenin or TCF/LEF will inhibit β -catenin-dependent signaling. Wnt/ Ca^{2+} signaling, induced by Wnt stimulation or calcium influx, has been observed to inhibit canonical signaling through a poorly understood mechanism that may involve β -catenin degradation (Topol et al. 2003). Similarly, nemo-like kinase, activated through the Wnt/ Ca^{2+} pathway, can directly phosphorylate and inhibit TCF/LEF in the nucleus (Ishitani et al. 1999). JNK activation via Wnt can have a positive or negative effect on β -catenin signaling depending on cell context (Liao et al. 2006; Saadeddin et al. 2009). The relationship between canonical/non-canonical signaling is incompletely understood in the CNS, but it is likely highly complex due to the importance of these pathways in fetal and adult brain. A variety of Wnt ligands are expressed in the CNS, particularly in the subgranular zone of the hippocampus and the subventricular zone of the lateral ventricles in the forebrain, where they are involved in regulating proliferation and differentiation of the progenitor pool (Alvarez-Buylla and Lim 2004; Inestrosa and Arenas 2010). Greater understanding of the interplay between β -catenin and other signaling pathways that regulate cell proliferation, differentiation, and survival

may provide tools for enhancing function and survival of neurons and glia as well as manipulating HIV replication in the CNS reservoir.

Proposed model of β -catenin/HIV interaction in HIV disease

Based on these collective studies, we propose a model depicted in Fig. 3 illustrating the impact of the Wnt/ β -catenin pathway on HIV and consequences to its dysregulation in the CNS. Briefly, HIV crosses the blood–brain barrier, either via HIV-infected monocytes that migrate into the CNS and differentiate into virus-producing macrophages, or possibly as cell-free virus that infects macrophages/microglia in the brain parenchyma. These cells begin to produce viral particles, secreted viral proteins (Tat, gp120, Vpr), and inflammatory mediators that lead to further activation and/or infection of local macrophages and microglia. Increase in IFN γ in particular may contribute to dysregulated Wnt/ β -catenin signaling in neurons, astrocytes, and macrophages/microglia that (a) enhances HIV replication in macrophages/microglia and primes astrocytes to support productive HIV infection and (b) contributes to dysregulation and/or apoptosis of CNS cells through loss of β -catenin signaling. Increased HIV replication in the CNS enhances release of inflammatory mediators and neurotoxic viral proteins that lead to further dysregulation/death of neurons and glia, as well as promoting lymphocyte infiltration into the CNS. Dysregulated Wnt/ β -catenin signaling therefore contributes to a positive feedback loop that exacerbates HIV CNS viral load and neuronal/glial dysfunction.

HIV infection of astrocytes, while generally low and restricted, is greatly enhanced by signals that downregulate β -catenin signaling. This is especially relevant because astrocytes can serve as a reservoir for latent HIV. When β -catenin signaling is diminished, due to inflammatory mediators or any other signals, astrocytes under these circumstances would release HIV into the CNS that can possibly promote an underlying CNS microenvironment of inflammation. This inflammation may be low or high and persistent or sporadic depending on the degree of HIV replication at any given time. HIV from the CNS can also seed the periphery with HIV. Therefore, controlling HIV replication in the CNS will be highly relevant to efforts to purge the latent reservoir.

Therapeutic implications for harnessing Wnt/ β -catenin

Can Wnt/ β -catenin be manipulated to inhibit HIV replication or induce HIV replication to purge the latent reservoir? Suppressing β -catenin signaling in infected cells increases HIV replication, suggesting that the endogenous level of β -catenin suppresses HIV replication and without it the level of HIV in this system would be much higher. Conversely, activating β -catenin signaling potently inhibits HIV replication. It is a see-saw interaction that is well documented in a number of cell types (Fig. 4). Therefore, this pathway provides an intricate balance for HIV load that can be manipulated toward a specific clinical endpoint. For example, among individuals with maximum viral load suppression under HAART, inhibiting β -catenin can lead to activation of HIV replication, which will expose HIV to the action of HAART in an effort to purge the latent reservoir. Evidence for this rationale exists from studies in our lab, whereby monocytes, which do not support productive HIV and are a critical latent reservoir for HIV, have high levels of β -catenin, and when it is suppressed, HIV replication is enhanced to levels that are similar to that observed when these cells differentiate to macrophages (Aljawai et al., submitted for publication). Alternatively, in patients with uncontrolled viremia or resistance to current antiretroviral therapy, activating the β -catenin pathway can be used as a salvage therapy. Further, activating β -catenin can be used as an additional approach in combination with HAART among patients that do not achieve complete HIV suppression. This pathway shows molecular therapeutic promise in regulating HIV, but further studies are warranted to determine the exact mechanism by

which it interfaces with HIV and evaluate any negative consequences of inducing this pathway. Uncontrolled induction of β -catenin signaling is linked to a number of malignancies; however, it is the uncontrolled induction of this pathway that is likely to lead to enhance proliferative capacity of cells. Nonetheless, exploring HIV/ β -catenin interaction can inform novel molecular approaches for HIV therapeutic intervention.

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Fig. 1.

The canonical (Wnt/ β -catenin) pathway. **a** In the absence of a Wnt signal, cytosolic β -catenin is phosphorylated by a destruction complex comprised of Axin, adenomatous polyposis coli (*APC*), casein kinase-1 (*CK1*), and glycogen synthase kinase-3 (*GSK3*). Phosphorylation targets β -catenin for Trcp-mediated ubiquitination and degradation by the proteasome. In the nucleus, T cell factor/ lymphoid enhancer factor (*TCF/LEF*) transcription factors are bound to co-repressors such as transducin-like enhancer protein (*TLE*), c-terminal binding protein (*CTBP*), and histone deacetylases (*HDACs*). Nuclear β -catenin is inhibited from binding *TCF/LEF* through interaction with inhibitor of β -catenin and *TCF-4* (*ICAT*). Canonical signaling may also be inhibited by binding of Dickkopf-1 (*DKK-1*) to the co-receptor low-density lipoprotein receptor-related protein (*LRP*) 5/6. **b** When Wnt signaling is initiated, Axin is recruited to the plasma membrane and *LRP* 5/6 is phosphorylated by *CK1* and *GSK3*, leading to activation of Dishevelled (*Dvl*) and destabilization of the destruction complex. Hypophosphorylated β -catenin accumulates in the cytoplasm and is able to translocate to the nucleus, where it interacts with *TCF/LEF* to displace its co-repressors and recruit positive co-factors including Bcl9 and Pygopus (*Pygo*) to activate transcription of Wnt target genes

Fig. 2.

Signaling pathways interface with Wnt/ β -catenin signaling. Akt and p38 can both activate the canonical pathway by inhibiting GSK3 activity, preventing it from phosphorylating β -catenin. Akt phosphorylates GSK3 at the N terminus on Ser9, while P38 phosphorylates the C-terminal residue Ser839. Conversely, activation of the non-canonical Wnt/JNK pathway has been observed to inhibit nuclear accumulation of β -catenin under some circumstances. Release of calcium from intracellular stores, such as occurs when the Wnt/ Ca^{2+} pathway is activated, can lead to activation of nemo-like kinase (NLK), which directly phosphorylates TCF to inhibit interaction with β -catenin. Induction of nuclear factor of activated T cells (NFAT) via the Wnt/ Ca^{2+} pathway has also been observed to promote GSK3-independent degradation of β -catenin, but the mechanism is poorly understood. MKKK mitogen-activated protein kinase (MAPK) kinase kinase, MKK MAPK kinase, PI3K phosphoinositide-3-kinase, PLC phospholipase C, PKC protein kinase C, CaMKII calcium/calmodulin-dependent kinase II, JNK c-Jun N-terminal kinase

Fig. 3.

Consequences of dysregulated Wnt/b-catenin signaling on HIV replication in the CNS. HIV crosses the blood–brain barrier either through migration of HIV-infected monocytes or possibly transcytosis of cell-free virus that infects macrophages/microglia in the brain parenchyma. Infected cells begin to produce viral particles, inflammatory mediators (*IFN*), and viral proteins (Tat, gp120, Vpr) that lead to further activation and/or infection of local macrophages and microglia and contribute to dysregulation of the Wnt/ -catenin pathway in neurons and glia. Dysregulated Wnt/ -catenin signaling (a) primes astrocytes to support productive HIV infection and enhances HIV replication in macrophages/microglia and (b) contributes to dysregulation and/or apoptosis of neurons and glia. Active HIV replication, in turn, amplifies production of inflammatory mediators and HIV verotoxins that cause further dysregulation and/or apoptosis of CNS cells as well as recruitment of leukocytes into the brain

Fig. 4.

Model of the inverse relationship between Wnt/ β -catenin signaling and HIV replication. When the Wnt/ β -catenin pathway is active, HIV replication is inhibited in astrocytes, peripheral blood lymphocytes (*PBLs*), and monocytes/macrophages. When β -catenin signaling is inactive or suppressed, as occurs when monocytes differentiate into macrophages or astrocytes are exposed to IFN γ , HIV replication is enhanced

Table 1

Examples of Wnt target genes

Wnt target genes	Function
c-myc	Cell growth and proliferation
Cyclin D	Cell proliferation/differentiation
Axin2	Negative regulator of Wnt signaling; Formation of β -catenin destruction complex
TCF-4	Transcription of Wnt target genes; may function as a transcriptional repressor in the absence of co-factor β -catenin
DKK-1	Negative regulator of Wnt/ β -catenin signaling
Bcl-XL	Anti-apoptotic protein
PPAR	Nuclear hormone receptor involved in differentiation, lipid storage, cell polarization; shown to have anti-inflammatory functions in models of experimental allergic encephalitis
L1 neural adhesion	Neural cell adhesion molecule involved in axon growth
Survivin	Inhibitor of apoptosis

Table 2Examples of biologic properties of β -catenin in the CNS

Function	Region	Dependent/ independent of β - catenin/TCF-mediated gene transcription	Reference
Enhanced dendritic arborization	Hippocampus	Independent	Yu et al. 2003
Increase in synapse numbers in response to enriched environment Synaptogenesis	Hippocampus fetal hippocampus	Undetermined; requires release of Wnt7a/b at the synapse	Gogolla et al. 2009; Davis et al. 2008
Enhanced clustering of presynaptic vesicle proteins	Hippocampus	Independent	Cerpa et al. 2008; Varela-Nallar et al. 2009
Enhanced formation of presynaptic inputs in fetal brain	Hippocampus	Undetermined	Davis et al. 2008
Enhanced release of neurotransmitters enhanced recycling of neurotransmitters	Fetal hippocampus: CA3–CA1 synapses	Undetermined	Cerpa et al. 2008; Varela-Nallar et al. 2009
Promotion of long-term potentiation	Hippocampus	Requires induction of Wnt/ β -catenin in postsynaptic neurons	Chen et al. 2006
Memory consolidation	Amygdala	Undetermined	Maguschak and Ressler 2008
Resistance to Ab-induced apoptosis		Dependent	Garrido et al. 2002; Alvarez et al. 2002; De Ferrari et al. 2003; Farias et al. 2004; Inestrosa and Toledo 2008