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Gliomas: Role of Wnt/β-Catenin/ Tcf Signaling Pathway

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1. Introduction

1.1 Gliomas

Gliomas are the most common primary brain tumors and accounts for about 1.7% of all human neoplasms and 77% of all brain tumors. Gliomas have an annual incidence of 5-10 cases per 100,000 in western population and are a leading cause of death among children and adults diagnosed with a neoplasia of the brain. Gliomas are the heterogeneous group of tumors that are broadly classified into oligodendrogliomas, ependymomas, astrocytomas and mixed oligoastrocytomas based on the specific type of cell to which they closely resemble. According to World Health Organization (WHO) astrocytomas are divided into four clinical grades on the basis of analysis of the most malignant tumor region, number of mitoses, nuclear atypia, microvascular proliferation and presence of necrosis. These includes pilocytic astrocytoma (WHO grade I), diffuse astrocytoma (WHO grade II) anaplastic astrocytoma (WHO grade III) and glioblastoma multiforme (WHO grade IV). Glioblastoma multiforme (GBM) is most malignant, aggressive and devastating form with a worse prognosis. Patients with GBM have a mean survival of about 1 year, whereas patients with anaplastic astrocytomas survive for 2-3 years, and those with diffuse astrocytomas can survive for as long as 10-15 years (Ohgaki and Kleihues., 2009; Louis *et al*., 2007).

Grade I tumors, also known as pilocytic astrocytomas are childhood brain tumors, benign in nature with circumscribed and well differentiated tumor margins. These tumors are curable by surgery and might represent a separate disease from the astrocytomas of other grades. Diffuse astrocytomas (grade II) are well differentiated and slow-growing tumors, predominantly manifested in young adults (~34 years). They exhibit consistent tendency to diffusely infiltrate surrounding brain structures and tends to progress to anaplastic astrocytoma and eventually to GBM. Characteristic molecular genetic features of diffuse astrocytomas are point mutations in the TP53 tumor suppressor gene (50-80%) and overexpression of PDGF-A and PDGFR-α is observed in astrocytic tumors of all stages (60%), but gene amplification was only detected in a small subset (<10%) of secondary GBM (Nupponen *et al.,* 2006). Anaplastic astrocytoma (grade III) arise *de novo* or from less malignant diffuse astrocytoma and shows tendency for malignant progression to GBM. Males are frequently affected and the mean age is 41 years. Anaplastic astrocytoma possesses higher frequency of TP53 mutations and other genetic changes include p16 and p19 deletion, RB alterations, and LOH on chromosome 19q (50%) (Nupponen *et al.,* 2006).

Glioblastomas are the heterogeneous intraparenchymal masses and the most common malignant primary brain tumors in adults. These tumors show the histological evidences of cellular polymorphism, nuclear atypia, mitotic activity, microvascular proliferation, vascular thrombosis and necrosis. Microscopically, they consists of several cell types: the glioma cell proper, hyper proliferative endothelial cells, macrophages and trapped cells of normal brain structures that are overrun by the invading glioma cells. Several histological characteristics are used to grade and define gliomas. These include regions of necrosis, in which necrotic areas are surrounded by densely packed tumor cell nuclei and are referred as "pseudopalisading" necrosis. In addition, the blood vessels both within and adjacent to the tumor are hypertrophied. Furthermore, the nuclei of tumor cells are extremely variable in size and shape, a characteristic called nuclear polymorphism. Tumor cells characteristically invade the adjacent normal brain parenchyma, migrating through the white matter tracts to collect around blood vessels, neurons and at the edge of the brain parenchyma in the subpial region (Kleihues and Ohgaki., 1999).

GBM may develop *de novo* (primary GBM) or from less malignant precursor lesion (secondary GBM). However, the majority of develop *de novo* with short clinical history usually less than 3 months. They may manifest at any age, but are more common in adults (~55 years) and males are more frequently affected. The secondary GBM occur in younger age group (~39 years), show a slightly more favorable outcome and develop far less often than primary GBM. The time interval for progression from diffuse low-grade astrocytoma to secondary GBM varies considerably (~4-5 years). However, with regard to histopathological and immunohistochemical features there are no differences between primary and secondary GBM (Kleihues and Ohgaki., 1999). Comparative gene expression analysis showed that primary and secondary GBM have distinct expression profiles, however, half of the clinically determined primary GBM has a similar pattern to the secondary GBM. These suggest that primary GBM may originate from a clinically undiagnosed lower grade lesion.

1.2 Genetic alterations associated with primary and secondary GBM

Primary and secondary GBM are distinct in their molecular features. Chromosomal aberrations of gliomas have been studied using karyotyping, comparative genomic hybridization (CGH), or chromosome painting. The chromosomal aberrations identified in primary GBM are distinct from those of secondary GBM. However, there are also chromosomal aberrations that are shared by both primary and secondary GBM. The most chromosomal aberrations associated with primary GBM are amplifications and gains of 7p, 12q13-21, and chromosome 19. The main chromosomal regions showing losses are 10q, 9p, 13q, and 22q. In addition primary GBM may harbors additional aberrations such as losses of 18q, 16p, and 19q, and gains of 20q, and 12q. Anaplastic astrocytomas associated with losses of 9p, 10q, 13q and gains of 1q, 7p, 11q, and Xq. Diffuse astrocytomas associated with gains of 3q, 4q, 7q, 12p, and 19p, and losses of Xp, 1p, and 19q.

The genetic hallmark of primary GBM that typically lack a TP53 mutation is MDM2 amplification/overexpression (50%). Additional genetic changes in primary GBM includes EGFR amplification (40% of cases) and/or overexpression (60%), CDKN2-A, CDKN2-B and PTEN mutations (30%), RB alteration, p16 deletion (30-40%) LOH on the entire chromosome 10 (50-80%). The sequence in which gene alterations are acquired is not known since these neoplasms develop very rapidly, without a clinically or histopathologically identifiable precursor lesion. The TP53 mutations are less common in primary GBM (<10%). Secondary

GBM frequently associate with mutations of gene TP53. These mutations in more than 90% cases are already present in the first biopsy of diffuse low grade or anaplastic astrocytoma. Most likely, the TP53 mutation is the initial gatekeeper lesion in astrocytic tumors, which then, through genetic instability undergoes malignant progression. The pathway to secondary glioblastoma is further characterized by LOH on chromosomes 19q and 10q (but not on the entire chromosome 10 as it is seen in primary GBM). Recently it was pointed out that genomic alterations of LOH 1p and 19q, which are observed in the majority of oligodendroglioma, may be observed in GBM. However, in contrast to oligodendroglioma, in GBM loss of 19q is more likely to be partial than complete and loss of 1p is uncommon (~10%). It was suggested that combined losses of chromosome arms 1p and 19q may indicate better prognosis and potential sensitivity to chemotherapy in GBM patients, while isolated loss of either 1p or 19q is of no prognostic significance. The IDH1 gene at 2q33 encodes isocitrate dehydrogenase 1 (IDH1) catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate, predominantly located in the cytosol. Recent studies demonstrated that IDH1 mutations are very early and frequent genetic alteration in low grade astrocytomas (80%), anaplastic astrocytomas and secondary glioblastomas. In contrast, IDH1 mutations are very rare (<5%) or absent in pilocytic astrocytomas and primary glioblastomas. Almost 60% of the low grade astrocytomas have both TP53 and IDH1 mutations (Ohgaki and Kleihues., 2009).

The progression of astrocytomas to more malignant forms results from the stepwise accumulation of genetic alterations and the consequent disruption of apoptotic pathway and augmentation of survival signaling. These genetic alterations in astrocytomas ultimately result in the abnormal activation of signal transduction pathways, downstream of receptor tyrosine kinases or disruption of cell cycle arrest pathways. For example, amplification or activating mutations of EGFR, over-expression of FGF, FGFR, PDGF and PDGFR due to either gene amplification or other epigenetic mechanisms, all lead to constitutive activation of corresponding receptor tyrosine kinase signaling. Subsequently, a number of downstream signal transduction pathways are activated, including the PI3K/AKT pathway, RAS/MAP kinase pathway, C-MYC pathway, protein kinase C pathway, and STAT pathways (Ohgaki and Kleihues., 2007).

1.3 Wnt signaling pathway

The Wnt proteins (the name derived from mouse Int-1 and Drosophila wingless) comprise a large family of protein ligands that affect diverse processes such as embryonic induction, generation of cell polarity, and the specification of cell fate (Logan *et al*., 2004), tissue homeostasis and cancer. A number of Wnt genes, including Wnt2, Wnt7b and Wnt 5a, have been associated with abnormal proliferation of human breast tissue and other tumors. The Wnt receptor complex that activates the canonical pathway contains two components: a member of the Frizzled family and either one of two single-span transmembrane proteins, low density-lipoprotein receptor related proteins (LRP5 and LRP6). Once bound by their cognate ligands, the Fzd/LRP receptor complex activates the canonical signaling pathway. The central player of the canonical signaling pathway is β-catenin, a cytoplasmic protein whose stability is regulated by the "destruction complex". Within this complex the Axin and APC proteins form a scaffold that facilitates β-catenin phosphorylation by CK1α and GSK3β. Phosphorylated β-catenin is subsequently recognized and ubiquitinylated, and

degrades in proteasomes. The resulting low levels of β-catenin allow the DNA binding Tcf/Lef proteins to interact with transcriptional co-repressors to block target genes expression. Interaction of Wnt ligand with its specific receptor complex containing a Frizzled family member and LRP5 or LRP6 triggers the formation of Dvl-Fzd complexes and the phosphorylation of LRP by CK1γ facilitating relocation of Axin to the membrane and inactivation of the destruction box. This allows β-catenin to accumulate and enter the nucleus, where it interacts with members of the Tcf/Lef family and converts them into potent transcriptional activators by displacing groucho/TLE proteins and recruiting an array of co-activator proteins including CBP, TBP, BRG1, BCL9/PYG, Legless, Mediator and Hyrax. This ensures efficient activation of Tcf target genes such as c-myc, n-myc, cyclinD1, c-jun, MMP7, VEGF, IL-8 etc (Moon *et al*., 2004. The overview of Wnt signaling pathway is illustrated in figure 1.

1.4 Wnt signaling-neural stem cells-gliomas

During the development of nervous system neural precursor or progenitor cells (NPC) act as a source of various types of specialized cells in the brain. Several studies have suggested that these cells are able to self-renew, a hall mark of stem cells. Wnt signaling is a candidate pathway in controlling neural stem cells self-renewal and differentiation. Wnt signaling is required at several stages of central nervous system development. The neural stem cells and progenitor cells in the brain are at a risk of malignant transformation, presumably because of the fact that most of the oncogenic and developmental pathways that responsible for tumor formation are critical for the functions like cell survival, self renewal, proliferation and differentiation and neural stem cells exhibit least resistance in tumorigenesis, since they already have the ability to bypass apoptosis and senescence. The developmental pathways in particular Wnt signaling pathway critically regulate the self-renewal, proliferation and differentiation of neural stem cells and other progenitor cells in the brain. Deregulation of/or abnormal operation of this pathway potentially leads to the development of brain tumors.

2. Wnt/β-catenin/Tcf signaling pathway components in gliomas

The progression of low grade astrocytomas to higher grades results from the stepwise accumulation of genetic alterations and the consequent disruption of the apoptotic pathways and augmentation of survival signaling. Here investigations on the possible role of Wnt signaling pathway components were described in detail.

2.1 Extracellular Wnt signaling inhibitors

Wnt signaling pathway is inhibited by extracellular Wnt antagonists which are divided into two functional classes, the sFRP (secreted Frizzled Related Protein) class and the Dickkopf class. sFRP class includes sFRP family (sFRP1, sFRP2, sFRP3, sFRP4, and sFRP5), sizzled, sizzled2, crescent, WIF-1 (Wnt inhibitory factor-1) and cerebrus, which directly binds to Wnts, thereby altering their ability to bind to the receptor complex. Roth *et al*. (2000) investigated the role of sFRPs in glioma cell growth and motility. sFRP-1 and sFRP-2 are produced by the majority of malignant glioma cell lines and ectopic expression of sFRPs increased clonogenecity and enhanced resistance to serum starvation. In contrast, sFRPs do

not modulate glioma cell susceptibility to apoptosis induced by the cytotoxic cytokines and cytotoxic drugs. sFRP-2 strongly promotes the growth of intracranial glioma xenografts in nude mice. In contrast, enhanced expression of sFRPs inhibits the motility of glioma cells *in vitro*. sFRP-mediated effects on glioma cells are accompanied by decreased expression and activity of matrix metalloproteinase-2 (MMP-2) and decreased tyrosine phosphorylation of β-catenin. This suggested that sFRPs promote survival under non-supportive conditions and inhibit the migration of glioma cells. Dickkopf family comprises four members (Dkk-1 to Dkk-4) and a unique Dkk-3-related protein named Soggy (sgy). The DKK family of glycoproteins curtails Wnt induced signals by binding to co-receptors LRP 5 and LRP 6. The human Dkk-1 (hDkk-1) gene, a transcriptional target of the p53 tumor suppressor, encodes a potent inhibitor of the Wnt signaling pathway and regulates the spatial patterning/morphogenesis of the mammalian central nervous system. Its induction was greatly enhanced following DNA damage and in response to other chemotherapeutic agents through p53 dependent mechanism. Shou *et al*. (2002) reported that over expression of DKK-1 significantly reduced the Wnt2 dependent β-catenin expression and sensitizes the glioblastoma cell lines to apoptosis in response to various chemotherapeutic agents that cause DNA alkylation and DNA damage. Muller *et al*. (2005) analyzed the DKK1 in series of 73 brain tumors for structural alterations in the entire coding sequence by single-strand conformation polymorphism and direct sequencing. They detected several sequence variants but no obvious mutations that affecting DKK1. REIC/Dkk-3 acts as a suppressor in various human cancers. Studies by Mizobuchi *et al*. (2007) showed that the expression levels of Dkk-3 were lower in human malignant glioma tissues compared to normal brain tissues and inversely correlated with the degree of malignancy. Dkk-3 expression levels were lower in glioma cell lines compared to normal human astrocytes. Knockdown of Dkk-3 using si-RNA resulted in increased survival cell index compared to control cells. Further, ectopic expression of Dkk-3 in glioblastoma cell lines showed the decreased survival index in a time dependent manner. In contrast forced expression of Dkk-3 does not alter the survival cell index in normal astrocytes. Overexpression of Dkk-3 induced the apoptosis in GBM cell lines through the activation of phosphor-JUN, caspase-9 and caspase-3 and also resulted in the reduction and degradation of β-catenin.

Wnt inhibitory factor-1 (WIF-1) is one of the secreted antagonists that can directly bind to Wnt proteins and inhibits the Wnt/β-catenin signaling. Down-regulation and promoter hypermethylation of WIF-1 have been reported in several human malignancies. Yang *et al*. (2010) reported that expression levels of WIF-1 mRNA and protein were significantly decreased in human astrocytomas compared to normal brain tissues and expression levels were negatively correlated with the histological malignancy astrocytomas. Further this reduced WIF-1 gene expression was associated with the hypermethylation of WIF-1 gene promoter. In contrast, no hypermethylation was observed in normal brain tissues. Hypermethylation was observed in 54.72% of astrocytoma samples, especially in 70% of high grade astrocytomas. Gotze *et al*., (2009) investigated gliomas of different malignancy grades for promoter hypermethylation in members of the secreted frizzled-related protein (*SFRP1, SFRP2*, *SFRP4*, *SFRP5*), dickkopf (*DKK1, DKK3*) and naked (*NKD1, NKD2*) families of Wnt pathway inhibitors. They found that frequent promoter hypermethylation of Wnt pathway inhibitor genes in astrocytomas of varying grades. Hypermethylation of *SFRP1, SFRP2* and *NKD2* each occurred in more than 40% of the primary glioblastomas, while

DKK1 hypermethylation was found in 50% of secondary glioblastomas. Further, treatment of *SFRP1*-, *SFRP5*-, *DKK1*-, *DKK3*-, *NKD1*- and *NKD2*-hypermethylated U87-MG glioblastoma cells with demethylating agent 5-aza-2-deoxycytidine and with histone deacetylase inhibitor Trichostatin A resulted in increased expression of each gene. Furthermore, *SFRP1*-hypermethylated gliomas showed significantly lower expression of the respective transcripts when compared with unmethylated tumors. Similarly, Foltz *et al*., (2010) studied epigenetic inactivation of Wnt pathway inhibitors in glioblastomas using a large-scale whole-genome approach. They found that three genes DKK1, SFRP1, and WIF1 were epigenetically silenced in glioblastomas and confirmed the decreased expression of these genes in GBM tumor tissue samples relative to normal brain tissue samples. Further, the expression of these genes is restored in T98G GBM cells by treatment Trichostatin A, but only DKK1 expression is restored by treatment with the 5-azacytidine. Ectopic expression of DKK1 significantly reduces colony formation and increases chemotherapy-induced apoptosis in T98G glioblastoma cells. While, ectopic expression of WIF1 and SFRP1 shows a relative lack of response. Chronic Wnt3a stimulation only partially reverses growth suppression after DKK1 reexpression, whereas a specific inhibitor of the JNK pathway significantly reversed the effect of DKK1 reexpression on colony formation and apoptosis in T98G cells. This support the potential growth-suppressive function for epigenetically silenced DKK1 in GBM and suggests that DKK1 restoration could modulate Wnt signaling through both canonical and noncanonical pathways. These investigations suggest an important role of epigenetic silencing of Wnt pathway inhibitor genes in gliomas, particularly in glioblastomas, with distinct patterns of hypermethylated genes distinguishing primary from secondary glioblastomas.

2.2 Wnt ligands and frizzled receptors

Wnt proteins are secreted glycoproteins which are modified through post-translational modifications such as palmitoylation, required for correct secretion and N-linked glycosylation which increases the stability of Wnts. These are categorized according to canonical and noncanonical based on the pathway activated by Wnts. It was reported that a number of Wnt genes, including Wnt1, Wnt2, Wnt3a, Wnt5a, Wnt7a, Wnt7b, Wnt 10b and Wnt13, have been associated with tumor development. There were several studies investigated the expression of Wnts in gliomas. Howng *et al*. (2002) studied the mRNA expression of Wnt1, Wnt5a, Wnt10b and Wnt13 in brain tumors. It was observed that Wnt 5a, Wnt10b, Wnt13 were expressed in most of the brain tumors, whereas Wnt1 was less expressed. Further no correlations were observed between Wnts expression and histopathological grading. Yu *et al*. (2007) showed that Wnt5a was upregulated in glioblastomas than that of lowgrade tumors and normal brain samples and overexpression of Wnt5a in glioblastoma cell lines increased the proliferation. Knockdown of Wnt5a using siRNA resulted in the reduction of proliferation of glioblastoma cell lines and also reduced the tumor growth *in vivo*. Pu *et al*. (2009) studied the mRNA expression of Wnt1, Wnt2, Wnt3, Wnt4, Wnt5a, Wnt10b and Wnt13 and frizzled receptors Fzd2 and Fzd5. The expression levels of Wnt2, Wnt5a and Fzd2 mRNA and protein were overexpressed in astrocytomas but not expressed in normal brain tissues and exhibited significant positive correlation with the degree of malignancy. Whereas, Wnt1 and Fzd5 were not expressed in gliomas and Wnt3, Wnt4, Wnt10b, Wnt13 expression were almost equally expressed in

tumor and normal brain tissues. Knockdown of Wnt2 in glioma cell lines significantly downregulated the expression of Wnt2, β-catenin as well as expression of Fzd2, p-GSK3β, cyclin D1, PI3K, and p-Akt. Further, Wnt2 knockdown also resulted in the reduced cell viability, cell cycle arrest in Go/G1 phase with lowered S phase proportion, increase in apoptotic cell population and reduced invasive ability. In nude mice experiments of xenograft tumors, Wnt2 siRNA treatment slowdown the tumor growth and the tumor size was significantly lower than control treated. Also in this subcutaneous model the expression levels of Wnt2, fzd2, β-catenin and p-GSK3β were decreased following Wnt2 siRNA treatment and induce tumor cell apoptosis. Zhang *et al*. (2006) studied the role of Frizzled 9 in astrocytoma samples and reported that Frizzled 9 was upregulated in astrocytomas.

2.3 Dishevelled and FRAT-1

Dishevelled (Dvl) identified as positive regulator of Wnt signaling pathway positioning downstream of the frizzled receptor and upstream of β-catenin. Overexpression or constitutive activation of dishevelled promotes neoplastic transformation and its involvement has been reported in various human cancers. Dvl performs dual functions, on one hand it transduces Wnt signals to stabilize the β-catenin and on the other hand it relays the signals for the activation of Jun kinases. Three variants of Dvls were identified to date and their prominent role in tumor progression was reported in several human cancers. In canonical Wnt signaling Dvl interacts with Axin and dissociates the destruction complex by relocating the Axin to the cytoplasmic tail of LRP. Also by interaction with FRAT1/GBP it inhibits the GSK3β activity. These mechanisms lead to the stabilization of β-catenin levels in the cytoplasm. We studied (Sareddy *et al*., 2009a) the expression status of Dvl variants in astrocytoma tissues and found that Dvl-3 was upregulated in tumor tissues while very low expression was observed in normal brain sample. The expression levels were progressively increased from low grade to high grade astrocytomas and showed significant positive correlation with the pathological grade of astrocytomas.

FRAT1 (frequently arranged in advanced T-cell lymphomas-1) is an inhibitor of GSK-3β and was identified as a positive regulator of Wnt/β-catenin pathway by inhibiting the GSK-3β activity there by stabilizing β-catenin. Its oncogenic role was established in several human cancers. In response to Wnt signaling activation Dishevelled protein recruits FRAT1 into the destruction complex which facilitates the dissociation of GSK3β from destruction complex, leading to the stabilization of β-catenin. Guo *et al*. (2010) found that FRAT1 was overexpressed in several human astrocytoma samples and showed the significant positive correlation with the pathological grading of astrocytomas. In normal brain samples FRAT1 expression was very low compared to tumor samples. FRAT1 immunoreactivity was also positively correlated with the β-catenin immunoreactivity.

2.4 AXIN-APC-GSK3β

Axin, a tumor suppressor was originally identified as an inhibitor of Wnt signaling and is central to the down regulation of β-catenin. Axin acts as a scaffolding protein which facilitates the phosphorylation of β-catenin by GSK3β and CK1α and subsequent proteasomal degradation. It is a core component of destruction complex and binds directly to β-catenin, APC, GSK3 β, CK1α and Dvl. It is often mutated in several human

malignancies. Nikuseva Martic *et al*. (2010) examined the changes of Axin1 in 72 neuroepithelial brain tumors. LOH experiments showed the LOH of AXIN1 in 11.1% of brain tumors and majority proportion was distributed to glioblastomas (6.3%). Compared to healthy brain tissues Axin1 expression was down regulated in 65.5% of brain tumors, of which majority of them are astrocytomas. In most of the samples Axin was localized in the cytoplasm. It was also observed that β-catenin was localized mainly in nucleus or cytoplasm and nucleus. They also demonstrated that there was no difference in Axin protein levels in patients with AXIN1 LOH and patients without it. In contrast, relative β-catenin levels were significantly higher in patients with AXIN LOH in comparison to without it. Comparison of relative levels of β-catenin and AXIN1 revealed that they were significantly reversely proportional.

Adenomatous polyposis coli gene (APC) is a tumor suppressor and is often mutated in wide variety of human cancers. It was demonstrated that most of the colon and other human malignancies having highly constitutive β-catenin due to mutations in APC or β-catenin itself. Mutations in APC and β-catenin genes were noticed in the childhood brain tumor medulloblastoma but till to date no mutations were found in gliomas. However, recent reports provided the evidence that the APC mutations are in secondary brain metastasis rather than in primary tumors. Pecina-Slaus *et al*. (2010) analyzed exon 15 of the APC in a 49-year-old male patient with brain metastasis and its primary site was lung carcinoma. PCR method and direct DNA sequencing of the metastasis and autologous lymphocyte samples identified the presence of a somatic mutation at position 5883 G–A in the metastasis tissue. Interestingly, these authors observed that the lack of protein expression of APC, however, β-catenin localization was observed in the nucleus. The mutation is a silent mutation that might have consequences in the creation of a new splice site. To date no prominent mutations were observed in β-catenin but it was reported that β-catenin is mutated at S33 as sporadic event in a brain metastasis (Lee *et al*., 2009). Further, this mutated β-catenin enhances the Tcf dependent promoter activity.

Glycogen synthase kinase 3 is a serine/threonine kinase regulates diverse signaling pathways involved in proliferation, apoptosis and cell cycle control. GSK3 has two isoforms include GSK3α, GSK3β which are functionally independent. It performs independent functions based on the cell type, and it described as a prosurvival factor in pancreatic cancer and as a pro-apoptotic factor in colon cancer and interconnects several pathways which plays major role in several human disorders. Korur *et al*. (2009) analyzed the role of GSK3β in malignant gliomas and reported that mRNA and protein levels of GSK-3β were overexpressed in human glioblastoma tissues compared to normal brain tissues. Direct inhibition of GSK3β activity by siRNA, the specific inhibitor SB216763, or lithium chloride (LiCl) induced tumor cell differentiation, tumor cell apoptosis and reduced the clonogenecity.

2.5 β-Catenin

β-Catenin was first identified as a member of Wnt signaling pathway. The β-catenin gene *CTNNb1* was localized at 3p2.1. β-catenin existed in two pools: one is located in the cytomembrane which involved in cell adhesion and other is in the cytoplasm or/and nucleus which is mainly involved in the regulation of Wnt pathway. It was identified that mutations in the β-catenin gene that affect specific serine and threonine residues abrogates

the phosphorylation dependent degradation of β-catenin. These regulatory sequences are often mutated in wide variety of human cancers. It was noticed that colorectal tumors that contain APC mutations harbors low β-catenin mutations where as in tumors lacking APC, mutations in CTNNb1 was greatly increased. Dissociation of cytoplasmic inhibitory complex owing to the upstream activity of Wnt signals or mutations in APC and Axin leads to the cytosolic accumulation of β-catenin. The Oncogenic potential of β-catenin was extensively studied in wide variety of human cancers. We studied the expression levels of β-catenin mRNA and protein in 32 human astrocytoma samples and found that the mRNA and protein levels of β-catenin were elevated in astrocytoma samples while very low levels were observed in normal brain tissues. The expression levels of β-catenin were progressively increased form low grade astrocytomas to higher grades and positively correlated with the histological grading of astrocytomas. In most of the tumor sections β-catenin was accumulated in the nucleus and cytosol which is crucial in mediating Wnt pathway activity (Sareddy *et al*. 2009a). Moreover, we examined the β-catenin levels in the progression of ENU-induced gliomas in rat model. In this study, we identified that the levels of β-catenin were increased as tumor grows and correlated with malignant progression of gliomas (Sareddy *et al*. 2009b). Pu *et al*. (2009) also showed that knockdown of β-catenin using siRNA downregulated the expression of β-catenin, p-GSK3β, cyclin D1, PI3K, and pAKT and reduced the cell viability, arrest the cell cycle at G0/G1 phase with lowered S phase cells, increased the apoptotic cell population and inhibited the invasion of glioma cell lines. Further, treatment with β-catenin siRNA in subcutaneous xenograft model reduced and slowed down the tumor size and growth respectively. In these tumors β-catenin siRNA treatment caused the reduction in the levels of β-catenin, pGSK3β, PI3K, Wnt2 and Fzd2 and induced the apoptosis. Similar results were also observed by Liu *et al*. (2010), stating that expression levels of β-catenin mRNA and protein were elevated in astrocytomas in comparison with normal brain tissues and showed the positive correlation with the grade of the astrocytomas. β-catenin siRNA transfection into glioma cell lines inhibited the cell proliferation, induced the apoptosis, arrested the cell cycle in G0/G1 phase and downregulated the expression of β-catenin targets such as cyclin D1, c-jun and c-Myc. Zhang *et al*. (2009) studied the correlation between β-catenin distribution, tumor grade and patient 2 year survival. They found that there was no correlation between grade of the tumor and distribution of β-catenin. However, significant positive correlation was observed between levels of β-catenin and 2-year patient survival. The expression of β-catenin was not correlated with other clinicopathological characteristics such as tumor age, tumor size, and sex and tumor location. Further, survival analysis showed that patients with astrocytoma showing less expression of β-catenin tend to be associated with good prognosis, whereas, astrocytoma patients with high β-catenin expression associated with poor prognosis.

2.6 Lef-1 and Tcf-4

Lef/Tcf family transcription factors consist of four members: Lef-1, Tcf-1, Tcf-3 and Tcf-4. Lef/Tcf transcription factors belong to high mobility group (HMG) domain proteins that recognize the same DNA consensus motif through HMG box DNA-binding domain. Tcf-1 is predominantly expressed in cells of T cell lineage and Lef-1 is expressed in pre-B and T cells. Lack of Tcf-1 and Lef-1 augment the T cell development and arrest the development at earlier stages. Tcf-3 is expressed in somatic epithelium, keratinocytes of the skin. Tcf-4 is

expressed in midbrain and in epithelium of intestine and mammary glands. Constitutive expression of Tcf-4 is essential for maintenance of gut epithelium, however, constitutive active β-catenin-Tcf-4 complexes were observed in colon carcinoma. In the absence of β-catenin, Tcf/Lef factors suppress the Wnt target gene expression by binding with members of the Groucho (Grg/TLE) family of transcriptional co-repressors. Translocation of β-catenin converts Tcf family proteins into potent transcriptional activators by displacing Groucho/TLE proteins and recruiting an array of co-activator proteins including CBP, TBP, BRG1, BCL9/PYG, Legless, Mediator and Hyrax (Barker and Clevers., 2006). β-catenin does not have a DNA binding domain, but it has a potent transcription activation domain. In general, Lef/Tcf transcription factors do not have a strong transcription activation domain, but they have a good DNA binding/bending domain. Thus, when β-catenin binds to Lef/Tcf proteins, a potent transcription regulatory complex is formed. We examined the expression levels of Lef1 and Tcf4 in human astrocytic samples and found that compared to normal brain tissues Lef1 and Tcf-4 expression levels were significantly elevated in astrocytomas and in most of the tumors these proteins were localized in the nucleus. The increased Tcf4 and Lef1 levels were significantly correlating with the pathological grade of astrocytomas. We also demonstrated the interaction of β-catenin with Tcf4 in the nuclear fractions of GBM tissues and in cell lines. In addition, we also found that the Lef-1 and tcf-4 levels were elevated in ENU-induced rat gliomas compared to control rats and protein levels were progressively increased form initial stage to advanced stages. Oncogenic activity of Wnt signaling is mediated through overexpression of their target genes cyclin D1, c-Myc, c-Jun, MMP2, N-Myc etc. These proteins play essential roles in the cell cycle progression, cell proliferation and survival. In our study we observed the elevated levels of cyclin D1, c-Myc, c-jun, N-Myc in astrocytomas of different clinical grade in comparison with normal brain samples. The protein levels were significantly correlating with the histological grading of astrocytomas and similar results were also observed in ENU-induced gliomas.

2.7 Pygopus 2

When β-catenin is accumulated and translocated to nucleus, β-catenin relieves the action of corepressors and recruits the array of coactivators to Tcf/Lef factors such as CBP, TBP, BRG1, Legless, Mediator, Hyrax, Bcl-9 (B-cell lymphoma-9), and pygopus. These coactivators are essential for β-catenin/Tcf dependent transcription. Wang *et al*. (2010) examined the pygopus 2 expression in human astrocytoma samples. Pygopus 2 levels were overexpressed in astrocytoma samples compared to controls and exhibited a positive correlation with tumor grade. Knockdown of pygopus with small hairpin RNA (shRNA) resulted in inhibition of cell proliferation, colony forming ability, BrdU incorporation and invasiveness of human and rat glioma cell lines. Knockdown of pygopus 2 also resulted in the arrest of cell cycle at G1 stage and this was associated with reduction of S phase cell population. Knockdown of pygopus 2 also leads to the decreased expression of Wnt target gene cyclin D1 without altering the β-catenin levels and its nuclear translocation. Further, Chen *et al*. (2010) examined the pygopus 2 role in rat glioma cell lines. Overexpression of pygopus significantly enhances the cell proliferation and cell cycle progression from G1 to S and this was associated with the elevation of cyclin D1 levels without altering the β-catenin levels. Moreover, pygo2 expression levels were significantly correlated with the expression of cyclin D1 in human glioma samples.

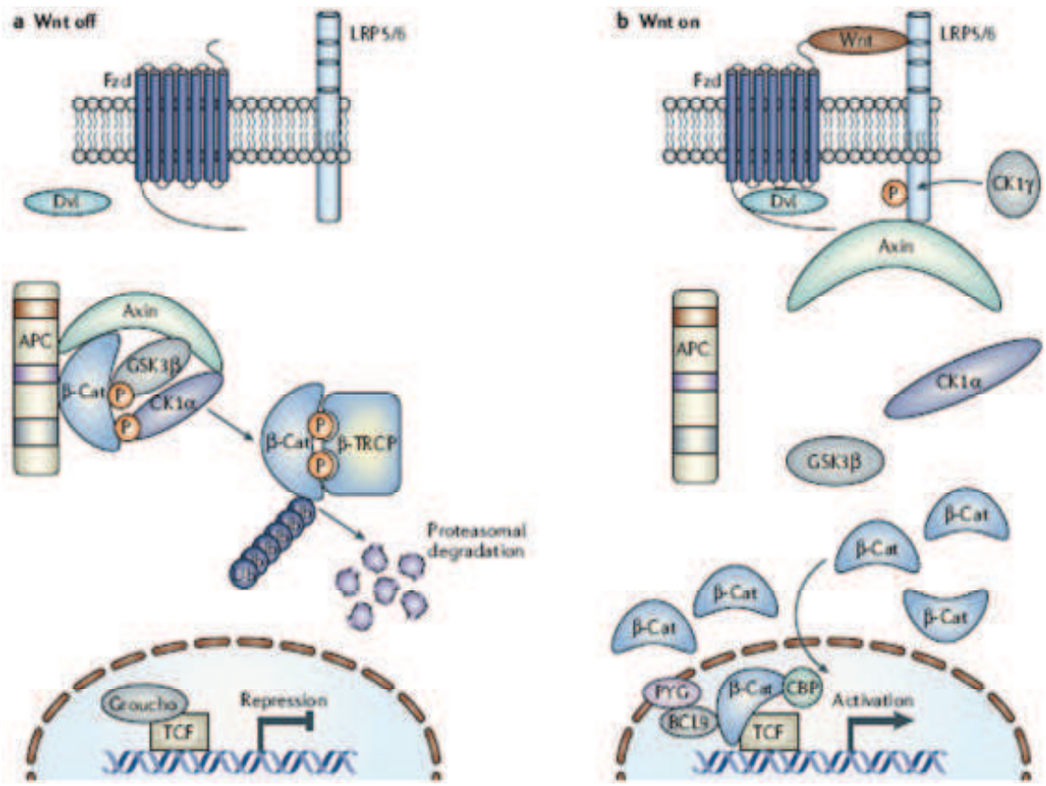


Fig. 1. **An overview of the Wnt signaling pathway**. **(a)** In the absence of a Wnt signal, β-catenin is captured by APC and axin within the destruction complex, facilitating its phosphorylation by the kinases CK1α and GSK3β. CK1α and GSK3β then sequentially phosphorylate a conserved set of serine and threonine residues at the amino terminus of β-catenin. This facilitates binding of the β-TRCP, which subsequently mediates the ubiquitinylation and efficient proteasomal degradation of β-catenin. The resulting β-catenin 'drought' ensures that nuclear DNA-binding proteins of the Tcf/Lef transcription factor family (TCF1, TCF3, TCF4 and LEF1) actively repress target genes by recruiting transcriptional co-repressors (Groucho/TLE) to their promoters and/or enhancers. **(b)** Interaction of a Wnt ligand with its specific receptor complex containing a Frizzled family member and LRP5 or LRP6 triggers the formation of Dvl–Fzd complexes and the phosphorylation of LRP by CK1γ, facilitating relocation of axin to the membrane and inactivation of the destruction box. This allows β-catenin to accumulate and enter the nucleus, where it interacts with members of the Tcf/Lef family. In the nucleus, β-catenin converts the Tcf proteins into potent transcriptional activators by displacing Groucho/TLE proteins and recruiting an array of coactivator proteins including CBP, TBP, BRG1, BCL9/PYG, Legless, Mediator and Hyrax. This ensures efficient activation of Tcf target genes such as c-MYC, which instruct the cell to actively proliferate and remain in an undifferentiated state. Following dissipation of the Wnt signal, β-catenin is evicted from the nucleus by the APC protein and Tcf proteins revert to actively repressing the target gene program. β-TRCP, β-transducin repeat-containing protein; APC, adenomatous polyposis coli; BCL9, B-cell lymphoma 9; CK1α, casein kinase 1α; CK1γ, casein kinase 1γ; CBP, CREB-binding protein; Fzd, Frizzled; Lef, lymphoid enhancer factor; LRP, low-density lipoprotein receptor-related protein; PYG, Pygopus; Tcf, T-cell factor. (This illustration is reproduced from Barker *et al*., 2006).

3. Conclusion

In summary, recent activities that deal with the study of Wnt/β-catenin/Tcf signaling pathway in gliomas insist that this pathway is activated in human gliomas and involved in the malignant progression of gliomas. This pathway attracted as candidate therapeutic target and inhibition of this pathway may curtail the malignant progression of gliomas.

4. References

Barker, N., and Clevers, H. 2006. Mining the Wnt pathway for cancer therapeutics. Nat. Rev. Drug. Discov. 5: 997-1014.

Chen, Y. Y., Li, B. A., Wang, H. D., Liu, X. Y., Shen, S. H., Zhu, H. W., and Wang, H. D. 2011. The role of pygopus 2 in rat glioma cell growth. Med. Oncol.28:631–64.

Foltz, G., Yoon, J. G., Lee, H., Ma, L., Tian, Q., Hood, L., and Madan, A., 2010. Epigenetic regulation of Wnt pathway antagonists in human glioblastoma multiforme. Genes & Cancer 1: 81-90.

Gotze, S., Wolter, M., Reifenberger, G., Muller, O., and Sievers, S. 2010. Frequent promoter hypermethylation of Wnt pathway inhibitor genes in malignant astrocytic gliomas. Int. J. Cancer 126: 2584-2593.

Guo, G., Mao, X., Wang, P., Liu, B., Zhang, X., Jiang, X., Zhong, C., Huo, J., Jin, J., and Zhuo, Y. 2010. The expression profile of FRAT1 in human gliomas. Brain Res. 1320: 152-158.

Howng, S. L., Wu, C. H., Cheng, T. S., Sy, W. D., Lin, P. C., Wang, C., and Hong, Y. R. 2002. Differential expression of Wnt genes, beta-catenin and E-cadherin in human brain tumors. Cancer Lett. 183: 95-101.

Kleihues, P., and Ohgaki, H. 1999. Primary and secondary glioblastomas: From concept to clinical diagnosis. Neuro Oncol. 1: 44-51.

Korur, S., Huber, R. M., Sivasankaran, B., Petrich, M., Morin, P. Jr., Hemmings, B. A., Merlo, A., and Lino, M. M. 2009. GSK3beta regulates differentiation and growth arrest in glioblastoma. PLoS One 4: e7443.

Lee, C. I., Hsu, M. Y., Chou, C. H., Wang, C., Lo, Y. S., Loh, J. K., Howng, S. L., and Hong, Y. R. 2009. CTNNB1 (beta-catenin) mutation is rare in brain tumours but involved as a sporadic event in a brain metastasis. Acta Neurochir. 151: 1107-1111.

Liu, X., Wang, L., Zhao, S., Ji, X., Luo, Y., and Ling, F. 2010. beta-Catenin overexpression in malignant glioma and its role in proliferation and apoptosis in glioblastoma cells. Med. Oncol. 16: 75-79.

Logan, C. Y., and Nusse, R. 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell. Dev. Biol. 20: 781-810.

Louis, D. N., Ohgaki, H., Wiestler, O. D., Cavenee, W. K., Burger, P. C., Jouvet, A., Scheithauer, B. W., and Kleihues, P. 2007. The 2007 WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol. 114: 97–109.

Mizobuchi, Y., Matsuzaki, K., Kuwayama, K., Kitazato, K., Mure, H., Kageji, T., and Nagahiro, S. 2008. REIC/Dkk-3 induces cell death in human malignant glioma. Neuro Oncol. 10: 244-253.

Moon, R. T., Kohn, A. D., De Ferrari, G. V., and Kaykas, A. 2004. WNT and beta-catenin signalling: diseases and therapies. Nat. Rev. Genet. 5: 691-701.

Muller, W., Lass, U., Wellmann, S., Kunitz, F., and von Deimling, A. Mutation analysis of DKK1 and in vivo evidence of predominant p53-independent DKK1 function in gliomas. Acta Neuropathol. 109: 314-320.

Nikuseva Martic, T., Pecina-Slaus, N., Kusec, V., Kokotovic, T., Musinovic, H., Tomas, D., and Zeljko, M. 2010. Changes of AXIN-1 and beta-catenin in neuroepithelial brain tumors. Pathol. Oncol. Res. 16: 75-79.

Nupponen, N. N., and Joensuu, H. 2006. Molecular pathology of gliomas. Curr. Diagn. Pathol. 12: 394-402.

Ohgaki, H., and Kleihues, P. 2007. Genetic pathways to primary and secondary glioblastoma. Am. J. Pathol. 170: 1445-1453.

Ohgaki, H., and Kleihues, P. 2009. Genetic alterations and signaling pathways in the evolution of gliomas. Cancer Sci. 100: 2235-2241.

Pecina-Slaus, N., Majic, Z., Musani, V., Zeljko, M., and Cupic, H. 2010. Report on mutation in exon 15 of the APC gene in a case of brain metastasis. J. Neurooncol. 97: 143-148.

Pu, P., Zhang, Z., Kang, C., Jiang, R., Jia, Z., Wang, G., and Jiang, H. 2009. Downregulation of Wnt2 and beta-catenin by siRNA suppresses malignant glioma cell growth. Cancer Gene Ther. 16: 351-361.

Roth, W., Wild-Bose, C., Platten, M., Grimmel, C., Melkonyan, H.S., Dichgans, J., and Weller, M. 2000. Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. Oncogene 19: 4210-4220.

Sareddy, G. R., Challa, S., Panigrahi, M., and Babu, P. P. 2009b. Wnt/beta-catenin/Tcf signaling pathway activation in malignant progression of rat gliomas induced by transplacental N-ethyl-N-nitrosourea exposure. Neurochem. Res. 34: 1278-1288.

Sareddy, G. R., Panigrahi, M., Challa, S., Mahadevan, A., and Babu, P. P. 2009a. Activation of Wnt/beta-catenin/Tcf signaling pathway in human astrocytomas. Neurochem. Int. 55: 307-317.

Shou, J., Ali-Osman, F., Muttani, A. S., Pathak, S., Fedi, P., and Srivenugopal, K. S. 2002. Human Dkk-1, a gene encoding a Wnt antagonist, responds to DNA damage and its overexpression sensitizes brain tumor cells to apoptosis following alkylation damage of DNA. Oncogene 21: 878-889.

Wang, Z. X., Chen, Y Y., Li, B. A., Tan, G. W., Liu, X. Y., Shen, S. H., Zhu, H. W., and Wang, H. D. 2010. Decreased pygopus 2 expression suppresses glioblastoma U251 cell growth. J. Neurooncol. 100: 31-41.

Yang, Z., Wang, Y., Fang, J., Chen, F., Liu, J., Wu, J., and Wang, Y. 2010. Expression and aberrant promoter mehtylation of Wnt inhibitory factor-1 in human astrocytomas. J. Exp. Clin. Cancer Res. 29: 26.

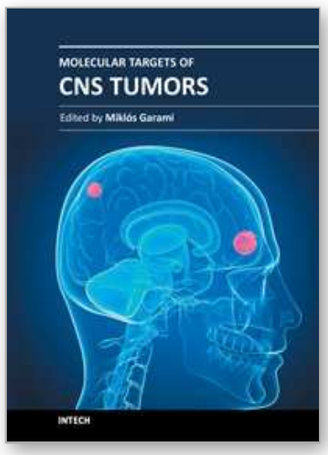
Yu, J. M., Jun, E. S., Jung, J. S., Suh, S. Y., Han, J. Y., Kim, J. Y., Kim, K. W., and Jung, J. S. 2007. Role of Wnt5a in the proliferation of human glioblastoma cell lines. Cancer Lett. 257: 172-181.

Zhang, L. Y., Jiang, L. N., Li, F. F., Li, H. Liu, F., Gu, Y., Song, Y., Zhang, F., Ye, J., and Li, Q. 2009. Reduced beta-catenin expression is associated with good prognosis in astrocytoma. Pathol. Oncol. Res. 16: 253-257.

Zhang, Z., Schittenhelm, J., Guo, K., Buhring, H. J., Trautmann, K., Meyermann, R., and Schluesener, H. J. 2006. Upregulation of frizzled 9 in astrocytomas. Neuropathol. Appl. Neurobiol. 32: 615-624.

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Molecular Targets of CNS Tumors

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Molecular Targets of CNS Tumors is a selected review of Central Nervous System (CNS) tumors with particular emphasis on signaling pathway of the most common CNS tumor types. To develop drugs which specifically attack the cancer cells requires an understanding of the distinct characteristics of those cells. Additional detailed information is provided on selected signal pathways in CNS tumors.

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