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Vital Elements of the Wnt-Frizzled Signaling Pathway in the Nervous System

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Abstract

Wnt proteins are cysteine-rich glycosylated proteins named after the *Drosophilia Wingless* (*Wg*) and the mouse *Int-1* genes that play a role in embryonic cell patterning, proliferation, differentiation, orientation, adhesion, survival, and programmed cell death (PCD). Wnt proteins involve at least two intracellular signaling pathways. One pathway controls target gene transcription through β-catenin, generally referred to as the canonical pathway and a second pathway pertains to intracellular calcium (Ca^{2+}) release which is termed the non-canonical or Wnt/Ca^{2+} pathway. The majority of Wnt proteins activate gene transcription through the canonical signaling pathway regulated by pathways that include the Frizzled transmembrane receptor and the co-receptor LRP-5/6, Dishevelled, glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), and β-catenin. In contrast, the non-canonical Wnt signaling pathway has two intracellular signaling cascades that consist of the Wnt/Ca^{2+} pathway with protein kinase C (PKC) and the Wnt/PCP pathway involving Rho/Rac small GTPase and Jun N-terminal kinase (JNK). Through a series of signaling pathways, Wnt proteins modulate cell development, proliferation, and cell fate. In regards to cell survival and fate through PCD, Wnt may be critical for the prevention of tissue pathology that involves cytokine and growth factor control during disorders such as neuropsychiatric disease, retinal disease, and Alzheimer's disease. Elucidation of the vital elements that shape and control the Wnt-Frizzled signaling pathway may provide significant prospects for the treatment of disorders of the nervous system.

Keywords

Adenomatous polyposis coli; Akt; alzheimer's; β-catenin; dishevelled; erythropoietin; frizzled; GSK-3β; neurons; psychiatric; retinal disease; stem cells; vascular endothelial growth factor

FUNCTIONAL CLASSES OF THE WNT FAMILY

Named after the *Drosophilia Wingless* (*Wg*) and the mouse *Int-1* genes, Wnt proteins are secreted cysteine-rich glycosylated proteins that play a role in embryonic cell patterning, proliferation, differentiation, orientation, adhesion, survival, and apoptosis (Chong, ZZ and Maiese, K, 2004, Melkonyan, HS *et al*., 1997, Nelson, WJ and Nusse, R, 2004, Nusse, R and Varmus, HE, 1982, Patapoutian, A and Reichardt, LF, 2000, Smalley, MJ and Dale, TC, 1999, Wodarz, A and Nusse, R, 1998). Wnt proteins are divided into two functional classes based on their ability to induce a secondary body axis in *Xenopus* embryos and to activate

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certain signaling cascades that consist of the Wnt1 class and the Wnt5a class. The members of the Wnt1 class lead to a secondary body axis in *Xenopus* and include Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8 and Wnt8a. Wnt proteins of this class facilitate activation of the Frizzled transmembrane receptor and the co-receptor lipoprotein related protein 5 and 6 (LRP-5/6). This leads to the activation of the typical canonical Wnt/β-catenin pathway. The Wnt5a class cannot induce secondary axis formation in *Xenopus* and includes the Wnt proteins of Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a and Wnt11. These Wnt proteins bind the Frizzled transmembrane receptor to activate heterotrimeric G proteins and increase intracellular calcium levels. In addition, the Wnt proteins can induce Rho-dependent changes in the actin cytoskeleton.

The receptors of the Wnt proteins consist of at least 10 family members termed the Frizzled proteins after the first member, *Drosophila* tissue polarity gene *Frizzled* (Adler, PN *et al*., 1990, Vinson, CR *et al*., 1989). Members of the Frizzled protein family have several characteristics. These include a N-terminal signal peptide, an extracellular domain that contains a 120-amino acids, a cysteine-rich domain followed by a hydrophilic linker region that shows little sequence similarity among family members, a highly conserved seven-transmembrane domain separated by short extracellular and cytoplasmic loops, and a cytoplasmic domain of variable size and little sequence homology among family members (Adler, PN *et al*., 1990, Hsieh, JC, 2004, Vinson, CR *et al*., 1989, Wang, Y *et al*., 1996, Wodarz, A *et al*., 1998).

The Wnt proteins bind to the activity sites of Frizzled receptor proteins that are relevant to either the canonical and non- canonical Wnt-Frizzled signaling pathways leading to specific biological functions. In addition to the Frizzled protein receptors, other obligate co-receptors also are necessary for canonical Wnt-Frizzled signaling pathway. An additional single-pass transmembrane protein named as LRP-5/6 from the low-density-lipoprotein receptor family is required for this process (Pinson, KI *et al*., 2000, Tamai, K *et al*., 2000, Wehrli, M *et al*., 2000). In the canonical Wnt-Frizzled signaling pathway, Wnt binds to both the Frizzled transmembrane receptor and the co-receptor LRP-5/6 (Wehrli, M *et al*., 2000) resulting in the inhibition of the downstream component glycogen synthase kinase-3β (GSK-3β) (Ikeda, S *et al*., 1998, Papkoff, J and Aikawa, M, 1998). Wnt signaling also can be transmitted through the binding of extracellular domain of LRP-5/6 to Axin, a key component in the GSK-3β complex, indicating that the LRP-5/6 receptor is an important part of the Wnt-Frizzled signaling pathway (Mao, J *et al*., 2001, Tolwinski, NS *et al*., 2003).

Another co-receptor for the canonical and non- canonical Wnt-Frizzled signaling pathway is Ryk that belongs to one of divergent members of the receptor tyrosine kinase family. Ryk not only can form a complex with Frizzled proteins such as the co-receptor LRP-5/6 resulting in activation of the canonical Wnt-Frizzled signaling pathway, but also can regulate the non-canonical Wnt-Frizzled signaling pathway through Frizzled-independent pathways (Bejsovec, A, 2005, Cheyette, BN, 2004). The molecular structure of all Ryk genes is characterized by an extracellular domain with homology to Wnt inhibitory factor-1 (WIF1), a single transmembrane-spanning sequence to which Wnt proteins bind (Patthy, L, 2000, Schneider, S *et al*., 1999). In addition, a conserved intracellular PDZ-binding motif exists which links Ryk to downstream molecules of Wnt-Frizzled signaling pathway, such as Dishevelled (Bejsovec, A, 2005, Cheyette, BN, 2004, Lu, W *et al*., 2004). Wnt proteins can bind to the extracellular domain of the Ryk receptor through the intracellular PDZ-binding domain in the Ryk receptor to result in regulating cell proliferation, differentiation, migration, polarity, survival, and death through either the canonical or the non- canonical Wnt-Frizzled signaling pathway.

THE CANONICAL AND NON-CANONICAL PATHWAYS

Wnt involves at least two intracellular signaling pathways. One pathway controls target gene transcription through β-catenin, generally referred to as the canonical pathway that involves

Wnt1, Wnt3a, and Wnt8 and functions through β-catenin-dependent pathways. Another pathway pertains to intracellular calcium (Ca^{2+}) release which is termed the non-canonical or Wnt/Ca^{2+} pathway consisting primarily of Wnt-4, Wnt-5a, and Wnt-11 that functions through non-β-catenin-dependent pathways, such as the planar cell polarity (PCP) pathway (Nusse, R, 1999, Patapoutian, A *et al*., 2000, Salinas, PC, 1999, Tada, M and Smith, JC, 2000) and the Wnt-Ca^{2+}-dependent pathways (Katoh, M, 2002, Kuhl, M *et al*., 2000, Nusse, R, 1999, Patapoutian, A *et al*., 2000, Salinas, PC, 1999, Slusarski, DC *et al*., 1997).

Upon binding to either the Frizzled receptor or a receptor complex consisting of Frizzled and LRP5/6, Wnt protein can activate one of three different signaling cascades. These cascades include the canonical Wnt signaling pathway (Nusse, R, 1999, Rattner, A *et al*., 1997), the Wnt/PCP pathway (Nusse, R, 1999, Rattner, A *et al*., 1997, Veeman, MT *et al*., 2003), or the Wnt/Ca^{2+} pathway (Katoh, M, 2002, Kuhl, M *et al*., 2000, Nusse, R, 1999, Patapoutian, A *et al*., 2000, Salinas, PC, 1999, Slusarski, DC *et al*., 1997). Each of pathways, although distinct, appears to be transduced initially through Dishevelled, a cytoplasmic multi-functional phosphoprotein (Axelrod, JD *et al*., 1998, Boutros, M and Mlodzik, M, 1999, Boutros, M *et al*., 1998). In mammals, the Dishevelled protein family members contains Dishevelled-1, Dishevelled-2, Dishevelled-3 in all organs. These family members have three highly conserved domains that include an N-terminal DIX domain named for Dishevelled and Axin, a central PDZ domain termed for Postsynaptic density-95, Discs-large and Zonula occludens-1, and a C-terminal DEP that is named for Dishevelled, Egl-10 and Pleckstrin (Habas, R and Dawid, IB, 2005, Wharton, KA, Jr., 2003). At the level of Dishevelled, the Wnt signaling pathway can be separated along one of three different cascades that are dependent upon the three highly conserved domains of Dishevelled. As a result, Dishevelled is a key transducer of the Wnt signal that acts at the plasma membrane or in the cytoplasm in all three Wnt-Frizzled signaling pathways. However, new work has suggested that Dishevelled also acts within the nucleus and nuclear location of Dishevelled is essential for its function in the Wnt-Frizzled signaling pathway (Itoh, K *et al*., 2005, Weitzman, JB, 2005).

The majority of Wnt proteins activate gene transcription through the canonical signaling pathway controlled by β-catenin. In general, all Wnt signaling pathways are initiated by interaction of Wnt proteins with Frizzled receptors, but in this pathway, the Wnt signaling pathway will only be activated if the binding of the Wnt protein to the Frizzled transmembrane receptor takes place in the presence of the co-receptor LRP-5/6 (Mao, J *et al*., 2001, Pinson, KI *et al*., 2000, Wehrli, M *et al*., 2000) resulting in the formation of a Wnt-Frizzled-LRP5/6 trimolecular complex. Once Wnt protein binds to the Frizzled transmembrane receptor and the co-receptor LRP-5/6, this is followed by recruitment of Dishevelled. Dishevelled is phosphorylated by casein kinase Iε to form a complex with Frat1 and inhibit GSK-3β activity (Ikeda, S *et al*., 1998, Kishida, M *et al*., 2001, Lee, E *et al*., 2001, Lee, JS *et al*., 1999, Papkoff, J *et al*., 1998). In addition, the formation of the Wnt-Frizzled-LRP5/6 complex also promotes the LRP5/6-mediated degradation of Axin (Mao, J *et al*., 2001).

The combined inhibition of GSK-3β activity with the degradation of Axin blocks the formation of the protein complex consisting of GSK-3β, Axin, and adenomatous polyposis coli (APC) tumor suppressor protein. Yet, during the absence of Wnt signaling, β-catenin is associated with the protein complex of GSK-3β, Axin and APC tumor suppressor protein. β-catenin is phosphorylated by activation of GSK-3β leading to its ubiquitination and subsequent degradation by proteosomes (Aberle, H *et al*., 1997, Hart, M *et al*., 1999, Latres, E *et al*., 1999, Patapoutian, A *et al*., 2000, Winston, JT *et al*., 1999). As a result, β-catenin cannot translocate into the nucleus and physically bind to DNA in order to activate the transcription of its target genes. In the absence of β-catenin in the nucleus, the T cell factor (Tcf) and lymphocyte enhancer factor (Lef) (Tcf/Lef) family members are associated with transcriptional inhibitors, such as Groucho (Cavallo, RA *et al*., 1998, Roose, J *et al*., 1998).

Yet, without the formation of the protein complex of GSK-3β, Axin and APC tumor suppressor protein, phosphorylation of β-catenin with its subsequent degradation does not occur and the accumulation of free β-catenin results for translocation to the nucleus (Akiyama, T, 2000, Cavallo, RA *et al*., 1998, Ikeda, S *et al*., 1998, Roose, J *et al*., 1998). Once positioned in the nucleus, the free β-catenin acts as a transcription factor and activates Tcf and Lef by forming nuclear complexes with members of the Tcf/Lef transcription factor family (Ishitani, T *et al*., 2003). This leads to the transcription and expression of a variety of Wnt-responsive target genes such as c-Myc (He, TC *et al*., 1998), cyclin D1 (Nusse, R, 1999, Shtutman, M *et al*., 1999, Tetsu, O and McCormick, F, 1999), and Axin 2 (Jho, EH *et al*., 2002, Lustig, B *et al*., 2002).

The non-canonical Wnt signaling pathway, also termed the atypical Wnt-Frizzled signaling pathway, has two intracellular signaling cascades that consist of the Wnt/Ca^{2+} pathway and the Wnt/PCP pathway. In the Wnt/Ca^{2+} pathway, Wnt protein binds to Frizzled transmembrane receptors on the cell surface resulting in several cellular processes that involve stimulation of heterotrimeric G proteins, increased intracellular Ca^{2+} release, decreased cyclic guanosine mono-phosphate (cGMP) levels, and activation of the two kinases Ca^{2+}-calmodulin-dependent protein kinase II (CamKII) or calcineurin (CaCN) and protein kinase C (PKC). These processes can stimulate nuclear factor (NF)-AT and other transcription factors (Kuhl, M, 2004, Veeman, MT *et al*., 2003, Wang, HY and Malbon, CC, 2003). In the Wnt/PCP pathway, Wnt proteins bind to Frizzled transmembrane receptors on the cell surface followed by activating Rho/Rac small GTPase (Habas, R *et al*., 2003) and Jun N-terminal kinase (JNK) (Moriguchi, T *et al*., 1999) to assist in the subsequent regulation of cytoskeletal organization and gene expression (Moulin, N and Widmann, C, 2004).

WNT AND NEURONAL DEVELOPMENT

The Wnt-Frizzled signaling pathway leads to the development of the brain, spinal cord, and the extension of numerous sub-populations of sensory and motor neurons. The canonical Wnt-Frizzled signaling pathway is required for anterior neural patterning in studies with *Xenopus* embryos (Kiecker, C and Niehrs, C, 2001). *XIdax*, an inhibitor of the canonical Wnt-Frizzled signaling pathway, can reduce the expression of anterior neural markers, indicating that the canonical Wnt-Frizzled signaling pathway is crucial for the anterior neural development in *Xenopus* (Michiue, T *et al*., 2004). The co-expression of Wnt1 and Wnt3a may be necessary for the development of the dorsal neural tube since loss of these two Wnt proteins results in fewer dorsal lateral neural precursors, suggesting that the Wnt-Frizzled signaling pathway plays a vital role in regulating dorsal neural patterning (Chizhikov, VV and Millen, KJ, 2005, Ikeya, M *et al*., 1997, Muroyama, Y *et al*., 2002). Several cellular proteins in the Wnt-Frizzled signaling pathway that have been shown to be involved in the dorsal-ventral patterning of the neural tube also directly regulate patterning of the telencephalon (Grove, EA and Tole, S, 1999) and also can contribute to forebrain patterning in the developing brain (Abu-Khalil, A *et al*., 2004, Braun, MM *et al*., 2003). For example, Wnt 3a, Wnt 5a, and Wnt 2b contribute to the development of the cortical hem which forms the boundary between the hippocampus and choroids plexus in the embryonic cerebral cortex (Grove, EA *et al*., 1998, Lee, SM *et al*., 2000). Wnt genes, genes encoding Frizzled Wnt receptors, or secreted Frizzled-related proteins and Tcf/Lef-1 transcription factors, also are expressed in postnatal mouse cerebral cortex lasting into young adulthood, further indicating that the Wnt/β-catenin signaling pathway represents a major cortical input during embryonic brain development (Shimogori, T *et al*., 2004).

The Wnt-Frizzled signaling pathway also functions as a regulator of specific precursor cells in the developing brain (Panhuysen, M *et al*., 2004). Additional work also demonstrates that autoregulation of canonical Wnt/β-catenin signaling pathway can control midbrain development through the expression of transcription factor Tcf-4 isoforms that require Wnt2b,

but also control Wnt2b (Kunz, M *et al*., 2004). Lef1/Tcf proteins also regulate the generation of dentate gyrus granule cells and the development of the hippocampus (Galceran, J *et al*., 2000). Other work further demonstrates roles for Dishevelled, Rac, and JNK signaling pathways during neuronal development. Wnt7b and Dishevelled can activate Rac and JNK signaling pathways to promote dendritic branching growth in cultured hippocampal neurons, since application of dominant-negative Rac, administration of dominant-negative JNK, or inhibition of JNK activity can inhibit Dishevelled-mediated dendritic growth (Rosso, SB *et al*., 2005).

WNT AND NEURONAL INJURY

Programmed cell death (PCD) (also known as apoptosis) is considered to be a significant component of cell death that contributes to neuronal destruction. Dysfunctions in the regulation or execution of apoptosis are implicated in a wide range of developmental abnormalities and diseases (Maiese, K and Chong, ZZ, 2004, Mattson, MP, 2004). Apoptosis also serves as a central pathway that can lead to a cell's demise in a variety of tissues (Maiese, K, 2001, Maiese, K and Chong, ZZ, 2003) and has recently been identified in organisms as diverse as plants (Hatsugai, N *et al*., 2004). PCD consists of membrane phosphatidylserine (PS) exposure and DNA fragmentation (Maiese, K *et al*., 2004) (Fig. **1**). PCD can contribute significantly to a variety of disease states that especially involve the nervous system such as cerebral ischemic disease, Alzheimer's disease, and trauma (Chong, ZZ *et al*., 2004, Doonan, F and Cotter, TG, 2004, Ferretti, P, 2004, Koyama, R and Ikegaya, Y, 2004, Li, F *et al*., 2004). As an early event in the dynamics of cellular apoptosis, PS exposure may be required for embryogenesis (Bose, J *et al*., 2004). Yet, in mature tissues, membrane PS externalization can become a signal for the phagocytosis of cells (Hong, JR *et al*., 2004) (Fig. **1**). In the nervous system, cells expressing externalized PS may be removed by microglia (Li, F *et al*., 2004, Lin, SH and Maiese, K, 2001) (Fig. **2**). An additional role for membrane PS externalization in the vascular cell system is the activation of coagulation cascades (Chong, ZZ *et al*., 2002, Chong, ZZ *et al*., 2004). The externalization of membrane PS residues in endothelial cells can promote the formation of a procoagulant surface (Bombeli, T *et al*., 1997).

Independent from the early externalization of membrane PS residues, the cleavage of genomic DNA into fragments is considered to be a delayed event that occurs late during apoptosis (Dombroski, D *et al*., 2000, Jessel, R *et al*., 2002, Kang, JQ *et al*., 2003, Maiese, K and Vincent, AM, 2000). Several enzymes responsible for DNA degradation have been differentiated based on their ionic sensitivities to zinc (Torriglia, A *et al*., 1997) and magnesium (Sun, XM and Cohen, GM, 1994). Calcium, a critical independent component that can determine cell survival (Weber, JT, 2004), also may determine endonuclease activity through calcium/magnesium - dependent endonucleases such as DNase I (Madaio, MP *et al*., 1996). Other enzymes that may degrade DNA include the acidic, cation independent endonuclease (DNase II) (Torriglia, A *et al*., 1995), cyclophilins (Montague, JW *et al*., 1997), and the 97 kDa magnesium - dependent endonuclease (Pandey, S *et al*., 1997). In the nervous system, three separate endonuclease activities are present that include a constitutive acidic cation-independent endonuclease, a constitutive calcium/magnesium-dependent endonuclease, and an inducible magnesium dependent endonuclease (Vincent, AM and Maiese, K, 1999). The physiologic characteristics of the magnesium dependent endonuclease, such as a pH range of 7.4−8.0, a dependence on magnesium, and a molecular weight of 95−108 kDa, are consistent with a recently described constitutive 97 kDa endonuclease in non-neuronal tissues.

Oxidative stress can lead to apoptosis in neurons through multiple cellular pathways (Chong, ZZ *et al*., 2005). Oxidative stress results in nuclei condensation and DNA fragmentation (Chong, ZZ *et al*., 2003, Goldshmit, Y *et al*., 2001, Pugazhenthi, S *et al*., 2003, Vincent, AM *et al*., 1999). In neurons, free radical exposure produces apoptotic death in hippocampal and

dopaminergic neurons (Chong, ZZ *et al*., 2003, Sharma, SK and Ebadi, M, 2003, Vincent, AM and Maiese, K, 1999, Witting, A *et al*., 2000). Externalization of membrane PS residues also occurs in neurons during anoxia (Chong, ZZ *et al*., 2002), free radical exposure (Chong, ZZ *et al*., 2003), or during the administration of agents that induce the production of reactive oxygen species, such as 6-hydroxydopamine (Salinas, M *et al*., 2003).

The Wnt-Frizzled signaling pathway can regulate PCD through a variety of mechanisms that include the Wnt-bone morphogenetic protein (BMP) signaling loop (Ellies, DL *et al*., 2000, Golden, JA *et al*., 1999), secreted Frizzled-related protein-2 (SFRP2) expression (Ellies, DL *et al*., 2000, Jones, SE *et al*., 2000), Wnt-β-catenin signaling (Ahmed, Y *et al*., 2002, Brault, V *et al*., 2001, Galceran, J *et al*., 2000, Hari, L *et al*., 2002), c-Jun N-Terminal kinase signaling (Grotewold, L and Ruther, U, 2002, Lisovsky, M *et al*., 2002, Yeo, W and Gautier, J, 2004), GSK-3β-NF-κB signaling (Bournat, JC *et al*., 2000, Kozlovsky, N *et al*., 2002) and gene expression that involves human Dickkopf-1 (hDkk-1) (Shou, J *et al*., 2002), *nemo* (Mirkovic, I *et al*., 2002), *sox 10* (Honore, SM *et al*., 2003), and tau (Jackson, GR *et al*., 2002).

Wnt1 signaling has been associated with the control of apoptosis during injury in some cell systems. Wnt1 prevents apoptosis through β-catenin / Tcf transcription mediated pathways (Chen, S *et al*., 2001, Rhee, CS *et al*., 2002). Overexpression of exogenous Wnt1 results in the protection of cells against c-myc induced apoptosis through induction of β-catenin, cyclooxygenase-2, and Wnt1 induced secreted protein (WISP-1) (You, Z *et al*., 2002). Wnt1 signaling also can inhibit apoptosis through prevention of cytochrome c release from mitochondria and the subsequent inhibition of caspase 9 activation (Chen, S *et al*., 2001). The APC gene also appears to represent another mechanism that regulates apoptosis. The APC gene functions to cleave β-catenin leading to the down-regulation of transactivation of Tcf/Lef (Munemitsu, S *et al*., 1995). Without Tcf/Lef activity, APC is then permitted to increase the activities of caspase 3, caspase 7, and caspase 9 and lead to the cleavage of poly (ADP-ribose) polymerase (PARP) to enhance the vulnerability of cells to apoptosis (Chen, T *et al*., 2003).

The Wnt-β-catenin signaling pathway regulates cell proliferation and differentiation, cell polarity, and specification of cell fate, such as apoptosis. Yet, different from the Wnt-BMP signaling loop, the Wnt-β-catenin signaling pathway can prevent apoptosis through the regulation of β-catenin and Tcf/Lef. In β-catenin mutant embryos, the removal of β-catenin can lead to apoptotic loss of the hindbrain, the melanocyte lineage, neural crest cells, sensory neurons and dorsal root ganglia (Brault, V *et al*., 2001, Hari, L *et al*., 2002). Over-expression of exogenous Wnt-1 results in the protection of cells against c-Myc induced apoptosis through induction of β-catenin, cyclooxygenase-2, and Wnt-1 induced secreted protein (WISP-1) (You, Z *et al*., 2002).

The Wnt signaling pathway also can decrease apoptosis and increase survival of neurons or neuronal cell lines by activating NF-κB (Bournat, JC *et al*., 2000), inhibiting GSK-3β (Kozlovsky, N *et al*., 2002), or blocking the release of cytochrome c (Fig. **1**). In the absence of Wnt activity, GSK-3β phosphorylates β-catenin at serine or threonine residues of the N-terminal region to predispose degradation of β-catenin through ubiquination (Fig. **2**). GSK-3β dependent phosphorylation of β-catenin can be promoted through phosphorylation of Axin (Yamamoto, H *et al*., 1999). In studies with chemotherapeutic agents, Wnt1 signaling also can inhibit apoptosis through prevention of cytochrome c release from mitochondria and the subsequent inhibition of caspase 9 activation (Chen, S *et al*., 2001).

The Wnt pathway also uses protein kinase B (Akt) to promote cellular differentiation and survival (Chong, ZZ *et al*., 2005) (Fig. **1**). Since Wnt can inactivate GSK-3β and block the phosphorylation of β-catenin (Ikeda, S *et al*., 1998, Papkoff, J *et al*., 1998), this leads to the activation of β-catenin followed by transcription of its target genes for cellular protection. Akt

may be necessary in pathways that involve Wnt1, since Akt inhibits the activity of GSK-3β through phosphorylation of this protein to promote cell survival (Crowder, RJ and Freeman, RS, 2000). Furthermore, neuronal cell differentiation that is dependent upon Wnt signaling appears to become stalled without Akt phosphorylation and the subsequent inactivation of GSK-3β (Fukumoto, S *et al*., 2001). In addition, Wnt has been demonstrated through WISP-1 to activate the anti-apoptotic signaling pathway of Akt following genomic DNA damage (Su, F *et al*., 2002) and to block cell injury during serum withdrawal through increased Akt phosphorylation and activity (Longo, KA *et al*., 2002).

Wnt and Neuropsychiatric Disorders, Retinal Disease, and Alzheimer's Disease

Wnt1 expression has been demonstrated in the brains of individuals affected by neuropsychiatric disorders (Miyaoka, T *et al*., 1999). In addition, retinal degeneration during retinitis pigmentosa with the progressive loss of photoreceptors has been associated with increased secretion of Frizzled-related protein-2, a Wnt inhibitory protein, suggesting that loss of Wnt signaling may contribute to retinal neurodegeneration (Jones, SE *et al*., 2000). Other studies demonstrate that a mutation in the membrane-type Frizzled-related protein gene may be involved in retinal photoreceptor degeneration (Kameya, S *et al*., 2002).

In Alzheimer's disease, neurotoxicity of Aβ in hippocampal neurons has been linked to increased levels of GSK-3β and loss of β-catenin. Decreased production of Aβ can occur during the enhancement of PKC activity (Savage, MJ *et al*., 1998) which may be controlled by the Wnt pathway (Garrido, JL *et al*., 2002). The proteolytic processing of amyloid precursor protein (APP) during Alzheimer's disease also has been closely linked to the Wnt pathway through presenilin 1 (PS1) and Dishevelled. PS1 is required for the processing of APP and has been shown to down-regulate Wnt signaling and interact with β-catenin to promote its turnover (Soriano, S *et al*., 2001). Dishevelled also can regulate the α-secretase cleavage of APP through PKC/mitogen-activated protein kinase dependent pathways, increasing soluble production of APP (sAPP) (Mudher, A *et al*., 2001). Overexpression of mouse Dishevelled-1 and −2 inhibits GSK-3β mediated phosphorylation of tau protein and may thus prevent formation of neurofibrillary tangles during Alzheimer's disease (Wagner, U *et al*., 1997).

Potential Therapeutic Modalities Involving the Wnt Pathway

Several cytokines and trophic factors may have some dependence on the Wnt signaling pathway (Maiese, K *et al*., 2004, Maiese, K *et al*., 2005). Vascular endothelial growth factor (VEGF) can stimulate new vessel formation by promoting tyrosine phosphorylation of β-catenin (Cohen, AW *et al*., 1999, Roura, S *et al*., 1999). In addition, fibroblast growth factor 2 (FGF2) can inhibit GSK-3 activity, augment nuclear levels of β-catenin, and enhance Tcf/Lef-dependent transcription of a cyclin D1-luciferase construct, suggesting that the angiogenic properties of FGF2 are tightly regulated by β-catenin activation in the Wnt-Frizzled signaling pathway (Dono, R *et al*., 2002, Holnthoner, W *et al*., 2002). Another potential candidate is erythropoietin (EPO) (Li, F *et al*., 2004, Maiese, K *et al*., 2005). Both cell culture and animal model work have demonstrated neuronal protection with EPO (Chong, ZZ *et al*., 2002, Chong, ZZ *et al*., 2002, Genc, S *et al*., 2004). Systemic administration of EPO before or immediately after a retinal insult can protect retinal ganglion cells from apoptosis (Grimm, C *et al*., 2002) and can improve functional outcome and reduce lipid peroxidation during spinal cord injury (Kaptanoglu, E *et al*., 2004). EPO also can block microglial cell activation and proliferation to prevent phagocytosis of injured cells through pathways that involve cellular membrane PS exposure (Chong, ZZ *et al*., 2004) and the regulation of caspases (Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003). EPO can prevent cellular inflammation by inhibiting several pro-inflammatory cytokines, such as IL-6, tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein 1 (Chong, ZZ *et al*., 2002, Genc, S *et al*., 2004).

Components of the Wnt pathway that are utilized by EPO, such as Akt, can offer cellular protection (Maiese, K *et al*., 2003). EPO can phosphorylate Akt and is dependent upon the activation of PI 3-K and Janus Kinase 2 (Jak2) (Chong, ZZ *et al*., 2002, Witthuhn, BA *et al*., 1993). One of the principal pathways through which EPO prevents cellular apoptosis is through the activation of Akt (Maiese, K *et al*., 2004, Maiese, K *et al*., 2005) During anoxia or free radical exposure, expression of the active form of Akt (phospho-Akt) is increased (Kang, JQ *et al*., 2003, Kang, JQ *et al*., 2003). EPO can significantly enhance the activity of Akt during oxidative stress and prevent inflammatory activation of microglia (Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003). This up-regulation of Akt activity during injury paradigms appears to be vital for EPO protection, since prevention of Akt phosphorylation blocks cellular protection by EPO (Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003). Through the regulation of the PI 3-K/Akt dependent pathway, EPO can prevent cellular apoptosis following N-methyl-D-aspartate toxicity (Dzietko, M *et al*., 2004), hypoxia (Chong, ZZ *et al*., 2002), and oxidative stress (Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003, Chong, ZZ *et al*., 2003).

CONCLUSION

Wnt-Frizzled signaling consists of Wnt/β-catenin, Wnt/Ca^{2+}, and Wnt/planar cell polarity pathways and are involved with the development of the brain, spinal cord, and the extension of sub-populations of sensory and motor neurons. The Wnt-Frizzled signaling pathway also modulates both early and late apoptotic injury paradigms in a variety of cell populations during the development of an organism as well as during acute and chronic injury. Diseases of the nervous system, such as retinal degeneration and Alzheimer's disease, may be dependent upon the Wnt pathway. In addition, cytokines and growth factors may use Wnt proteins to exert their biological effects. Identifying the vital elements that shape and control the Wnt-Frizzled signaling pathway may provide significant prospects for the treatment of disorders of the nervous system.

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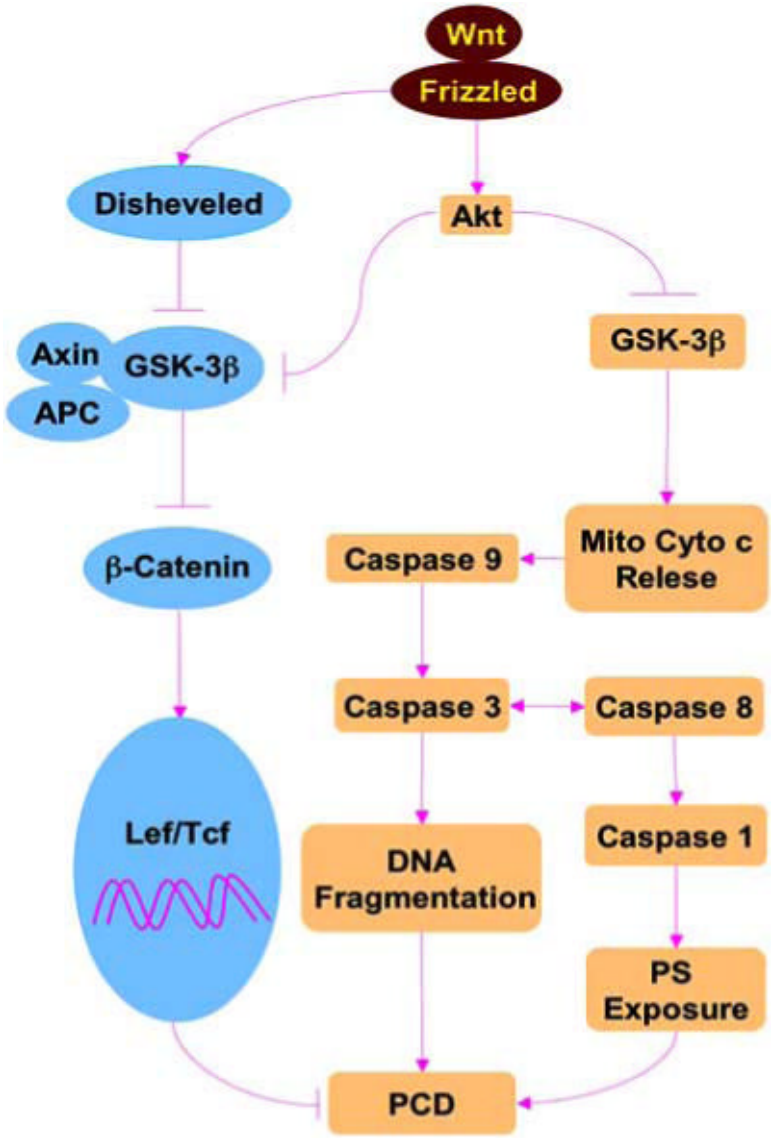

Fig. (1). The Wnt pathway controls mitochondrial cytochrome c release, caspase activity, and programmed cell death (PCD)

The Wnt protein binds to its Frizzled receptors activating Dishevelled followed by the inhibition of GSK-3β (glycogen synthase kinase-3β), Axin and APC (adenomatous polyposis coli) tumor suppressor protein complex. The GSK-3β, Axin and APC complex blocks phosphorylation of ß-catenin. β-catenin translocates to the cell nucleus and contributes to the formation of Lef/Tcf (lymphocyte enhancer factor/T cell factor) and β-catenin complex that can lead to cellular proliferation, differentiation, and survival. The binding of Wnt to Frizzled receptors also can activate Akt that prevents the activity of GSK-3β, prevents Mito (mitochondrial) Cyto c (cytochrome c) release, caspase activity, and PCD that involves DNA fragmentation and phosphatidylserine (PS) exposure.

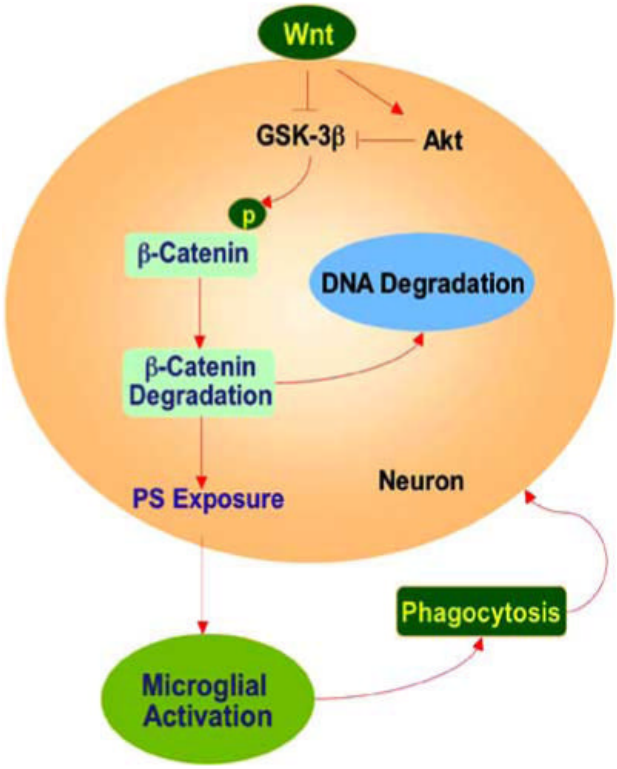

Fig. (2). Wnt preserves neuronal survival and may prevent microglial activation

Through its receptor Frizzled, Wnt inhibits glycogen synthase kinase-3β (GSK-3β). In the absence of Wnt, GSK-3β can degrade β-catenin, promote membrane phosphatidylserine (PS) exposure, and lead to microglial activation and the phagocytosis of neurons.