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Wnt Signaling Network in Homo Sapiens

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1. Introduction

Signaling is a part of system communication among living cells by processing biological information that governs basic cellular activities and coordinates cell actions. The signals are transmitted by means of signaling molecules, and this process ends up with altering of gene transcription in the nucleus resulting in many cellular processes such as differentiation and proliferation. Systems biology research helps us understand the underlying structure of cell signaling networks and how changes in these networks affect the transmission and flow of information. Here we thoroughly investigate Wnt signaling, a major signaling system known to have role in regulating processes such as embryonic development and growth, cell differentiation, proliferation, migration and polarity.

There are two major types of Wnt signaling (Figure 1) classified as canonical (Wnt/β-catenin) and non-canonical (planar cell polarity (PCP) and the Wnt-calcium (Wnt/Ca^{2+})) pathways (Katoh, 2005; Nusse, 2005; DasGupta, 2005). In canonical Wnt signaling, the

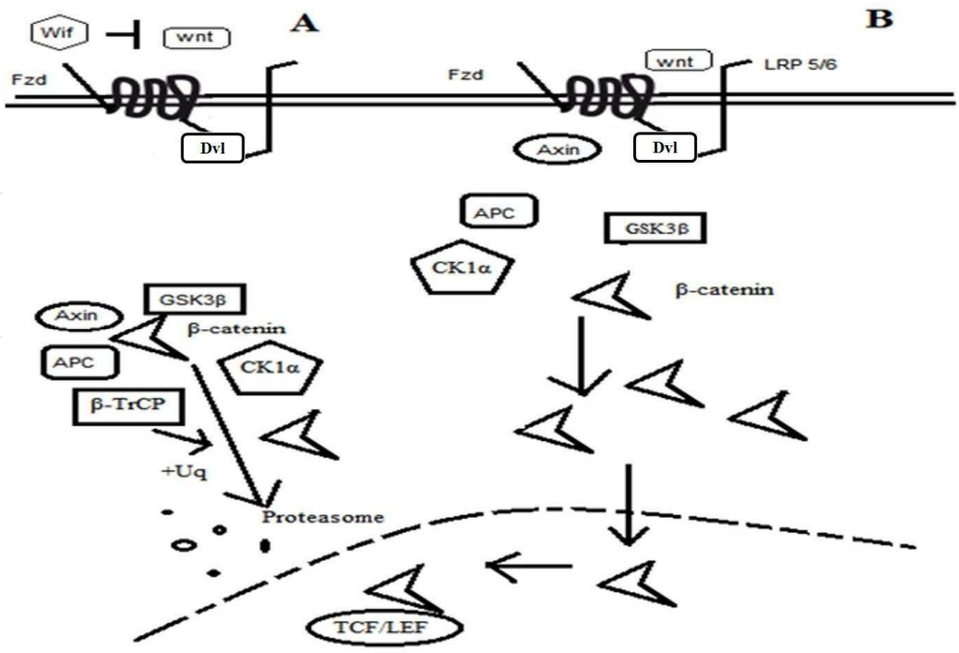
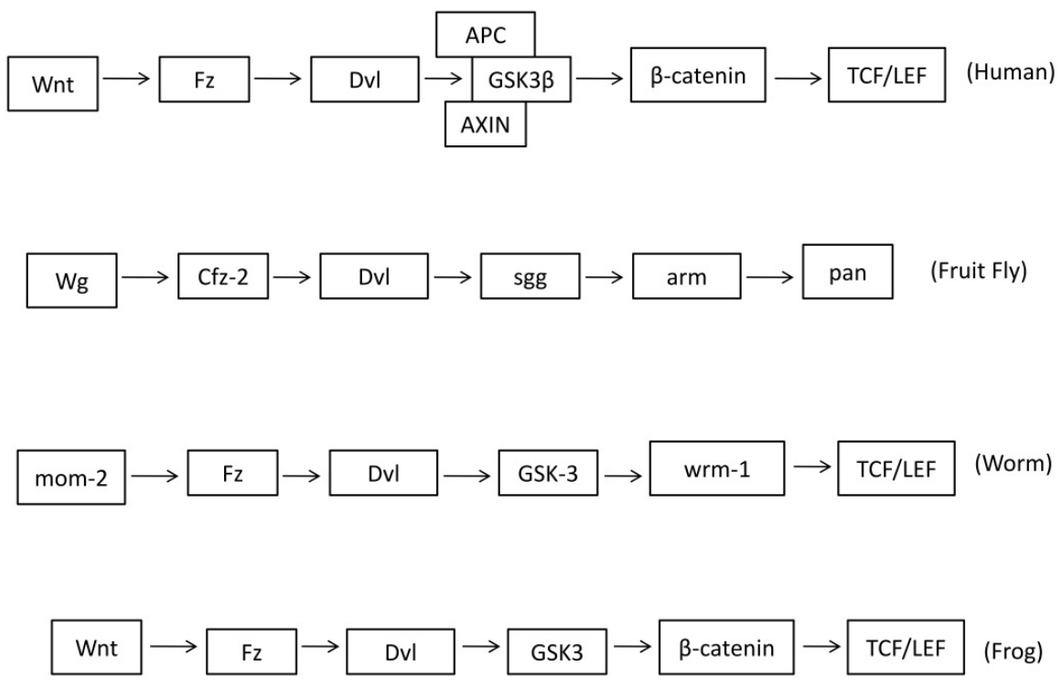


Fig. 1. Regulation of β-catenin stability by Wnt signaling.

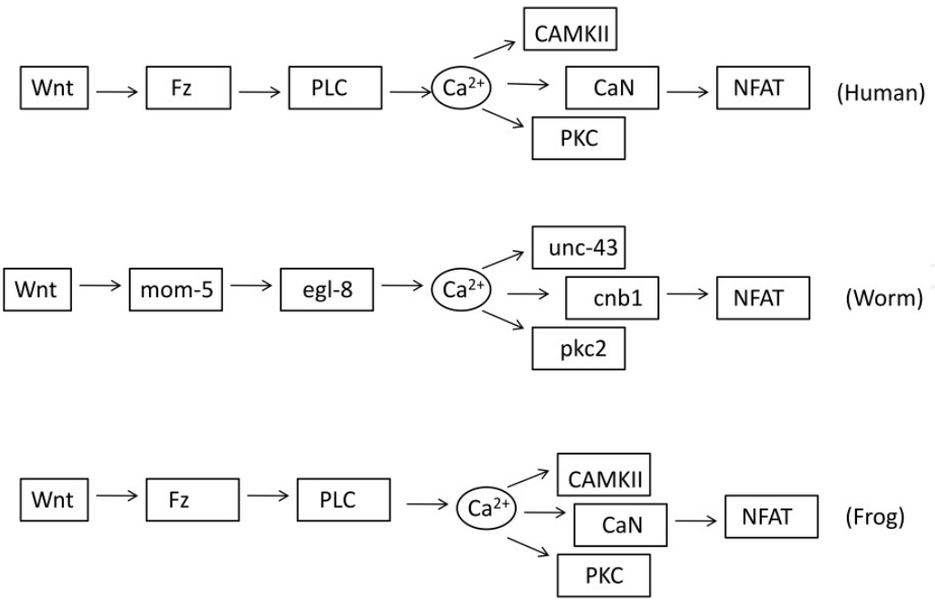
absence of the Wnt ligand, the signal transduction is at non-operating state (off), the protein complex that involves APC and AXIN proteins facilitates the phosphorylation of β-catenin by glycogen synthase kinase (GSK3β). Then β-catenin binds to a protein called β–TrCP (beta-transducin repeat containing protein) that mediates the ubiquitination of CTNNB1 and the proteins tagged with ubiquitin are degraded by the proteosome that results in β-catenin destruction by the proteosomal degradation. When Wnt proteins bind to Frizzled receptors, this receptor activation works counter to destruction complex (APC-AXIN-GSK3β) by preventing β-catenin phosphorylation via Dishvelled protein. Then β–catenin accumulates and enters into the nucleus where it binds to a DNA binding protein of the TCF/LEF family and then activates new gene expression programs (Cadigan, 2008).

The illustration (Figure 1) depicts a cell in the absence (A) and in the presence (B) of Wnt protein. In unstimulated cells, β-catenin not complexed with the cadherin adhesion complex is phosphorylated by CKI and GSK3β, leading to β-TrCP dependent ubiquitination and proteosomal degradation. The presence of Wnt, on the hand, promotes LRP (lipoprotein receptor-related protein), cell-surface coreceptor, and Fz association, leading to recruitment of Dvl to the complex, followed by GSK3β and CKI casein kinase phosphorylation of LRP. This stabilizes recruitment of AXIN to the receptor, which in turn may disrupt the activity of the destruction complex, and hence allows accumulation of β-catenin and nuclear translocation (Cadigan, 2008). An overview of types of Wnt signaling in different species is illustrated in Figure 2.

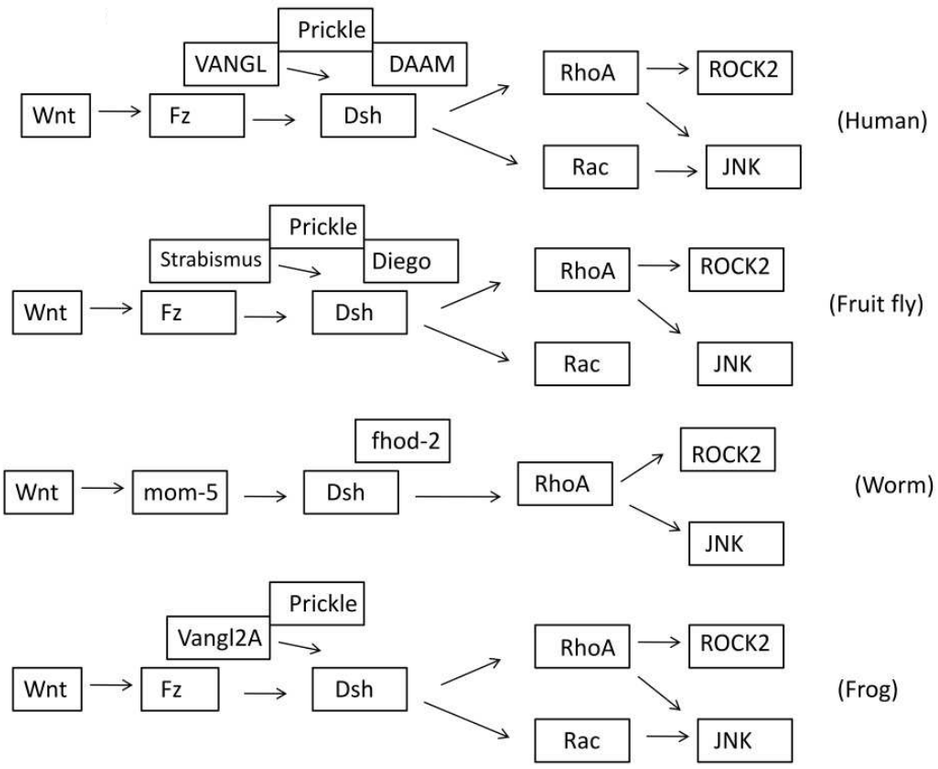


(a) Wnt/β-catenin

Fig. 2. (Continued)



(b) Wnt/Ca^{2+}



(c) Wnt/PCP

Fig. 2. An overview of Wnt signaling pathways:(a)Wnt/ β-catenin (b) Wnt/calcium (c) Wnt/Planar cell polarity in different species (KEGG: Kyoto Encyclopedia of Genes and Genomes; http://www.genome.jp/kegg/)

The number of non-canonical pathways is unclear (Veeman *et al*., 2003; Eisenmann, 2005; Pandur, 2005). A Wnt signalling pathway that increases intracellular Ca^{2+} levels has been proposed as Wnt/Ca^{2+} pathway in vertebrates (Kuhl *et al*., 2000). There is also a Wnt/JNK (Wnt/c-Jun N terminal kinase) pathway in vertebrates that controls cell polarity (Veeman *et al*., 2003; Pandur 2005). A new Wnt signalling pathway called Wnt-5A/Ror2 signalling has been proposed in *X. leavis* (Schambony and Wedlich, 2007). In flies, the Wnt/PCP pathway (Figure 2c), which controls planar cell polarity, is the best-known non-canonical Wnt signalling pathway so far (Veeman *et al*., 2003, Eisenmann, 2005). Processes such as P2/EMS signalling, T cell polarity, Z1/Z4 polarity and B cell polarity utilize non-canonical Wnt signalling in *C. elegans*. Furthermore, a non-canonical pathway, a PCP like pathway, has been proposed to control the polarity of B cells (Wu and Herman, 2006). Recently a novel non-canonical Wnt pathway, which is also β-catenin independent, in vertebrate and fly has been proposed to act in neuronal specification (Hingwing *et al*., 2009).

Wnt-planar cell polarity (PCP) and Wnt-calcium(Ca^{2+}) pathways that are independent of β-catenin are considered the two most well-recognized non-canonical pathways in Wnt signaling. The activation of kinase C (PKC), Jun kinase (JNK), RHOA and nemo-like kinase (NLK) mediates the Wnt-planar cell polarity (PCP) pathway which is related to tissue polarity control and cell movement. The Wnt/PCP signaling pathway is mostly characterized in *D. Melanogaster*. Frizzled, Van Gogh, Stan/Flamingo, Dishevelled, Prickle and Diego proteins are the major proteins of Drosophilia Wnt/PCP signaling. Diego activates the pathway with binding to Dishevelled whereas Prickle prevents this association and negatively regulates the pathway. The tissue polarity is established by asymmetrical localization of FZD-Dsh-Diego-Stan complex and Vang-Prickle complex. The main proteins of the human Wnt/PCP pathway are mostly the homologs of the *Drosophilia* proteins. VANGL1, VANGL2 (Van Gogh homologs), DVL1, DVL2, DVL3 (Dishevelled homologs), PRICKLE1, PRICKLE2 (Prickle homologs) and ANKRD6 (Diego homolog) are the core proteins having essential roles in human Wnt/PCP signaling cascade. In Wnt/PCP signaling, Wnt ligands (WNT5A, WNT5B and WNT11) first bind to Frizzled receptors (FZD3, FZD6). Then, the association of Prickle, Vangl and Dishevelled proteins result in RHOA activation following the binding of Dishevelled proteins to Daam proteins which are implicated in actin-cytoskeleton re-organization. Moreover, by Dvl-dependent manner, the JNK cascade is activated by the association of dishevelled proteins with MAPK3. On the other hand, NLK signaling cascade is activated by Dvl-independent manner in which the Ca^{2+} release by FZD proteins activates MAP3K7 (TAK1). NLK protein is known to be an activator of Wnt/PCP signaling as well as the inhibitor of canonical Wnt pathway (Katoh, 2005; Katoh 2003; Wu and Maniatis, 1999).

Calcium has been implicated as an important messenger in Wnt pathway and recent studies showed that higher frequencies of calcium transients were associated with faster rates of outgrowth (Veeman *et al.*, 2003; Pandur 2005; Cadigan, 2008). WNT5a also activates the Wnt/Ca^{2+} signaling. The binding of Wnts to Frizzled receptors leads to an activation of heterotrimeric G-proteins and subsequent activation of phospholipase C by the G-protein beta/gamma dimer. This enzyme cleaves phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 is released from the membrane, and binds to the IP3 receptor which subsequently releases calcium ions from intracellular stores. This locally restricted calcium release subsequently activates calcium-sensitive proteins like protein kinase C (PKC), calcium-calmodulin dependent kinase II

(CamKII), and/or calcineurin (CaCN). IP3 is then degraded to inositol by means of specific phosphatases and recycled to PIP2 (Kuhl et al., 2000; Kohn and Moon, 2005; Kuhl, 2004; Slusarskia et al., 1997). Activated CaCN dephosphorylates NF-AT (nuclear factor of activated T-cells), which then translocates to nucleus to regulate gene expression. Studies performed for Xenopus showed that this signaling is active in the development of ventral cell fate which inhibits Wnt/β-catenin pathway (Saneyoshi et al., 2002; Veeman et al., 2003; Pandur, 2005). In addition to that, recent studies reported that there is a crosstalk between canonical and non-canonical Wnt signaling through GSK3b since it can phosphorylate NF-AT (Beals et al., 2003; Ohteki et al., 2000). The Wnt/PCP and Wnt/Calcium signaling pathways have overlaps since both of them are mediated by calcium release, and Dishevelled proteins play essential roles in activation of these cascades.

The deregulations and mutations in this signaling pathway cause several human diseases including lung, breast, colon and colorectal cancers. Recent works have demonstrated that Wnt-activated excessive β-catenin accumulation in nucleus plays an important role in tumour formation. On the other hand, one of the β-catenin independent signalling pathways; the Wnt/Ca^{2+} signalling pathway, is proposed to antagonize Wnt/β-catenin signalling pathway. Therefore, Wnt/Ca^{2+} cascade may have the ability to act as a tumour suppressor. However, still much more work has to be done on this subject as the mechanism of this antagonism is not well known. (Wang and Malbon, 2003; Giles *et al.*, 2003; Veeman *et al.*, 2003; Kohn and Moon, 2005; Slusarski and Pelegri, 2007; Maiese *et al.*, 2008).

Investigating Wnt signaling is therefore attractive for researchers to identify the suitable drug targets for therapeutic intervention in cancer treatment (Cadigan and Liu, 2006; Cong et al., 2004; Widelitz, 2005; Nusse, 2005). This signaling pathway has been studied and analyzed using different species such as worm (*Caenorhabditis elegans*), fly (D*rosophila melanogaster*), frog (*Xenopus leavis*) etc. in order to enucleate the governing mechanisms through essential components (DasGupta, 2005; Kuhl et al., 2000; Kohn and Moon, 2005). However, the whole Wnt mechanism is not well understood in human due to the restrictions on experiments with human, and more attention should be devoted to this organism.

In this study, the reconstruction of Wnt signaling network was computationally performed for *Homo Sapiens* via integration of interactome data and Gene Ontology annotations. The graph theoretical analysis was then performed for analyzing the topological properties of the network proteins. Moreover, the linear paths in which the signal is transferred from ligands (input) to transcription factors (output) were identified in order to follow signal transmittal as well as to identify the specific proteins of canonical and non-canonical Wnt pathways. Furthermore, the crosstalk analysis was applied in order to detect the significant bridging proteins in all these pathways. Finally, the proteins which might be targets of drugs against diseases involving Wnt pathways are indicated and these proteins are compared with the ones reported in literature in terms of topological properties and their roles in canonical and non-canonical Wnt pathways.

This work is aimed to give an insight on the role of Wnt signalling in maintaining the homeostasis as well as reacting to cellular stress. Understanding the molecular basis underlying the ability of Wnt proteins to perform antagonistic or similar signalling activities would eventually lead us to new ideas about how to suppress cancer cells in human metabolism.

2. Materials and methods

2.1 Network reconstruction by GO annotations

The reconstruction of Wnt signaling network in human was performed by integrating Gene Ontology (GO) annotations and PPI data. First of all, the core proteins that are known to have roles in Wnt signaling in *Homo Sapiens* were obtained from literature. Then an annotation collection table was prepared by using GO annotations (biological process, cellular component and molecular function) of the core proteins. All the GO annotations of all human proteins were downloaded from GO website (http://www.geneontology.org/GO.current.annotations.shtml) by October 2010. Next, the human proteins whose all three GO terms match with those in the annotation collection table were included into the network. Thereafter, the physical protein-protein interaction data among these human proteins were extracted from MINT (Ceol *et al*., 2009) HPRD (Prasad *et al*., 2009) and BioGRID (Stark *et al*., 2010) by January 2011. Finally, the interaction partners of the proteins that passed the GO annotation filter were obtained and these protein pairs were used to reconstruct the network (Figure 3).

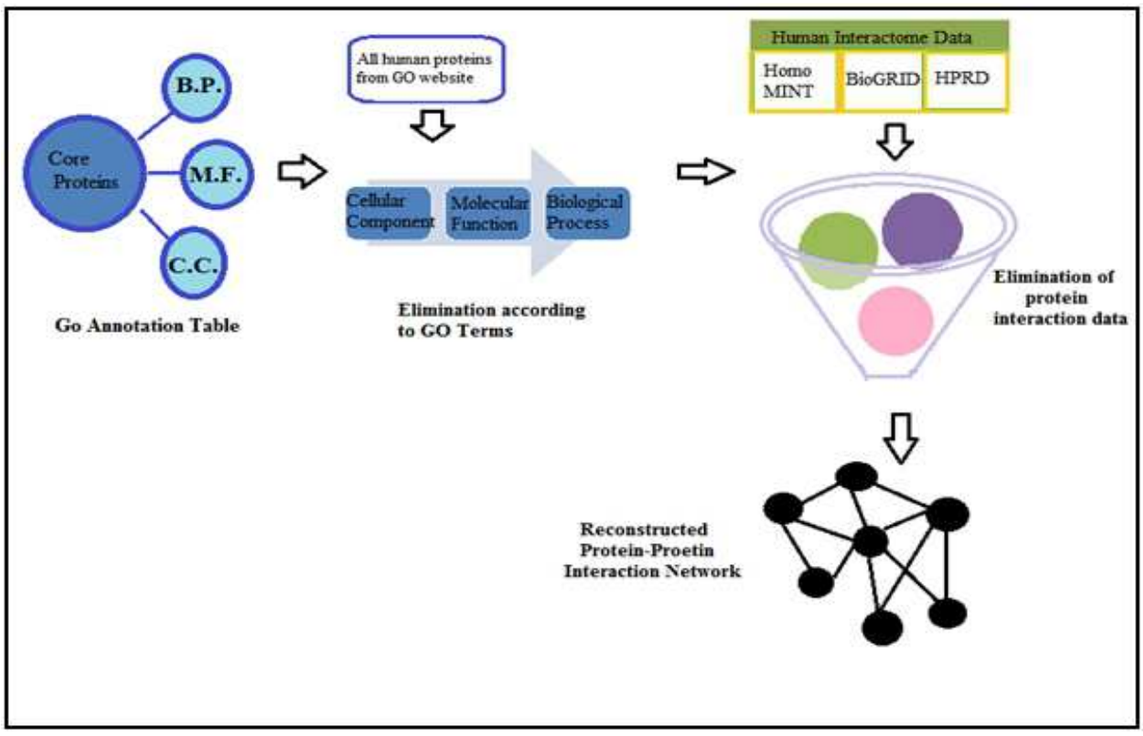


Fig. 3. The schematic representation of the algorithm used for the network reconstruction.

2.2 Graph theoretical analysis

Graph theoretical analysis enables better understanding of the structure of the complex networks and the distribution of the components via topological properties. The topology of the reconstructed network was determined by Network Analyzer Plug-in of Cytoscape (ver. 2.7.0). In the network analysis, the proteins are nodes whereas the interactions between the

nodes are edges. The input of the calculations is the binary protein interactions and the output is the topological parameters such as degree distribution of the nodes, number of highly connected nodes (hubs), network diameter, mean path length, clustering coefficient, number of shortest paths and average shortest path length.

The network diameter is the maximum node eccentricity. The eccentricity here can be defined as the maximum non-infinite length of a shortest path from a node to another node in the network. The average shortest path length (or the characteristic path length) is the expected distance between two connected nodes. The betweenness centrality is another topological aspect and it is defined as the number of shortest paths going through a node. The proteins with high betweenness centrality values are called as bottleneck proteins. Another parameter, clustering coefficient of a node *N* can be defined as the ratio of the number of edges between the neighbors of *N* to the maximum number of edges that could possibly exist between the neighbors of *N*. The network clustering coefficient is the average of the clustering coefficients for all nodes in the network. This value is always between 0 and 1.

2.3 Module detection and analysis

In order to get an insight on the cellular role of the network, a Cytoscape plug-in, MCODE (1.31) was used to identify these highly interconnected regions in the present protein-protein interaction network. After the detection of the modules, the statistically overrepresented Gene Ontology functions of the proteins in the modules are obtained by the Biological Networks Gene Ontology tool (BINGO 2.44). BiNGO combines the relevant GO annotations and relates them upwards through the GO hierarchy. The output of this analysis gives the most significant GO annotations with the lowest P-value (Maere *et al*., 2005).

2.4 Network decomposition analysis

In network decomposition analysis, the protein interaction networks are decomposed into linear paths in which ligands are inputs and transcription factors are outputs (Tekir et al., 2008). This analysis enables us to investigate the network communication route via linear paths. The linear paths of the reconstructed network were found by the NetSearch program (Steffen *et al*., 2002). The participation frequencies of the proteins were calculated at a specified length for determining the essentiality of the proteins in the network. Next, crosstalk analysis, in which the network crosstalk value of a node is obtained by the difference in degree of the node in all considered networks and the maximum degree of this node in any individual pathway, was performed to detect the components that connect the pathways in signal transduction. A high path crosstalk value implies that a node is more important in the combined network than it was in the individual pathways (Zielinski *et al*., 2009).

3. Results

In the present study, the protein-protein interaction sub-networks of Wnt signaling in *Homo sapiens* were reconstructed using the system biology approach in which many types of biological data and analysis techniques are integrated. Also, the topological analyses of these sub-networks were performed.

3.1 Reconstruction of Wnt signaling networks

In the first step, the proteins that are known to be involved in human Wnt signaling were identified through literature search. These selected Wnt proteins that belong to both canonical and non-canonical Wnt pathways in human were taken as the core proteins of the sub-networks to be constructed (Tables 1 and 2). The algorithm explained in section 2.1 was followed during the reconstruction process.

For canonical Wnt pathway 68 core proteins were identified. For PCP and Ca++ pathways these numbers were 33 and 32, respectively. For canonical pathway, 10592 physical protein interactions, which were obtained from MINT, BioGRID and HPRD databases, were accepted to the network. For PCP network 5928 protein-protein interactions and for Ca++ network 6080 protein-protein interactions were accepted to these sub-networks.

After removing the isolated smaller parts a network of 3251 nodes and 9304 edges was obtained for Wnt/β-catenin pathway; 1952 nodes and 5001 edges were obtained for PCP pathway; 2112 nodes and 5293 edges were obtained for Wnt/calcium pathway.

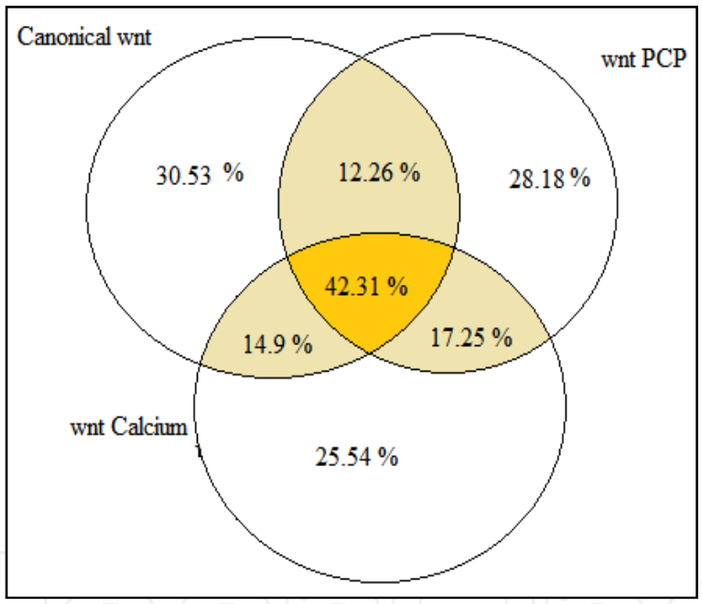


Fig. 4. Venn Schema Representation of Common Nodes.

Finally, the sub-networks were integrated and the whole Wnt network was obtained with 3489 nodes and 10092 edges. The common proteins of each sub-network were identified (Figure 4) and among them the proteins such as dishvelled (DVL1, DVL2, DVL3), AXIN (AXIN 1, AXIN 2), casein kinase (CSNK1A1, CSNK1D, CSNK1E, CSNK2A1, CSNK2B), β-catenin (CTNB1), frizzled (FZD1, FZD2, FZD4, FZD7, FZD8, FZD9), adenomatous polyposis coli protein (APC), glycogen synthase kinase-3 beta (GSK3β), mitogen-activated protein kinase (MAP3K2, MAP3K4, MAP3K7, MAP3K8), nuclear factor of activated T-cells (NFATC1, NFATC2), Smad family (SMAD 1, SMAD 2, SMAD 3, SMAD 4, SMAD 5, SMAD 6, SMAD 7, SMAD 9), transcription factor 7-like 2 (TCF7L2), cellular tumor antigen p53 (TP53), protein kinase C inhibitor protein 1 (YWHAZ) are found to be related to diseases like cancer, Alzheimer's, diabetes andosteoarthritis (Table 3).

General Name(Canonical)-Uniprot ID			
Wnt1	P04628	SENP2	Q9HC62
Wnt2	P09544	DKK1	O94907
Wnt2B	Q93097	NKD2	Q969F2
Wnt3	P56703	NKD1	Q969G9
Wnt3A	P56704	CXXC4	Q9H2H0
Wnt4	P56705	SKP1	P63208
Wnt7A	O00755	CUL1	Q13616
Wnt10B	O00744	NLK	Q9UBE8
FZD1	Q9UP38	RUVBL1	Q9Y265
FZD2	Q14332	SMAD4	Q13485
FZD4	Q9ULV1	SMAD3	P84022
FZD5	Q13467	CTBP1	Q13363
FZD7	O75084	CTBP2	P56545
LRP5	O75197	MAP3K7	O43318
LRP6	O75581	LEF1	Q9UJU2
DVL1	O14640	TCF7	P36402
DVL2	O14641	TCF7L1	Q9HCS4
DVL3	Q92997	BTRC	Q9Y297
FRAT1	Q92837	SIAH1	Q8IUQ4
FRAT2	O75474	EP300	Q09472
GSK3B	P49841	FBXW11	Q9UKB1
AXIN1	O15169	PSEN1	P49768
AXIN2	Q9Y2T1	WIF1	Q9Y5W5
APC2	O95996	PORCN	Q9H237
APC	P25054	CER1	O95813
PPP2CA	P67775	SFRP1	Q8N474
CSNK1A1	P48729	SFRP2	Q96HF1
CSNK1A1L	Q8N752	SFRP4	Q6FHJ7
CSNK1D	P48730	SFRP5	Q5T4F7
CSNK1E	P49674	SOX17	Q9H6I2
CSNK2A2	P19784	CHD8	Q9HCK8
CSNK2B	P67870	TBL1X	O60907
CTNNB1	P35222	CTNNBIP1	Q9NSA3

Table 1. Core proteins of canonical Wnt signaling pathway

General Name (PCP)-Uniprot ID		General Name (Ca++)-Uniprot ID	
Wnt5A	P41221	Wnt5A	P41221
Wnt5B	Q9H1J7	Wnt11	O96014
Wnt11	O96014	Wnt1	P04628
FZD3	Q9NPG1	PLCB1	Q9NQ66
FZD2	Q14332	PLCB2	Q00722
FZD6	O60353	PLCB3	Q01970
MAGI3	Q5TCQ9	PLCB4	Q15147
ROR1	Q01973	CAMK2A	Q9UQM7
ROR2	Q01974	CAMK2B	Q13554
PTK7	Q13308	CAMK2D	Q13557
VANGL1	Q8TAA9	CAMK2G	Q13555
VANGL2	Q9ULK5	CHP	Q99653
CELSR1	Q9NYQ6	PPP3CA	Q08209
CELSR2	Q9HCU4	PPP3CB	Q8N1F0
CELSR3	Q9NYQ7	PPP3CC	P48454
DVL1	O14640	PPP3R1	P63098
DVL2	O14641	PPP3R2	Q96LZ3
DVL3	Q92997	CHP2	O43745
PRINCKLE1	Q96MT3	PRKCA	P17252
PRINCKLE2	Q7Z3G6	PRKCB	P05771
NKD1	Q969G9	PRKCG	P05129
NKD2	Q969F2	NFAT5	O94916
ANKRD6	Q9Y2G4	NFATC1	O95644
DAAM1	Q9Y4D1	NFATC2	Q13469
DAAM2	Q86T65	NFATC3	Q12968
RHOA	P61586	NFATC4	Q14934
ROCK1	Q13464	FZD2	Q14332
ROCK2	O75116	FZD3	Q9NPG1
RAC1	P63000	FZD4	Q9ULV1
RAC2	P15153	FZD6	O60353
MAPK8	P45983	NLK	Q9UBE8
MAPK9	P45984		
MAPK10	P53779		

Table 2. Core proteins of non-canonical Wnt signaling pathway

1.1.1 Protein	1.1.2 Disease	1.1.3 References
β-catenin	Carcinogenesis, hepatocellular carcinomas Wilms' tumors	Klaus and Birchmeier,2008;Maiti *et al*.,2000
DVL	Lung cancer	Yang *et al*.,2010
FZDs	Gastric cancer,colorectal cancer& carcinogenesis	Kirikoshi, Sekihara and M. Katoh,2001;Ueno et al,2008
APC	Colorectal cancer, carcinogenesis	Klaus andBirchmeier, 2008; Ueno et al, 2008
KC1AL	Alzheimer Disease	Li,Yin and Kuret,2004
YWHAZ	Breast cancer, Obesity, Diabetes	Peng, Wang and Shan, 2009
sFRP(s)	colon cancer, mesothelioma, bladder cancer	Tan and Kelsey, 2009; Paul and Dey, 2008; Gehrke, Gandhirajan and Kreuzer, 2009.
GSK-3β	colorectal cancer	Ge and Wang, 2010
Smad3	Osteoarthritis	Valdes *et al,* 2010

Table 3. The common proteins found to be related to diseases.

3.2 Graph theoretical analysis

In order to gain insight on the characteristics of canonical and noncanonical pathways of Wnt signaling, the mean degree (average number of interactions per protein), clustering coefficients (normalized number of interactions between neighbors of each protein), mean path lengths, network diameters (longest path between any two nodes), power-law distribution exponents (γ), and centrality values were estimated using Network Analyzer. The degree distribution of each sub-network have scale-free topology and approximates a power law model ($P(k) \cong k^{-\gamma}$) with few nodes having high degree (hub proteins) and the others having low degree (Table 4). The network diameter value indicates the speed of signal flow. The diameters are 14, 13, and 15 for Wnt β-catenin, PCP and calcium signaling networks, respectively. The network diameter of the whole Wnt signaling network in which these three sub-networks are integrated, is found to be 15. The network diameter and the shortest path length distribution indicate small-world properties of the analyzed network. In addition to that, the average (mean) connectivity values are 5.72, 5.12 and 5.01 for β-catenin, PCP and calcium pathways. The topological properties of the present networks are consistent with many networks reported in literature (Table 4).

The hubs of the canonical pathway are obtained as KC1AL (Casein kinase I isoform alpha-like), YWHAZ (Protein kinase C inhibitor protein 1) and TBL1XR1 (F-box-like/WD repeat-containing protein). Casein kinase-1-alpha forms β-catenin destruction complex when connected to the proteins of APC, β-catenin and glycogen synthase kinase-3-beta (GSK3-β) (Faux *et al.*, 2008). KC1AL has interactions with the core proteins, AXIN1, AXIN2, CSNK1A1, CSNK1D and CSNK1E (String database). TBL1XR1, also a core protein of canonical Wnt signaling, is involved in signal transduction and cytoskeletal assembly and plays an essential role in transcription activation mediated by nuclear receptors and has effects on cytotypic differentiation. Besides, low levels of TBL1XR1 gene expression cause

Model	Number of Nodes	Number of Interactions	Power Law exponent(γ)	Mean Path Length	Network Diameter	Reference
Wnt/β-catenin (H. Sapiens)	3251	9304	1.78	4.46	14	Present work
Wnt/PCP (H. Sapiens)	1952	5001	1.80	4.61	13	Present work
Wnt/Ca^{+2} (H. Sapiens)	2112	5293	1.68	4.56	15	Present work
Wnt (whole) (H. Sapiens)	3489	10092	1.75	4.40	15	Present work
Wnt/β-catenin (D.melanogaster)	656	1253	1.78	4.80	13	Toku et al., 2010
Hedgehog (D.melanogaster)	568	975	1.75	4.80	14	Toku et al., 2010
EGFR (Oda et., 2005)	329	1795	1.86	4.70	11	Tekir et al., 2009
Signaling (S. cerevisiae)	1388	4640	1.76	6.81	9	Arga et al., 2007
DIP (C.elegans)	2638	4030	-	4.80	14	Wu et al., 2005
Sphingolipid (H. Sapiens)	3097	11064	1.68	4.10	13	Özbayraktar, 2011
Insulin_glucose transporting (H. Sapiens)	498	2887	1.53	2.9	5	Tekir et al., 2010
Ca-signaling (H. Sapiens)	1826	10078	1.49	3.57	11	Tiveci et al., 2011

Table 4. Graph theoretical properties of the protein interaction networks.The hubs of the Wnt/Ca^{2+} pathway are PRKCB (Protein kinase C beta type), PRKCA (Protein kinase C alpha type) and also YWHAZ (Protein kinase C inhibitor protein 1). Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and second messenger diacylglycerol. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. PRKCA also binds to RHOA which is another core protein in Wnt/PCP signaling. PRKCB, calcium-activated and phospholipid-dependent serine/threonine-protein kinase, is involved in various processes such as regulation of the B-cell receptor (BCR) signalosome, apoptosis and transcription regulation and it has an interation with the core protein, dishevelled 2 (DVL2) and the common hub protein YWHAZ. These hub proteins were also detected as the bottleneck proteins of the networks, due to their high betweenness centrality values. The topological properties of the hubs are listed in Table 5.

breast cancer (Kadota et al., 2009). YWHAZ (14-3-3 protein zeta/delta /Protein kinase C inhibitor protein 1), which is a member of highly conserved 14.3.3 proteins that are involved in many vital cellular processes such as metabolism, protein trafficking, signal transduction, apoptosis and cell cycle regulation, is a key component in both canonical and non-canonical Wnt signaling. In addition to its interaction with canonical pathway core protein of CSNK1A1, YWHAZ also has interactions with core proteins of NFATC2, NFATC4 and MAPK8 of non-canonical Wnt signaling. YWHAZ protein is the common hub and also a bottleneck protein in all reconstructed Wnt signaling sub-networks. YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer (Ralhan et al., 2008).

Model	Uniprot ID (Name)	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree	Average Shortest Path Length
Wnt/Canonical	Q8N752 (KC1AL)	0.168	0.356	0.0060	241	2.817
	P63104 (YWHAZ)	0.124	0.350	0.0071	189	2.855
	Q9BZK7 (TBL1XR1)	0.052	0.289	0.0071	107	3.464
Wnt/PCP	P63104 (YWHAZ)	0.182	0.351	0.0094	133	2.850
Wnt/Ca^{2+}	P17252 (PRKCA)	0.160	0.353	0.0122	129	2.830
	P63104 (YWHAZ)	0.136	0.343	0.0099	125	2.917
	P05771 (PRKCB)	0.135	0.334	0.0074	149	2.997

Table 5. Topological properties of bottleneck proteins in human Wnt signaling.

3.3 Module detection and analysis

Scale-free networks are known to be composed of clustered regions and in biological networks these clustered regions correspond to molecular complexes named as modules (Bader and Houge, 2003). The canonical pathway was clustered into 75 complexes. Many of the proteins in the modules have roles in binding, catalytic activity and transcriptional regulation. The modules with significant molecular functions directly related to Wnt signaling were then detected by GO enrichment analysis. Some examples are as follows: The proteins in one module of Wnt/β-catenin (canonical) pathway were enriched in Wnt protein binding. NADH dehydrogenase (ubiquinone) activity was dominant in another module. In Wnt/Planar Cell Polarity (PCP) sub-network, a module showed potassium channel activity. The proteins of a module in Wnt/Ca^{2+}subnetwork were enriched in calcium ion binding.

The information obtained by module analysis such as finding of proteins behaving functionally similar in modules enabled us to confirm the present Wnt signaling network reconstructed using an integrated approach of interactomics and GO annotations.

3.4 Network decomposition analysis

The linear paths in the reconstructed Wnt signaling network as a whole and those in each canonical and noncanonical Wnt pathway were determined via NetSearch algorithm (Steffen et al. 2002) in order to examine the signal transmittal steps. In this algorithm, the membrane (ligand) proteins were set as input whereas the transcription factors were set as output components (Table 6) of Wnt signaling network in *Homo Sapiens.*

In the Wnt signaling network as a whole, the shortest path length is found to be 4, which includes 5 proteins connected by 4 linear interactions for two linear paths from Wnt3A to LEF1 (Table 7). The path length is increased in order to cover all the proteins in the network. However, a maximum number of 12 steps that has 1 086 956 linear paths in which only 59 (50%) of 118 core proteins and 1244 (34%) of 3676 proteins are covered, is achieved due to computer capacity. The linear paths were found to reach to LEF1 (Q9UJU2) in canonical subnetwork and NFATC1 (O95644), NFATC2 (Q13469), NFATC3 (Q12968) in noncanonical subnetwork.

Input Protein (Uniprot_ID)	Protein Name	Output Protein (Uniprot_ID)	Protein Name
P04628	Wnt1	O94916	NFAT5
P09544	Wnt2	O95644	NFATC1
Q93097	Wnt2B	Q13469	NFATC2
P56703	Wnt3	Q12968	NFATC3
P56704	Wnt3A	Q14934	NFATC4
P56705	Wnt4	Q9UJU2	LEF1
P41221	Wnt5A	P36402	TCF7
Q9H1J7	Wnt5B	Q9HCS4	TF7L1
O00755	Wnt7A		
O00744	Wnt10B		
O96014	Wnt11		

Table 6. Input and output proteins of the linear paths.

Path Length	Input Protein				Output Protein
4	*P56704* (Wnt 3A)	*Q07954* (LRP1)	*P12757* (SKIL)	*Q13485* (SMAD4)	*Q9UJU2* (LEF1)
	P56704 (Wnt 3A)	*Q07954* (LRP1)	*P12757* (SKIL)	*Q15796* (SMAD2)	*Q9UJU2* (LEF1)

Table 7. The linear paths at path length of 5.

3.4.1 Canonical vs non-canonical Wnt pathways

Network decomposition analysis was performed for canonical and non-canonical Wnt pathways separately. A maximum number of 12 steps that has 815627 linear paths, in which

only 33 of 68 core proteins (42%) and 1115 of 3251 proteins (32%) are participated, can be obtained for canonical pathway. The number of linear paths at 12 steps is found to be 29082 for non-canonical pathway, in which 546 of 2547 nodes and only one core protein of 60 core proteins are covered. It has 1098373 linear paths at 14 steps, and 27 of 60 core proteins (48%) and 817 of 2547 proteins (34%) are covered. This result seems to be logical since the diameter of the non-canonical pathway is found to be larger than that of canonical pathway, which implies that the signal transfer is slower in non-canonical pathway. A minimum number of 4 steps (5 proteins) was necessary to reach the end transcription factor in canonical pathway whereas the signal has to pass at least 7 proteins in case of non-canonical pathways such as PCP or Wnt/Ca2+ signaling. In general, the information flow preferring short routes is faster in canonical pathways.

3.4.2 Participation of proteins in linear paths

For identification of the significant proteins in the whole Wnt network, the percentages of each protein contributing to linear paths were calculated (Table 8) and the proteins having participation percentages higher than 20 are discussed below. T cell specific transcription factor 1-alpha (LEF1) has the highest percentage since it is one of the output proteins. WNT7A and WNT1 are the input proteins. These three proteins (WNT7A, WNT1 and LEF1)

Uniprot ID	Protein Name	Recommended Name	Canonical/ Noncanonical	Participation in linear paths (%)	Degree
Q9UJU2	LEF1	T cell-specific transcription factor 1-alpha	Canonical	56.19	17
O00755	WNT7A	Protein Wnt-7A	Canonical	51.91	2
O00144	FZD9	Frizzled-9	Canonical/PCP/Ca^{2+}	51.91	4
Q99750	MDFI	MyoDfamilyinhibitor	Canonical/PCP/Ca^{2+}	50.94	50
P04628	WNT1	Proto-oncogene Wnt-1	Canonical/Ca^{2+}	47.20	10
Q9HD26	GOPC	Golgi-associated PDZ andcoiled-coil motif-containing protein	Canonical/PCP	46.89	18
Q9H461	FZD8	Frizzled-8	Canonical/PCP/Ca^{2+}	46.87	4
P33992	MCM5	DNA replicationlicensingfactor MCM5	Canonical/Ca^{2+}	42.94	6
Q14566	MCM6	DNA replicationlicensingfactor MCM6	Canonical/Ca^{2+}	38.83	28
Q15797	SMAD1	SMAD familymember 1	Canonical/PCP/Ca^{2+}	29.23	60
P28070	PSB4	Proteasomesubunit beta type-4	Canonical	28.75	19

Table 8. Proteins with the highest participation percentages in Wnt signaling pathway.

are also the core proteins of the canonical Wnt signaling sub-network and they bind to essential proteins, which are common to many paths in the network. Frizzled 9 (FZD9), which is a receptor for Wnt proteins, is common to all three sub-networks of Wnt signaling. It leads to the activation of dishevelled proteins, inhibition of GSK-3β kinase, nuclear accumulation of β-catenin and activation of Wnt target genes. It was hypothesized that FZD9 may be involved in transduction and intercellular transmission of polarity information during tissue morphogenesis and/or in differentiated tissues (www.uniprot.org). Another protein common to all three Wnt sub-networks is MyoD family inhibitor protein (MDFI), which regulates the transcriptional activity of TCF7L1/TCF3 by direct interaction to it, and it prevents TCF7L1/TCF3 from binding to DNA. The DNA replication licensing factor proteins (MCM5 and MCM6) have interaction with each other and MCM5 also binds to MDFI and β-catenin, which is an essential protein for Wnt signaling pathway. Besides that, SMAD1-OAZ1-PSMB4 complex mediates the degradation of the CREBBP/EP300 repressor SNIP1.

When the proteins with low participation percentages in linear paths are evaluated according to the criteria of low betweenness and high closeness centrality values, four proteins (LRSAM1, MLTK, MARK1 and miyosin 9) seem to be important for consideration as putative drug targets (either by activation or inhibition) and need further examination (Table 9).

	Protein _ID	**Name**	**Protein _ID**	**Name**	**Protein _ID**	**Name**	**Protein _ID**	**Name**
Input Protein	O00755	Wnt7A	P04628	Wnt1	P04628	Wnt1	P04628	Wnt1
	O00144	FZD9	Q9H461	FZD8	Q9H461	FZD8	Q9H461	FZD8
	Q99750	MDFI	Q9HD26	GOPC	Q9HD26	GOPC	Q9HD26	GOPC
	Q12906	ILF3	P13569	CFTR	P13569	CFTR	P13569	CFTR
	Q8N752	KC1AL	P08670	VIME	P08670	VIME	P08670	VIME
	Q9UQM7	CAMK2A	O43353	RIPK2	Q12873	CHD3	O43353	RIPK2
	Q13554	CAMK2B	P05771	PRKCB	Q14974	IMB1	P05771	PRKCB
	P48443	RXRG	**Q9P0L2**	**MARK1**	Q00722	PLCB2	**Q9NYL2**	**ZAK**
	Q6UWE0	**LRSAM1**	P31947	SFN	Q96QT4	TRPM7	P31947	SFN
	Q99816	TS101	P63104	YWHAZ	**P35579**	**MHY9**	P63104	YWHAZ
	Q13464	ROCK1	P30291	WEE1	P19838	NFKB1	P30291	WEE1
	Q15796	SMAD2	P84022	SMAD3	P17252	PRKCA	P84022	SMAD3
Output Protein	Q9UJU2	LEF1	Q9UJU2	LEF1	O95644	NFAC1	Q9UJU2	LEF1
Path Length	12		12		12		12	

Table 9. Linear paths of lowest participant proteins.

LRSAM1 (leucine rich repeat and sterile alpha motif containing1), also called RIFLE and TAL (TSG101-associated ligase), is an E3 type ubiquitin ligase. TSG101 itself is a tumor suppressor gene, which has a role in maturation of human immunodeficiency virus, and LRSAM1 is implicated in its metabolism directly by polyubiquitination (Guernsey et al., 2010). The functional disruption of TSG101 led both to cellular transformation and to tumors that metastasized spontaneously in nude mice (Li and Cohen, 1996). In addition to that, although genomic alterations in TSG101 are rare in human cancer, functional inactivation of the gene enhances metastatic growth of murine fibroblasts (Li and Cohen 1996). Another protein is ZAK (MLTK - Q9NYL2) which inhibits human lung cancer cell growth via ERK and JNK activation in an AP-1-dependent manner (Yang et al., 2010). Also, overexpression of ZAK results in apoptosis (OMIM).

Another protein is serine/threonine-protein kinase MARK1. Cellular studies showed that overexpression of MARK1 resulted in shorter dendrite length and decreased transport speed. MARK1 overexpression in individuals with autism may underlie subtle changes in synaptic plasticity linked to dendritic trafficking (Maussion et al., 2008; OMIM). The last protein is miyosin9. Fechtner syndrome, which is an autosomal dominant disorder characterized by the triad of thrombocytopenia, giant platelets, and Dohle body-like inclusions in peripheral blood leukocytes, with the additional features of nephritis, hearing loss, and eye abnormalities, mostly cataracts, is caused by heterozygous mutation in the gene encoding nonmuscle myosin heavy chain-9 (MYH9; 160775) on chromosome 22q11 (Peterson et al., 1985; OMIM). ZAK and MARK1 both bind to SFN which has interaction with YWHAZ. YWHAZ is found to be hub and bottleneck protein in these reconstructed canonical and noncanonical Wnt pathways due to its high degree and betweenness centrality value, respectively. YWHAZ also has a low participation percentage of 0.95 in linear paths. YWHAZ is found to be a key mediator protein in various diseases involving various types of cancers, heart diseases, obesity, diabetes and autism (Nguyen and Jordá, 2010). Key mediators are proteins that bind to significant proteins (mostly hubs) and so they can be chosen as the drug targets.

3.4.3 Specific proteins in linear paths

The proteins in the linear paths ending at transcription factors specific to canonical and noncanonical pathways were further examined in detail. The proteins, which participate in the linear paths leading to one transcription factor only, are called specific proteins of that particular pathway.

286 specific proteins were obtained where 262 of them belong to canonical (transcription factor LEF1) and 24 of them belong to non-canonical pathway (transcription factor NFATC). They were then investigated according to their topological properties such as lower betweenness centrality, higher closeness centrality and higher clustering coefficient than the average for drug target identification. As a result, 51 proteins (48 canonical, 3 noncanonical) meet these criteria. Among 51 proteins 4 proteins in canonical pathway seem to be important since they are either related to important diseases or connected to significant proteins in the network. These proteins are Myc proto-oncogene protein (MYC), TGF-beta receptor type-2 (TGFR2), cyclin-dependent kinase inhibitor 3 (CDKN3) and F-box-like/WD repeat-containing protein TBL1X (canonical).

MYC is a protein that participates in the regulation of gene transcription. The mutations and overexpressions seen in MYC resulted in cell proliferation and consequently formation of cancer. The translocations such as t (8:14) are the reasons of the development of Burkitt's lymphoma. Soucek *et al.,* 2008 demonstrated that the temporary inhibition of MYC selectively killed lung cancer cells in mouse, making it a potential drug target in cancer (Gearhart *et al.,* 2007; Soucek *et al*., 2008). TGFR2 is the receptor protein of TGF-beta and also known to be involved in tumor suppression. It forms receptor complexes with serine/threonine protein kinases and has role in activation of SMAD transcriptional regulators. The mutations and defects seen in this protein are associated with Lynch sendrome, Loeys-Deitz aortic aneurysm syndrome, Osler-Weber-Rendu syndrome, hereditary non-polyposis colorectal cancer type 6 (HNPCC6) and esophageal cancer (Tanaka *et al*. , 2000; Lu *et al*., 1998).

TBL1X is a protein that plays an essential role in transcription activation mediated by nuclear receptors. Besides, it is a component of E3 ubiquitin ligase complex containing UBE2D1, SIAH1, CACYBP/SIP, SKP1, APC and TBL1X proteins. It has interactions with essential proteins of Wnt signaling such as APC and β-catenin and it is also a core protein of reconstructed canonical Wnt signaling pathway (Matsuzawa and Reed, 2001). CDKN3 is a member of cyclin-dependent kinases (CDKs) which have roles in regulating cell cycle, transcription, mRNA processing, and differentiation of nerve cells (Gyuris *et al*., 1993). The overexpression and defects seen in this protein leads to prostate cancer and hepatocellular carcinoma (HCC) (Yeh *et al*., 2003; Lee *et al*., 2000).

These specific proteins except TBL1X are related to cancer and they are suitable for drug target applications according to their topological properties. Hence, they need more attention with further experimental investigation.

3.4.4 Crosstalk of proteins in Wnt sub-networks

Signaling networks are communicating systems and they interact with each other rather than behaving in isolation. If a node has a high network crosstalk value, which is defined as the difference in degree of the node in all considered networks and the maximum degree of this node in any individual pathway, it means that this component is a branch node connecting two or more pathways. The network crosstalk analysis indicated 239 proteins that are found to be common among Wnt sub-networks.

One of the highest crosstalk values belongs to YWHAZ protein (Table 10). This is rational since this protein was obtained as the hub and bottleneck protein of all canonical and non-canonical Wnt pathways. Besides, DVL2 has a significant crosstalk value. Dishevelled proteins also have high participation in the subnetworks since they interact with the core proteins such as frizzled receptors and GSK3β in Wnt/β-catenin sub-network, and with frizzled receptors and DAAM1 in Wnt/PCP sub-network. Smad proteins also have considerable crosstalk value since they have interactions with AXIN, beta-catenin and LEF1 proteins. PRKCA, which was found as hub and core protein in Wnt/calcium sub-network, has a non-zero crosstalk value. AXIN protein is also a significant protein that has participation in β-catenin destruction complex with APC, GSK3β and CKIα. Detecting these connector proteins by network crosstalk analysis is a promoter step for further experimental studies towards cancer treatment. However, further elaboration on the crosstalk mechanism is difficult due to the fact that the reconstructed networks are undirected.

Proteins		Network crosstalk values
YWHAZ	Hub-Core protein (all sub-networks)	11
DVL2	Core protein (β-catenin and Wnt/ PCP sub-networks)	11
CAMK2A	Core protein (Wnt/Ca^{2+} sub-network)	4
SMAD3-4	Core proteins (β-catenin subnetwork)	4
GSK3B	Core protein (β-catenin sub-network)	2
PRKCA	Hub-Core protein (Wnt/ Ca^{2+} subnetwork)	2
RAC1	Core protein (Wnt/PCP sub-network)	2
NFATC2	Core protein (Wnt/Ca^{2+} subnetwork)	1
AXIN1	Core protein (β-catenin sub-network)	1

Table 10. Proteins and network crosstalk values

4. Discussion

4.1 Wnt signaling in maintaining homeostasis and managing cellular stress

Homeostasis, balance of cellular processes, is an important phenomenon since cells are the factories that maintain the intracellular environment and keep the conditions stable. Therefore, it is essential for cells to maintain homeostasis for the organism to remain healthy. Wnt signaling, being related to embryonic development, generation of cell polarity and specification of cell death, is highly effective in maintaining homeostasis in adults (Peifer and Polakis, 2000). In canonical Wnt pathway, for example, the stabilization of β-catenin plays an essential role in cellular homeostasis. In the absence of Wnt ligands, a destruction complex is formed by AXIN, APC, GSK-3β and β-catenin, that results in β-catenin phosphorylation by GSK-3β followed by ubiquitination and degradation that keeps β-catenin level low in cytoplasm. Wnt ligands, on the other hand, enhance the β-catenin accumulation via inhibition of GSK-3β by dishevelled proteins and free β-catenin is transferred into the nucleus where it interacts with transcription factors. Therefore, AXIN, APC and GSK-3β proteins are significant players for homeostasis.

The mutations seen in AXIN result in hepatocellular carcinoma, which implies that, it has a multi-objective position in tumorigenesis and embryonic axis formation. It is also reported that the main role of AXIN, beside controlling β-catenin level, is to down-regulate cell growth and help sustain cellular homeostasis (Zhang et al., 2001). AXIN is known to be is a "switch" protein for JNK and Wnt signaling pathways. It binds to MEKK1 and activates JNK signaling. MEKK1 is related to microtubule cytoskeletal stress and apoptosis. During JNK activation, AXIN-MEKK1-APC-β-catenin complex transduces the cytoskeletal stress signals for apoptosis (Yujiri et al., 1999; Zhang et al., 2001).

4.2 Wnt/Ca^{2+}-Wnt/β-catenin antagonistic mechanism in *H. Sapiens*

The non-canonical Wnt signalling pathways do not signal through β-catenin and they can antagonize the functions of canonical Wnt pathway (Mc Donald and Silver, 2009). Wnt5a is known to activate non-canonical signalling via cGMP(cylic guanosine-3'5'-monophosphate) that actives protein kinase G. This leads to an increase in the cellular concentration of Ca^{2+}

and this Ca^{2+} increase triggers activation of calcium sensitive proteins. Wnt5a also inhibits the activation of canonical signalling via activation of NFAT which is mediated by activation of PLC (phospolipase C). PLC increases the calcium level that results in activation of CaCN (calcineurin) which activates NFAT.

Wnt/Ca^{2+} signalling pathway can inhibit Wnt/β-catenin pathway in two different ways: CACN-NFAT branch and CAMKII-TAK1-NLK branch (Figure 5). CACN-NFAT branch for inhibiting β-catenin function is mediated by PLC activation, which involves the β/γ subunits of heterotrimeric G-proteins leaving its α unit behind. PLC activation generates diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3) which eventually increases Ca^{+2} concentration in the cell. The calcium increase sets off the CaCN activation that results in dephosphorylation of NFAT (nuclear factor of activated T-cells). NFAT then translocates to nucleus to regulate gene expression. This CaCN-NFAT activated way inhibiting the canonical Wnt signalling pathway is covered in our reconstructed network (Saneyoshi et al., 2002; Veeman et al., 2003; Pandur, 2005). Moreover, the reconstructed network successfully

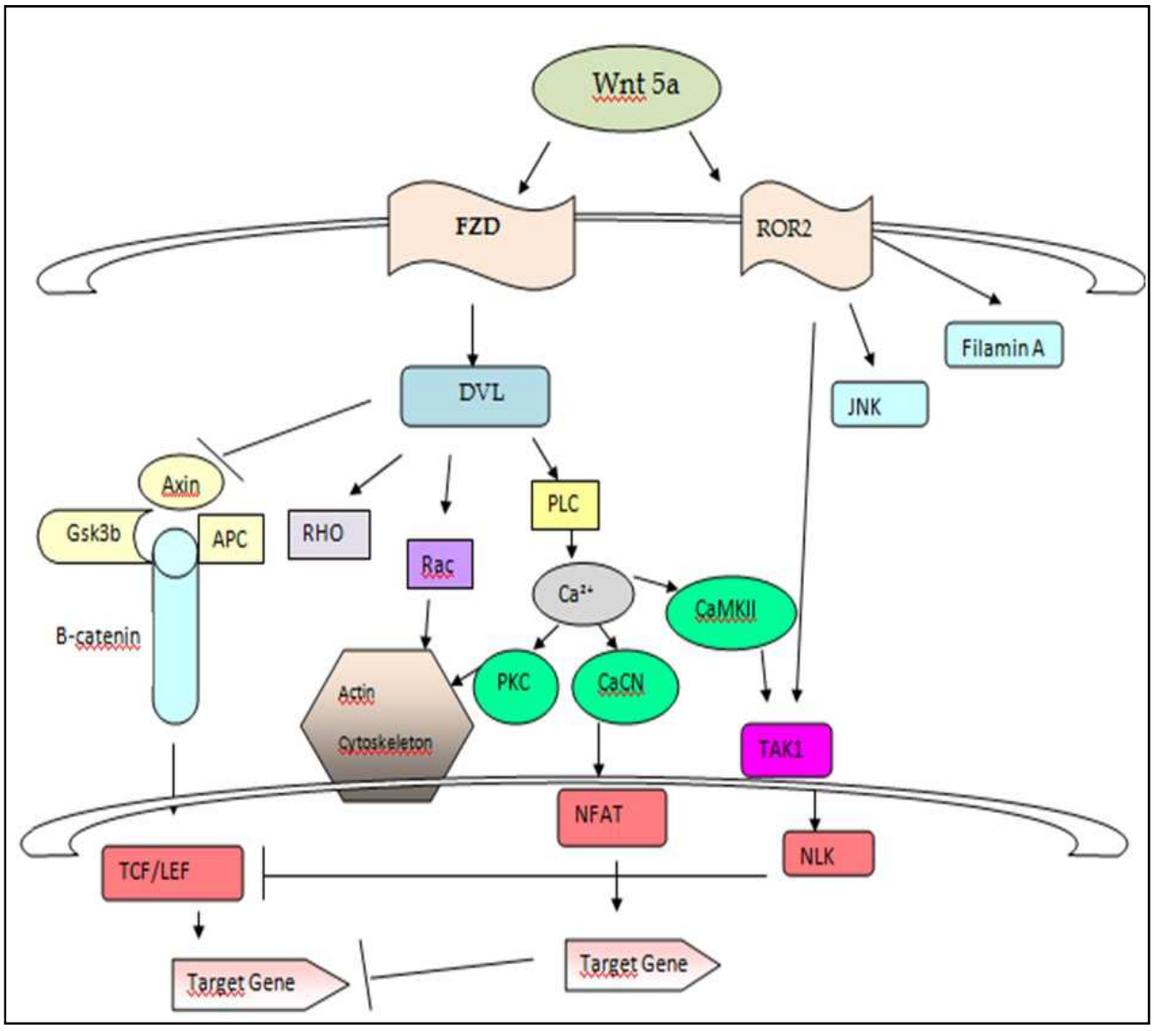


Fig. 5. General representation of Wnt signaling pathway in vertebrates.

covers CAMKII-TAK1-NLK branch, which is known to inhibit Wnt/β-catenin signalling pathway. As it is mentioned above, the PLC activation results in calcium release. The increase seen in Ca^{2+} level may trigger activation of another calcium sensitive protein; Ca^{2+}-calmodulin-dependent protein kinase II (CamKII) which further activates TGF-β activated kinase 1 (TAK1). TAK1 then stimulates nemo-like kinase (NLK), which has a role in TCF phosphorylation. Afterwards, the phosphorylation of TCF inhibits TCF/β–catenin complex (Kuhl et al., 2000; Pandur, 2005).

Besides stimulating non-canonical signaling and inhibiting canonical signalling through CamkII mentioned above, Wnt-5a can also inhibit the activation of canonical signalling through ROR2 signalling pathway that stimulates TAK1-NLK pathway in turn. ROR2 receptor also actives the actin binding protein Filamin A and JNK pathway (Mc Donald and Silver, 2009).

As a consequence it can be said that Wnt5a exhibits tumor suppressor activity through inhibiting the activation of canonical Wnt signalling. Recent research showed that, in HTC116 and HT-29 colon cancer cell lines, the activation of β-catenin-mediated transcription is reduced by Wnt5a (Macleod et al., 2007; Ying et al., 2008). Additionally, the reconstructed network provides a chance to investigate the antagonism between Wnt/Ca^{2+} and Wnt/β-catenin signalling pathways. Although the static nature of the network cannot directly explain the interaction characteristics between these pathways, a dynamic model can enlighten the antagonism between Wnt/Ca^{2+} and Wnt/β-catenin pathways.

4.3 Potential drug targets in the reconstructed Wnt signaling networks

Wnt signaling pathways regulate many cellular processes such as proliferation, migration and differentiation in embryonic development and maintenance of homeostasis in matured tissues. The deregulations and mutations in Wnt signaling pathway are known to result in cancer. Unfortunately, there is no selective inhibitor for the deficiencies in Wnt signaling. That is why targeting key components, such as SFRPs, WIF-1, DKK-1, APC, AXIN2, ICAT, LEF1 and β-catenin, of the Wnt signaling seems to be reasonable in cancer treatment (Aguilera et al., 2007).

The topological parameters such as centrality values or participation percentages in linear paths are important criteria in identification of putative target proteins. Therefore, the nodes that have lower average shortest path length, higher clustering coefficient, higher closeness centrality, lower betweenness centrality and higher participation percentages than the average values are further investigated (Table 11).

In our reconstructed networks, FZD9, WNT7A and LEF 1 proteins are found to be essential due to their high participation percentages in linear path analysis. Albers et al. (2011) show that the Wnt receptor Frizzled-9 (FZD-9) can be a new potential target for the treatment of osteoporosis by promoting bone formation. Also, it is known that the re-expression of WNT7A and signaling through FZD9 are associated with increased differentiation and used in the lung cancer treatment (Winn et al., 2005). Frizzled proteins are the receptors for Wnt ligand, and they are structurally similar to G protein-coupled receptors (GPCRs) which are targets of more than 50% of chemically applicable drugs (Yanaga and Sasaguri, 2007). So targeting frizzled proteins seems to be logical in cancer treatment. In addition to that, β-catenin has a connectivity value of 40 and participation percentage of 4.36%. β-catenin is

Uniprot ID	Name	Average Shortest Path Length	Betwenness Centrality	Closeness Centrality	Clustering Coefficient	Degree	Participation Percentage
P35222	β-catenin	**3.42**	1.43×10^{-2}	**0.292**	1.92×10^{-2}	40	$\mathbf{4.36}$
Q9UJU2	LEF1	**3.58**	4.52×10^{-3}	**0.279**	4.41×10^{-2}	17	$\mathbf{56.2}$
P49841	GSK3b	**3.42**	2.97×10^{-3}	**0.292**	8.57×10^{-2}	15	2.08×10^{-1}
P25054	APC	**3.66**	$\mathbf{7.45 \times 10^{-4}}$	**0.273**	$\mathbf{1.67 \times 10^{-1}}$	4	5.47×10^{-2}
Q8N474	SFRP1	6.49	$\mathbf{6.93 \times 10^{-4}}$	0.154	0	4	-
O94907	DKK-1	**4.22**	$\mathbf{5.94 \times 10^{-4}}$	0.237	0	2	-
Q9Y2T1	AXIN2	4.63	$\mathbf{5.73 \times 10^{-4}}$	0.216	$\mathbf{3.33 \times 10^{-1}}$	3	1.54×10^{-3}
Q14332	fzd2	4.57	$\mathbf{2.42 \times 10^{-4}}$	0.219	0	2	-
Q9NSA3	ICAT	4.41	$\mathbf{3.36 \times 10^{-5}}$	0.227	0	2	1.30×10^{-3}
Q9Y5W5	WIF-1	6.49	0	0.154	0	1	-
Q6UWE0	LRSAM1	**4.18**	8.73×10^{-4}	**0.239**	0	5	8.10×10^{-5}
Q9P0L2	MARK1	**3.90**	4.78×10^{-5}	**0.256**	0	2	8.10×10^{-5}
Q9NYL2	ZAK	**3.90**	6.21×10^{-4}	**0.256**	0	3	8.10×10^{-5}
P35579	MHY9	**3.58**	5.61×10^{-4}	**0.280**	1.11×10^{-1}	10	8.10×10^{-5}
O00144	FZD9	4.80	4.71×10^{-3}	0.208	0	4	$\mathbf{51.9}$
O00755	WNT7A	5.57	$\mathbf{4.27 \times 10^{-5}}$	0.179	0	2	$\mathbf{51.9}$
Q8N752	KC1AL	**2.83**	1.40×10^{-1}	0.354	6.09×10^{-3}	241	4.81
P63104	YWHAZ	**2.83**	1.18×10^{-1}	**0.353**	6.97×10^{-3}	200	9.46×10^{-1}
Q9BZK7	TBL1XR1	**3.46**	4.62×10^{-2}	**0.289**	7.14×10^{-3}	107	1.60×10^{-1}
Q16667	CNKD3	**4.31**	0	**0.232**	**1**	2	8.10×10^{-5}
P37173	TGFR2	**4.31**	$\mathbf{1.57 \times 10^{-4}}$	**0.232**	$\mathbf{6.0 \times 10^{-1}}$	5	4.69×10^{-2}
P01106	MYC	**4.14**	$\mathbf{1.53 \times 10^{-4}}$	**0.242**	$\mathbf{2.0 \times 10^{-1}}$	5	2.00×10^{-3}
Average		4.40	9.76×10^{-4}	0.232	1.13×10^{-1}	5.8	$7.7.78 \times 10^{-1}$

Table 11. The topological values of the target proteins.

encoded by an oncogene and has functions in the maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. β-catenin also anchors the actin cytoskeleton (Peifer and Polakis, 2000; Zhang et al., 2001). Luu et al. (2004) suggested that targeting β-catenin could be a rational approach in cancer treatment.

In the present Wnt network, there are two essential proteins (AXIN2 and APC) that have higher clustering coefficient values than the average and it is known that essential proteins tend to be more cliquish within the interaction network (Yu *et al.,* 2004; Estrada E., 2006). Ranking proteins according to their centrality measures can additionally be useful in selecting possible drug targets. Consequently, GSK3β and APC can be seen as potential drug targets in Wnt signaling for having higher closeness centrality value than the average. APC is related with colorectal cancer and APC-activating mutations are very common in colorectal cancer (Estrada E., 2006; Garber, 2009; Yanaga and Sasaguri, 2007).

Moreover, the betweenness centrality and bridging centrality (nodes between modules and connecting clusters defined by the ratio of the number of interactions of a neighboring node over the number of remaining edges) are also effective in identifying the drug targets due to their position in communication (Hopkins, 2008, Hwang et al., 2008). In order to prevent side effects and high lethality, the essential nodes with lower betweenness centrality values are chosen as drug targets on the purpose of not affecting the neighbors of the targeted protein. It is seen that APC, DKK1, AXIN2, FZD2, Wnt7A, ICAT and WIF1 are consistent with this fact (Table 11). SFRP1 protein needs special attention since its loss causes breast cancer (Klopocki et al., 2004).

It is further seen that the nodes which have low participation percentages as well as low degrees (LRSAM1, MARK1, ZAK, MHY9), the nodes which are defined as specific proteins (CNKD3, TGFR2 and MYC) and the nodes which are detected as hub proteins (YWHAZ, TBL1XR1, KC1AL) have the quality of conformance since they have lower average shortest path length and higher closeness centrality values than the average. These proteins can also be suggested as potential drug targets and more attention should be given through experimental analysis.

The gene expression data (microarray data) belonging to these proteins are within reach via several database sources. However, due to the disease heterogeneity, the expression level of a gene /protein can be up-regulated as well as down-regulated in cancer and the expression type may also differ according to the cancer type. Hence, it is difficult to obtain a right answer for the expression level of a gene/protein in diseases like cancer.

5. Conclusion

Recently, the evolutionarily conserved signaling pathways which are involved in embryonic development are on the march for many researches since the deregulations seen in the mechanism of these pathways results in several diseases, especially in cancer. Hence, interaction networks have begun to be appreciated because it may be useful to understand the general principles of biological systems by means of systems biology. Wnt signaling is a major signaling pathway which has important roles in embryonic development of many species. Hence, in this study, Wnt signaling pathway is investigated with the aim of getting an insight on the role of Wnt signalling in maintaining homeostasis as well as managing cellular stress, understanding the molecular basis underlying the ability of Wnt proteins to perform antagonistic or similar signalling activities and identifying the suitable drug targets for therapeutic intervention in cancer treatment.

The reconstruction of Wnt signaling network was performed for *Homo sapiens* via integration of interactome data and Gene Ontology annotations. The reconstruction process was applied to both canonical (Wnt/β-catenin) and non-canonical Wnt signaling pathways (Wnt/planar cell polarity; Wnt/calcium). The reconstructed whole Wnt signaling network contains 3489 nodes and 10092 interactions. AXIN, APC and GSK-3β proteins are found to be significant players for homeostasis. Moreover, AXIN-MEKK1-APC-β-catenin complex is important in transducing the cytoskeletal stress signals leading to apoptosis.

The ligand Wnt5a has dual role; it activates non-canonical signalling and also inhibits the activation of canonical signalling through a calcium dependent mechanism. This antagonism between noncanonical Wnt/Ca^{2+} and canonical Wnt/β-catenin signalling

pathways is successfully covered in our reconstructed network. CNKD3, TGFR2 and MYC, which are the specific proteins in linear paths leading to specific transcription factors in canonical pathway, are proposed as potential drug targets for cancer. The reconstructed large-scale protein-protein interaction network of Wnt signaling in *H. sapiens* will allow system biologist to see the global picture and guide them in designing experiments. For further research, experimental and clinical studies can be carried out for the validation of the proposed drug targets leading to design novel drugs.

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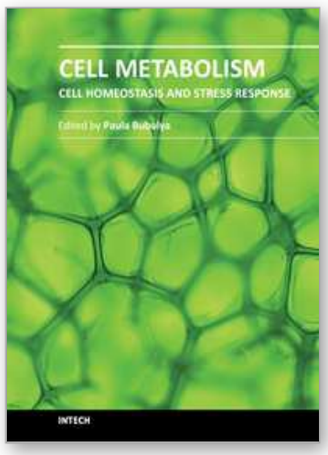
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