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ORIGINAL ARTICLE

In Silico Analysis of Molecular Interactions of FZD10 in Wnt Signaling Pathway Involved in Wound Healing

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ABSTRACT

Background: Wnt signaling pathway is a complex network involving important molecular and functional interaction molecules. It has a key role in the wound healing process.
Aim*:* To identify the molecular interactions of FZD10 in Wnt signalling pathway in wound healing.
Study Design: Computation based in silico study
Place and Duration of Study: Department of Biochemistry, Swat Medical College (STMC), Saidu Shareef, Swat, Pakistan from 1st March 2021 to 31st August 2021.
Methodology: We applied Search Tool for the Retrieval of Interacting Gene/Proteins (STRING) analysis, using specific parameters including (1) Textmining (2) Experiments (3) Databases (4) Co-expression (5) Neighbourhood (6) Gene fusion and (7) Co-occurrence for the identification of molecular interactions of the Wnt signaling pathway. The maximum number of interactors was set at 20 and highest confidence level at 0.900.
Results: STRING network analysis revealed that Frizzled Homologue 10 (FZD10) protein interacts with Hypoxia-Inducible Lipid Droplet-Associated Protein(HILPDA), Wnt1,Wnt3A, Wnt5A,Wnt7A,Wnt7B, Wnt16, Low-density lipoprotein receptor-related protein 5 (LRP5), LRP6, and Dishevelled Segment Polarity Protein 1 (DVL1) proteins in Wnt signaling pathway and contributes in various cellular activities, including cell proliferation, cellular migration, differentiation, apoptosis and stem cell regeneration, which are crucial for the wound healing process.
Conclusion: Functional enrichment analysis revealed that FZD10 protein is a key player in Wnt signaling pathway and has the potential to be considered as a candidate molecule for the therapeutics of wound healing process in future.
Keywords: Wnt Signaling pathway, STRING, molecular interactions, Frizzled-10, Cell proliferation, Cell migration

INTRODUCTION

Wnt signaling pathway is a highly intricate and active pathway in adults, playing a role in stem cell maintenance and self-renewal of normal tissue homeostasis and regeneration after injury.[1] Wnt signaling pathway regulates the key cellular functions including proliferation, differentiation, migration, apoptosis, and stem cell renewal.[2] Translated products of *WNT* gene are cysteine-rich glycoproteins, secreted by the cells into the extracellular matrix.[3] Wnt binds to the N-terminal extracellular cysteine-rich domain of a Frizzled family receptor, which is a member of the super family of G-protein coupled receptors.[2]

Frizzled (FZD) proteins belong to a family of Wnt receptors.[4] FZD10 contains one FZ (frizzled) domain. FZD10 protein is a cell surface receptor, which is activated by Wnt proteins and is involved in the regulation of cellular function.[5] Most of the frizzled receptors are coupled to the beta-catenin canonical signaling pathway, which leads to the activation of disheveled proteins, inhibition of GSK-3 kinase, nuclear accumulation of beta-catenin and activation of Wnt target genes. FZD10 is also known as FzE7 or CD350, which belongs to the G-protein coupled receptor Fz/Smo family.[6] FZD10 protein has been studied in various cancers[4], but is not extensively studied for its role in Wnt signaling pathway. Therefore, in this study, we aim to understand the molecular interactions of FZD10 in WNT signaling pathway through *In silico* analysis.

PATIENTS AND METHODS

This study was conducted from March 2021 to Aug 2021, at the Department of Biochemistry, Swat Medical College (STMC), SaiduShareef, Swat, Pakistan, following the approval by the Ethical Review Board (ERB). The *In silico* bioinformatics technique " STRING" version 11.5[7] was used to predict and analysed the molecular interactions of Wnt signaling pathway. STRING analysis was computed, based on particular set criteria including (1) Textmining (2) Experiments (3) Databases (4) Co-expression (5) Neighbourhood (6) Gene fusion and (7) Co-occurrence. The maximum number of all possible protein interactors were selected in the network. The highest confidence level was set at 0.900 as network interaction score. Result was evaluated for network enrichment p-value and average local clustering coefficient value. The network interactions were also assessed for cellular functions.[8]

RESULTS

Network interactions of FZD10 protein were studied by using STRING computing software. The name of protein was used as "query protein" in string software, which provided the network associations around the query protein from the set available protein databases. These associations were curated on the basis of (1) Textmining (2) Experiments (3) Databases (4) Co-expression (5) Neighbourhood (6) Gene fusion and (7) Co-occurrence of interaction protein with the query protein. Reliability of network and its enrichment was evaluated by inbuilt

statistical analysis in STRING software. The strength of the network was measured as log 10 (observed/expected), which described the enrichment affect. The log 10 (observed/expected)was the ratio between (i) the number of proteins in network that are annotated with a term and (ii) the number of protein that was expected to be annotated with this term in a random network of the same size. The average local clustering coefficient came out to be 0.982 for 11 nodes (including one query protein and 10 interactive proteins) and 46 edges that supported the confidence of analysis. The nodes in STRING analysis network represented the proteins, which were produced by a single coding region of gene locus. Small-sized node represented the indicated protein of unknown three dimensional structures (3D), whereas large-sized nodes, represented the protein with known or predicted three dimensional structure. Our results showed that resultant network consisted of only large nodes with known 3D structure. Red coloured node represented the FZD10 protein (query protein) and first shell of interactions with other proteins, whereas the white node symbolized the second shell of interactions. The network edges represented the protein-protein associations. Known interactions were extracted from curated database and experimental analysis, whereas predicted interactions were based upon gene neighbourhood, gene fusion and gene co-occurrence. Results also revealed some interactions were obtained from analysis of Textmining, co-expression and protein homology. String analysis showed FZD10 protein's interaction with ten other proteins including (1) HILPDA (Hypoxia-inducible lipid droplet-associated protein), (2) Wnt7A (Protein Wnt-7a), (3) Wnt7B (Protein Wnt-7b), (4) Wnt1 (Proto-oncogene Wnt-1), (5) Wnt16 (Protein Wnt-16), (6) Wnt5A (Protein Wnt-5a), (7) LRP5 (Low-density lipoprotein receptor-related protein 5), (8) LRP6 (Low-density lipoprotein receptor-related protein 6), (9) Wnt3A (Protein Wnt-3a), (10) DVL1 (Dishevelled Segment Polarity Protein1) (Fig. 1).The molecular interaction enrichment of FZD10 protein with 10 interactors was also tested statistically and results showed that network interactions were significant, having a Protein-Protein Interaction (PPI) enrichment p-value 4.77e-15.

These interactions revealed functional enrichment of FZD10 in biological process, molecular function, cellular component, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, PFAM (Protein family) protein domains, INTERPRO (InterPro Protein sequence analysis and classification) protein domains and GO (Gene ontology) features. Based on these associations, the result showed interaction of FZD10 in total fifteen networks. Out of these networks, Wnt signaling pathway was selected and exploredfor FZD10 associations in Wnt pathway (Table 1).

Wnt signaling pathway showed significant set of interactions of FZD10 protein (p< 1.45e-11) with 6 interactors out of 11. These 6 interactors belong to the proteins of Wnt family such as Wnt1, Wnt3A, Wnt5A, Wnt7A, Wnt7B and Wnt16 (Fig. 2).

Subcellular compartment analysis revealed that LRP5, Wnt3A and LRP6 interact and form the Wnt-Frizzled-LRP5/6 complex, which is membrane bound and is involved in receptor binding and subcellular interaction (p< 4.84e-06) (Fig. 3).

Interactions of FZD10 protein were evaluated in different cellular activities such as cell proliferation, differentiation, migration, apoptosis and stem cell renewal (Fig. 4).

Table 1: STRING networks of FZD10 protein

Pathway	Description	Count in network	Strength	False discovery rate
hsa05217	Basal cell carcinoma	8 of 62	2.36	2.19e-16
hsa04916	Melanogenesis	8 of 95	2.18	4.11e-15
hsa05226	Gastric cancer	10 of 114	2.09	3.35e-18
hsa05224	Breast cancer	10 of 145	2.09	3.35e-18
hsa04150	mTOR signalling pathway	10 of 151	2.07	3.35e-18
hsa04310	Wnt signalling pathway	10 of 154	2.06	3.35e-18
hsa05225	Hepatocellular carcinoma	10 of 160	2.05	3.35e-18
hsa04550	Signaling pathways regulation pluripotency of stem cell	8 of 140	2.01	7.20e-14
hsa04934	Cushing syndrome	8 of 153	1.97	1.17e-13
hsa04390	Hippo signalling pathway	8 of 153	1.97	1.17e-13
hsa05205	Proteoglycans in cancer	7 of 196	1.8	1.20e-10
hsa05010	Alzheimer disease	10 of 355	1.7	3.15e-15
hsa05165	Human papillomavirus infection	8 of 325	1.64	3.55e-11
hsa05200	Pathways in cancer	10 of 517	1.54	8.96e-14
has04928	Parathyroid hormone synthesis, secretion and action	2 of 103	1.54	0.0460

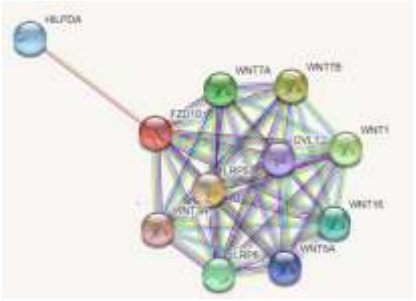


Fig. 1: Functional enrichment of FZD10 protein

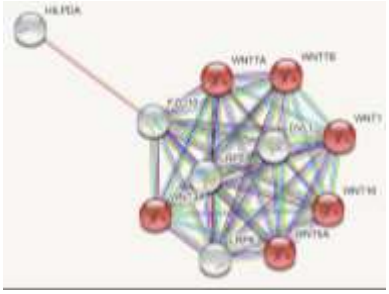


Fig. 2: Interactions of FZD10 protein in Wnt pathway

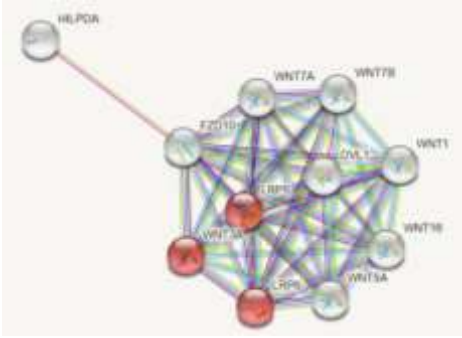
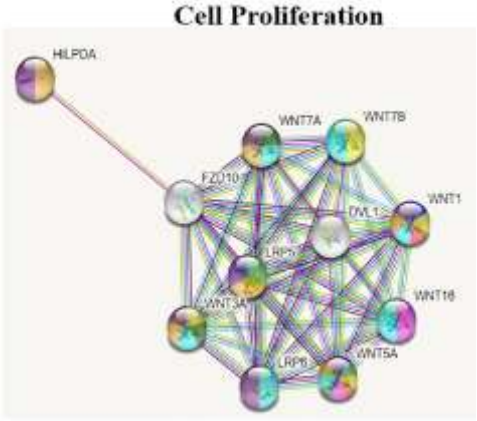
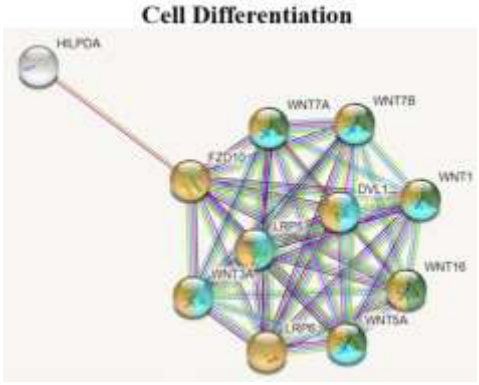
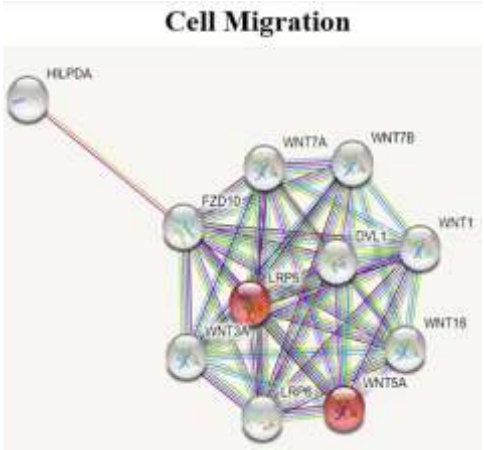
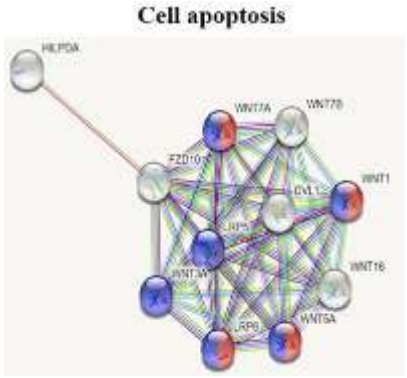


Fig. 3: Wnt-Frizzled-LRP5/6 complex









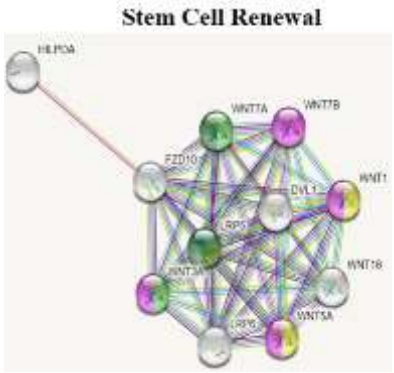


Fig. 4. Cellular interaction networks of FZD10 protein

RESULTS

of STRING analysis showed that FZD10 protein was also involved in cellular functions through its association with various interacting proteins. FZD10 interacts with HILPDA, Wnt7A, Wnt7B Wnt1, Wnt16, Wnt5A, LRP5, LRP6, DVL1 and Wnt3A and contributes in cellular proliferation and regulation, particularly in fibroblasts ($p<0.0179$), neural ($p<0.0007$) and epithelial cells ($p<0.435$). FZD10 interacts with all proteins except HILPDA and its positive association with nine proteins is involved in cell differentiation ($p<8.19e-05$). The interactions of FZD10 with Wnt1, Wnt7A, Wnt7B, Wnt16 and Wnt5A contributed in epithelial cellular differentiation ($p<0.0014$) and its association of Wnt7A, Wnt7B, Wnt1, Wnt16, Wnt5A, LRP5 and Wnt3A regulated the cell proliferation process ($p<0.0013$). LRP5 and Wnt5A interacted with FZD10 and was involved in cell migration during early embryonic development ($p<0.0028$). Network analysis showed that FZD10 regulated the apoptosis process in the cells by its interaction with Wnt7A, Wnt1, LRP5, LRP6, Wnt3A and Wnt5A ($p< 0.0046$).Furthermore, this analysis revealed the role of FZD10 interactions in stem cell renewal, such as Wnt7B, Wnt1, Wnt5A and Wnt3A associations result in stem cell proliferation ($p<7.09e-06$). FZD10, interactions with Wnt1 and Wnt5A specifically were involved in Hematopoietic stem cell proliferation ($p<0.0025$). Wnt7A, Wnt3A interaction with FZD10 affected cell division in stem cells ($p<0.0014$).

DISCUSSION

We performed network interaction analysis by using computational tool “STRING software” to understand the molecular and cellular functions and association of FZD10

protein. Comprehensive analysis showed the important role of FZD10 in many different pathways including Wnt signaling which has a key role during wound healing. This functional network analysis translates the importance and vitality of FZD10 protein within the biological systems and regulatory networks at molecular and protein level. It was also noticed during network analysis that FZD10 formed "Wnt-Frizzled-LRP5/6 complex" in plasma membrane and contributed in receptor function and downstream signaling. This complex also plays an important role in regeneration.[9] Interestingly among all these interaction, the common association between FZD10 protein and Wnt protein revealed strong bond at functional and molecular level. A previous study on a mouse model showed that Wnt signaling mediated by FZD10 was involved in the activation of vascular endothelial and smooth muscle cell that contributed positively in wound healing process.[10] STRING analysis revealed that FZD10 interactions contributed in various cellular functions which are crucial for the wound healing process such as cell proliferation, cellular migration, differentiation, apoptosis and stem cell regeneration.[11,12] Due to the important role of FZD10-Wntsignalingparticularly in cellular functions and stem cell regeneration, this pathway can be used for the development of novel therapeutic models which can induce wound healing by targeting the Wnt pathway.[13]

CONCLUSION

Functional enrichment analysis revealed that FZD10 protein is a key player in Wnt signalling pathway and has a potential to be considered as a candidate molecule for further studies related to the therapeutics of wound healing.

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