**Elucidating the potential role of Wnt Inhibitory Factor-1 (WIF-1) as a Wnt signalling antagonist in cancer therapeutics**

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**Abstract:**

Cancer is a complex disease characterized by abnormal gene expression and aberrant cellular signaling pathways. The Wnt/β-catenin signaling is a prominent contributor to cell survival, EMT and MDR in several cancers. The WIF1 protein is a potent Wnt pathway antagonist but is downregulated in major cancers, and the connections of WIF1 protein with Wnt pathway components have not been thoroughly investigated. In this current study, firstly, a computational strategy including gene ontology and expression analysis has been performed to gain insights into the function of the WIF1 protein in various cancer subtypes. Further, the PPI analysis was performed, which highlighted Wnt ligands (Wnt1, Wnt3a, Wnt4, Wnt5a, Wnt8a, and Wnt9a), along with the Frizzled receptors (Fzd1 and Fzd2) and the low-density lipoprotein complex (Lrp5/6) as the foremost interactors of WIF1 protein. Molecular docking studies and molecular dynamics simulations were performed to evaluate the binding energy, dynamics, and stability of the interacting proteins with the WIF1 domain of the protein. The results highlight WIF1-Lrp6 and WIF1-Wnt8a complexes as the most stable interactions, while Wnt3a-WIF1 and Wnt5a-WIF1 complexes as moderately stable. This study contributes to developing an interaction nexus and plausible role of WIF1 protein in inhibiting cancer progression. Further, it is known that the activated Wnt signaling pathway plays a key role in the proliferation and metastasis of TNBC. *In silico* study using the TCGA datasets revealed that the WIF1 gene is significantly downregulated in breast cancer. Therefore, to modulate the Wnt signaling in TNBC, recombinant WIFI protein has been successfully cloned in the pET28a vector and confirmed with a legitimate band at 1.14kb for the WIF1 gene. Further, the overexpression of His–WIF1 has been optimized and confirmed by SDS page with a band at around 42 kDa. Eventually, cellular assays will be performed, which would help establish the WIF1 protein as a potent molecule to inhibit the Wnt signaling-induced TNBC progression and invasion.

**Keywords:** Cancer, Protein interactions, WIF1, Wnt signaling pathway, Cloning, MD simulations