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Research Article

An integrated approach for herbal drugs to target GSK3B and its linkage with melanoma and type-2 diabetes

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Abstract: Glycogen synthase kinase-3 (GSK3) is known to be among the busiest kinase in most cells which have more than 100 known substrates. The current understanding of GSK3 regulated mechanisms are emerging topics in the actions of GSK3, particularly its interactions with receptors and receptor-coupled signal transduction events, and differential actions and regulation of the two GSK3 isoforms, GSK3α and GSK3β. To understand the control of GSK3 control in terms of selective phosphorylation of the substrate and why does it appear evolutionarily favorable for GSK3 to assume such a large responsibility is potentially challenging main in case of human diseases. GSK3 must be particularly adaptable for incorporating new substrates into its repertoire, and we focused on it in unique way where it focused on the critical pathways and their major components which are common in the diseases type-2 diabetes and melanoma mediated by GSK3β that may contribute to its capacity to fulfill its roles in multiple signaling pathways and finally, we have targeted these GSK3 associated proteins with the herbal drugs Diosmin and Fisetin. Here, we unravel the potential links between GSK3β targets, melanoma, and type-2 diabetes and further presented pivotal herbal drugs and the targets for both the diseases mediated by GSK3β leading to the conclusion that there is no direct linkage between melanoma and type-2 diabetes but GSK3β mediates them.

Keywords: GSK3β; gene expression; herbal drugs; Diosmin and Fisetin; type-2 diabetes; and melanoma

1. Introduction

GSK3 is a widely expressed serine/threonine kinase that comes in two forms: GSK3 and GSK3 isoforms, both of which are active in the absence of phosphorylation by separate upstream kinases. GSK3 was first found as a glycogen synthesis regulator, but it's now implicated in a number of signaling pathways that govern a variety of activities. Hepatocellular carcinoma, cholangiocarcinoma, breast cancer, prostate cancer, leukaemia, and melanoma are all known to be linked to GSK3. It has been demonstrated to trigger apoptosis in some situations and prevent apoptosis in others, as well as to promote cancer development or limit tumor cell proliferation, indicating that various GSK3 modulators may target distinct targets). It also works on autophagy regulation, evaluating signaling pathways in neurodegenerative and liver illnesses, as well as oxidative stress and autophagic cell death, with an emphasis on liver injury. GSK3 is also thought to be a potential therapeutic target for natural and synthetic inhibitors in a variety of disorders, a regulator of mammalian ageing, and a link between metabolic changes in senescent cells and age-related diseases[1-4].

GSK3 inhibitors have previously been demonstrated to be effective in the treatment of SARS-CoV2. The phosphorylation of key serine residues in SARS-CoV2 nucleocapsid proteins by GSK3, which is necessary for viral replication, suggests that GSK3 may play a role in influencing innate and adaptive immune responses. GSK3 inhibition dampens hepatic and renal failure in animal models of hemorrhagic shock, according to studies, by upregulating anti-inflammatory IL-10 and downregulating IL-12p40 and IL-6, a cytokine implicated in the cytokine release syndrome found in patients with severe SARS-CoV2. GSK3 inhibition lowers the systemic inflammatory response in models of sepsis and ischaemia/reperfusion injury through modulating the NF-kB-induced inflammatory response. SIR and the prevalent diffuse intravascular vascular coagulopathy found with SARS-CoV2 infection may be affected by these results. GSK3 inhibition lowered mRNA expression of IL-1, IL-6, and inducible NO synthase in a lipopolysaccharide (LPS)-mediated inflammatory model (iNOS)[3, 5-7].

Diabetes is a high-risk illness that is connected to heart disease and stroke. According to the WHO, diabetes will affect 511 million people by 2030. Many targets have been found in the hunt for new targets for type-2 diabetes, including GSK3, Dipeptidyl Peptidase, PPAR, Glucosidase, Amylase, GLP-1, and SGLT. Among the targets, GSK3 has been found to be an effective therapy for diabetes. GSK3 is a glycogen synthesis regulating enzyme in glycogen metabolism (glycogenesis). It catalyzes the creation of 1,4-glycosidic linkages in a linear, nonbranched molecule. GSK3 is a family of semi-conservative multifunctional serine/threonine kinase enzymes that comprises two isoenzymes with different N- and C-terminal sequences. Previous research has also revealed that there are distinct routes for diabetes and melanoma, as well as how GSK3 alteration may influence both. A mutation in the GSK3 complex causes diabetes. Synthetic and natural scaffolds affect GSK3, which has led to its optimization for the development of GSK3 inhibitors[8-14].

There are a number of previous research work focused on the role of GSK3 signaling in melanoma carcinogenesis. According to immunohistochemistry, GSK3 was shown to be focally expressed in the invasive portions of 12 and 33 percent of primary and metastatic melanomas, respectively. GSK3 inhibitors and siRNA knockdown of GSK3 were reported to reduce the motile behavior of melanoma cells in scratch wound, three-dimensional collagen-implanted spheroid, and modified Boyden chamber tests. GSK3 signaling inhibition was connected to lower transcription factor expression Slug and was shown to restrict N-cadherin expression at the messenger RNA and protein levels. Forced overexpression of N-cadherin reversed the impact of pharmacological and genetic GSK3 signaling ablation, which inhibited melanoma cells from adhering to endothelial cells and fibroblasts and preventing transendothelial migration. The ability of GSK3 inhibitors and siRNA knockdown to prevent FAK phosphorylation and increase the number of focal adhesions shows that GSK3 signaling plays another function in invasion. To summarize, we have discovered a previously undiscovered role for GSK3 in changing the motile and invasive behavior of melanoma cells via N-cadherin and FAK, to our knowledge. According to this study, inhibiting GSK3 may have therapeutic relevance in certain kinds of melanoma[15, 16].

With over 100 identified substrates to deal with, GSK3 may be the busiest kinase in most cells. How does GSK3 preserve its ability to selectively phosphorylate each substrate, and why was it advantageous for GSK3 to take on such a big role in evolution? GSK3 must be very versatile to add new substrates to its repertoire, and we examine the unique characteristics of GSK3 that may contribute to its ability to play diverse roles in signaling circuits. The regulatory mechanisms governing GSK3 (primarily post-translational modifications, substrate priming, cellular trafficking, and protein complexes) have previously been discussed, as well as how each of these regulatory mechanisms contributes to GSK3's ability to select which substrates to phosphorylate, and how these mechanisms may have contributed to its adaptability as new substrates evolved. Most researchers' current focus is on the periphery of their understanding of the mechanisms regulating GSK3 and emerging topics in GSK3's actions, particularly its interactions with receptors and receptor-coupled signal transduction events, as well as the differences in the actions and regulation of the two GSK3 isoforms, GSK3 and GSK3. GSK3 is also known for its participation in a variety of illnesses, including mental and neurological disorders, inflammatory diseases, cancer, and others. We have presented the therapeutic potential of targeting GSK3. We have concentrated on known interactors based on the protein-protein interaction (PPI) network database, with a particular focus on the link with melanoma and prospective herbal medicines Diosmin and Fisetin to target. We've also looked at the link between GSK3 and T2D, although our main focus is still on GSK3 and melanoma[1-3, 7, 17-31].

2. Methods

Here, the main goal was to study the linkage of GSK3ß in case of mainly melanoma and T2D in terms of the gene expression and their functional impact, for which gene expression datasets were collected from Gene Expression Omnibus (GEO). The primary source of our data is GSE41662 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41662[32]), TCGA database for melanoma and GSE121 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121[33]). Since, we were mainly interested in understanding the potential linkage of GSK3ß with melanoma and also with T2D and the difference between gene expression pattern. We used GEO2R to generate a list of DEGs by dividing samples into two groups: control and infected, and uninfected and infected, and then calculating fold changes and p-values. The thresholds for fold changes and p-values used to select genes as differentially expressed were +/- 2 and 0.05, respectively. We used the KEGG database for pathway research and developed our own algorithm for pathway and network analysis. KEGG Pathways was used for associated pathways with GSK3β, Diosmin and Fisetin[25, 34-37] and their interactors (STITCH database) followed by the protein classes (SwissTargetPrediction), and (c) Fisetin and its interactors (STITCH database) followed by the protein classes (SwissTargetPrediction)[20, 23, 26, 28, 38-40].

MATLAB algorithms, such as mattest, have been used for statistical analysis and differential gene expression prediction. The KEGG database and proprietary code for pathway and network analysis were used for pathway analysis. Throughout the project, FunCoup2.0 was utilised to generate the DEGs networks, and Cytoscape was utilised to visualise the networks. Four sorts of functional couplings or linkages, including protein complexes, physical interactions

between proteins, metabolic processes, and signalling pathways, are predicted by FunCoup. The majority of our computations and coding have been done using MATLAB[20, 23, 26-30, 34, 39-45].
After researching the DEGs and enriched pathways, the next step was to understand the network and the connections between the genes inside the DEG network. This was done using the FunCoup2.0 database, and the network was viewed using Cytoscape. MATLAB code was also used for figure plotting and analysis. MATLAB was used to generate the scripts for network level analysis, including how many connections each gene has and how many pathways each gene belongs to. MATLAB routines were also used to display the data. Swissmodel will be used to predict the 3D protein structure of the selected genes, and Swissdock will be utilised for docking[20, 34, 41, 45, 46].

3. Results

3.1. GSK3β controls a number of critical biological pathways and functions: Here, we have mapped out all the pathways associated where GSK3β is the direct component (Figure 1a). Among these pathways, there are a number of pathways which directly controls human diseases mainly cancers and infectious diseases or the disease associated with immune system. The critical pathways such as Wnt signaling, cell cycle chemokine signaling, BCR/TCR signaling, PI3K-AKT signaling, and insulin signaling appear common to both the diseases type-2 diabetes and melanoma. The other pathways are well-defined in case of different types of cancers including melanoma (melanogenesis, pathways in cancer, colorectal cancer, endometrial cancer, prostate cancer) (Figure 1a). Based on these preliminary outcome, it appears that GSK3β –focused study could lead to potential therapeutic findings so we have applied in-silico approach where used two herbal drugs Diosmin and Fisetin to target the potential pathway(s) components to understanding the binding possibility of the two selected drugs (Diosmin and Fisetin). As an initial step, we have evaluated the binding targets of these two herbal drugs by using SwissTargetPrediction and classified the target proteins (Figure 1b and 1c). As compared to Fisetin, Diosmin shows much larger number of interactors. In terms of protein classes also both these drugs have different protein classes in dominance. In case of Diosmin, lyase and family A, G-protein-coupled receptor, secreted proteins, and enzyme are 26.7%, 26.7%, 13.3%, and 13.3%, respectively. Enzyme (33.3%), kinase (26.7%), and oxidoreductase (13.3%) are dominantly present as interactors of Fisetin (Figure 1b and 1c).

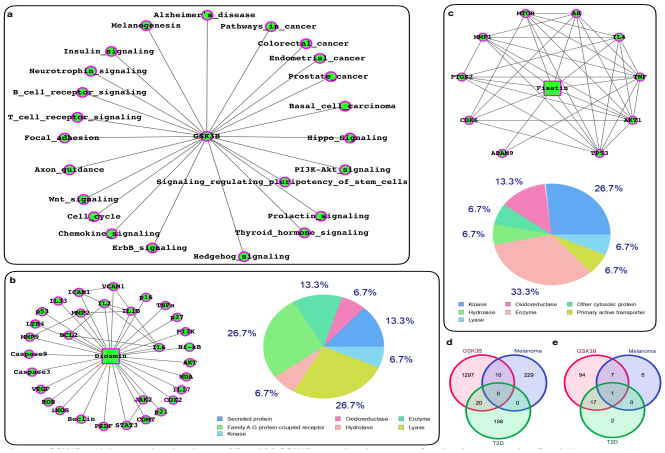

Figure 1. Basic analysis of GSK3β, Mimosin, and Fisetin. (a) KEGG Pathways associated with GSK3β, (b) Mimosin and its interactors (STITCH database) followed by the protein classes (SwissTargetPrediction), and (c) Fisetin and its interactors (STITCH database[51]) followed by the protein classes (SwissTargetPrediction).

3.2. Genes and pathways shared between the GSK3β interactors, type-2 diabetes, and melanoma: In the next step, we have performed the mapping of overall GSK3β-associated genes, DEGs of melanoma, and DEGs of T2D and pathways enrichment analysis for the respective genes lists and finally performed comparative analysis of all the three lists in terms of genes and pathways (Figure 1d and 1e). From the comparative study of the three genes lists, we observe that there were 10 genes shared between GSK3β and melanoma, 20 genes shared between GSK3β and T2D while melanoma and T2D does not show any shared gene and also we could not see even a single gene common between all three lists. So, we could conclude that there could be shared genes between GSK3β and melanoma and GSK3β and T2D which could act as bridge between them while there is no common gene between T2D and melanoma (Figure 1d). Similar to gene comparison, we have also performed the comparative study of the biological pathways and the functions for the three genes lists and we observe that there is only one pathway which is common between all the three lists (Figure 1e and Table 1). There were seven pathways common between GSK3β and melanoma and 17 pathways common between GSK3β and T2D and there was not a single pathway shared between T2D and melanoma. Cytokine-cytokine receptor interaction appears to be commonly enriched for GSK3β genes list, melanoma, and T2D. Purine metabolism, starch and sucrose metabolism, retinol metabolism, metabolism of

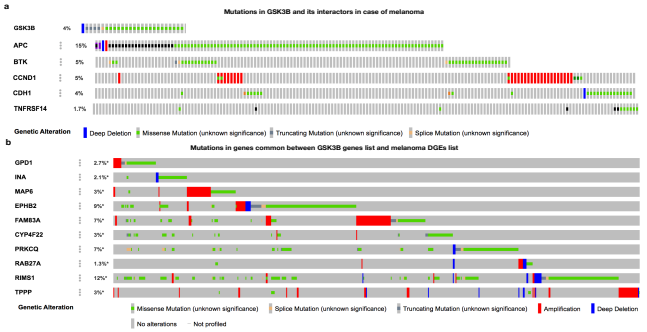

Figure 2. (a) Mutations in GSK3B and its interactors in case of melanoma and (b) Mutations in genes common between GSK3B genes list and melanoma DGEs list.

xenobiotics by cytochrome P450, NF--kB signaling, Toll-like receptor signaling, adipocytokine signaling between GSK3B and melanoma (Table 1). ErbB signaling, cAMP signaling, FoxO signaling, Phagosome, PI3K-Akt signaling, apoptosis, adrenergic signaling in cardiomyocytes, wnt signaling, osteoclast differentiation, hippo signaling, focal adhesion, pathways regulating pluripotency of stem cells, platelet activation, Jak-STAT signaling, regulation of actin cytoskeleton, insulin signaling pathway, oxytocin signaling between GSK3β and T2D (Table 1).

Table 1. Enriched pathways for GSK3B, melanoma, and type-2 diabetes.

Enriched pathways for GSK3B-associated genes

Enriched pathways	p-values
KEGG_04010_MAPK_signaling	5.42E-20
KEGG_04012_ErbB_signaling	5.42E-20
KEGG_04014_Ras_signaling	5.42E-20
KEGG_04015_Rap1_signaling	5.42E-20
KEGG_04020_Calcium_signaling	5.42E-20
KEGG_04022_cGMP-PKG_signaling	5.42E-20
KEGG_04024_cAMP_signaling	5.42E-20
KEGG_04066_HIF-1_signaling	5.42E-20
KEGG_04068_FoxO_signaling	5.42E-20
KEGG_04071_Sphingolipid_signaling	5.42E-20
KEGG_04072_Phospholipase_D_signaling	5.42E-20
KEGG_04110_Cell_cycle	5.42E-20
KEGG_04114_Oocyte_meiosis	5.42E-20
KEGG_04120_Ubiquitin_mediated_proteolysis	5.42E-20
KEGG_04151_PI3K-Akt_signaling	5.42E-20
KEGG_04152_AMPK_signaling	5.42E-20
KEGG_04211_Longevity_regulating_pathway	5.42E-20
KEGG_04261_Adrenergic_signaling_in_cardiomyocytes	5.42E-20
KEGG_04310_Wnt_signaling	5.42E-20
KEGG_04360_Axon_guidance	5.42E-20
KEGG_04370_VEGF_signaling	5.42E-20
KEGG_04371_Apelin_signaling	5.42E-20
KEGG_04380_Osteoclast_differentiation	5.42E-20
KEGG_04392_Hippo_Signaling	5.42E-20
KEGG_04510_Focal_adhesion	5.42E-20
KEGG_04520_Adherens_junction	5.42E-20
KEGG_04530_Tight_junction	5.42E-20
KEGG_04540_Gap_junction	5.42E-20
KEGG_04550_Signalings_regulating_pluripotency_of_stem_cells	5.42E-20
KEGG_04611_Platelet_activation	5.42E-20
KEGG_04650_Natural_killer_cell_mediated_cytotoxicity	5.42E-20
KEGG_04660_T_cell_receptor_signaling	5.42E-20
KEGG_04662_B_cell_receptor_signaling	5.42E-20
KEGG_04664_Fc_epsilon_RI_signaling	5.42E-20
KEGG_04666_Fc_gamma_R-mediated_phagocytosis	5.42E-20

KEGG_04668_TNF_signaling	5.42E-20
KEGG_04713_Circadian_entrainment	5.42E-20
KEGG_04720_Long-term_potentiation	5.42E-20
KEGG_04721_Synaptic_vesicle_cycle	5.42E-20
KEGG_04722_Neurotrophin_signaling	5.42E-20
KEGG_04750_Inflammatory_mediator_regulation_of_TRP_channels	5.42E-20
KEGG_04810_Regulation_of_actin_cytoskeleton	5.42E-20
KEGG_04910_Insulin_signaling	5.42E-20
KEGG_04912_GnRH_signaling	5.42E-20
KEGG_04914_Progesterone-mediated_oocyte_maturation	5.42E-20
KEGG_04915_Estrogen_signaling	5.42E-20
KEGG_04916_Melanogenesis	5.42E-20
KEGG_04917_Prolactin_signaling	5.42E-20
KEGG_04919_Thyroid_hormone_signaling	5.42E-20
KEGG_04920_Adipocytokine_signaling	5.42E-20
KEGG_04921_Oxytocin_signaling	5.42E-20
KEGG_04145_Phagosome	4.11E-19
KEGG_04270_Vascular_smooth_muscle_contraction	4.11E-19
KEGG_04723_Retrograde_endocannabinoid_signaling	4.11E-19
KEGG_04730_Long-term_depression	4.11E-19
KEGG_04210_Apoptosis	8.22E-18
KEGG_04620_Toll-like_receptor_signaling	8.22E-18
KEGG_03010_Ribosome	1.56E-16
KEGG_04670_Leukocyte_transendothelial_migration	1.56E-16
KEGG_04144_Endocytosis	2.81E-15
KEGG_04918_Thyroid_hormone_synthesis	2.81E-15
KEGG_00230_Purine_metabolism	4.78E-14
KEGG_04141_Protein_processing_in_endoplasmic_reticulum	4.78E-14
KEGG_00020_Citrate_cycle_(TCA_cycle)	7.65E-13
KEGG_00030_Pentose_phosphate_pathway	7.65E-13
KEGG_00051_Fructose_and_mannose_metabolism	7.65E-13
KEGG_00190_Oxidative_phosphorylation	7.65E-13
KEGG_04150_mTOR_signaling	7.65E-13
KEGG_00500_Starch_and_sucrose_metabolism	1.15E-11
KEGG_00620_Pyruvate_metabolism	1.15E-11
KEGG_04340_Hedgehog_signaling	1.15E-11
KEGG_04350_TGF-beta_signaling	1.15E-11
KEGG_04630_Jak-STAT_signaling	1.15E-11
KEGG_03013_RNA_transport	1.61E-10
KEGG_04622_RIG-I-like_receptor_signaling	2.09E-09
KEGG_00520_Amino_sugar_and_nucleotide_sugar_metabolism	2.51E-08
KEGG_04064_NF-kappa_B_signaling	2.51E-08

KEGG_04070_Phosphatidylinositol_signaling_system	2.51E-08
KEGG_04924_Renin_secretion	2.51E-08
KEGG_00052_Galactose_metabolism	2.76E-07
KEGG_00562_Inositol_phosphate_metabolism	2.76E-07
KEGG_03320_PPAR_signaling	2.76E-07
KEGG_04140_Regulation_of_autophagy	2.76E-07
KEGG_04740_Olfactory_transduction	2.76E-07
KEGG_00071_Fatty_acid_metabolism	2.76E-06
KEGG_00240_Pyrimidine_metabolism	2.76E-06
KEGG_04060_Cytokine-cytokine_receptor_interaction	2.76E-06
KEGG_04115_p53_signaling	2.76E-06
KEGG_04960_Aldosterone-regulated_sodium_reabsorption	2.76E-06
KEGG_00564_Glycerophospholipid_metabolism	2.48E-05
KEGG_04130_SNARE_interactions_in_vesicular_transport	2.48E-05
KEGG_00710_Carbon_fixation_in_photosynthetic_organisms	4.18E-05
KEGG_04962_Vasopressin-regulated_water_reabsorption	1.88E-04
KEGG_00590_Arachidonic_acid_metabolism	1.98E-04
KEGG_00970_Aminoacyl-tRNA_biosynthesis	1.98E-04
KEGG_03040_Spliceosome	1.98E-04
KEGG_03050_Proteasome	1.98E-04
KEGG_04146_Peroxisome	1.98E-04
KEGG_04330_Notch_signaling	1.98E-04
KEGG_04621_NOD-like_receptor_signaling	1.98E-04
KEGG_00260_Glycine__serine_and_threonine_metabolism	1.39E-03
KEGG_00561_Glycerolipid_metabolism	1.39E-03
KEGG_00980_Metabolism_of_xenobiotics_by_cytochrome_P450	1.39E-03
KEGG_04742_Taste_transduction	1.39E-03
KEGG_04913_Ovarian_steroidogenesis	1.39E-03
KEGG_00330_Arginine_and_proline_metabolism	8.33E-03
KEGG_00480_Glutathione_metabolism	8.33E-03
KEGG_00650_Butanoate_metabolism	8.33E-03
KEGG_03420_Nucleotide_excision_repair	8.33E-03
KEGG_00250_Alanine__aspartate_and_glutamate_metabolism	4.17E-02
KEGG_00270_Cysteine_and_methionine_metabolism	4.17E-02
KEGG_00280_Valine__leucine_and_isoleucine_degradation	4.17E-02
KEGG_00340_Histidine_metabolism	4.17E-02
KEGG_00380_Tryptophan_metabolism	4.17E-02
KEGG_00450_Selenoamino_acid_metabolism	4.17E-02
KEGG_00640_Propanoate_metabolism	4.17E-02
KEGG_00830_Retinol_metabolism	4.17E-02
KEGG_04514_Cell_adhesion_molecules_(CAMs)	4.17E-02
KEGG_04612_Antigen_processing_and_presentation	4.17E-02

Enriched pathways for GSK3B-associated genes Melanoma

KEGG_00830_Retinol_metabolism	2.09E-09
KEGG_00983_Drug_metabolism_-_other_enzymes	2.09E-09
KEGG_00980_Metabolism_of_xenobiotics_by_cytochrome_P450	2.51E-08
KEGG_00982_Drug_metabolism_-_cytochrome_P450	2.51E-08
KEGG_00140_Steroid_hormone_biosynthesis	2.76E-07
KEGG_00500_Starch_and_sucrose_metabolism	2.76E-06
KEGG_00860_Porphyrin_and_chlorophyll_metabolism	2.76E-06
KEGG_00040_Pentose_and_glucuronate_interconversions	2.09E-05
KEGG_04060_Cytokine-cytokine_receptor_interaction	2.48E-05
KEGG_04064_NF-kappa_B_signaling	1.39E-03
KEGG_04620_Toll-like_receptor_signaling	8.33E-03
KEGG_00230_Purine_metabolism	4.17E-02
KEGG_04920_Adipocytokine_signaling	4.17E-02

Enriched pathways for GSK3B-associated genes T2D

KEGG_04151_PI3K-Akt_signaling	2.51E-08
KEGG_04810_Regulation_of_actin_cytoskeleton	2.76E-06
KEGG_04510_Focal_adhesion	2.48E-05
KEGG_04024_cAMP_signaling	8.33E-03
KEGG_04068_FoxO_signaling	8.33E-03
KEGG_04145_Phagosome	8.33E-03
KEGG_04261_Adrenergic_signaling_in_cardiomyocytes	8.33E-03
KEGG_04310_Wnt_signaling	8.33E-03
KEGG_04380_Osteoclast_differentiation	8.33E-03
KEGG_04392_Hippo_Signaling	8.33E-03
KEGG_04611_Platelet_activation	8.33E-03
KEGG_04910_Insulin_signaling	8.33E-03
KEGG_00310_Lysine_degradation	4.17E-02
KEGG_04012_ErbB_signaling	4.17E-02
KEGG_04060_Cytokine-cytokine_receptor_interaction	4.17E-02
KEGG_04210_Apoptosis	4.17E-02
KEGG_04512_ECM-receptor_interaction	4.17E-02
KEGG_04550_Signalings_regulating_pluripotency_of_stem_cells	4.17E-02
KEGG_04630_Jak-STAT_signaling	4.17E-02
KEGG_04921_Oxytocin_signaling	4.17E-02

3.3. Genes and pathways shared between the GSK3β interactors, type-2 diabetes, and melanoma: Now, we have analyzed the genes which were common between the three lists and observe that GPD1, INA, MAP6, EPHB2, FAM83A, CYP4F22, PRKCQ, RAB27A, RIMS1, and TPPP were common between GSK3β genes list and melanoma DGEs list. ACVR1B, APC, ARHGAP44, BTK, CAMK2B, CCND1, CDH1, EIF5B, GAP43, GLIPR1, OGT, PAK2, PPP1CA, PPP2R5E, PRKAR2A, RAB3GAP2, STX7, TNFRSF14, TRIM3, and TUBA1B were common between GSK3β genes list and the T2D DEGs list (Table 1).

We have further explored the genes common between GSK3β genes list and melanoma DGEs list, genes common between GSK3β genes list and the T2D DEGs list, Diosmin and Fisetin interactors. For this purpose, we have used the

TCGA database mainly to investigate the potentials of these genes in melanoma. Here, we have explored the mutations in the inferred genes of GSK3β interactors, GSK3β genes list and melanoma DGEs list, genes common between GSK3β genes list and the T2D DEGs list, and the mutations in inferred genes of Diosmin and Fisetin interactors in case of melanoma. We observe that in case of inferred genes of GSK3β interactors, APC appear highly mutated in case of melanoma, BTK mutated in 5%, CCND1 mutated in 5%, CDH1 mutated in 4%, and TNFSF14 mutated in 1.7% of total selected melanoma samples (Figure 2a).

In case of the genes common between GSK3β genes list and melanoma DGEs list, much higher number of melanoma patients shows mutations in RIMS1 (12%), followed by EPHB2 (9%), FAM83A (7%), and PRKCQ (7%). MAP6, TPPP, CYP4F22, INA, and RAB27A appear mutated in ≤ 3% of the total selected melanoma patients (Figure 2b). Moreover, the mutations have been deeply explored to understand the mutation regions (domains/motifs) and the type of mutations (Figure 3). None of these genes display driver and SV/fusion mutation while VUS (variant of uncertain (or unknown) significance) which is a genetic variant that has been identified through genetic testing but whose significance to the function or health of an organism is not known and missense mutations are dominantly present. In case of the genes common between GSK3β genes list and T2D DGEs list, APC and ARHGAP44 appear mutated in 11% and 8% of the total melanoma patients, respectively. BTK, CCND1, and RAB3GAP2 were mutated in 5%, 5%, and 4% of the total melanoma patients, respectively and the rest of the genes were mutated in less than 4% of the melanoma patients (Figure 4). Similarly, we have also explored the types of mutations and their locations further where we observe opposite to the genes common between GSK3β genes list and melanoma mainly in case of driver mutations which shows driver mutations are dominantly present in several genes (APC (64), CCND1 (5), CDH1 (7), and STX7 (1)) in case of the genes GSK3β genes list and T2D DEGs list (Figures 5--6).

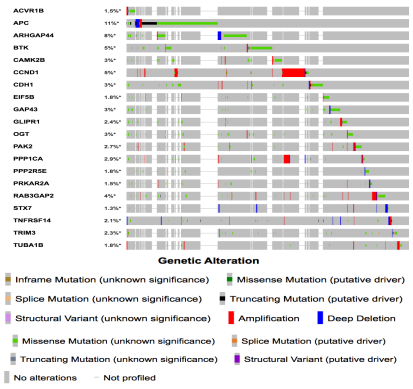

Figure 4. Mutations in the genes common between GSK3β genes list and the T2D DEGs list with their respective 3D protein structures.

Finally, we have explored the mutations in the inferred genes of Diosmin and Fisetin interactors in case of melanoma patient samples. Here, there are a number of genes which were highly mutated in melanoma patients. CDKN2A appear mutated in 32% of the total patients mostly were deep

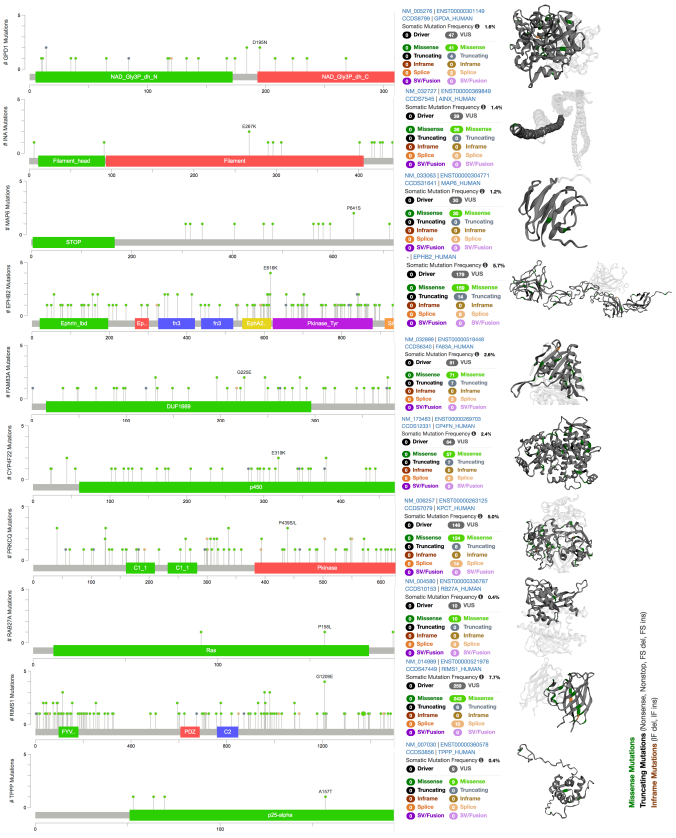

Figure 3. Mutations in the genes common between GSK3B genes list and melanoma DGEs list.

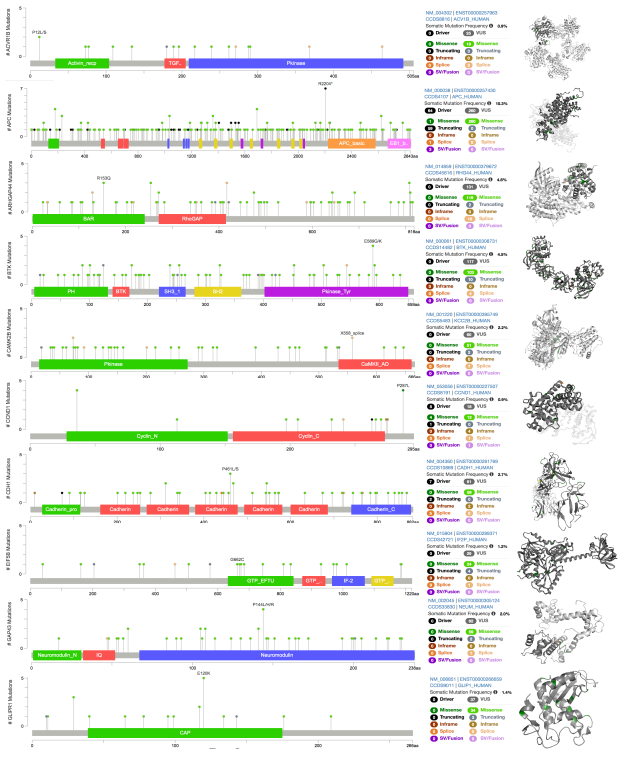

Figure 5. Mutations in the genes common between GSK3B genes list and T2D DGEs list.

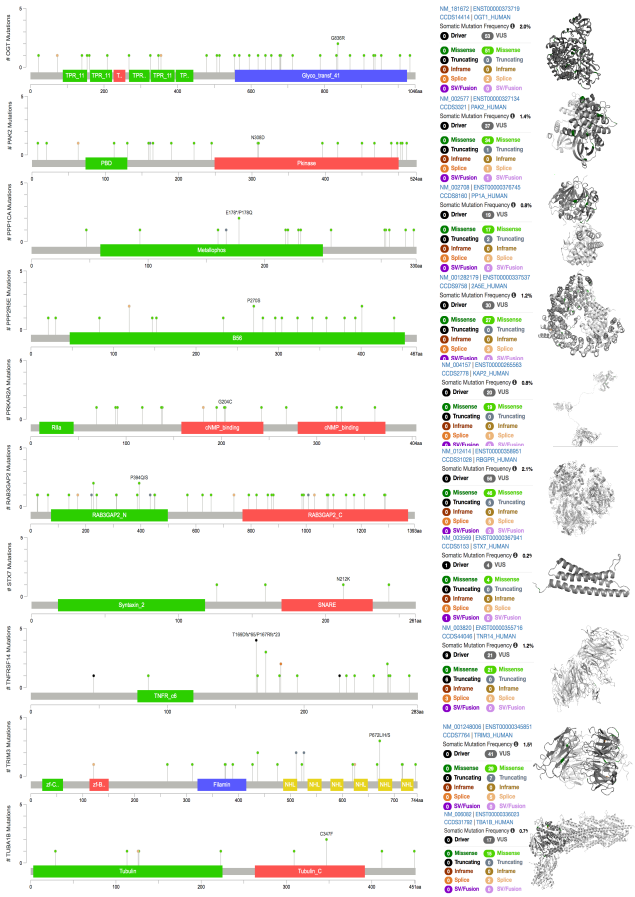

Figure 6. Mutations in the genes common between GSK3B genes list and T2D DGEs list.

GSK3 is a widely expressed serine/threonine kinase that comes in two forms: GSK3 and GSK3 isoforms, both of which are active in the absence of phosphorylation by separate upstream kinases. GSK3

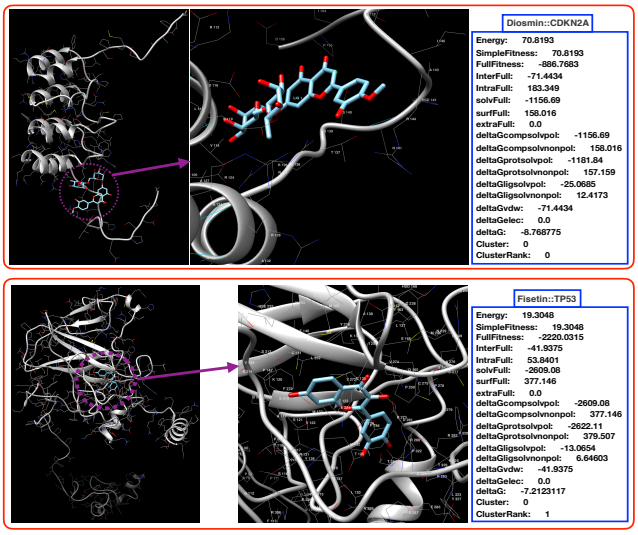

Figure 8. Docking of CDKN2A with Diosmin and TP53 with Fisetin.

deletions (Figure 7) and this gene is inferred for Diosmin interactor. TP3 and MTOR of Fisetin was mutated in 18% and 9% of the patients, respectively. Moreover, CDKN2A and TP53 were docked with Diosmin and Fisetin, respectively and we observe that Diosmin has much higher binding affinity (delta G = -8.76kcal/mol) compared to TP53 docked with Fisetin (delta G = -7.21kcal/mol) while the delta G of Fisetin with TP53 is also quite significant (Figure 8). This result leads to the conclusion that for such critical proteins CDKN2A and TP53, Diosmin and Fisetin could be the pivotal drugs to target melanoma.

4. Dicussion: We focused on known interactors based on the PPI network database, with a specific focus on the relationship with melanoma and possible herbal drugs to target, in order to debate and show the potentials of GSK3 in the case of melanoma. Although our major emphasis is still GSK3 and melanoma, we've looked at the link between GSK3 and T2D. Such research takes a novel approach to GSK3 and its link to melanoma, followed by T2D, which is highly unusual in terms of its nobility.

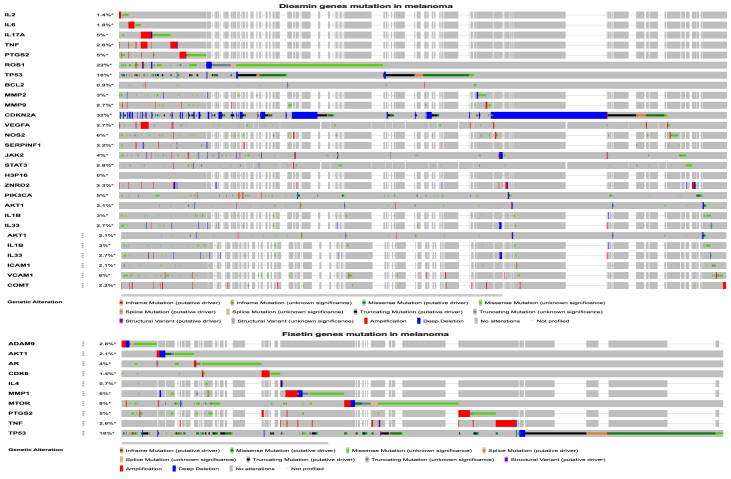

Figure 7. Mutations in the inferred genes of Diosmin and Fisetin interactors.

was first found as a glycogen synthesis regulator, but it's now implicated in a number of signaling pathways that govern a variety of activities. It is involved in a variety of disorders, including cancer and T2D. The active form of GSK3 has been demonstrated to trigger apoptosis in some situations and prevent apoptosis in others, as well as to promote cancer development or limit tumor cell proliferation, implying that various GSK3 modulators may target distinct targets[2, 4, 5, 9, 18, 19, 22, 47].

The involvement of GSK3β, a multifunctional serine/threonine kinase found in all eukaryotes, in insulin-dependent glycogen synthesis was initially discovered. GSK3 has been shown to be involved in a number of cellular functions, including proliferation, differentiation, motility, and survival. GSK3β malfunction has been associated to non-insulin-dependent diabetes, cardiovascular disease, many neurodegenerative illnesses, and bipolar disorder, among other things. As a result, GSK3β inhibitors' medicinal potential has been a prominent research focus. However, GSK3β has a role in tumor growth and neoplastic transformation. The role of GSK3 in carcinogenesis and cancer progression is still being contested; it may operate as a "tumor suppressor" in certain tumors while encouraging growth and development in others. GSK3β

controls drug sensitivity and resistance in cancer treatment. As a result, while GSK3β is a prospective therapeutic target for a range of human illnesses, its impact on cancer development and therapy must be carefully examined. This gene produces a serine-threonine kinase that belongs to the glycogen synthase kinase subfamily. It is a negative regulator of glucose homeostasis and has a role in energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways. Mutations in this gene have been associated to Parkinson's disease and Alzheimer's disease[1, 48-55].

Table 2. Mutual exclusivity.

Gene A	Gene B	Neither	Gene A Not Gene B	Gene B Not Gene A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
PRKCQ	RIMS1	1262	62	145	49	2.782	<0.001	<0.001	Co-occurrence
CYP4F22	RIMS1	1298	26	165	29	>3	<0.001	<0.001	Co-occurrence
CYP4F22	PRKCQ	1371	36	92	19	2.975	<0.001	<0.001	Co-occurrence
INA	CYP4F22	1443	20	45	10	>3	<0.001	<0.001	Co-occurrence
EPHB2	RIMS1	1215	109	153	41	1.579	<0.001	<0.001	Co-occurrence
GPD1	RIMS1	1298	26	175	19	2.438	<0.001	<0.001	Co-occurrence
EPHB2	PRKCQ	1285	122	83	28	1.829	<0.001	<0.001	Co-occurrence
GPD1	CYP4F22	1427	36	46	9	2.955	<0.001	<0.001	Co-occurrence
RAB27A	TPPP	1448	16	48	6	>3	<0.001	<0.001	Co-occurrence
INA	RIMS1	1306	18	182	12	2.258	<0.001	<0.001	Co-occurrence
INA	PRKCQ	1386	21	102	9	2.542	<0.001	<0.001	Co-occurrence
INA	EPHB2	1348	20	140	10	2.267	<0.001	0.001	Co-occurrence
EPHB2	CYP4F22	1327	136	41	14	1.736	<0.001	0.002	Co-occurrence
FAM83A	RIMS1	1246	78	170	24	1.173	0.001	0.005	Co-occurrence
FAM83A	TPPP	1372	92	44	10	1.761	0.002	0.007	Co-occurrence
FAM83A	PRKCQ	1320	87	96	15	1.245	0.005	0.015	Co-occurrence

GPD1	FAM83A	1379	37	94	8	1.665	0.008	0.021	Co-occurrence
EPHB2	FAM83A	1284	132	84	18	1.06	0.009	0.021	Co-occurrence
FAM83A	CYP4F22	1370	93	46	9	1.527	0.009	0.022	Co-occurrence
GPD1	EPHB2	1333	35	140	10	1.444	0.01	0.022	Co-occurrence
MAP6	CYP4F22	1414	49	49	6	1.821	0.013	0.027	Co-occurrence
MAP6	PRKCQ	1361	46	102	9	1.384	0.016	0.032	Co-occurrence
MAP6	RIMS1	1282	42	181	13	1.132	0.017	0.034	Co-occurrence

T2D is a non-communicable disease that causes blood glucose levels to remain consistently high outside of the normal range. Diabetes and insulin resistance are the world's major causes of illness and death in humans. A variety of enzymes and hormones have a role in this illness, the most significant of which are GSK3, a key enzyme, and insulin, a critical hormone. GSK3, the main enzyme, controls and influences cellular shape, growth, motility, and apoptosis by a number of mechanisms, including phosphorylation, protein complex formation, and other cellular distribution. GSK3 enzyme dysfunction can cause a number of disorders, including insulin resistance and diabetes, as well as neurological ailments including Alzheimer's disease and cancer. Fluoroquinolones are the most common class of drugs that cause dysglycemia through interacting with the GSK3 enzyme. As a result, understanding GSK3's actions and processes is crucial, notably its function in glucose homeostasis via effects on glycogen synthase.

Obesity and T2D have both been related to an increased cancer risk and are becoming more prevalent. Insulin resistance and dyslipidemia are metabolic diseases that have been associated to obesity and type 2 diabetes, as well as the obesity-cancer relationship. Increased insulin/IGF-1 signaling, lipid and glucose uptake and metabolism, changes in cytokine, chemokine, and adipokine profiles, as well as changes in the adipose tissue immediately adjacent to cancer sites, have all been hypothesized as pathways associating obesity and diabetes with cancer progression.

5. Conclusions: Here, we have performed GSK3ß based study with the potential focus on melanoma and additional focus on type-2 diabetes and the two herbal drugs in integration with GSK3ß oriented study in melanoma. This study leads to the conclusion that. GSK3β controls a number of critical biological pathways and functions: Here, we have mapped out all the pathways associated where GSK3β is the direct component. Among these pathways, a number of them influence human diseases directly, namely malignancies and infectious diseases, as well as disorders connected with the immune system. Wnt signaling, cell cycle chemokine signaling, BCR/TCR signaling, PI3K-AKT signaling, and insulin signaling believed to be shared by the illnesses type 2 diabetes and melanoma. The GSK3 interactors, type-2 diabetes, and cancer all have genes and pathways in common, whereas T2D and melanoma did not. In the case of melanoma, CDKN2A and TP53 might be the main medicines addressed by Diosmin and Fisetin, respectively.

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