Original Article

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Comprehensive bioinformatic analysis of Wnt1 and Wnt1-associated diseases

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SUMMARY Wnt1 is the first member of the Wnt family that was identified. It is phylogenetically conserved and essential for oncogenesis and multiple developmental processes. This study has summarized diseases and mutations related to *Wnt1*. *Wnt1* is involved in various cancers, genetic type XV osteogenesis imperfecta, osteoporosis, and neurological diseases. The expression of *Wnt1* in normal tissues and different types of cancers and the potential survival of cancer were analyzed using experiment-based bioinformatic analysis. Systematic analysis indicated that abnormal expression of *Wnt1* is significantly associated with cancers, such as kidney renal carcinoma, hepatocellular carcinoma, thyroid carcinoma, head and neck squamous cell carcinoma, and uterine corpus endometrial carcinoma. GeneMANIA and STRING predicted that 32 proteins were involved with Wnt1 in Wnt signaling pathways and sorting and secretion of Wnts. These interacting molecules significantly co-occurred according to cBioPortal analysis. Thirty-three genes with an alteration frequency of more than 50% were observed in several cancers like esophageal squamous cell carcinoma, melanoma, and non-small cell lung cancer. Functional and experiment-based bioinformatics indicated that Wnt1 may act as a target of a potential biomarker for various types of human cancers. Wnt1 and other Wnt1-related proteins and signaling pathways may be ways to treat osteoporosis.

Keywords *Wnt1*, *Wnt1* mutations, *Wnt1* expression, co-occurrence, bioinformatics, type XV osteogenesis imperfecta, cancers

1. Introduction

Wnts are secreted lipid-modified glycoproteins that transmit a signal through one more of different signaling pathways including canonical Wnt-β-catenin signaling and non-canonical pathways. Aberrant components of Wnt signaling are related to various human diseases, including genetic diseases and complex diseases such as cancer (*1,2*). *Wnt1*, the first member of the Wnt family to be identified, is a gene that was activated by integration of mouse mammary tumor virus (MMTV) proviral DNA in virally induced breast tumors in 1982 (*3*). It is evolutionarily conserved and adjacent to the *Wnt10b* gene on chromosome 12 in homo sapiens (*4,5*). Wnt1 is reported to be vital for the development of the embryonic brain and central nervous system (CNS) (*6-8*). *Wnt1* expression was mapped at the dorsal p1 midline and mesencephalon (*9*). Knockout mice of the homozygous Int-1 displayed a severe phenotype, ranging from death to ataxia (*10*). Conditional knockout of *Wnt1* in mesenchymal progenitors led to severe fractures in mice resembling severe osteogenesis imperfecta (OI) (*11*). Overexpression of *Wnt1* induces duplication of the embryonic axis (*6,12*). Moreover, Wnt1 plays an essential role in osteoblast functions, bone development, and bone homeostasis (*13-15*). *Wnt1* mutations are reported to be associated with type XV OI or early-onset osteoporosis (*13,14,16,17*). The current study has summarized the expression, mutation, and functions of the Wnt1 based on a comprehensive bioinformatic analysis.

2. Materials and Methods

2.1. Phylogenetic analysis of Wnt1

The sequence of the Wnt1 protein was retrieved from an NCBI database and analyzed with the software TBtools.

Multiple sequence alignment was performed with Clustal W. A phylogenetic tree were drawn with the software Molecular Evolutionary Genetics Analysis (MEGA) and FigTree v1.4.3 using the neighbor-joining method. In total, 259 species were collected for phylogenetic analysis.

2.2. *Wnt1* and human diseases

Wnt1-related human diseases were summarized based on information from the Gene-Cloud of Biotechnology Information (GCBI) website (*https://www.gcbi.com.cn/gclib/html/index*). *Wnt1* variations were identified from St. Jude Cloud (*https://platform.stjude.cloud/requests/diseases*). *Wnt1* mutations that are responsible for type XV OI were identified from an OI mutation database (*https://oi.gene.le.ac.uk/home.php*). Mutations and copy number variations of *Wnt1* were analyzed with cBioPortal (*http://www.cbioportal.org*) (*18,19*); 46,697 samples from 44,347 patients with cancer in 176 studies were analyzed.

2.3. The expression of *Wnt1* in normal and cancer tissues and analysis of cancer survival

Wnt1 expression in different normal tissues was analyzed using a human protein atlas database (*https://www.proteinatlas.org/*). A total of 55 tissues and six different types of blood cells were analyzed along with 18 different types of blood cells and peripheral blood mononuclear cells (PBMCs). These data were normalized based on HPA, GTEx, and FANTOM5 transcriptomic analysis. The expression of *Wnt1* in human cancer was analyzed with Firebrowse (*http://firebrowse.org/*), which includes 37 different cancer types and 28 normal tissues as controls.

2.4. Prediction of the protein-protein interaction network and proteins co-occurring with Wnt1

GeneMANIA (*https://genemania.org/*) and STRING (*https://string-db.org/*) servers were used to analyze interaction proteins (*20,21*). These interactions include both physical and functional associations. cBioPortal was used to analyze the spectrum of mutations and copy number alterations of *Wnt1* and its interacting proteins in different cancers (all cancer types in TCGA data). The co-occurrence of Wnt1 and other proteins was predicted with cBioPortal.

2.5. Prediction of transcription factors and pathways involved in Wnt1

Pathways involving Wnt1 were predicted with KEGG (*http://www.kegg.jp*) and AmiGO2 (*http://amigo.geneontology.org/amigo*) and then used for gene ontology analysis (*22,23*)

3. Results

3.1. Phylogenetic analysis of the Wnt1 protein

Based on multiple sequence alignment, a phylogenetic analysis was performed to explore the likely similarities in and the relationship between the *Wnt1* protein in species from different genera and families. On the family level, hominids (*Homo sapiens, Pan paniscus, Pan troglodytes, Callithrix jacchus*) were clustered with gorillas (*Gorilla gorilla gorilla*) and Cercopithecidae (*Macaca nemestrina, Mandrillus leucophaeus*). Hamster and murine families were clustered together. These families were distinct in primates and rodents (dark blue in the figure). A total of 64 different species of mammals, which including mostly terrestrial organisms, some aquatic organisms, a few primates from Cercopithecidae, lemurids, and hominids, were cross-clustered, as represented by the light blue branch of the tree. Species from the feline family of Carnivora were grouped together. Different genera and species of ungulates, Carnivora, bats, and cetaceans were cross-clustered. Unlike species clustered in dark blue, rodents were all from the murine family; the yellow branch included other families which were predominantly squirrels, guinea pigs moles, and mole rats. Fish, birds, and amphibians are all grouped together to form two large individual branches, fish in light red were mainly bass, while Gymnotiformes, Clupeiformes, and Cyprinidae species were grouped together as a red branch (Figure 1).

3.2. *Wnt1* is related to multiple human diseases and the distribution of variants

Wnt1 mutations were found in different types of neoplasms including adenomatous polyposis coli, neoplasm metastasis, colorectal neoplasm, carcinoma, and lung neoplasms. *Wnt1* causes neurological conditions as well as OI (Figure 2A). Twelve mutations with ExAC frequencies were found in the ClinVar database (Figure 2B). The Catalogue Of Somatic Mutations In Cancer (COSMIC) database contained a total of 83 mutations, most of which were missense mutations (n = 49), followed by 24 frame shift mutations, 13 silent mutations, 4 splice region mutations, 4 nonsense mutations, and 1 splice mutation (Figure 2C). The frame shift mutation c.500delG was noted 17 times, mostly in colon and cecum cancer (Supplementary Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=56*, Figure S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=62*). A high alteration frequency was observed in ovarian cancer, colon adenocarcinoma, salivary cancer, prostate cancer, adrenocortical carcinoma, and mature T and NK neoplasms. A high percentage of *Wnt1* mutations was observed in ovarian cancer and colon adenocarcinoma. Copy number alterations of *Wnt1* were prevalent in

salivary cancer, esophageal squamous cell carcinoma, and prostate cancer (Supplementary Figure S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=62*). OI databases worldwide contained 36 mutations (Figure 2D).

3.3. The expression of *Wnt1* in normal and cancer tissues and survival analysis for patients with cancer

Results revealed that *Wnt1* was highly expressed in the placenta, basal ganglia, cerebral cortex, lymph node, and bone marrow but less expressed in other types of blood cells, granulocytes, skin, and the rectum, parathyroid glands, fallopian tubes, and adrenal glands (Figure 3A and 3B). *Wnt1* was up-regulated (fold-change > 2) in several cancers including kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), pan-kidney cohort (KIPAN), and sarcoma (SARC). In contrast, down-regulation of *Wnt1* was noted in glioma (GBMLGG), skin cutaneous melanoma (SKCM), glioblastoma multiforme (GBM), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), lung squamous cell carcinoma (LUSC), and colon adenocarcinoma (COADREAD) tumor tissues (Figure 3C, Supplementary Table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=57*).

The average level of *Wnt1* expression was lower in tumor tissues than that in normal tissues. The association of *Wnt1* expression with survival rates ($p < 0.05$) of patients with different cancers is shown in Figure 4. In kidney renal papillary cell carcinoma and renal clear cell carcinoma, patients with a higher level of *Wnt1* expression ($n = 56$ and 249, respectively) had a significantly lower overall survival compared to those with a lower level of *Wnt1* expression ($n = 121$ and 281, respectively) (Figure 4I, 4K). This was also observed in patients with cervical squamous cell carcinoma (Figure 4B). Lower *Wnt1* expression was associated with lower survival in patients with some cancers, and especially liver hepatocellular carcinoma, thyroid carcinoma, head and neck squamous cell carcinoma, and uterine corpus

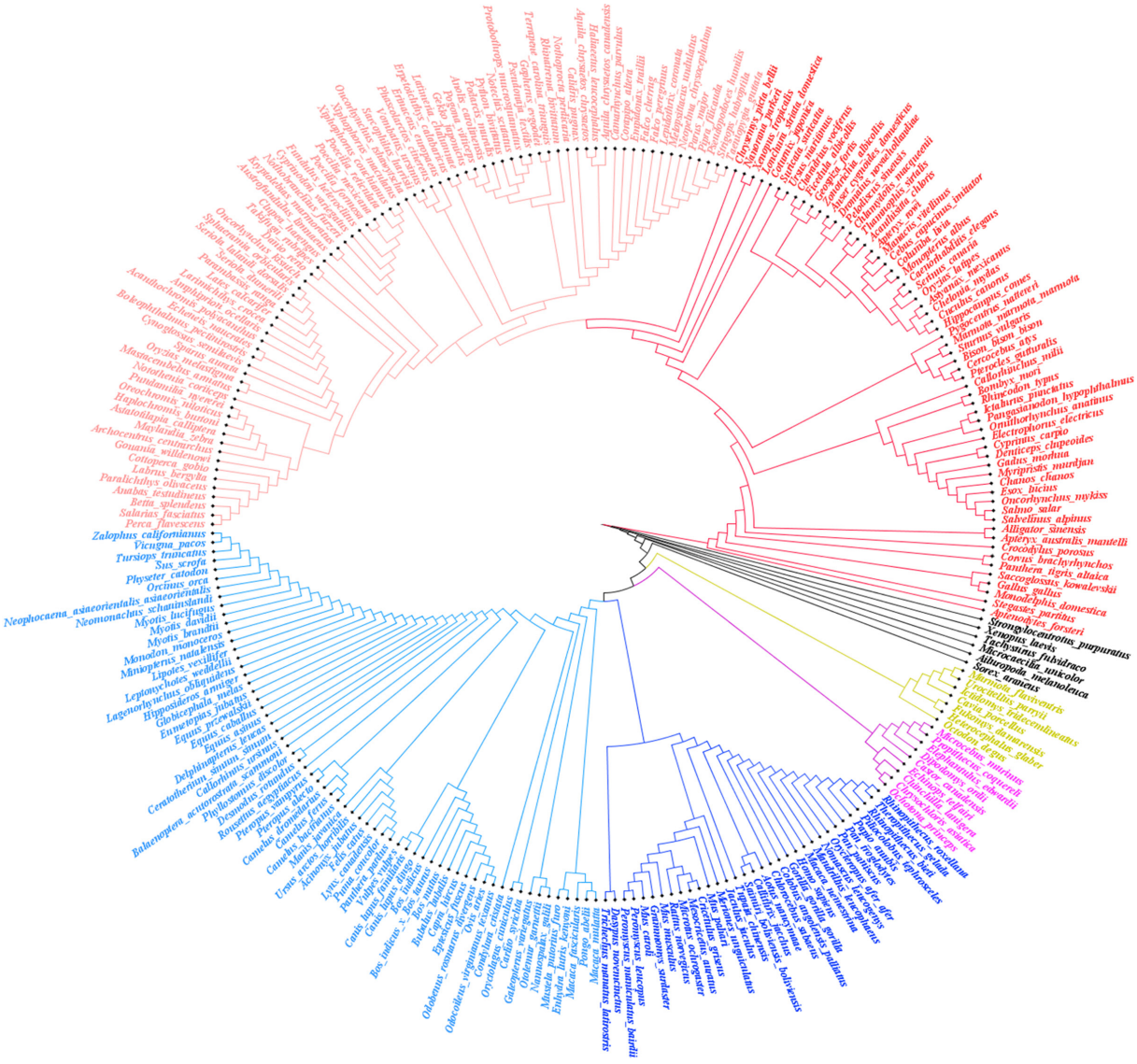

Figure 1. Phylogenetic analysis of Wnt1. In total, 259 different species were analyzed, and Wnt1 is highly conserved.

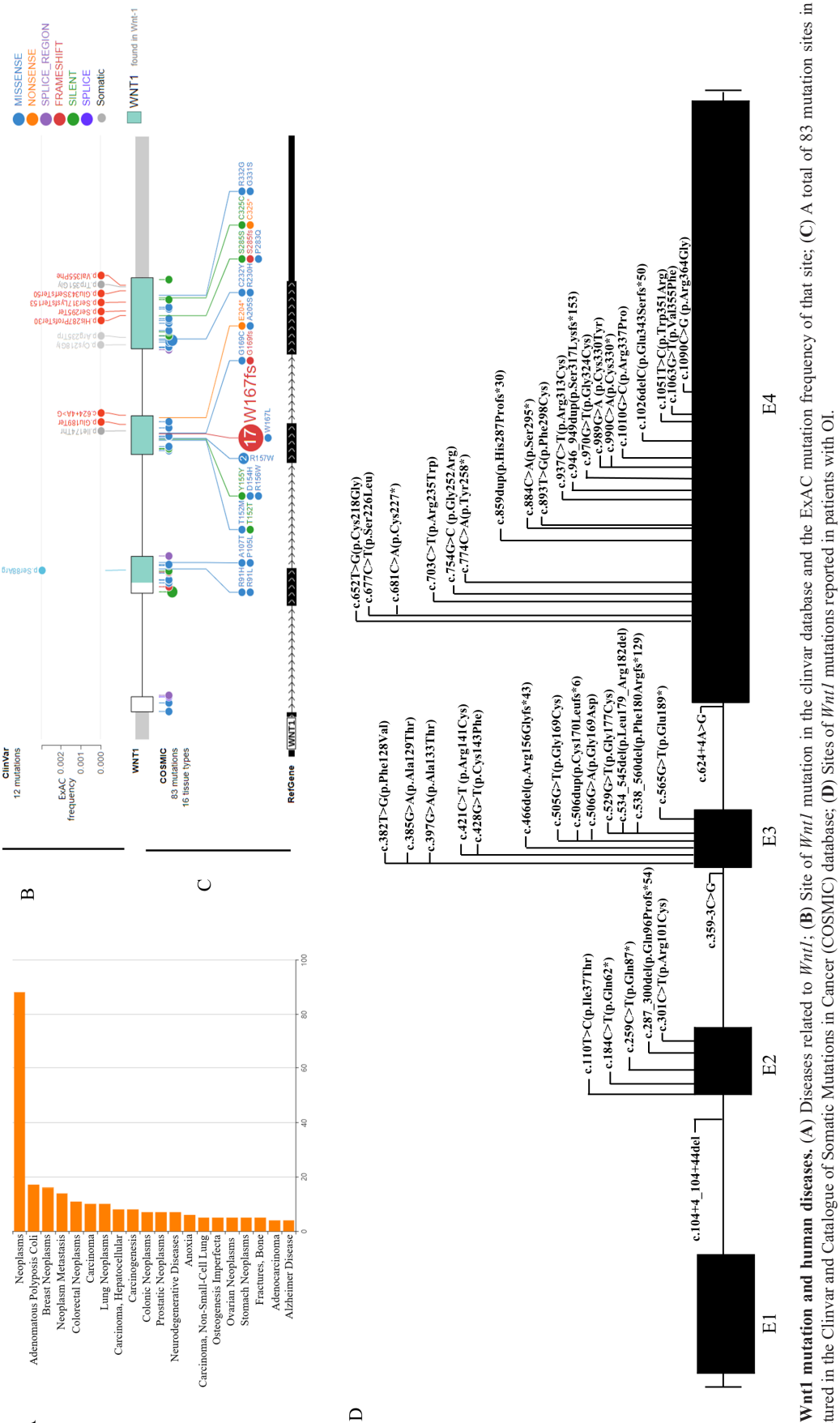

Figure 2. Wnt1 mutation and human diseases. (**A**) Diseases related to *Wntl*; (**B**) Site of *Wntl* mutation in the clinvar database and the ExAC mutation frequency of that site; (**C**) A total of 83 mutation sites in human tissues featured in the Clinvar and Catalogue of Somatic Mutations in Cancer (COSMIC) database; (**D**) Sites of *Wntl* mutations reported in patients with OI.

endometrial carcinoma (Figure 4A, 4D, 4E, and 4J).

3.4. Prediction of protein-protein interaction and cross-cancer analysis of *Wnt1* mutations and copy number alterations

GeneMANIA and String analysis of protein-protein interaction predicted a total of 21 and 25 proteins, respectively (Figure 5A, B, Supplementary Table S3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=58* and Table S4, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=59*). Both programs predicted interaction with DKK1, FZD1, FzZD5, FZD8, LRP5, LRP6, PORCN, ROR2, RYK, SFRP1, WIF1, Wnt1, and Wnt3A. Most physically interacted with Wnt1, excluding MACF1, TCF4, TIMM17B, KL, FZD3, and NKD1. TIMM17B was predicted to be co-localized with Wnt1. These interacting proteins are involved in the Wnt signaling pathway except for TIMM17B and KL (Supplementary Table S4, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=59*).

All 33 genes, including *CDC5L, DKK1, DVL1, FZD1, FZD10, FZD2, FZD3, FZD5, FZD6, FZD7, FZD8, KL, LRP5, LRP5L, LRP6, MACF1, NKD1, NKD2, NOTUM, PORCN, PPP2CA, PPP2R1A, ROR2, RYK, SFRP1, SFRP2, TCF4, TIMM17B, UBR3, WIF1, WLS, Wnt1*, and *Wnt3A*, were submitted to cBioPortal for alteration frequency analysis. The alteration spectrum of these 33 genes varied in 33 cancer types, including mutation, fusion, amplification, deep deletion, and multiple alteration in cancer. An alteration frequency of over 50% was observed in esophageal squamous cell carcinoma (71.58%), melanoma (68.02%), non-small cell lung cancer (64.01%), bladder urothelial carcinoma (62.77%), esophagogastric adenocarcinoma (61.26%), endometrial carcinoma (61.09%), ovarian epithelial tumor (59.76%), mature B-Cell neoplasms (52.08%), head and neck squamous cell carcinoma (51.05%), and invasive breast carcinoma (50.65%). The lowest alteration frequency (< 10%) was noted in miscellaneous neuroepithelial tumors, leukemia, and well-differentiated thyroid cancer (Figure 5C). All of these genes interacting with *Wnt1* significantly co-occurred according to co-occurrence analysis with cBioPortal (Supplementary Table S5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=60*).

3.5. Prediction of transcription factors and signaling pathways involving *Wnt1*

A total of 34 transcription factors and 10 motifs were predicted as shown in Figure 6. Some transcription factors do not have obvious annotated motifs or conserved motifs and regions. Wnt1 is involved in 14 KEGG pathways including mTOR, Wnt, Hippo, different types of cancer pathways, and signaling pathways as shown in Table 1. Gene ontology (GO) annotation annotated a total of 10 molecular functions, 19 different cellular components, and 70 biological process (Supplementary Table S6, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=61*). The main molecular functions associated with Wnt1 are receptor ligand activity, morphogen activity, protein domain-specific binding, and transcription regulatory region

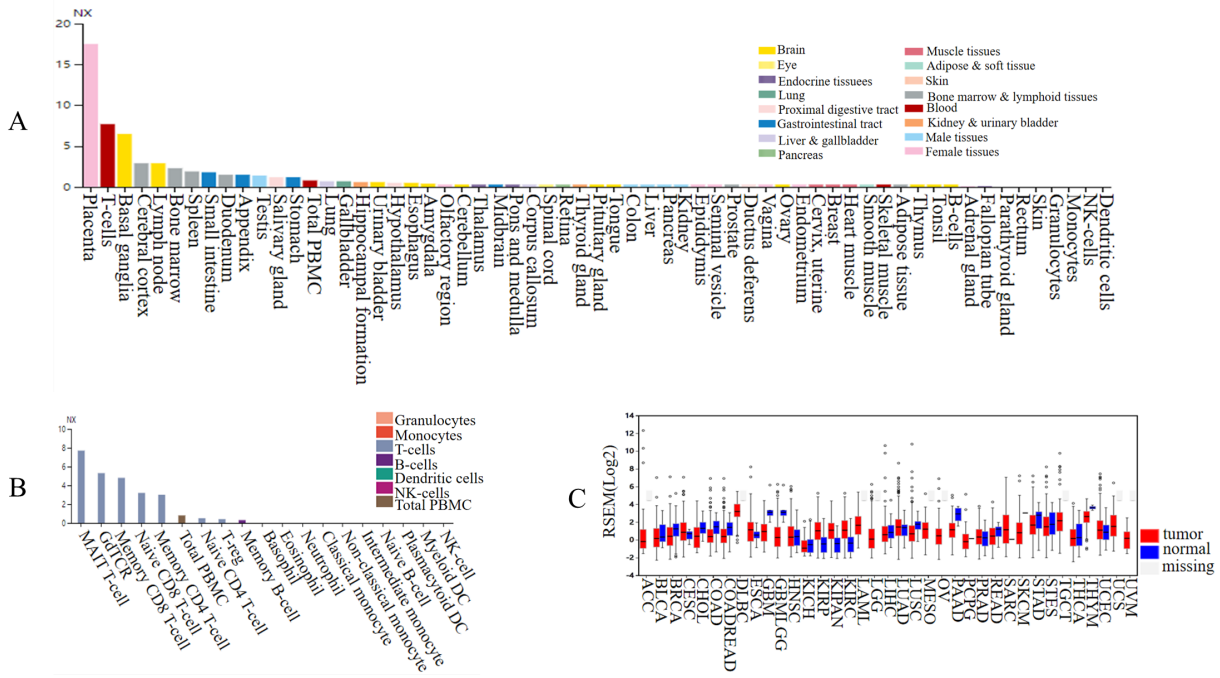

Figure 3. The level of *Wnt1* expression in different normal and cancer tissues. (**A**) A total of 55 tissues and 6 different types of blood cells; (**B**) Different types of blood cells and PBMCs; (**C**) Expression in 37 different cancer types and 28 normal tissues.

DNA binding. As a morphogen, Wnt1 is secreted as an extracellular matrix protein through the plasma membrane. Wnt1 is involved in the canonical Wnt signaling pathway and planar cell polarity pathway and it plays a role in cell fate commitment, cell proliferation, cell adhesion, cell-cell signaling, bone development, diencephalon development, embryonic brain development, and some other functions.

Table 1. KEGG pathway involved in Wnt1

KEGG ID	KEGG term
ko04150	mTOR signaling pathway
ko04310	Wnt signaling pathway
ko04390	Hippo signaling pathway
ko04391	Hippo signaling pathway – fly
ko04550	Signaling pathways regulating pluripotency of stem cells
ko04916	Melanogenesis
ko04934	Cushing syndrome
ko05165	Human papillomavirus infection
ko05200	Pathways in cancer
ko05205	Proteoglycans in cancer
ko05217	Basal cell carcinoma
ko05224	Breast cancer
ko05225	Hepatocellular carcinoma
ko05226	Gastric cancer

4. Discussion

Wnt1 is the first member of the Wnt family that was identified. It is evolutionarily conserved according to a phylogenetic analysis, and this is especially true in primates and rodents. Cross-talk among different species of fish, birds, and amphibians was observed in phylogenetic analysis. *Wnt1* is associated with various human diseases including cancers, CNS diseases, and bone diseases (early-onset osteoporosis and OI) (*15*). Altered expression of *Wnt1* and proteins it interacts with in Wnt signaling pathways and regulation were associated with oncogenesis, epithelial-to-mesenchymal transition, and the invasion of and prognosis for various cancers (*24-29*). Wnt1 is a potential prognostic factor for renal cell carcinoma and cutaneous squamous cell carcinoma (*30-32*).

OI is a genetically heterozygous disease characterized by frequently fractures and decreased bone mass. Patients with this diseases usually have blue sclera, dentinogenesis imperfecta, scoliosis, and a short stature. Type XV OI is an autosomal recessive form of OI, with biallelic mutations of Wnt1. A heterozygous mutation of Wnt1 leads to a dominant form of early on-set

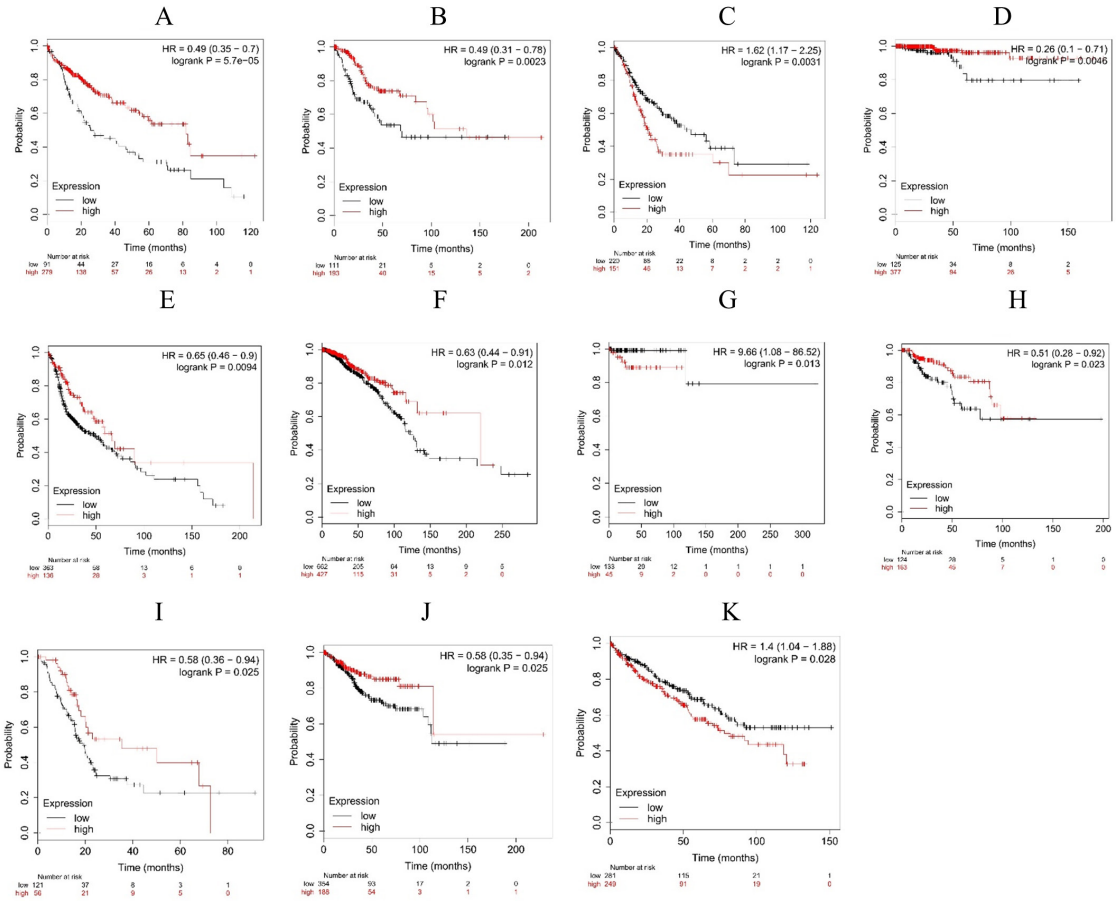

Figure 4. The survival curve of patients with high (red) and low (black)expression. (**A**) Liver hepatocellular carcinoma; (**B**) Cervical squamous cell carcinoma; (**C**) Stomach adenocarcinoma; (**D**) Thyroid carcinoma; (**E**) Head-neck squamous cell carcinoma; (**F**) Breast cancer; (**G**) Pheochromocytoma and paraganglioma; (**H**) Kidney renal papillary cell carcinoma; (**I**) Pancreatic ductal adenocarcinoma; (**J**) Uterine corpus endometrial carcinoma; (**K**) Kidney renal clear cell carcinoma.

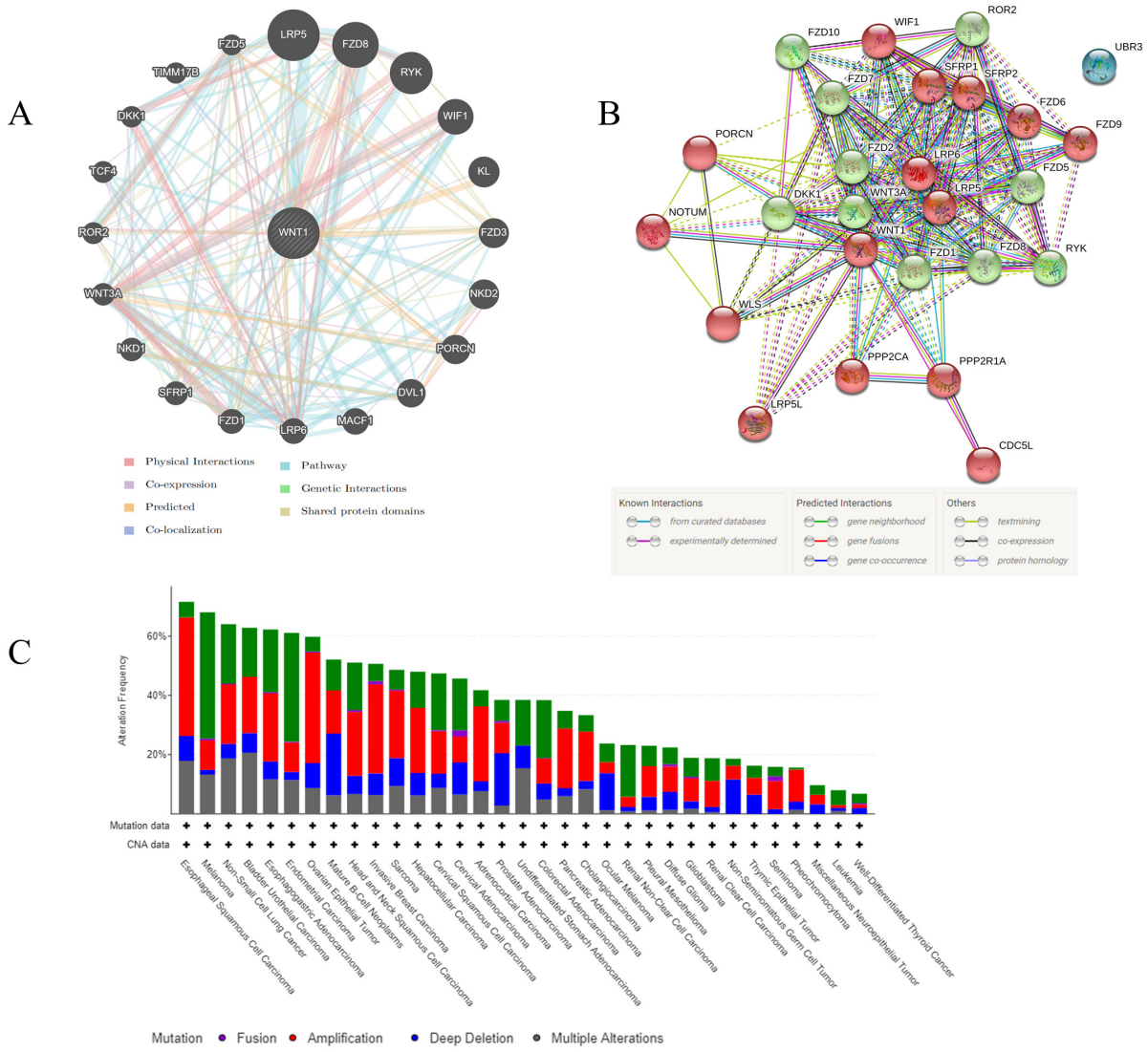

Figure 5. Proteins interacting and co-occurring with Wnt1. (A,B) Predicted proteins associated with Wnt1 according to GeneMANIA and STRING; **(C)** The alteration frequency of 33 genes as predicted in different cancers. The alteration frequency included mutations (green), fusions (violet), amplifications (red), deep deletions (deep blue), and multiple alterations (grey).

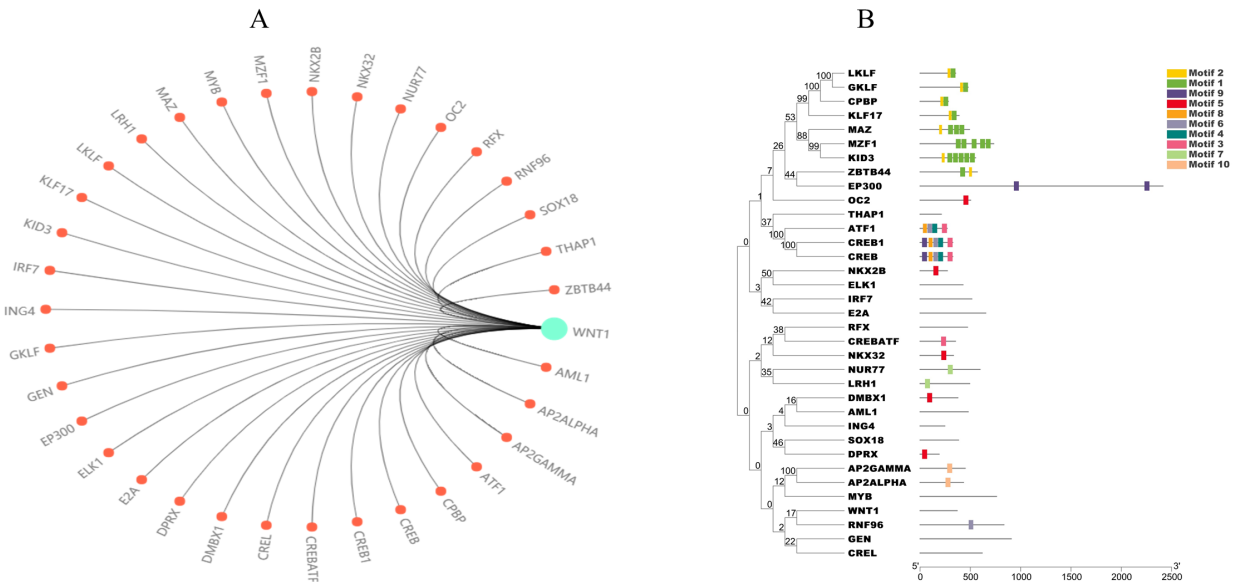

Figure 6. Prediction of Wnt1 transcription factors. (**A**) Predicted transcription factors highly associated with Wnt1; (**B**) Domain analysis of transcription factors using TBtools.

osteoporosis (*14*). Hence, some parental carriers suffer from osteoporosis (*13*). Patients with XV OI usually have severe long-bone deformities, though no fracture or deformity is noted at birth as is true in dominant forms of OI. Severe vertebral compression, developmental delay, and brain abnormalities are the main phenotypes that differ from those of other OI types (*15*). A neurological phenotype is also involved in Wnt1-induced OI (*33*). Therefore, the overlap in phenotypes between OI and other CNS diseases suggests crosstalk mechanisms related to *Wnt1* mutations.

A highly interesting finding is that the same mutation sites could lead to different diseases. G to A substitution at position 385 was found in both type XV OI in a homozygous or compound heterozygous form (*15,34*) and colorectal carcinoma (*35*). Different types of mutations in the same nucleic acids were observed in different diseases. The missense mutation c.466T was reported in stomach carcinoma. Deletion of this site led to a truncated protein with 156 amino acids found in a Chinese patient with OI whose parents were carriers (*15*). The mutations c.506dup and c.506G>A were both found in patients with XV OI (*17,34*). Deletion of this site is associated with colon and cecum carcinoma (*35,36*). *Wnt1* S88R is reported to be related to autism (*37*), though autism is also reported to be part of the phenotype for patients with type XV OI (*33*). Figure 1 shows that *Wnt1* mutations from cancers and OI are all clustered together and in the same Wnt1 domain, though most mutation sites differ. Thus, the challenge is diagnosing the condition with no obvious clinical phenotypes, especially in prenatal screening. Further research on the relationship between the phenotype and genotype could help determine the molecular mechanisms for *Wnt1*-induced diseases and guide diagnosis of the condition.

Proteins interacting with Wnt1 are related to maturation, secretion, and signaling pathways of Wnt. Like other Wnt proteins, Wnt1 is a protein that depends on O-acyltransferase porcupine (PORCN) and Wntless (WLS) for secretion (*38,39*). Notum acts as a negative regulator of Wnt signaling pathway by specifically mediating depalmitoleoylation of Wnts (*2*). Wnt1 activates canonical Wnt/β-catenin signaling *via* LRP5/6 receptors by cell-cell physical contact and regulates osteoclastogenesis with OPG in a juxtacrine manner (11). By binding to cell surface receptors, Wnt1 activates a canonical signaling pathway that increases cellular β-catenin activity. A mutation in Wnt1 or proteins it interacts with is associated with abnormal Wnt signaling and regulation; this affects oncogenesis, so Wnt1 could be a prognostic marker for cancers (*24,30,40-42*).

The current study systematically analyzed mutation, expression, and functions of Wnt1 in a number of human diseases. The expression of *Wnt1* and proteins interacting with it was involved in various cancers and is significantly related to survival in some cancers. The altered expression of *Wnt1* and proteins interacting with it may be a prognostic marker in some cancers.

Acknowledgements

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