

Original Research

In Silico Identification of Therapeutic Targets and Novel Drug Candidates for Malignant Peripheral Nerve Sheath Tumors

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Academic Editor: Graham Pawelec

Submitted: 28 December 2022 Revised: 20 April 2023 Accepted: 5 May 2023 Published: 24 September 2023

Abstract

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are an aggressive form of sarcomas with a poor prognosis and limited treatment options. Therefore, new therapeutic targets are urgently needed to identify novel drugs. **Methods:** Based on the Gene Expression Omnibus database, an integrated analysis was performed to identify differentially expressed genes (DEGs) in MPNSTs compared to neurofibromas (NFs). Then functional enrichment analyses, protein-protein interaction (PPI) network construction, and hub gene selection were conducted. We explored DEG-guided repurposable drugs to treat MPNST based on the Library of Integrated Network-Based Cellular Signatures (LINCS) database. Furthermore, the binding affinity between predicted drug candidates and the MPNST-associated hub gene was calculated using molecular docking. **Results:** We identified 89 DEGs in common with all three MPNSTs datasets. In the PPI networks, twist family bHLH transcription factor 1 (*Twist1*) with higher node degrees was further evaluated as a therapeutic target. Cytochalasin-d, cabozantinib, everolimus, refametinib, and BGT-226 were extracted from the LINCS database, which showed lower normalized connectivity scores (−1.88, −1.81, −1.78, −1.76, and −1.72, respectively) and was considered as drug candidates. In addition, the results of molecular docking between the five drugs and *Twist1* showed a binding affinity of −6.61, −7.03, −7.73, −3.94, and −7.07 kcal/mol, respectively. **Conclusions:** Overall, our results describe the importance of *Twist1* in MPNST pathogenesis. Everolimus was also found to be a potential therapeutic drug for MPNSTs.

Keywords: malignant peripheral nerve sheath tumors; twist family bHLH transcription factor 1; molecular docking; everolimus

1. Introduction

Malignant peripheral nerve sheath tumors (MPNSTs) are highly invasive cancers that account for approximately 10% of all soft tissue sarcoma; 50% of such tumors are associated with neurofibromatosis type 1 (NF1). These sarcomas generally have poor clinical outcomes, and are the leading cause of mortality and morbidity in adults with NF1. Complete surgical resection is still the main treatment method for MPNSTs due to the limited effectiveness of both chemotherapy and radiotherapy. However, a full resection may not be possible due to tumor size and location in many patients [1,2].

Several amplified genes that are important for the pathogenesis of MPNST have been identified. For example, the expression of epidermal growth factor receptor (*EGFR*) and *erbB2* is stronger in MPNSTs than in neurofibromas (NFs). Holtkamp *et al.* [3] valuated the effects of erlotinib and trastuzumab drugs targeting *EGFR* and *erbB2* in MPNST cell lines, and *EGFR* and *erbB2* have emerged as potential targets for the treatment of patients with MPNSTs. The progressive amplification of MET was found in the case of MPNST. NF1 ablated mice exhibit a strong MPNST phenotype without additional mutations, which are

consistently sensitive to the highly selective MET inhibitor capmatinib [4]. Frequent platelet-derived growth factor receptor alpha (*PDGFRα*) mutations in MPNST are typically associated with the co-amplification of KIT Proto-Oncogene (*c-KIT*). The tyrosine kinase inhibitor imatinib can inhibit the proliferation of MPNST cell lines *in vitro*, and it is known that imatinib can target *PDGFRα* and *c-KIT*. *PDGFRα* is a candidate for targeted therapy of MPNST [5]. Furthermore, the insulin-like growth factor 1 receptor (*IGF1R*) pathway is also a potential therapeutic target for MPNST patients. Inhibition of *IGF1R* in MPNST cell lines using small interference RNAs or *IGF1R* inhibitors leads to a significant decrease in cell proliferation, invasion, and migration [6]. Although these targeted drugs have proven to be effective in treating MPNSTs, there are currently no U.S. Food and Drug Administration (FDA)-approved drugs for the treatment of MPNSTs. Therefore, a better understanding of the initiation and progression is needed to identify new therapeutic strategies.

In this study, we screened druggable targets via integrated bioinformatics analysis and predicted therapeutic drugs for MPNSTs using the Library of Integrated Network-Based Cellular Signatures (LINCS) database, which identi-



fies the connection between gene expression profiles and FDA-approved drugs. Furthermore, molecular docking was used in the search for matching predicted potential drugs and screened targets. Our finding expands the current understanding of MPNST progression and identifies potential therapeutic drugs for NF1-related MPNSTs.

2. Materials and Methods

2.1 Data Collection and DEG Acquisition

The gene expression profiles of the microarray datasets GSE41747, GSE66743, and GSE141439 were obtained for analysis from the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/geo/>). Bioinformatics analyses of the data from online databases were carried out by R 4.1.1 software (R Foundation for Statistical Computing, Vienna, Austria). We performed differential expression analysis using the limma package (<https://bioconductor.org/packages/release/bioc/html/limma.html>) to determine differentially expressed genes (DEGs) with the criteria of $|\log_2FC| > 1$ and $p < 0.05$ and created volcano plots of the DEGs [7]. A Venn diagram and heatmap of DEGs were generated using the online database SangerBox website (<http://sangerbox.com/tool.html>).

2.2 Protein-Protein Interaction Network Construction, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes Pathway Analysis of DEGs

Search Tool for the Retrieval of Interacting Genes (STRING, <https://string-db.org/>) is an online tool for evaluating the information on protein-protein interaction (PPI) [8]. We use the STRING to find possible relations among the DEGs. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEGs were conducted with the online database SangerBox website.

2.3 PPI Network Module Analyses and Identification of Hub Genes

PPI network and module analyses were visualized using Cytoscape version 3.7.2 (Institute for Systems Biology, Seattle, WA, USA), and subsequent hub gene identification of the modules was performed [9]. First, the Cytoscape Molecular Complex Detection (MCODE) plug-in was used to identify the most important modules in the PPI networks. The criteria for selection were set as follows: MCODE score > 3 , number of nodes > 4 . Next, we use the CytoHubba plug-in of Cytoscape to filter hub genes from the PPI network with the Eccentricity, BottleNeck, and Closeness ranking methods [8]. The top 10 genes were considered hub genes.

2.4 Prognosis Analysis and Differential Expression of *Twist* Family bHLH Transcription Factor 1 in Pan-Cancer

We used the Kaplan-Meier method and Cox proportional hazards (CoxPH) regression model to explore the overall survival (OS) of patients with high and low *Twist*

family bHLH transcription factor 1 (*Twist1*) expression in 39 cancer types. Hazard ratios (HRs) with 95% confidence intervals (CIs) and log-rank p values were reported. $p < 0.05$ was considered statistically significant. The online database SangerBox website (<http://sangerbox.com/tool.html>) was used to analyze the expression of *Twist1* in different cancers and normal tissues [10]. The expression profiles of *Twist1* in different cancer and normal cell lines were obtained from the BioGPS database (<http://biogps.org>) [11].

2.5 Mutation Profiles

The c-BioPortal for Cancer Genomics (<http://cbiportal.org>) is a publicly available database for exploring multidimensional cancer genomics datasets [12]. The genetic alteration of *Twist1* in different cancers was analyzed by the c-BioPortal database [13].

2.6 MPNST-Associated Drug Prediction

We use The Connectivity Map, which is designed to identify biological pathways and expression modules by querying the gene lists of up- and down-regulated genes to rank small molecules, drugs and genes. A so-called connectivity score was used to evaluate the correlation between the drugs and input genes; a positive score indicates an inducement effect of the compound on the gene signatures, while a negative score reflects an inverse impact of a compound on the gene signatures. The L1000 database of the LINCS project includes 476,251 gene expression signatures gathered from 72 cell lines stimulated by 27,927 small molecule compounds. We identified the potential compounds based on the connectivity score (-2 to 2).

2.7 Molecular Docking Between MPNST Candidate Drugs and Hub Genes

The 3D structure files of the compounds were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The crystal structures of target proteins were retrieved from the RCSB Protein Data Bank (PDB) (<https://www.rcsb.org/>). AutoDockTools 1.5.7 software (Molecular Graphics Laboratory, The Scripps Research Institute, La Jolla, CA, USA) was used to prepare the required files, converting ligands and receptors structures from PDB file format to Pdbqt format. All water molecules were detached for predocking preparation, and then Kolmman charges and polar hydrogen atoms were added to the compounds using AutodockTools. Molecular docking calculations were performed by AutoDockTools. The docking results were expressed as binding affinity values of the obtained receptor/ligand complex (kcal/mol) according to hydrogen bonds, electrostatic, and hydrophobic interactions. PyMOL 2.6.0 software (PyMOL Molecular Graphics System, Schrödinger, LLC, Shanghai, China) was used to visualize the 3D structures of the protein and major receptor/ligand interaction in terms of hydrogen bonds binding to the key amino acid residues.

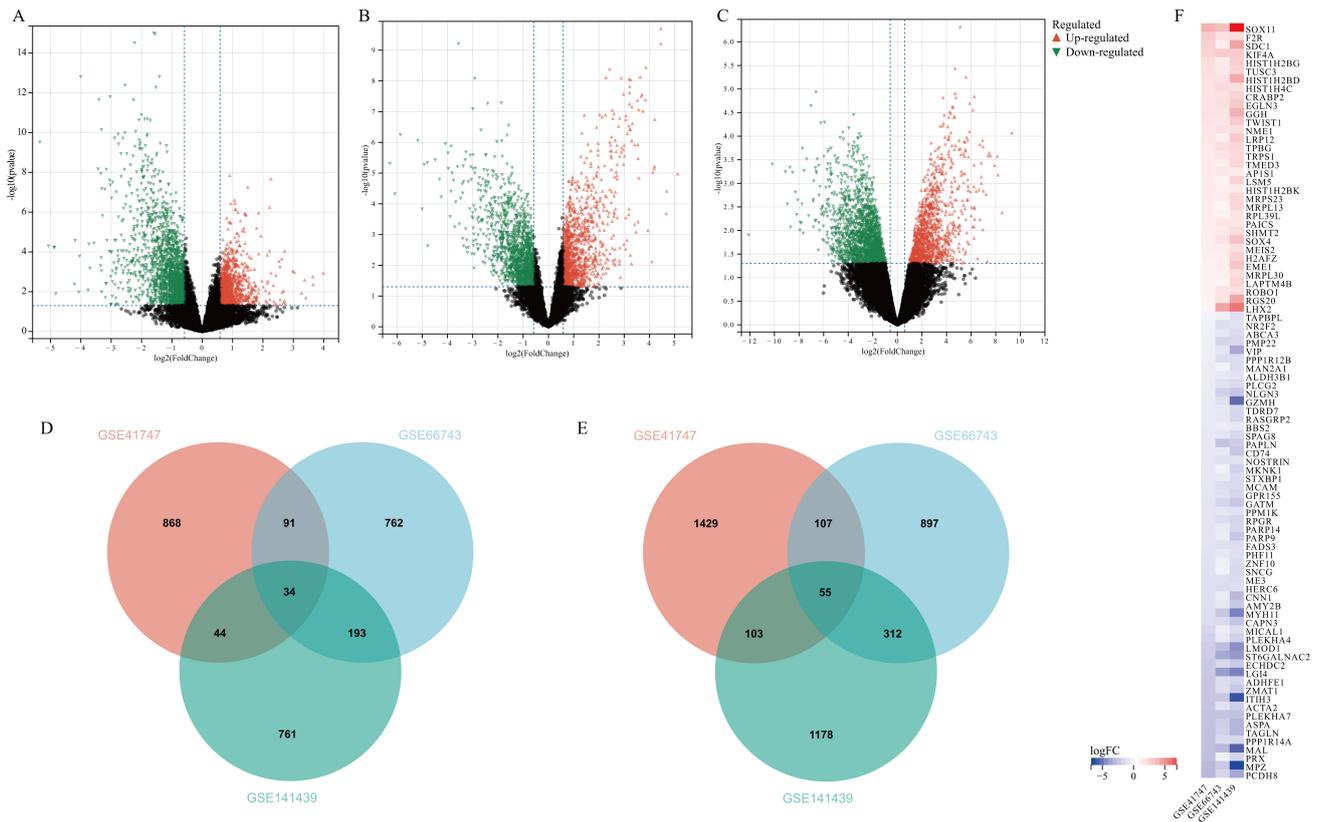


Fig. 1. Identification of overlapping DEGs. (A–C) Differential expression of genes between MPNST tissues and neurofibroma tissues in the microarray datasets (GSE41747, GSE66743, and GSE141439). (D) Venn diagram showing DEGs across the three datasets, with an overlap of 34 upregulated gene. (E) Venn diagram showing DEGs across the three datasets, with an overlap of 55 downregulated genes. (F) The heat map of overlap upregulated and downregulated genes. The columns and rows in the heat map represent GEO datasets and genes. MPNST, malignant peripheral nerve sheath tumors; DEGs, differentially expressed genes; GEO, Gene Expression Omnibus.

2.8 Statistical Analysis

The primary statistical analyses were performed in R 4.1.1 software. The normality test was performed with the Shapiro-Wilk test. The Kaplan-Meier curve was drawn to compare time to event distribution, and Univariate Cox regression analysis was used to evaluate the correlations between the DEGs and OS. All hypothetical tests were two-sided, and $p < 0.05$ was considered statistically significant with the following thresholds: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

3. Results

3.1 Identification of Overlapping DEGs in MPNST

Microarray datasets (GSE41747, GSE66743, and GSE141439) were retrieved from the GEO database to identify the DEGs between MPNST and NF samples (Fig. 1). There was a total of 80 samples, including 43 MPNST and 37 NF (GSE41747: 6MPNST/26NF; GSE66743:30MPNST/8NF; GSE141439: 7MPNST/3NF). Finally, 1037 DEGs with upregulation and 1694 DEGs with downregulation were identified in the GSE41747 dataset (Fig. 1A). 1080 DEGs with upregulation and 1371 DEGs

with downregulation were identified in the GSE66743 dataset (Fig. 1B). Additionally, 1032 DEGs with upregulation and 1648 DEGs with downregulation were identified in the GSE141439 dataset (Fig. 1C). We identified 89 common DEGs among the three GEO datasets, including 34 up-regulated genes and 55 downregulated genes (Fig. 1D–F).

3.2 Functional Enrichment Analyses

The GO enrichment analysis showed that biological process (BP) terms were related to the cellular process, single-organism process, metabolic process, and biological regulation. GO_cellular component (GO_CC) analysis showed enrichment in macromolecular complexes, membranes, and membrane-enclosed lumen. GO_molecular function (GO_MF) analysis showed enrichment in catalytic activity, DNA binding transcription factor activity, and structural molecular activity (Fig. 2A). The KEGG pathways were significantly enriched in antifolate resistance, proteoglycans in cancer, viral carcinogenesis, and the hypoxia inducible factor 1 (*HIF-1*) signaling pathway, which involved in multiple mechanism of tumorigenesis and related to occurrence and development of MPNSTs (Fig. 2B,C).

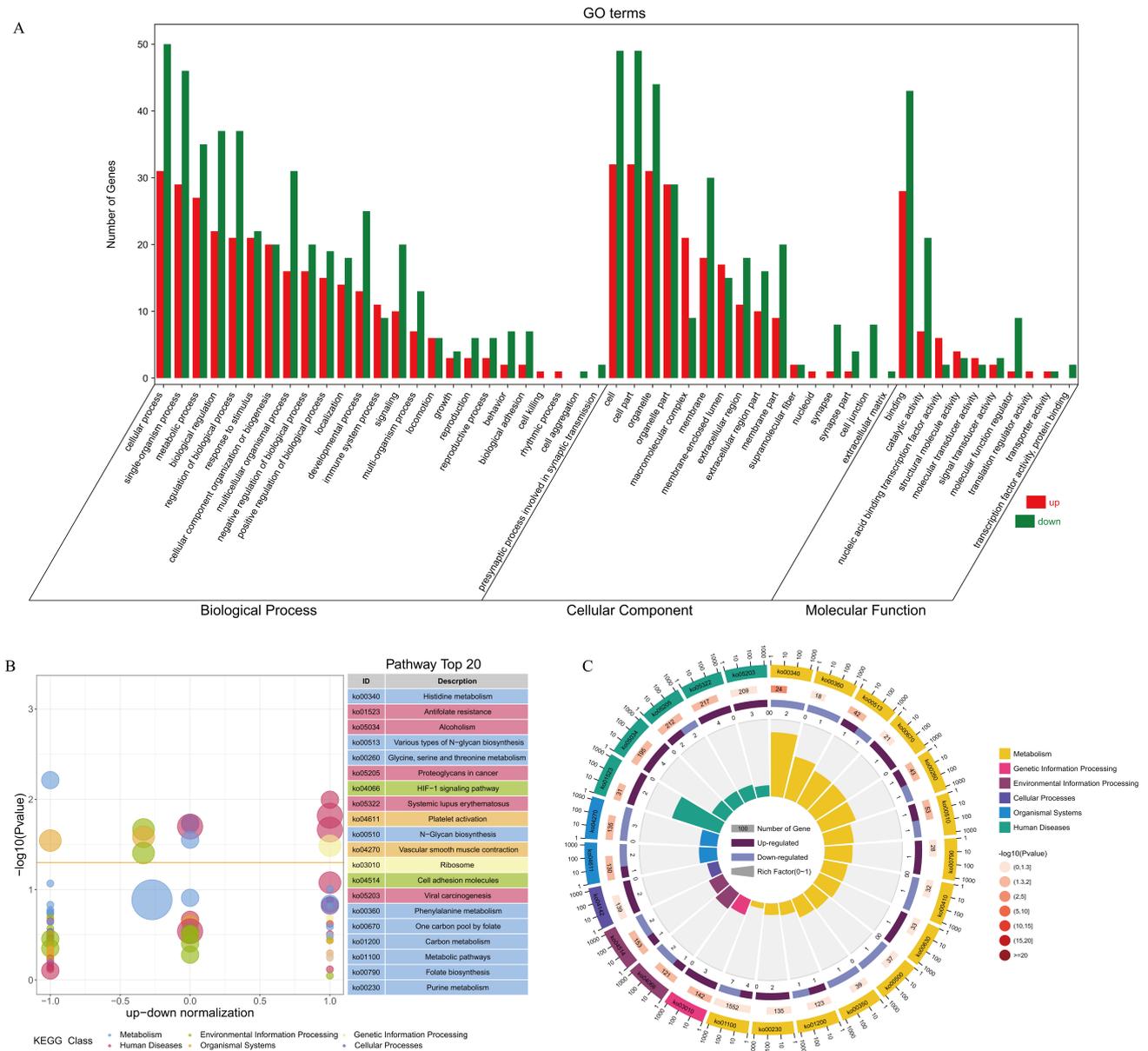


Fig. 2. Functional enrichment analyses of DEGs. (A) GO_MF analysis showed enrichment in catalytic activity, DNA binding transcription factor activity, and structural molecular activity. (B–C) The KEGG pathways were significantly enriched in antifolate resistance, proteoglycans in cancer, viral carcinogenesis, and the *HIF-1* signaling pathway, which involved in multiple mechanism of tumorigenesis and related to occurrence and development of MPNSTs. GO_MF, GO_molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

3.3 PPI Network Construction, Module Selection, and Identification of Hub Genes

The PPI network was constructed on STRING, which contained 287 nodes and 848 edges. The most significant module was obtained in the PPI network of the upregulated and downregulated genes using Cytoscape MCODE plugin. It was enriched in viral carcinogenesis, proteoglycans in cancer, and necroptosis (Fig. 3A).

Then, using the CytoHubba plugin of the Cytoscape, the top 10 hub genes were identified in the whole PPI network ranked in terms of different algorithm, includ-

ing *Twist1*, *HIST1H4C*, *SOX11*, *ACTA2*, *SOX4*, *H2AFZ*, *HIST1H2BD*, *HIST1H2BG*, *HIST1H2BK*, and *RPGR* by the EcCentricity method; *ACTA2*, *Twist1*, *HIST1H4C*, *H2AFZ*, *RPGR*, *MYH11*, *TAGLN*, *BBS2*, *PPP1R12B*, and *SOX11* by the BottleNeck method; and *ACTA2*, *HIST1H4C*, *Twist1*, *H2AFZ*, *MYH11*, *TAGLN*, *CNN1*, *LMOD1*, *HIST1H2BD*, and *HIST1H2BK* by the Closeness method (Table 1). Based on the high score of *Twist1* in various algorithms and combination with pan-cancer analysis, we selected it as the target gene.

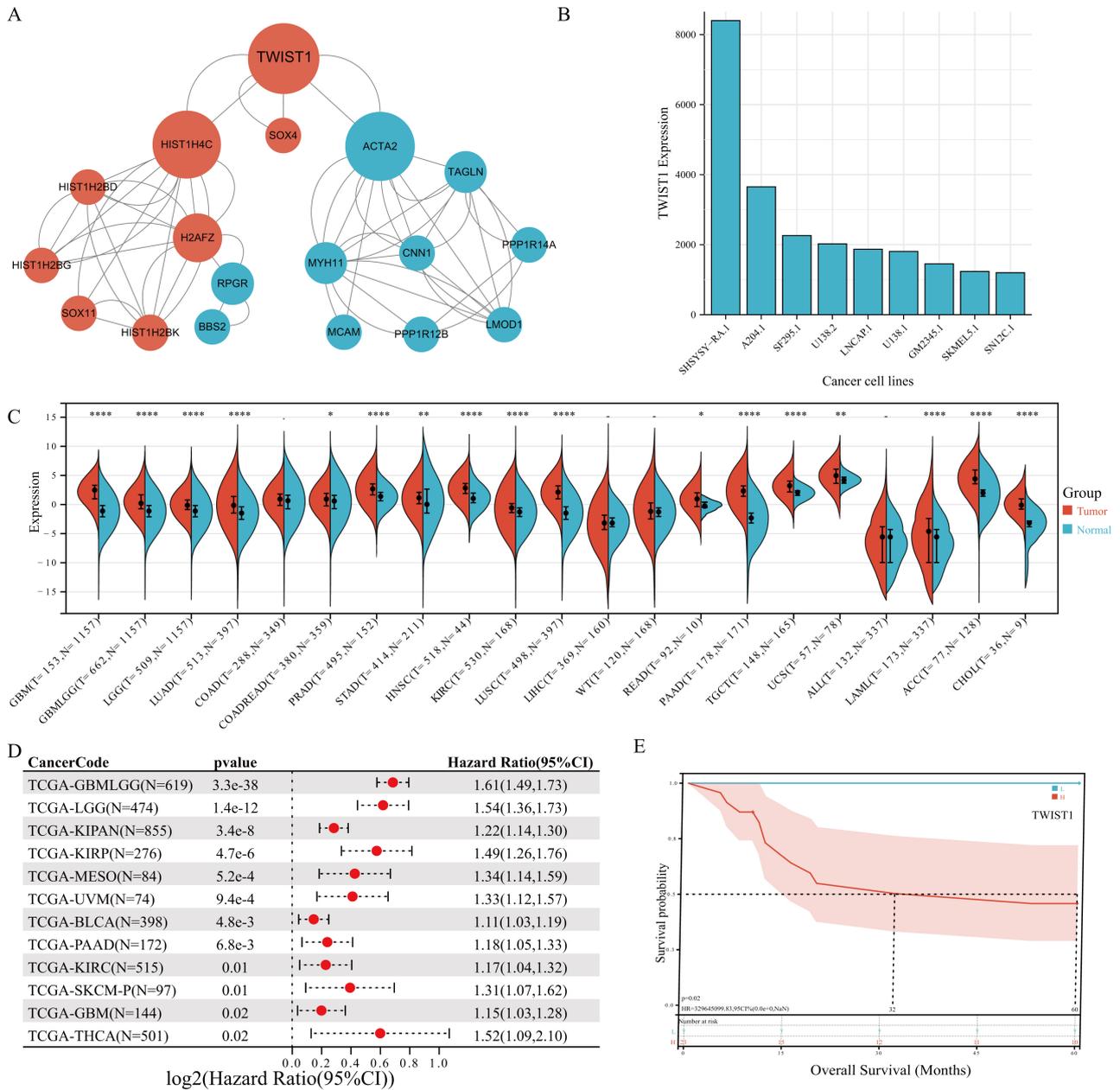


Fig. 3. Prognostic value and differential expression of *Twist1* across pan-cancer. (A) The protein-protein interaction network and hub genes. (B) *Twist1* expression in different cancer cell lines extracted from the BioGPS database. (C) *Twist1* expression in pan-cancers compared with normal tissues in the GEPIA database. (D) The correlation between the *Twist1* expression and prognosis in pan-cancer. (E) High *Twist1* expression was also associated with poor OS time in MPNST.

3.4 Prognostic Value and Differential Expression of *Twist1* across Pan-Cancer

We analyzed *Twist1* expression in various cancer cell lines and normal tissues extracted from the BioGPS database. The results revealed that *Twist1* expression increased not only in almost all cancer cells but also significantly varied among different cancer cell lines (Fig. 3B). Subsequently, we examined the expression levels of *Twist1* in pan-cancer using the online database SangerBox. The expression of *Twist1* was significantly increased in adrenocortical carcinoma (ACC), lower grade glioma (LGG), cholan-

giocarcinoma (CHOL), lung adenocarcinoma (LUAD), acute myeloid leukemia (LAML), stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), testicular germ cell tumors (TGCT), kidney renal clear cell carcinoma (KIRC), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), glioblastoma multiforme (GBM), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), and uterine carcinosarcoma (UCS) (Fig. 3C). These results suggested that *Twist1* is overexpressed in tumor tissues.

Table 1. Top 10 genes screened by the cytoHubba plugin of Cytoscape.

EcCentricity method		BottleNeck method		Closeness method	
Name	Score	Name	Score	Name	Score
<i>TWIST1</i>	0.25	<i>ACTA2</i>	18	<i>ACTA2</i>	10.11667
<i>HIST1H4C</i>	0.25	<i>TWIST1</i>	18	<i>HIST1H4C</i>	10
<i>SOX11</i>	0.2	<i>HIST1H4C</i>	18	<i>TWIST1</i>	9.25
<i>ACTA2</i>	0.2	<i>H2AFZ</i>	3	<i>H2AFZ</i>	8.816667
<i>SOX4</i>	0.2	<i>RPGR</i>	2	<i>MYH11</i>	8.783333
<i>H2AFZ</i>	0.2	<i>MYH11</i>	2	<i>TAGLN</i>	8.783333
<i>HIST1H2BD</i>	0.2	<i>TAGLN</i>	2	<i>CNN1</i>	8.283333
<i>HIST1H2BG</i>	0.2	<i>BBS2</i>	1	<i>LMOD1</i>	8.283333
<i>HIST1H2BK</i>	0.2	<i>PPP1R12B</i>	1	<i>HIST1H2BD</i>	8.15
<i>RPGR</i>	0.166667	<i>SOX11</i>	1	<i>HIST1H2BK</i>	8.15

The univariate Cox regression analysis was used to investigate the correlation between *Twist1* expression and prognosis, and a forest plot was drawn via R package “survival” (Fig. 3D) and Kaplan-Meier survival curves for tumors with significantly affected OS. High expression of *Twist1* was associated with poor OS time in LGG (n = 474, $p = 3.3 \times 10^{-38}$, HR = 1.61 [1.49, 1.73]), KIPAN (n = 855, $p = 3.4 \times 10^{-8}$, HR = 1.22 [1.14, 1.30]), KIRP (n = 276, $p = 4.7 \times 10^{-6}$, HR = 1.49 [1.26, 1.76]), MESO (n = 84, $p = 5.2 \times 10^{-4}$, HR = 1.34 [1.14, 1.59]), UVM (n = 74, $p = 9.4 \times 10^{-4}$, HR = 1.33 [1.12, 1.57]), BLCA (n = 398, $p = 4.8 \times 10^{-3}$, HR = 1.11 [1.03, 1.19]), PAAD (n = 172, $p = 6.8 \times 10^{-3}$, HR = 1.18 [1.05, 1.33]), KIRC (n = 515, $p = 0.01$, HR = 1.17 [1.04, 1.32]), SKCM (n = 97, $p = 0.01$, HR = 1.31 [1.07, 1.62]), GBM (n = 144, $p = 0.02$, HR = 1.15 [1.03, 1.28]), and THCA (n = 501, $p = 0.02$, HR = 1.52 [1.09, 2.10]). Furthermore, high *Twist1* expression was also associated with poor OS time in MPNSTs (Fig. 3E). Together, these data suggested that *Twist1* expression was associated with prognosis in most cancers, including MPNSTs.

3.5 *Twist1* Genomic Alterations in Different Cancer Groups

The results demonstrated that *Twist1* genomic alterations occurred in 1.5% of patients (Fig. 4A). The frequency of *Twist1* gene alterations was higher in different cancer types, especially in the type of amplification (Fig. 4B).

3.6 MPNST-Associated Drugs

Overlapping DEGs between MPNST and NF were used to search for drugs that could revert their expression signatures using the LINCS Query tool. Drug-gene combinations were ranked by search score. Drugs with low scores correspond to higher reversal potency and more significant potential for application. Finally, we selected five drugs (cytochalasin-d, cabozantinib, everolimus, refametinib, and BGT-226).

3.7 Molecular Docking Simulation

To know the interaction between predicted five drugs and *Twist1*, the molecular docking simulation study was performed by the AutoDockTools software. At the same time, we selected selumetinib, a classic mitogen-activated protein kinase (MAPK) inhibitor, as the reference drug for drug identification. The binding energy of cytochalasin-d, cabozantinib, everolimus, refametinib, BGT-226, and selumetinib with *Twist1* was -6.61 , -7.03 , -7.73 , -3.94 , -7.07 , and -6.22 kcal/mol, respectively (Table 2, Fig. 5). The molecular docking results revealed that everolimus interacted with *Twist1* via two H-bonds in close proximity of 2.1 Å and 2.2 Å with ARG-423 and TYR-430 residues, respectively.

4. Discussion

Only 8–13% of NF1 patients develop MPNSTs. The genetic alteration from NFs to MPNST formation is unclear [14]. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*)/p16 and polycomb repressive complex 2 (*PRC2*) gene mutations drive the transformation from NFs to MPNST [15–20]. p16 is a tumor suppressor protein encoded by the *CDKN2A* gene, which inhibits cyclin D-dependent protein kinases from entering the S phase of the cell cycle, thereby maintaining retinoblastoma protein (Rb) in a low-phosphorylated state and preventing its isolation from E2F transcription factor. Loss of p16 expression and homozygous deletion of the *CDKN2A* gene in NFs may be related to their transformation to MPNSTs [15]. The core *PRC2* complex comprises four components: EZH1/2, SUZ12, EED, and PcbAp46/48 [21]. Lee *et al.* [17] identified loss-of-function somatic alterations of the *PRC2* components (EED or SUZ12) in MPNSTs. MPNSTs with *PRC2* inactivation have been shown consistent and complete loss of trimethylation at lysine 27 of histone H3 (H3K27me3). The introduction of the missing *PRC2* component in a *PRC2*-deficient MPNST cell line restored H3K27me3 levels and reduced cell proliferation.

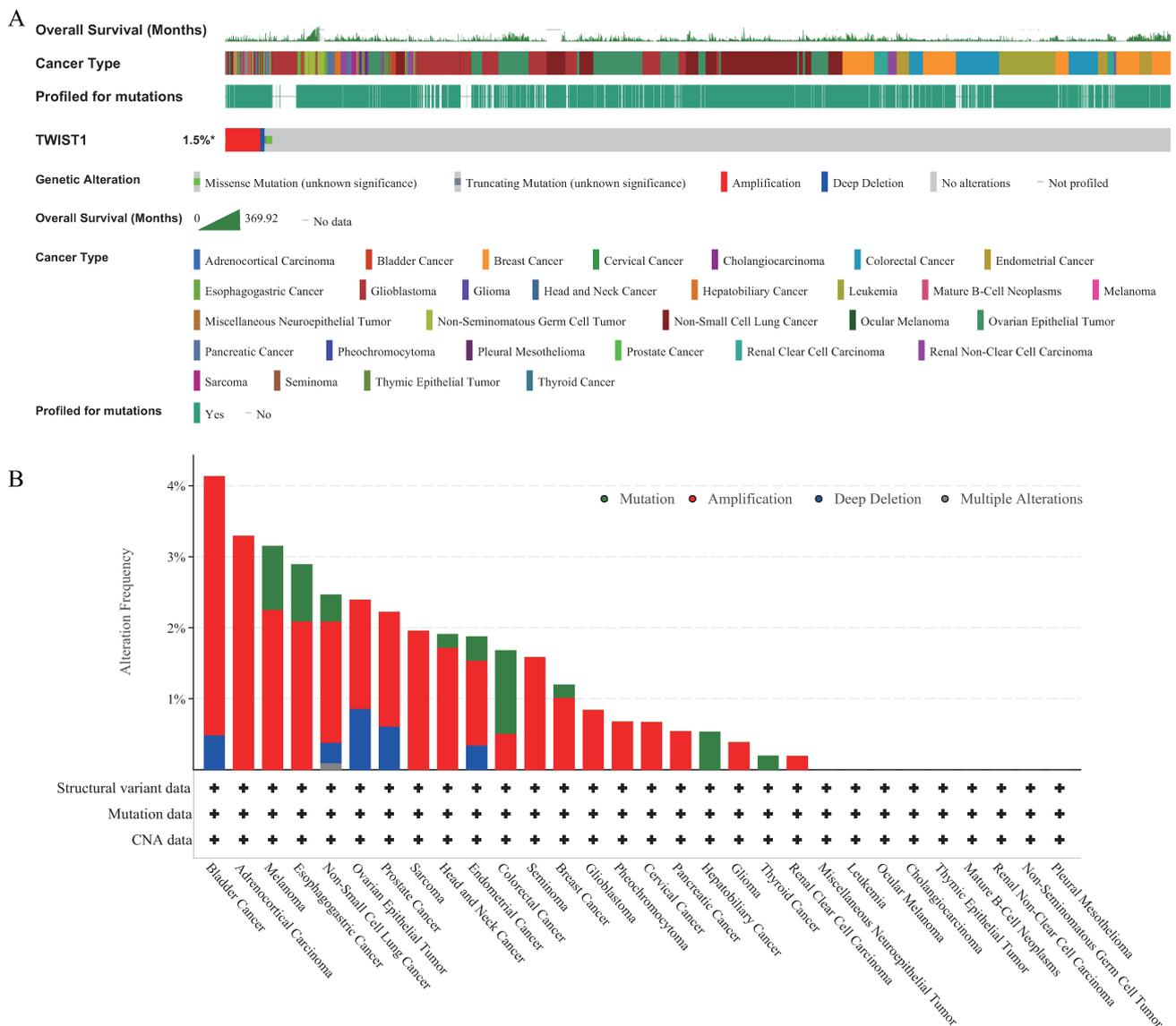


Fig. 4. GZMA Genomic Alterations in different cancer groups analyzed by the cBioPortal database. (A) *Twist1* genomic alterations occurred in 1.5% of patients. (B) The *Twist1* gene alterations frequency was higher in different cancer types, especially in the type of Amplification.

Some individuals with NF1 will develop MPNSTs that arise due to the malignant transformation of plexiform neurofibroma (pNF) [22]. In the transition from NFs to MPNSTs, a recently identified tumor is called Atypical neurofibromatosis neoplasms of uncertain biological potential (ANNUBP), which is hypothesized to be precursor lesions of MPNSTs [23]. However, the molecular mechanism underlying the transformation from pNF to MPNSTs is still unknown. In this study, we first identified DEGs between MPNSTs and NFs using three independent GEO databases. In total, 34 upregulated DEGs and 55 downregulated DEGs were revealed. Bioinformatics analysis revealed that the upregulated genes are mainly involved in proteoglycans in cancer, and viral carcinogenesis, and the HIF-1 signaling pathway. To identify the hub genes involved in the progres-

sion from NFs to MPNSTs, we constructed a PPI network, and *Twist1* was identified as a hub gene in the progression of MPNSTs. Further analysis revealed that higher expression of *Twist1* correlated with shorter survival time in MPNSTs as in pan-cancer. Thus it may be a potential biomarker for the prognosis of MPNSTs.

Cutaneous NFs are the most prevalent tumour in NF1 patients. Plexiform NFs, defined as NFs involving multiple peripheral nerve fascicles, are less frequent but lead to serious consequences. Malignant transformation develops in 5–10% of NF1 patients, typically in a plexiform NFs subtype [24]. The neural crest transcription (*SOX9*) is found to be a biomarker of MPNSTs, which is possibly a therapeutic target in NF1. Interestingly, <30% of cells in most cutaneous NFs are *SOX9* positive; plexiform NFs contain

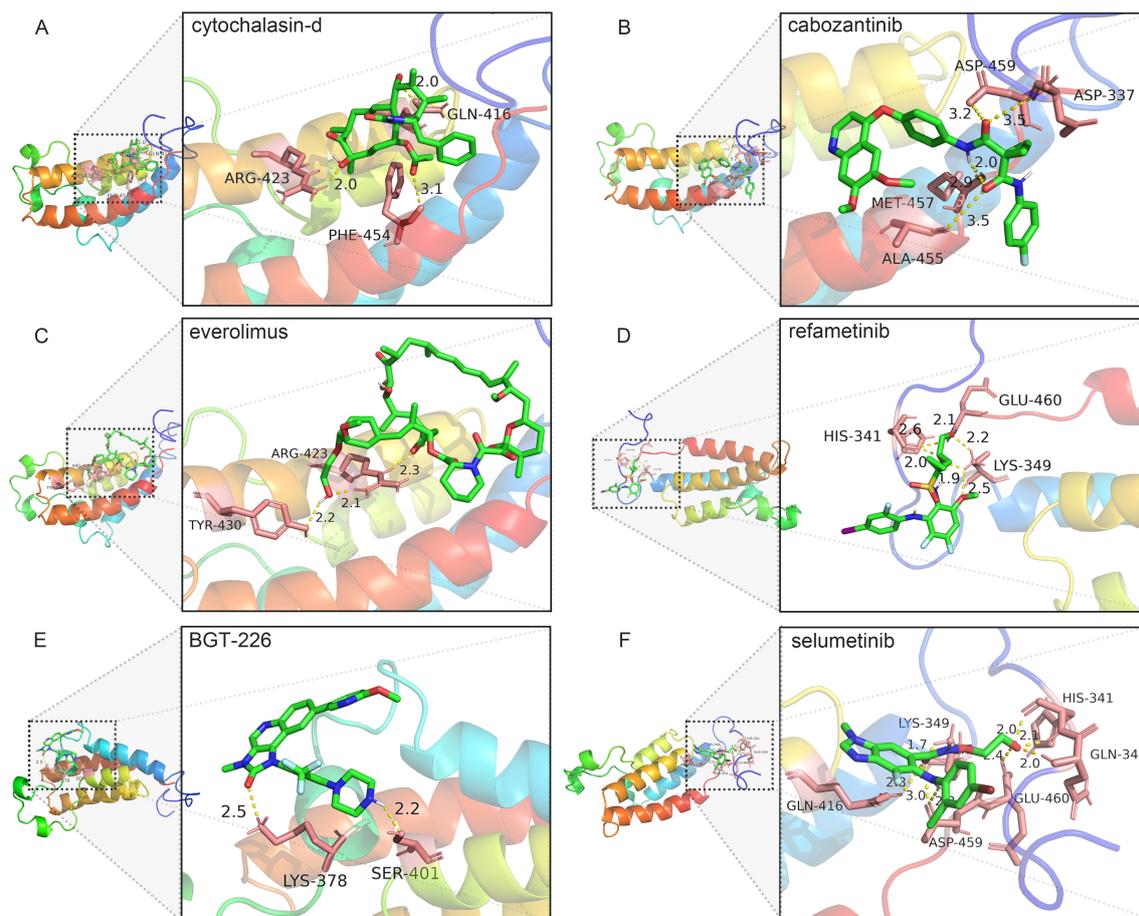


Fig. 5. Molecular docking profile of drug candidate with *Twist1*. (A) cytochalasin-d. (B) cabozantinib. (C) everolimus. (D) refametinib. (E) BGT-226. (F) selumetinib.

an average of 50% of *SOX9* positive cells, while in MPNSTs, >70% of cells are *SOX9* positive. This may indicate a potential evolutionary relationship among the three types of tumors [25,26].

It appears that plexiform NFs have a higher ability to evolve into MPNSTs than cutaneous NFs. But so far, it is still unknown whether there is a molecular difference between cutaneous NFs and plexiform NFs. In the GSE41747 dataset we used to analyse, transcripts that passed the ANOVA failed to distinguish the two NF subtypes [25,26]. The explanation is that cutaneous and plexiform NFs cells may be essentially the same but exposed to different tumor microenvironments. In addition, the other two datasets we used (GSE66743 and GSE141439) did not specify whether NFs were cutaneous NFs or plexiform NFs [27,28], so our analysis did not distinguish the two types of NFs. Due to the different potential of developing MPNSTs of two types of NFs, it is necessary to conduct gene differential analysis between different types of NFs and MPNSTs in future studies.

Twist1, a transcription factor and member of the basic helix-loop-helix protein family, is known to stimulate

epithelial-mesenchymal transition (EMT) and promote tumor invasion and metastasis. Dysregulation of *Twist1* is associated with the loss of epithelial markers, such as E-cadherin, and alpha/gamma-catenins, and the activation of mesenchymal markers, such as N-cadherin, suggesting that *Twist1* promotes the EMT [29]. *Twist1*-induced EMT is clinically associated with distant cancer metastasis and poor prognosis in multiple tumor types. *Twist1* transcription factors mediate EMT target genes involved in cell migration and invasion [30], multidrug resistance [31–33], cancer stem cell self-renewal [34,35], immune surveillance [36], and apoptosis [37].

Our study revealed that the expression of *Twist1* was significantly higher in GBM, LGG, LUAD, PRAD, STAD, HNSC, KIRC, LUSC, READ, PAAD, TGCT, UCS, LAML, ACC, and CHOL, consistent previous reports [29, 38–56]. *Twist1* might be a significant diagnostic and prognostic biomarker predicting poor prognosis, which has already been demonstrated in breast cancer [39], bladder cancer [57], esophageal squamous cell carcinoma [58], cervical cancer [59], head and neck cancer (HNC) [60], melanoma [61], colorectal cancer [62], hepatocellular cancer [63,64],

Table 2. The results of molecular docking between drug candidates with *Twist1*.

Ligand	Receptor	PDB ID	Binding Affinity (Kcal/mol)	Amino Acid Residue	Bonding Length	Interaction
cytochalasin-d	Twist1	2MJV	-6.61	ARG-423	2.0°	Hydrogen bond
				GLN-416	2.0°	Hydrogen bond
				PHE-454	3.1°	Hydrogen bond
cabozantinib	Twist1	2MJV	-7.03	ALA-455	3.5°	Hydrogen bond
				ASP-337	3.5°	Hydrogen bond
				ASP-459	3.2°	Hydrogen bond
				MET-457	2.9°	Hydrogen bond
				ARG-423	2.1°	Hydrogen bond
everolimus	Twist1	2MJV	-7.73	TYR-430	2.2°	Hydrogen bond
refametinib	Twist1	2MJV	-3.94	GLU-460	2.1°	Hydrogen bond
				HIS-341	2.0°	Hydrogen bond
				LYS-349	1.9°	Hydrogen bond
BGT-226	Twist1	2MJV	-7.07	LYS-378	2.5°	Hydrogen bond
				SER-401	2.2°	Hydrogen bond
selumetinib	Twist1	2MJV	-6.22	ASP-459	2.2°	Hydrogen bond
				GLN-340	2.0°	Hydrogen bond
				GLN-416	2.8°	Hydrogen bond
				GLU-460	2.4°	Hydrogen bond
				HIS-341	2.0°	Hydrogen bond
				LYS-349	1.7°	Hydrogen bond

PDB, Protein Data Bank.

nasopharyngeal cancer [51], and ovarian cancer [65,66]. The widespread involvement of *Twist1* in human cancer suggests that inhibition of *Twist1* is a promising therapeutic strategy for the prevention and treatment of cancer. We also showed that *Twist1* expression was linked to clinical prognosis in multiple cancers. In addition, high expression levels of *Twist1* had a significant association with poor OS in MPNSTs. *Twist1* is highly expressed in MPNSTs and has low expression in normal adult tissues, making it an promising therapeutic target for drug development. Therefore, the application of *Twist1* inhibitor has great clinical value for the treatment of MPNSTs, with few side effects.

We used LINCS datasets to identify novel MPNSTs drugs based on the overlapping DEGs. Finally, we selected five drugs (cytochalasin-d, cabozantinib, everolimus, refametinib, and BGT-226) with lower scores regarding their potential as drugs. To further predict the five drugs that might be direct *Twist1* inhibitors, the molecular docking analysis was performed using the AutoDockTools and PyMOL software. The results showed that everolimus interacting with *Twist1* has lowest binding energy, making everolimus an optimal potential drug.

The mammalian target of rapamycin (*mTOR*), which involve in the growth and proliferation of tumor cells and play a role in the switch between cell catabolism and anabolism, is dysregulated in many types of tumors [67]. This explains the potential use of *mTOR* inhibitors in oncology. Everolimus is a new *mTOR* inhibitor, which inhibits the growth and angiogenesis of tumor cells by continuously inhibiting *mTOR*. Everolimus blocking the bind-

ing of the raptor to *mTOR* can restore control of the activated PI3K/AKT/mTOR signalling pathway. Everolimus has been approved for treating advanced breast cancer with positive hormone receptors and negative human epidermal growth factor receptor 2 [68], advanced renal cell carcinoma after targeted treatment of vascular endothelial growth factor [69], and advanced neuroendocrine tumors of pancreatic, gastrointestinal or pulmonary origin [70,71]. In addition, it can be used to control the growth of subependymal giant cell astrocytoma, renal angiomyolipoma, and hamartoma in tuberous sclerosis complex (TSC) [72]. It can also be used for immunosuppression after heart or kidney transplantation [73].

Interestingly, *Twist1* promotes energy metabolism reprogramming (EMR) of glucose metabolism in breast cancer cells by activating the PI3K/AKT/mTOR pathway. EMR is crucial for the survival of cancer cells, as it can increase cancer proliferation, migration, and invasion [74]. In addition, previous studies have confirmed that *Twist1* promotes tumor invasion and metastasis by inducing EMT, and multiple signals that induce EMT often converge in the PI3K/AKT/mTOR signalling pathway. Xue *et al.* [75] found that complete EMT phenotype and morphological changes require PI3K/AKT mediated *Twist1* phosphorylation in breast tumor cells. *Twist1* may be a PI3K/AKT/mTOR signalling pathway member. Based on our research, everolimus could be a potential therapeutic drug for MPNSTs. The results of molecular docking show that everolimus has the lowest binding energy with *Twist1*, indicating that it may play an anti-tumor role by targeting

Twist1 in addition to targeting mTOR. Another possible explanation is that it indirectly regulates *Twist1* phosphorylation by targeting PI3K/AKT/mTOR pathway. However, our study results have not been experimentally validated and further experiments are needed.

This study is the first to systematically investigate the potential role of *Twist1* in MPNSTs, which may be a key gene related to the occurrence and progression of MPNSTs. In addition, through molecular docking, everolimus, a potential target drug for MPNSTs, has a clear binding site and good binding ability with *Twist1*. It is expected to provide new ideas and insights for exploring the treatment strategies and therapeutic drugs of MPNSTs.

5. Conclusions

Overall, our results describe the importance of *Twist1* in MPNST pathogenesis. everolimus was also found to be a potential therapeutic drug for MPNSTs. These findings require further experimental studies for confirmation.

6. Limitation

This study had limitations. First, we have not performed further experiment methods to verify the functional roles of *Twist1*. Therefore, functional experiments including cell proliferation, migration, gain- and loss-of-function assays, should be conducted in the future. Second, although we have identified the clinical significance of *Twist1* in MPNSTs in three independent datasets, verification in large clinical samples is still needed.

Abbreviations

MPNSTs, Malignant peripheral nerve sheath tumors; DEGs, differentially expressed genes; *Twist1*, twist family bHLH transcription factor; NFs, neurofibromas; pNF, plexiform neurofibroma; EMT, epithelial-mesenchymal transition.

Availability of Data and Materials

Publicly available datasets were analyzed in this study. The datasets include The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>) and the Genotype-Tissue Expression (GTEx) dataset (<http://www.gtexportal.org/home/>).

Author Contributions

HH and MW designed the research study. MW, JF and JX analyzed and interpreted the data. RP and XD performed the experiments. JF and JX contributed materials, analysis tools or data. HH and MW wrote the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was ethically approved by the ethics committee of Xiangya Hospital, Central South University [No. 202204065].

Acknowledgment

Not applicable.

Funding

This research was funded by the Hunan Provincial Science and Technology Department [Grant No. 2021JJ31065].

Conflict of Interest

The authors declare no conflict of interest.

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