Identification of potential target genes associated with the effect of propranolol on angiosarcoma via microarray analysis

SHIYONG ZHOU[1*], PENGFEI LIU[1*], WENHUA JIANG[2] and HUILAI ZHANG[1]

[1]Department of Lymphoma, Sino-US Center of Lymphoma and Leukemia,
Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer,
Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin 300060;
[2]Department of Radiotherapy, Second Hospital of Tianjin Medical University, Tianjin 300211, P.R. China

Abstract. The purpose of the present study was to explore the effect of propranolol on angiosarcoma, and the potential target genes involved in the processes of proliferation and differentiation of angiosarcoma tumor cells. The mRNA expression profile (GSE42534) was downloaded from the Gene Expressed Omnibus database, including three samples without propranolol treatment (control), three samples with propranolol treatment for 4 h and three samples with propranolol treatment for 24 h. The differentially expressed genes (DEGs) in angiosarcoma tumor cells with or without propranolol treatment were obtained via the limma package of R and designated DEGs-4 h and DEGs-24 h. The DEGs-24 h group was divided into two sets. Set 1 contained the DEGs also contained in the DEGs-4 h group. Set 2 contained the remainder of the DEGs. Functional and pathway enrichment analysis of sets 1 and 2 was performed. The protein-protein interaction (PPI) networks of sets 1 and 2 were constructed, termed PPI 1 and PPI 2, and visualized using Cytoscape software. Modules of the two PPI networks were analyzed, and their topological structures were simulated using the tYNA platform. A total of 543 and 2,025 DEGs were identified in angiosarcoma tumor cells treated with propranolol for 4 and 24 h, respectively, compared with the control group. A total of 401 DEGs were involved in DEGs-4 h and DEGs-24 h, including metallothionein 1, heme oxygenase 1, WW domain-binding protein 2 and sequestosome 1. Certain significantly enriched gene ontology (GO) terms and pathways of sets 1 and 2 were identified, containing 28 overlapping GO terms. Furthermore, 121 nodes and 700 associated pairs were involved in PPI 1, whereas 1,324 nodes and 11,839 associated pairs were involved in PPI 2. A total of 45 and 593 potential target genes were obtained according to the node degrees of PPI 1 and PPI 2. The results of the present study indicated that a number of potential target genes, including AXL receptor tyrosine kinase, coatomer subunit α, DR1-associated protein 1 and ERBB receptor feedback inhibitor 1 may be involved in the effect of propranolol on angiosarcoma.

Introduction

Angiosarcoma is a rare malignant vascular tumor and is difficult to diagnose and treat (1). It may be characterized by rapidly proliferating and extensively infiltrating anaplastic cells, which are derived from blood vessels, and lining irregular blood-filled spaces (2,3). Angiosarcoma is derived from mesenchymal cells and usually originates from the liver, breast, spleen, bone or heart (2,4-6). Angiosarcoma accounts for between 1 and 2% of all sarcomas, and its overall 5-year survival rate is <20%, owing to the high recurrence and distant metastasis rates (2). In addition, metastasis usually occurs in the liver, lung, bone and lymph nodes (7). Treatment of angiosarcoma is multifaceted and primarily consists of radiotherapy, surgery and chemotherapy (8).

Propranolol is a non-selective β-blocker and may inhibit the growth of angiosarcoma by affecting the proliferation and differentiation of angiosarcoma tumor cells, thus being considered a promising treatment to delay surgery (9-11). However, its underlying molecular mechanisms and pharmacodynamics of the effects on angiosarcoma remain obscure, and the potential target genes involved in the proliferation and differentiation processes of angiosarcoma tumor cells also require investigation.

Gene microarray is widely used as an effective technology to detect the gene expression in cells and tissues at different disease stages of cancer. Thus, it may aid in the identification of novel signaling pathways or molecular mechanisms associated with tumorigenesis.

In the present study, the differentially expressed genes (DEGs) in angiosarcoma tumor cells treated with propranolol compared with the control group were identified via a bioinformatics-based method. Furthermore, enenrichment analysis, protein-protein interaction (PPI) network construction and module analysis were performed. These analyses aided in the identification of essential genes associated with angiosarcoma, such as AXL receptor tyrosine kinase (AXL), coatomer subunit α, DR1-associated protein 1, ERBB receptor feedback inhibitor 1, family with sequence similarity 195 member A, expressed sequence AA467197, apoptosis-associated tyrosine kinase, ATP-binding cassette subfamily A member 7, acyl-CoA dehydrogenase family member 9 and acyl-CoA-binding domain containing 6. Thus, this may contribute to understanding the molecular mechanism underlying angiosarcoma in order to identify potential gene targets for the diagnosis and treatment of patients with angiosarcoma.

Materials and methods

mRNA expression microarray data. The standardized mRNA expression profile GSE42534 (9) was downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/) database, including 3 samples without propranolol treatment (the control group), 3 samples with propranolol treatment for 4 h and 3 samples with propranolol treatment for 24 h.

Identification and grouping of differentially expressed genes. The DEGs in angiosarcoma tumor cells of the propranolol treatment groups compared with the control group were obtained using the limma package of R (http://bioconductor. org/packages/release/bioc/html/limma.html) (12). They were designated DEGs-4 h and DEGs-24 h. The DEGs-24 h were divided into 2 sets. Set 1 contained those DEGs also contained in the DEGs-4 h group. Set 2 contained the remainder of the DEGs. For the sake of accuracy, all DEGs were identified according to the following criteria: $P<0.001$;$|log_2$ (fold-change) $|\geq1$.

Gene ontology (GO) and pathway enrichment analysis. In order to explore the potential biological processes that were altered, GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; david.abcc.ncifcrf.gov/) (13). The GO terms and the KEGG pathways were identified with the criterion $P<0.05$.

Construction of protein-protein interaction (PPI) networks. The two PPI networks for sets 1 and 2 were constructed using the Search Tool for the Retrieval of Interacting Genes/ Proteins (STRING) (14) database, termed PPI 1 and PPI 2, respectively, and visualized using Cytoscape software (version 3.4.0; http://www.cytoscape.org/) (15). STRING, which manipulates the interactions between genes or proteins from multiple sources, was used to identify the interactions of DEGs. A combined score (a representation of reliability of interactions) >0.4 was used as the threshold for the selection of interaction pairs. Modules of the two PPI networks were analyzed using the Multi Contrast Delayed Enhancement

plug-in of Cytoscape (16). When the combined score was >1.5, function enrichment analysis of all enrolled DEGs was performed using DAVID, and the GO terms and the KEGG pathways with $P<0.05$ were identified. Topological structures of the two PPI networks were analyzed using tYNA (tyna.gersteinlab.org/tyna) (17), and potential target genes, whereby the degree of node attributes was ≥10, were identified. Degree represents the number of direct interactions a node has with with other nodes.

Results

Identification of DEGs. A total of 543 DEGs (242 up- and 301 downregulated) and 2,025 DEGs (1,107 up- and 918 downregulated) were identified in angiosarcoma tumor cells treated with propranolol (DEGs-4 h and DEGs-24 h, respectively) compared with the control group. A total of 401 DEGs (set 1) were involved in DEGs-4 h and DEGs-24 h, including metallothionein 1, heme oxygenase 1, WW domain-binding protein 2 and sequestosome 1. Among set 1, 179 DEGs in the DEGs-4 h group were upregulated, of which 170 DEGs were upregulated and 9 DEGs (2410011G03Rik, 2810417H13Rik, ATPase inhibitory factor 1, G2 and S-phase expressed 1, LSM5 homolog U6 small nuclear RNA and mRNA degradation associated, non-SMS condensing I complex subunit H, Rp127, ubiquitin-40A ribosomal protein S27a precursor and zinc finger CCHC-type-containing 8) were downregulated in the DEGs-24 h group. Similarly, 222 DEGs of the DEGs-4 h group were downregulated, of which 196 DEGs were downregulated and 26 DEGs [D730049H07Rik, desert hedgehog, dual-specificity phosphatase 7, endothelin 1, ETS proto-oncogene 1, general receptor for phosphoinositides, mitogen-associated protein kinase 6, midnolin, myeloid-associated differentiation marker, lysophosphatidic acid receptor 6, platelet-derived growth factor subunit A, PDZ and LIM domain (Pdlim) 1, Pdlim7, plexin A2, phosphatidic acid phosphatase type 2B, Ppmlf, regulator of G-protein signaling 16, ras homolog family member B, roundabout guidance receptor 4, sterile α motif domain-containing 4, solute carrier family 2 member 1, solute carrier family 9 isoform A3 regulatory factor 2, tissue inhibitor of metalloproteinase 3, tumor necrosis factor-α-induced protein 2, trophoblast glycoprotein and WNT1-inducible signaling pathway protein 1] were upregulated in the DEGs-24 h group.

Functional and pathway enrichment analysis of sets 1 and 2. The top 20 most significantly enriched GO terms of sets 1 and 2 are presented in Table IA and B, respectively. Among them, 28 terms were coincident (Table IC). The enriched KEGG pathways of sets 1 and 2 are presented in Table IIA and B, respectively.

Construction of the PPI networks for sets 1 and 2 and analysis of modules. The PPI networks of PPI 1 and PPI 2 are presented in Figs. 1 and 2. A total of 121 nodes and 700 associated pairs were involved in PPI 1, whereas 1,324 nodes and 11,839 associated pairs were involved in PPI 2. Fig. 3 and Table IIIA present the module information of PPI 1. Fig. 4 and Table IIIB present the module information of PPI 2.

Table I. Significantly enriched and coincident GO terms in sets 1 and 2.

A, Top 20 most significantly enriched GO terms in set 1

GO ID	GO name	Gene number	P-value
GO:0005730	Nucleolus	23	0.000000006
GO:0016126	Sterol biosynthetic process	8	0.000000580
GO:0031974	Membrane-enclosed lumen	43	0.000000604
GO:0001525	Angiogenesis	12	0.000017900
GO:0070013	Intracellular organelle lumen	38	0.000025500
GO:0043233	Organelle lumen	38	0.000027000
GO:0006694	Steroid biosynthetic process	9	0.000027800
GO:0006695	Cholesterol biosynthetic process	6	0.000037100
GO:0048514	Blood vessel morphogenesis	14	0.000037200
GO:0016125	Sterol metabolic process	9	0.000050400
GO:0001568	Blood vessel development	15	0.000081700
GO:0031981	Nuclear lumen	31	0.000082000
GO:0001944	Vasculature development	15	0.000106000
GO:0005773	Vacuole	13	0.000118000
GO:0008610	Lipid biosynthetic process	16	0.000120000
GO:0005764	Lysosome	12	0.000148000
GO:0000323	Lytic vacuole	12	0.000156000
GO:0043232	Intracellular non-membrane-bounded organelle	51	0.000304000
GO:0043228	Non-membrane-bounded organelle	51	0.000304000
GO:0042127	Regulation of cell proliferation	22	0.000387000

B, Top 20 most significantly enriched GO terms in set 2

GO ID	GO name	Gene number	P-value
GO:0030529	Ribonucleoprotein complex	100	0.000000000000
GO:0005739	Mitochondrion	178	0.000000000000
GO:0005840	Ribosome	52	0.000000000000
GO:0044429	Mitochondrial part	84	0.000000000001
GO:0003735	Structural constituent of ribosome	39	0.000000000002
GO:0043233	Organelle lumen	142	0.000000000003
GO:0031974	Membrane-enclosed lumen	145	0.000000000005
GO:0070013	Intracellular organelle lumen	141	0.000000000006
GO:0043228	Non-membrane-bounded organelle	208	0.000000000014
GO:0043232	Intracellular non-membrane-bounded organelle	208	0.000000000014
GO:0006412	Translation	58	0.000000000047
GO:0031090	Organelle membrane	108	0.000000000050
GO:0005681	Spliceosome	33	0.000000000056
GO:0006396	RNA processing	70	0.000000000140
GO:0008380	RNA splicing	42	0.000000000438
GO:0031967	Organelle envelope	79	0.000000000456
GO:0031975	Envelope	79	0.000000000543
GO:0019866	Organelle inner membrane	54	0.000000001140
GO:0006397	mRNA processing	48	0.000000002120
GO:0016071	mRNA metabolic process	51	0.000000010600

C, Coincident enriched GO terms in sets 1 and 2

GO ID	GO name	GO ID	GO name
GO:0000166	Nucleotide binding	GO:0031981	Nuclear lumen

Table I. Continued.

C, Coincident enriched GO terms in sets 1 and 2

GO ID	GO name	GO ID	GO name
GO:0005730	Nucleolus	GO:0032553	Ribonucleotide binding
GO:0005773	Vacuole	GO:0032555	Purine ribonucleotide binding
GO:0005783	Endoplasmic reticulum	GO:0034404	Nucleobase, nucleoside and nucleotide biosynthetic process
GO:0005829	Cytosol	GO:0034654	Nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process
GO:0006334	Nucleosome assembly	GO:0034728	Nucleosome organization
GO:0006364	rRNA processing	GO:0042254	Ribosome biogenesis
GO:0006396	RNA processing	GO:0043228	Non-membrane-bounded organelle
GO:0009165	Nucleotide biosynthetic process	GO:0043232	Intracellular non-membrane-bounded organelle
GO:0016072	rRNA metabolic process	GO:0043233	Organelle lumen
GO:0017076	Purine nucleotide binding	GO:0044271	Nitrogen compound biosynthetic process
GO:0022613	Ribonucleoprotein complex biogenesis	GO:0046907	Intracellular transport
GO:0030529	Ribonucleoprotein complex	GO:0051726	Regulation of cell cycle
GO:0031974	Membrane-enclosed lumen	GO:0070013	Intracellular organelle lumen

GO, gene ontology; set 1, coincident differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 4 h and treated with propranolol for 4 h compared with treated without propranolol; set 2, differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 24 h compared with treated without propranolol, but not in angiosarcoma tumor cells treated with propranolol for 4 h compared with treated without propranolol.

Table II. Enriched KEGG pathways in sets 1 and 2.

A, Enriched KEGG pathways in set 1

Term	Count	P-value
mmu04115: p53 signaling pathway	9	0.000105
mmu00100: Steroid biosynthesis	5	0.000398
mmu00900: Terpenoid backbone biosynthesis	4	0.003012
mmu04142: Lysosome	9	0.004013
mmu00600: Sphingolipid metabolism	5	0.012340
mmu00240: Pyrimidine metabolism	7	0.017197
mmu00270: Cysteine and methionine metabolism	4	0.033547
mmu00650: Butanoate metabolism	4	0.044913
mmu05214: Glioma	5	0.048883

B, Enriched KEGG pathways in set 2

Term	Count	P-value
mmu03040: Spliceosome	37	0.000000
mmu00190: Oxidative phosphorylation	31	0.000001
mmu03010: Ribosome	22	0.000026
mmu04142: Lysosome	24	0.000291
mmu05211: Renal cell carcinoma	17	0.000350
mmu00480: Glutathione metabolism	13	0.001727
mmu05016: Huntington's disease	28	0.006229
mmu05012: Parkinson's disease	22	0.007000
mmu05222: Small cell lung cancer	16	0.007770
mmu03030: DNA replication	9	0.010537

Table II. Continued.

B, Enriched KEGG pathways in set 2

Term	Count	P-value
mmu04666: Fc gamma R-mediated phagocytosis	17	0.012796
mmu04114: Oocyte meiosis	19	0.013122
mmu03018: RNA degradation	12	0.016095
mmu04110: Cell cycle	20	0.018766
mmu05200: Pathways in cancer	41	0.020303
mmu04662: B cell receptor signaling pathway	14	0.024365
mmu05212: Pancreatic cancer	13	0.024948
mmu00600: Sphingolipid metabolism	9	0.030444
mmu00980: Metabolism of xenobiotics by cytochrome P450	12	0.031062
mmu00330: Arginine and proline metabolism	10	0.043757
mmu00860: Porphyrin and chlorophyll metabolism	7	0.046693
mmu00511: Other glycan degradation	5	0.048603
mmu04062: Chemokine signaling pathway	24	0.056122
mmu05010: Alzheimer's disease	24	0.056122
mmu05215: Prostate cancer	14	0.056323
mmu04620: Toll-like receptor signaling pathway	15	0.056726
mmu03410: Base excision repair	8	0.061961
mmu00230: Purine metabolism	21	0.066837
mmu00982: Drug metabolism	12	0.068874
mmu04920: Adipocytokine signaling pathway	11	0.073182
mmu04810: Regulation of actin cytoskeleton	27	0.075019

KEGG, Kyoto Encyclopedia of Genes and Genomes; set 1, coincident differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 4 h and treated with propranolol for 4 h compared with treated without propranolol; set 2, differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 24 h compared with treated without propranolol, but not in angiosarcoma tumor cells treated with propranolol for 4 h compared with treated without propranolol; DEGs, differentially expressed genes.
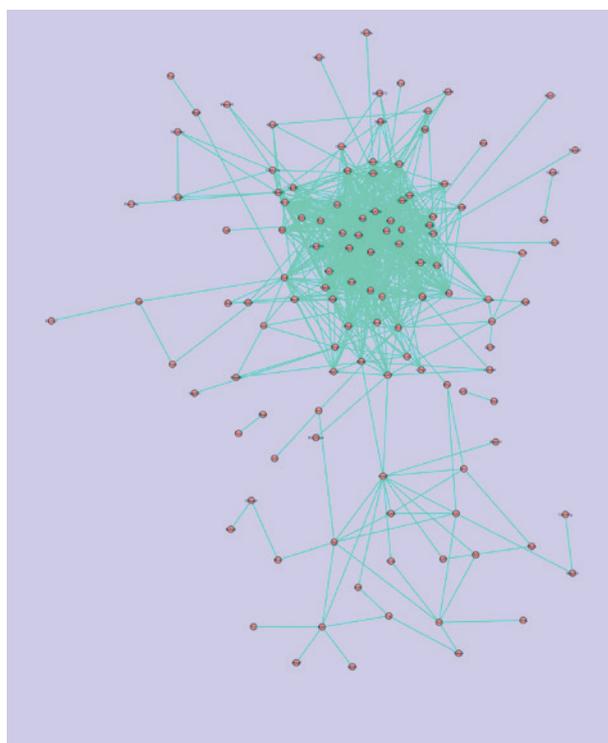

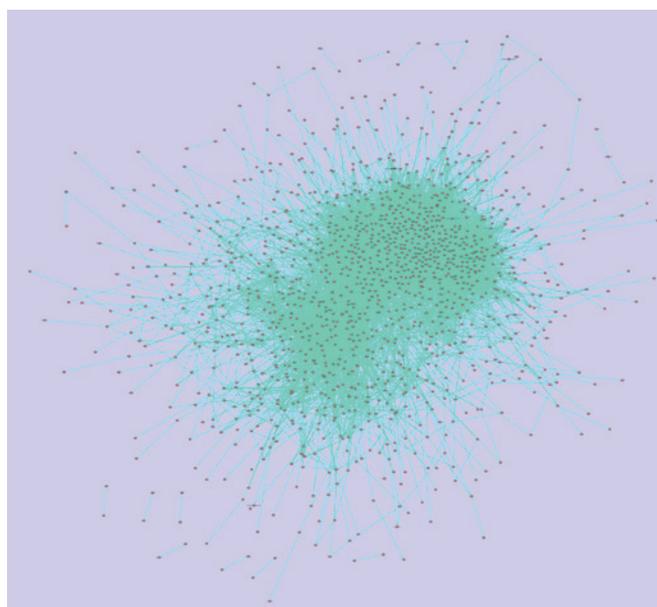Figure 1. Protein-protein interaction network of set 1.

Figure 2. Protein-protein interaction network of set 2.

Extent of enriched function and topological structure analysis of the PPI networks. There were 20 GO terms (including
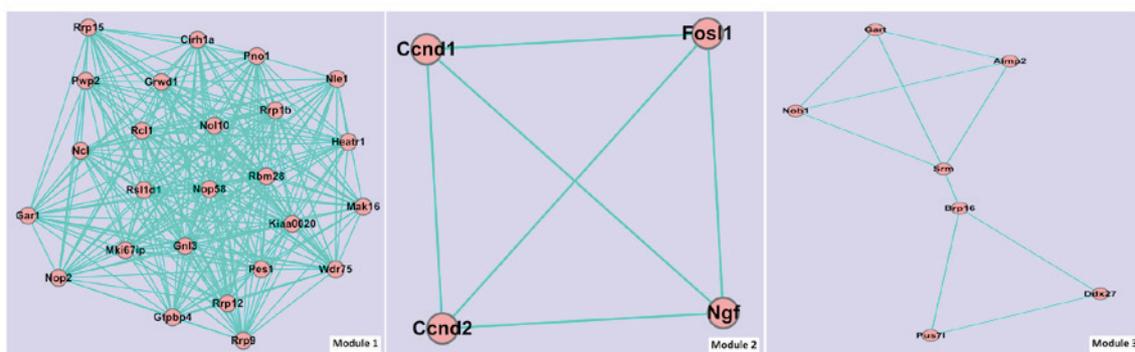
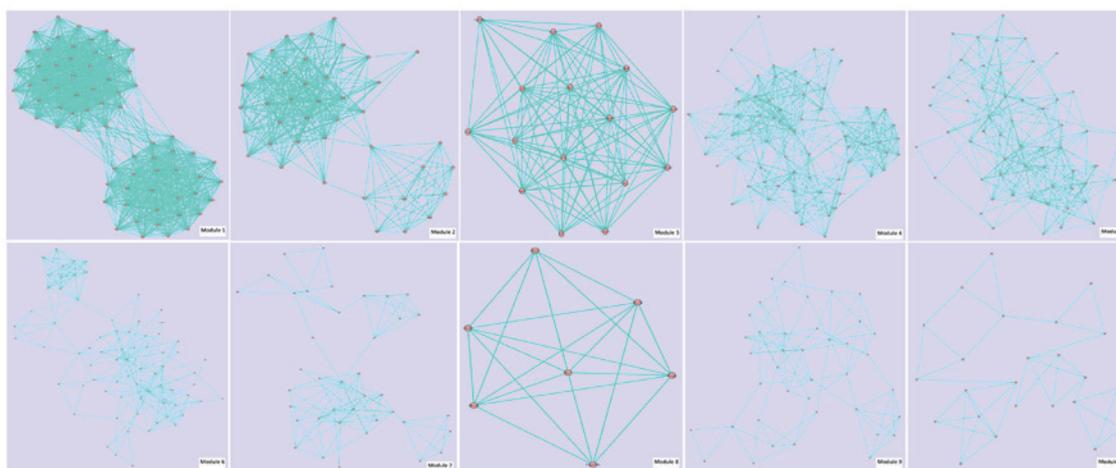Figure 3. Module information of protein-protein interaction set 1.

Figure 4. Module information of protein-protein interaction 2.

nucleolus, intracellular organelle lumen, membrane-enclosed lumen, ribosome biogenesis and RNA processing) and no KEGG pathways enriched in module 1 of PPI 1. The numbers of the enriched module functions of PPI 2 are presented in Table IV. The results identified that no enriched KEGG pathways appeared in modules 1 and 9 of PPI 2. A total of 45 and 593 potential target genes were obtained according to the node degrees of PPI 1 and PPI 2, and the top 10 nodes (potential target genes) which were associated with the other nodes in the PPI networks for sets 1 and 2 are presented in Table VA and VB, respectively.

Discussion

Numerous studies have demonstrated the selective cytotoxicity and relative safety of propranolol on vascular tumors, and laid the groundwork for the notable efficacy and the suppressive ability of propranolol on angiosarcoma (9-11,18-20). In the present study, it was found that the number of DEGs-24 h was higher compared with the number of DEGs-4 h. In addition, nearly all of the DEGs-4 h overlapped with and were contained in the DEGs-24 h group. Furthermore, differential expression (upregulated or downregulated) of DEGs-24 h was more evident compared with DEGs-4 h. This indicated that the 401 overlapping DEGs in set 1 were important in the effects of propranolol on angiosarcoma tumor cells. Notably,

9 upregulated DEGs of the DEGs-4 h group were downregulated in the DEGs-24 h group, whereas 26 downregulated DEGs of the DEGs-4 h group were upregulated in the DEGs-24 h group. It was possible that these genes perform multiple roles in the effect of propranolol on angiosarcoma; however, this conjecture requires additional experimental verification.

The enriched GO terms of set 1 primarily contained 'angiogenesis, blood vessel morphogenesis, vasculature development', 'sterol biosynthetic process, cholesterol biosynthetic process, lipid biosynthetic process', 'lysosome, lytic vacuole, vacuole', and 'nucleolus, intracellular non-membrane-bounded organelle, regulation of cell proliferation'. It is well known that lipid metabolism may affect the development of blood vessels (21-23) and various organelles involved in various biological processes (23,24). Cell proliferation is an essential process in the development of blood vessels (25). According to Table IA, the majority of enriched GO terms of set 1 were associated with the biological processes of blood vessels, whereas the enriched GO terms of set 2 were primarily associated with energy metabolism (including ribosome, structural constituent of ribosome), protein transfer (including ribonucleoprotein complex, ribosome, membrane-enclosed lumen) and compounds biosynthesis (including RNA processing, mRNA metabolic process and envelope). The overlapping enriched GO terms of sets 1 and 2 were primarily involved in nucleic acid metabolism, nucleotide biosynthesis and nucleic

Table III. Module information of the protein-protein interaction networks for sets 1 and 2.

A, Module information of the protein-protein interaction network of set 1

Module ID	Score	Gene number	Edge number
1	10.4	25	260
2	1.5	4	6
3	1.5	7	10

B, Module information of the protein-protein interaction network of set 2

Module ID	Score	Gene number	Edge number
1	17.1	70	1195
2	9.4	41	387
3	6.8	16	109
4	6.4	70	446
5	5.2	56	292
6	3.6	74	267
7	2.9	39	113
8	2.9	7	20
9	2.5	40	99
10	1.7	24	41

Set 1, coincident differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 4 h and treated with propranolol for 4 h compared with treated without propranolol; set 2, differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 24 h compared with treated without propranolol, but not in angiosarcoma tumor cells treated with propranolol for 4 h compared with treated without propranolol.

Table IV. Enriched function numbers of modules of the protein-protein interaction network of set 2.

Modules	Enriched GO term numbers	Enriched KEGG pathway number
Module 1	0	0
Module 2	47	1
Module 3	10	1
Module 4	122	6
Module 5	62	5
Module 6	90	11
Module 7	105	15
Module 8	12	1
Module 9	154	0
Module 10	22	6

Set 2, differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 24 h compared with treated without propranolol, but not in angiosarcoma tumor cells treated with propranolol for 4 h compared with treated without propranolol; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table V. Top 10 nodes most significantly associated with other nodes in the protein-protein interaction network of sets 1 and 2.

A, Top 10 nodes most significantly associated with other nodes in the protein-protein interaction network of set 1

Gene symbol	Degree	Clustering coefficient	Eccentricity	Betweenness centrality
AXL	45	0	6	0
COPA	44	0	7	0
DRAP1	44	0	2	0
ERRFI1	41	0	2	0
FAM195A	38	0	8	0
FAM98A	36	0	8	0
FASTKD5	36	0	8	0
FEZ2	33	0	2	0
FST	33	0	6	0
GADD45G	33	0	7	0

B, Top 10 nodes most significantly associated with other nodes in the protein-protein interaction network of set 2

Gene symbol	Degree	Clustering coefficient	Eccentricity	Betweenness centrality
AA467197	184	0	2	0
AATK	120	0	8	0
ABCA7	120	0	7	0
ACAD9	116	0	2	0
ACBD6	113	0	8	0
ACSL3	111	0	9	0
AFG3L1	109	0	7	0
AGAP1	109	0	8	0
AHNAK	106	0	9	0
ANGEL2	106	0	7	0

Set 1, coincident differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 4 h and treated with propranolol for 4 h compared with treated without propranolol; set 2, differentially expressed genes in angiosarcoma tumor cells treated with propranolol for 24 h compared with treated without propranolol, but not in angiosarcoma tumor cells treated with propranolol for 4 h compared with treated without propranolol.

acid binding. Therefore, it was concluded that propranolol affected angiosarcoma primarily by influencing the biological processes of blood vessels in the early stage and by effecting the biological metabolism and transfer processes in the later stage. The enriched KEGG pathways of set 1 were tumor-associated biological processes, including the p53 signaling pathway and cysteine and methionine metabolism. In the later stage, the enriched KEGG pathways were more extensive, including the ribosome signaling pathway, lysosome signaling pathway, Huntington's disease and Parkinson's disease.

According to the topological structure analysis of the PPI networks, certain potential biomarkers were identified,

including AXL, coatomer subunit α, DR1-associated protein 1, ERBB receptor feedback inhibitor 1, family with sequence similarity 195 member A, expressed sequence AA467197, apoptosis-associated tyrosine kinase, ATP-binding cassette subfamily A member 7, acyl-CoA dehydrogenase family member 9 and acyl-CoA-binding domain containing 6. According to Table VA, *AXL* was the most significantly meaningful gene in the early stage. *AXL* is a member of the tyrosine kinase receptor family and is associated with cell adhesion and recognition, cell proliferation, apoptosis, blood coagulation and inflammation (26). It performs important roles in the occurrence and development of various tumors, including the inhibition of tumor cell apoptosis, the involvement in tumor angiogenesis and cellular invasion (27-30). Following its original identification, the upregulation of *Axl* has been reported in a variety of hematopoietic tumors, including leukemia and melanoma (31-35). Furthermore, previous studies have demonstrated that *Axl* may also perform a role in a number of chemotherapy-resistant cancers (36,37). In the present study, it was proposed that *Axl* may be a potential target in the early stage of angiosarcoma treated with propranolol. This discovery may indicate an important direction for future studies. Similarly, *AA467197* may be a potential biomarker in the late stage of angiosarcoma treated with propranolol. It is a key point of the effects of propranolol on angiosarcoma to identify and develop small-molecule drugs with the potential to selectively inhibit *Axl* and *AA467197* expression and their signaling pathways.

Acknowledgements

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