Translational Oncology 19 (2022) 101385

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original Research

Differences in potential key genes and pathways between primary and radiation-associated angiosarcoma of the breast

Yuanfeng Wei [a], Xi Yang [a], Limin Gao [b], Yong Xu [a,*], Cheng Yi [a,*]

[a] Department of Medical Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, China
[b] Department of Pathology, West China Hospital, Sichuan University, Chengdu 610041, China

ARTICLE INFO

Keywords:
Radiation-associated angiosarcoma of the breast
Primary breast angiosarcomas
Differentially expressed genes
Molecular targeted therapy

ABSTRACT

Background: Angiosarcoma of the breast is a high-grade malignant soft tissue tumor, it can be divided into primary and radiation-associated angiosarcoma(secondary). However, the differences between primary and secondary angiosarcomas in terms of pathogenesis, clinical behavior, early diagnosis biomarkers, genetic abnormalities, and therapeutic targets remain to be fully elucidated. At the same time, due to its rarity, most of current information relating to angiosarcoma is provided by case reports. Therefore, exploring the mechanisms of primary and secondary breast angiosarcoma have important value for the discovery of new biomarkers and research into potential therapeutic targets.
Methods: The differentially expressed genes (DEGs) between 36 cases of primary angiosarcoma and 54 cases of secondary angiosarcoma were screened. Then, the DEGs were used to gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Then, a protein-protein interaction (PPI) network was constructed using the STRING database.
Results: A total of 18 DEGs were identified, of which 13 were upregulated and 5 were downregulated in secondary breast angiosarcoma. The GO enrichment analysis showed that the DEGs were most enriched in metabolism, energy pathways, and protein metabolism in biological processes. The enriched signaling pathways of DEGs were the transforming growth factor-β (TGF-β), Wnt, Hippo and PI3K-Akt signaling pathways. Then, the PPI network was conducted and hub genes were identified and they were involved in thyroid hormone, Hippo and other signaling pathways.
Conclusion: This study lay the foundation for the discovery of effective and reliable molecular biomarkers and essential therapeutic targets for these malignancies.

Background

Angiosarcoma is a high-grade malignant soft tissue tumor, originating from lymphatic or vascular endothelial cells, which exhibit rapid proliferation and invasion capacity that is associated with a poor prognosis [1,2]. Angiosarcoma may arise in any location of the body, such as bone, liver, heart or breast; it occurs most frequently in the skin and soft tissues [3]. When it occurs in the breast of younger women with no previous cancer history or any associated factor that is called primary breast angiosarcoma. It is most frequent in women between age 20 to 50 and usually present as a lump that appears in the parenchymal tissue of the breast without any changes in the skin [4]. Although the mechanisms underlying angiosarcoma remain to be fully clarified, recent studies have highlighted some definite risk factors, including UV irradiation, chronic lymphoedema, occupational exposure to vinyl chloride, and certain familial syndromes [5], as well as a history of radiotherapy, which is one of the most important factors. Breast cancer is one of most common malignancies and the second leading cause of cancer-related death in women [6,7]. With the rapid improvement of medical and health care, most breast cancer patients are diagnosed at an early stage. In the last few decades, breast conserving surgery combined with whole-breast radiotherapy (WBRT) has become the gold standard treatment for breast cancer [8–12]. However, radiation-associated angiosarcoma of the breast (secondary breast angiosarcoma) is a very serious complication of radiation exposure and occurs mainly in elderly women after a median period of 4–8 years post-radiotherapy [13]. Secondary breast angiosarcoma often occurs in the irradiated area after breast-conserving treatment [14]. It is an extremely rare malignant

* Corresponding authors.
E-mail addresses: xy868996@163.com (Y. Xu), yicheng6834@126.com (C. Yi).

https://doi.org/10.1016/j.tranon.2022.101385
Received 21 February 2022; Accepted 22 February 2022

Table 1
The differentially-expressed genes (DEGs) between secondary and primary AS in GSE52664 datasets.

DEGs	gene symbol					
Up-regulated genes	UNC5A	DPRX	HPDL	CTLA4	ISLR2	MYC
	ZSCAN10	KCNF1	COL22A1	PROM1	SCN9A	RET
	ICOS	CMBL	SCN11A	TCF15	RAB17	TCFL5
	GJC2	IQCA1	SULT1C4	PRR17	MEOX2	TRPM6
	KBTBD11	CA4	GCOM1	KCNIP1	TUSC3	WASF3
	RELN	N4BP3	CHSY3	PREX2	DPY19L2	HOXA9
	KIT	NLE1	DAND5	BCHE	RASGRP3	PGM5
	DNAH2	TRPC6	FLT4	MAF	SCHIP1	STK32B
	CDCA7L	LRFN4	CETP	MAST4	MYO5C	VWCE
Down-regulated genes	NTSR1	ANKRD1	P2RX6	NDP	TUBB4	NRCAM
	TGM2	NRGN	EFEMP1	ITPKA	APCDD1L	SPP1
	HS3ST1	ZNF667	PLCD3	NRIP3	NNMT	ODZ3
	PLAT	PMP22	LRP11	GK	NAGS	BNC2
	SORL1	MRAS	CDH15	TNFRSF12A	TYROBP	IER3
	APLP1	PPDPF	LXN	SERPINE1	SLC6A6	BAMBI
	PLEKHG5	SLC41A2	MT2A	ITGAX	SERPINB8	ALCAM
	ITGA3	IL17RA	CDKN2C	GPR153	DPYD	DST
	PIM1					

tumor, and its incidence is less than 1% of all soft tissue sarcomas [15]. Like primary breast angiosarcoma, secondary breast angiosarcoma also has a worse prognosis than breast cancer.

Although primary and secondary breast angiosarcomas share some similarities, such as the first symptom is the appearance of lumps in the breast [16,17], and have similar morphology [18], these entities are clinically and histologically different. Primary breast angiosarcoma is also very rare, accounting for 0.04% of malignant breast cancers and 8% of breast sarcomas. It usually occurs in the parenchyma of unirradiated breast and may or may not spread to the skin and subcutaneous tissue. It is manifested as a painless diffuse enlargement of the mass or no mass, and the median age of onset is approximately 40 years old [19]. In contrast, secondary breast angiosarcoma usually originates from the dermis and subcutaneous tissue of the irradiated breast and presents with ecchymosis, erythema, pruritus, skin thickening, or some combination of these features. Because secondary breast angiosarcoma is manifested clinically as ecchymosis or an area of thickened skin, features that are very similar to bruises, this malignancy is difficult to distinguish and the diagnosis is usually delayed [20,21]. Moreover, some studies have revealed differences in the pathogenesis and mechanism of development between primary and secondary angiosarcomas. The progression of primary angiosarcoma may be related to mesenchymal stem cells or progenitor cells and can therefore occur anywhere in the body. In contrast, secondary angiosarcoma develops due to external damage, and is mainly limited to the damaged area [22]. Furthermore, molecular studies have shown that MYC and KDR were significantly upregulated in secondary angiosarcomas compared to primary angiosarcomas [23]. However, the exact differences between primary and secondary angiosarcomas in terms of pathogenesis, clinical behavior, early diagnosis biomarkers, genetic abnormalities, and therapeutic targets remain to be fully elucidated. Due to its rarity, most of the current information relating to angiosarcoma is provided by case reports and single-institution retrospective cohort studies and the research with large-scale genomic studies published to date are very limited. Meanwhile, a growing number of studies found that a variety of signaling pathways were involved in the development of angiosarcoma. However, few studies have addressed the differences among the signaling pathways involved in primary and secondary angiosarcomas, which have limited the diagnosis and treatment of these two types of angiosarcoma. Therefore, identification of new biomarkers and therapeutic targets is important for improving the diagnosis and treatment of primary and secondary breast angiosarcomas.

In this study, we aimed to identify novel biomarkers, pathways, and potential therapeutic targets for primary and secondary breast angiosarcomas to facilitate future research. We downloaded the GSE52664 and GSE49790 datasets from the gene expression omnibus (GEO) database and identified the differentially expressed genes (DEGs) between primary and second breast angiosarcomas. Then, other approaches including gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and protein-protein interaction (PPI) network construction were performed to predict the potential regulatory mechanisms.

Materials and methods

Data collection

Two datasets (GSE52664 and GSE49790) were retrieved from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). The GSE52664 dataset consisted of 26 primary breast angiosarcomas and 29 secondary breast angiosarcomas, all of which developed following radiotherapy for primary breast cancer. The GSE49790 dataset consisted of 10 primary breast angiosarcomas and 25 secondary breast angiosarcomas. The two datasets have the same characteristics.

Data processing and DEG analysis

We screened the DEGs between primary and secondary breast angiosarcomas based on the following criteria: In the GSE49790 database, the criteria for DEGs were fold change (FC) >2.0 and the P-value $<$ 0.05. In the GSE52664 database, a false discovery rate (FDR) of 0% and a fold change (FC) of minimum 2.0 were considered statistically significant. VENNY (Version 2.1.0) was used to identify the overlapping DEGs in the two datasets.

Functional enrichment analyses

To analyze the function, the overlapping DEGs were subjected to GO and KEGG pathway analysis using the Functional Enrichment analysis tool (FunRich3.1.3) [24] and KEGG Orthology Based Annotation System (KOBAS) 3.0(kobas.cbi.pku.edu.cn/) [25], respectively. $P < 0.05$ was considered to indicate statistical significance. The top GO and KEGG pathway terms were depicted using the ggplot2 (version 3.1.1) package in R.

PPI network construction

The PPI network was predicted by the STRING database (version 11.0; http://www.string-db.org) to explore the functions of the overlapping DEGs. No more than 20 interactions were shown and the minimum required interaction score was 0.4 (medium confidence). The disconnected nodes in the identified network were hidden.

Table 2
The up-/down-regulated differentially-expressed genes (DEGs) between secondary and primary AS in GSE49790 datasets.

DEGs	gene symbol					
Up-regulated genes	PROX1	GPR1	PTX3	CTLA4	EYA1	MMP12
	CLDN10	SCN3A	GCOM1	MFNG	GNG4	RELN
	SCN3B	UCHL1	IQCA1	EFNA5	ISLR2	TBX1
	PDPN	TUBA4A	CXADRP3	MYC	CPNE7	HIST1H3J
	FABP5	DSP	PCLO	KIAA0114	SNX10	CHRNA5
	POTEE	DCHS2	IGF2BP1	EGLN3	PRKCQ	HOXD10
	PRSS21	ROPN1L	SNHG8	GAL	IL12RB2	UNC5A
	CHRNA1	SLC7A1	ALPK3	SEMA3A	SLC17A9	RPP25
	WASF3	C6orf141	ICOS	SEMA3D	PRKCZ	LRP8
	POTEF	ZSWIM5	PCBD1	GRAP	PLK1	PGM5P2
	YDJC	MAST1	CENPF	CETP	TSTA3	CD5L
	SFXN1	POLR3G	BCAT1	DNAJC12	PGM5	ODC1
	C1QBP	SLC19A1	KIAA1409	CBS	SMYD2	PVT1
	C12orf24	SNCAIP	EPB41	PTTG1	TRIP13	C10orf10
	CKS2	PRKAA2	MIR17HG	LOX	MYO7A	CENPV
	CDCA7L	HIST1H2BO	RAC3	SLC26A4	RBM38	CDCA7
	HIST1H2AJ	HIST1H4D	TERC	HIST1H4C	TAF4B	HIST1H2BG
	HIST1H2AE	CCNB1	PRDM8	TOP2A	FAM83D	UHRF1
	HIST1H2BM	PRR11	GRAPL	FSD1	ABCC4	ZNF556
	DHCR7	HIST1H2AL	CCNA2	CLDN5	GPR97	MAP4K2
	LEPR	NT5DC3	NOP16	NLN	HIST1H2BL	AKAP12
	KHK	TSPAN11	NOV	SGSM1	SLC7A5	SCML2
	TRIB3	LOXHD1	BIRC5	TMEM97	PCSK6	CCT3
	CMBL	TTLL12	S100A4	RPS6KA2	KRT18	CYTL1
	FLNC	HCP5	LRRC34	HIST1H3I	UBE2C	ABCA3
	NEK2	MPP6	TXN	SRPK1	HIST1H3F	HIST1H2BI
	XRCC2	NOS2	HIST1H3H	GPRIN3	ZDBF2	TRAP1
	BUB1B	SLC6A17	MCM10	IPCEF1	KIF20A	PAICS
	RASGRF2	EMID1	AHCY	SNHG1	CLIC2	SLC4A11
	STXBP6	IL7	ASPM	HIST1H4L	STON2	PNP
	SCN5A	TSPO	SLC2A1	MRC1	HMGA1	CDC20
	CCDC86	GCNT2	CABC1	MYBL2	POTEKP	ABCA4
Down-regulated genes	LXN	APLNR	F2RL2	TMEM150C	FOS	IGF2
	TNFAIP6	SNORD114	C8orf4	CHIC1	IGFBP3	VCAN
	LRRC17	TRO	PBX1	NRXN3	C3	PDE5A
	NCOA2	GPX8	GUCY1B3	NOSTRIN	ANO1	NR4A1
	APOD	SEMA6D	SVEP1	TSC22D3	EDIL3	LRRN3
	ECM2	EBF2	IL1R1	SPG20	FBN1	PDE3A
	PKD2	PLXDC2	PHGDH	COL15A1	HEY2	RBMS3
	PCDHB5	ADAMTS9	ARHGAP28	SERPINF1	COL12A1	TSHZ2
	GLI3	RUNX1	SPAG16	ENPP1	PRKXP1	MIPOL1
	BEX4	KCNE4	PCDH17	CTGF	PTGIS	ZNF135
	GLIPR1	MAMDC2	TGM2	PRKD1	DDX26B	PCDH7
	ERRFI1	MEIS3P1	KCNMB4	CYR61	C10orf72	PCOLCE
	FAM184A	SGCD	DLC1	VGLL3	FGD1	SELENBP1
	HOXB2	SRR	ABCC9	CHST11	PSD3	ZNF793
	NR4A2	BAMBI	SMARCA1	OPHN1	KAL1	CAMK2D
	PLEKHA5	ANXA1	SLC16A4	COL6A2	EPB41L3	GALC
	ZNF334	MAFB	ZNF347	FAS	GSN	RASGEF1B
	TBX3	PI15	JAM2	GUCY1A3	SFRP2	LPHN2
	CYYR1	ASPN	LDHB	STEAP4	MXRA5	TBX15
	CCDC80	CTSO	ISLR	HMCN1	EGR2	PRSS35
	OLFML1	BEND7	FOSB	OLFML3	PAPSS2	AKT3
	EGR1	CX3CR1	ZNF382	DDAH1	PALMD	PTGS2
	PALLD	OLFM2	LRFN5	ZNF462	LOXL2	ANGPTL1
	RERG	EFHD1	CYBRD1	SPON1	COL5A2	PLEKHH2
	NEO1	LAMA2	PRICKLE1	SNX7	ACTA2	HTRA1
	CRISPLD1	ZNF320	GALNTL1	PDE7B	ZNF528	NR4A3
	ZNF660	C2orf43	MYEF2	TPM1	CCDC36	NRP1
	HEYL	SULF1	NOTCH3	CDH2	SSPN	TMEM47
	ZC4H2	C7orf58	PRICKLE2	KCNJ2	KIAA1598	ELOVL2
	EPB41L4A	EMCN	SPARCL1	P2RY14	FSTL1	ATF3
	FGF7	ADAMTS1	ATL1	METTL7A	CD44	ADCY1
	SGCB	JUN	LARP6	DPT	DCBLD2	FBLN2
	TMEM30B	FAM66C	FAM13C	PCNXL2	RNF144B	SORT1
	THBD	PTH2R	FBLN7	TWSG1	CCDC7	LHX6
	DCN	CDH11	OGN	DACH1	SERPINE2	GEM
	GSTM5	NTRK2	MEG3	GATA6	CFH	CPE
	TSHZ3	SH3BGRL	BMPR1A	ADAM12	TSPYL5	FBLN5
	SESN3	IGFBP5	DACT1	PRRX1	THBS4	ENTPD1
	SERPINE1	COL1A2	EMG1	EFNB1	CDK14	SEMA5A
	CLMN	LOXL1	SYTL2	PCDH1	FMO1	AASS
	VSIG2	LPAR4	ZNF238	CORO2B	GUCY1A2	RNF180
	MAGED2	JPH1	GSTM1	MGP	PDGFRB	COL5A1

(*continued on next page*)

Table 2 (*continued*)

DEGs	gene symbol					
	ZEB2	Sep-04	FAM102B	OXCT1	FAT1	ZNF808
	THBS1	ANKS1B	LRP12	CYP4V2	PLXDC1	TGFB3
	EGR3	CROT	FAM26E	FXYD6	CNN1	PER3
	SCD5	SHOX2	PRR24	ALDH1L2	CCND2	PRKACB
	C11orf41	CTBS	PDE9A	KITLG	IFIT5	GABRE
	TBX18	ZNF429	GPR56	CDR1	SRPX2	HEPH
	PMEPA1	FAM59A	EFEMP2	BNC2	ZNF454	C18orf15
	RAPH1	HNMT	DISP1	C1orf21	C1orf51	LPAR6
	KIF13A	GFPT2	FRRS1	TMEM98	SPRY2	ZCCHC24
	EDNRA	RFTN2	FRZB	LPL	DCLK1	FGF2
	PTPRG	CAMK2N1	SYCP2	NEDD9	CRISPLD2	FNDC1
	DDR2	ITGA11	ADAMTSL3	RGS5	GTDC1	TAGLN
	THBS2	FMOD	BICC1	PYGO1	SLC16A2	NCALD
	ZNF880	EPS8	ENPEP	TNC	COL6A3	PODN
	COL1A1	SAMD9L	ADAMTS12	ANTXR1	TMEM119	PCDH18
	RGS4	TMCC3	FUT8	OSMR	CRYAB	OLFML2B
	CTTNBP2	RCAN2	TMTC1	ITM2A	STEAP2	MYOF
	EFHA2	ZFP36	CFHR1	SCN4B	IL13RA1	RASD2
	MSC	SDC2	PRSS23	MDFIC	RARB	NDRG2
	RUNX1T1	SMOC2	SLC26A10	TLR7	PTPN13	BHLHE40
	ABCG2	LEPREL1	RGS2	GPX7	MXRA8	KCNJ8
	DAAM2	NOTCH2	CHST1	CDH6	ARHGAP24	SMAD7
	DECR1	PDE10A	ZNF154	ZNF677	ACAA2	XAF1
	MAP1A	CHN2	LRRC3B	RNF135	IL34	ZNF641
	MRVI1	CYB5R2	IFITM1	DENND1B	ERV3	SPON2
	CC2D2B	TGFBR1	WISP1	ZHX2	FAM180A	TES
	MFGE8	FARP1	CD248	SRPX	NXT2	AADAT
	WNK3	DMD	CCDC146	AQP1	SLC25A27	PURA
	SPATA6	CYGB	IFI27L2	IFI44	PPFIBP2	TCEAL8
	EGFR	ARHGAP10	POPDC2	GRIK3	EHD3	ZNF585B
	PABPC4L	COL6A1	KCNIP3	STAT6	FOLH1B	TPST1
	ACPL2	ICAM1	NIPSNAP3B	DGKI	CRABP2	FRK
	SOCS5	ASPA	POSTN	GSTM2	NHS	ASAH1
	AXL	C18orf1	ABCA6	RGMA	LRRC32	BGN
	MOCS1	CRYBG3	ZNF415	ZNF676	MAGEH1	SLC30A4
	CAP2	DUSP1	MCC	PFN2	THY1	PCDHB18
	ZNF655	PCDHB3	ITPR1	SPATS2L	FAM82A1	ACSS3
	ZNF711	TTC39B	SLC44A1	LCA5	EGFLAM	ABI2
	RCBTB2	ZNF229	GNG7	GSTM4	PDZRN3	FAP
	PPM1K	KCNT2	EPAS1	BST2	ZNF558	TPPP3
	GPR34	PTGR1	CMAH	CD14	LEF1	GBP3
	GCA	GTF2A1L	DSC2	DNM3	PTGFRN	ITIH5
	CTSK	GHR	SPTLC3	PLAC9	FAM198B	CX3CL1
	SOX5	CREB3L1	SAMD9	VEGFC	NEXN	MORC4
	CLEC14A	ARHGAP32	TMEM133	CYP7B1	ERP27	PDK3
	CELF2	ARHGEF9	ATG4A	PTPLAD2	CA8	BMP4
	LRP1	FOLH1	C11orf63	SCARA3	C3orf59	ZBTB20
	TRPC1	PLVAP	HAS2	ADAM22	MYL9	ACVR2A
	TWIST1	EBF1	PLSCR4	SNAP25	IL1B	SLFN12
	C14orf144	PTGDR	C9orf150	SLC8A1	SERTAD4	VCAM1
	COL3A1	XYLT1	SNX19	AFAP1L2	LPAR1	BOC
	RNF152	ITGB3	ARSD	PCSK5	PCDHB10	CFI
	RASSF8	MMP16	CAPN11	PARM1	GULP1	VSIG4
	SORBS1	SHROOM4	CYTH3	PDZD2	PFKFB2	ZNF471
	MAMLD1	DNM1	TIMP4	EAF2	PLCL1	ZNF83
	SOBP	F3	CD200R1	PVRL3	KCNB1	ZNF436
	NFASC	JDP2	TRIM22	RNASEL	CYP2U1	PID1
	NOVA1	LIN7A	DNAJB5	HPSE2	TSPAN6	AFF2
	AEBP1	CACNA1D				

Subsequently, Cytoscape software (version 3.7.1) was used to visualize the PPI network. The Molecular Complex Detection (MCODE) application in Cytoscape was employed to select significant modules using the default parameters. CytoHubba was used to calculate the degree of each protein node and the top 10 genes were described as hub genes. Then, FunRich3.1.3 and KOBAS were used to conduct functional enrichment analyses of the hub genes in the modules.

Results

Identification of DEGs in the expression profiles

Compared to primary angiosarcoma, we screened 103 DEGs in secondary angiosarcoma, including 54 up-regulated and 49 down-regulated genes in the GSE52664 dataset (Table 1). For the GSE49790 dataset, a total of 794 DEGs were identified (192 upregulated and 602 downregulated) in secondary breast angiosarcomas compared to the primary breast angiosarcomas (Table 2). As shown in the Venn diagram (Fig. 1), 18 overlapping DEGs (5 downregulated and 13 upregulated) were identified among the two datasets (Table 3).

GO and KEGG pathway analysis of the overlapping DEGs

GO and KEGG pathway analysis were performed to gain a more in-depth understanding of the overlapping DEGs. Metabolism, energy pathways, and protein metabolism were dramatically enriched in

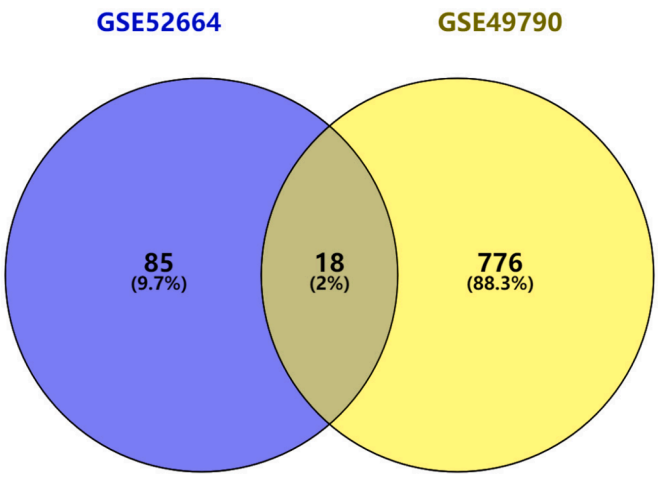

Fig. 1. Identification of overlapping DEGs in GSE52664 and GSE49790.

biological processes (BP) (Fig. 2). Perinuclear region of the cytoplasm, nucleolus and exosomes were mainly enriched in cellular component (CC) (Fig. 3). Transcription factor activity, serine-type peptidase activity and cytoskeletal protein binding were mainly enriched in molecular function (MF) (Fig. 4). In addition, the most enriched KEGG pathways were the TGF-β, T cell receptor, Wnt, Hippo and PI3K-Akt signaling pathways (Fig. 5).

PPI network and sub-modules analysis

STRING was used to construct the PPI network to further explore the underlying associations between the DEGs (Fig. 6A). The top two modules were identified with the MCODE application (Fig. 6B,C). Module 1 consisted of 15 nodes and 36 edges, with a score of 5.143. Module 2 consisted of four nodes and six edges. CytoHubba was used to screen out the top 10 genes as hub genes (Fig. 6D,E). MYC was identified as the most outstanding gene. Details of the top 10 hub genes are shown in Table 4. GO and KEGG pathway enrichment analysis were then conducted to investigate the functional associations of the top 10 hub genes. The hub genes were mainly related to protein metabolism and regulation of gene expression, epigenetic in biological process. Cellular component (CC) analysis showed that the hub genes were mainly enriched in transcription factor TFTC complex, STAGA complex, cell surface, and extracellular (Fig. 7). In addition, the most enriched KEGG pathways were complement and coagulation cascades, thyroid hormone, microRNAs in cancer and Hippo signaling pathways (Fig. 8).

Discussion

Angiosarcoma is a highly malignant soft tissue sarcoma that originates from vascular or lymphatic endothelial cells, accounting for about 1%-2% of soft tissue sarcomas. It occurs mainly in the head, face, neck and other parts, and its occurrence is related to chronic lymphedema and radiation [26,27]. Patients diagnosed with angiosarcoma lesions have poor survival, with 5-year disease-free survival and 5-year survival rates of <50% and <35%, respectively [28]. Studies have shown that more than half of patients with angiosarcoma have high prevalence of recurrence and distant metastases, and eventually die of the malignant tumor [29].

Angiosarcoma of the breast can be divided into primary and secondary malignancies. Primary angiosarcoma of the breast is a rare malignant tumor that occurs without a history of cancer or identifiable risk factors and usually occurs in women between the ages of 30 and 50 years [30]. Recently, multiple reports highlighted the increasing incidence in secondary breast angiosarcoma with the rise in the number of women with breast cancer treated with breast conservation therapy (BCT) and postoperative radiotherapy [22,30,31]. However, most current studies on primary and secondary angiosarcomas are case reports and little is known about the genetic abnormalities due to the lack of large-scale genomic studies, which seriously hinders the progress of diagnosis and treatment of angiosarcoma. Therefore, there is an urgent need to find effective biomarkers and therapeutic targets for early diagnosis and treatment of primary and secondary angiosarcomas.

In this study, we downloaded two datasets from the GEO database, which together contain 36 cases of primary angiosarcoma and 54 cases of secondary angiosarcoma. A total of 18 DEGs were identified, of which 13 were upregulated (UNC5A, CTLA4, ISLR2, MYC, ICOS, CMBL, IQCA1, GCOM1, WASF3, RELN, PGM5, CDCA7L, CETP) and five were downregulated (TGM2, BNC2, LXN, SERPINE1 and BAMBI) in secondary angiosarcoma compared to primary breast angiosarcoma. We then built the related PPI networks of these DEGs and identified hub genes, which showed that MYC, FOXP3 and SERPINE1 were the most outstanding genes.

MYC is a proto-oncogene that plays a key role in a variety of oncogenic pathways, such as cell proliferation and differentiation, adhesion, invasion and apoptosis [32]. Lae et al. reported that MYC amplification was detected in all 32 cases of breast radiation-induced angiosarcomas, but only one out of 15 cases of primary angiosarcoma [33]. Styring et al. found that MYC was upregulated in secondary angiosarcoma [34]. Thariat et al. demonstrated that C-myc overexpression can be used to identify radiation-induced angiosarcoma [35]. Mito et al. revealed that MYC overexpression is common among radiation-induced angiosarcomas compared with other angiosarcomas [36]. Requena et al. [37]

Table 3
The description of overlapped differentially-expressed genes (DEGs) between secondary and primary AS among the two datasets.

DEGs	Gene symbol	Description	Chromosome	Map location
Up-regulated genes	UNC5A	unc-5 netrin receptor A	5	5q35.2
	CTLA4	cytotoxic T-lymphocyte-associated protein 4	2	2q33
	ISLR2	immunoglobulin superfamily containing leucine-rich repeat 2	15	15q24.1
	MYC	v-myc avian myelocytomatosis viral oncogene homolog	8	8q24.21
	ICOS	inducible T-cell co-stimulator	2	2q33
	CMBL	carboxymethylenebutenolidase homolog (Pseudomonas)	5	5p15.2
	IQCA1	IQ motif containing with AAA domain 1	2	2q37.3
	GCOM1	GRINL1A complex locus 1	15	15q21.3
	WASF3	WAS protein family, member 3	13	13q12
	RELN	reelin	7	7q22
	PGM5	phosphoglucomutase 5	9	9q13
	CDCA7L	cell division cycle associated 7-like	7	7p15.3
	CETP	cholesteryl ester transfer protein, plasma	16	16q21
Down-regulated genes	TGM2	transglutaminase 2	20	20q12
	BNC2	basonuclin 2	9	9p22.2
	LXN	latexin	3	3q25.32
	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	7	7q22.1
	BAMBI	BMP and activin membrane-bound inhibitor	10	10p12.3-p11.2

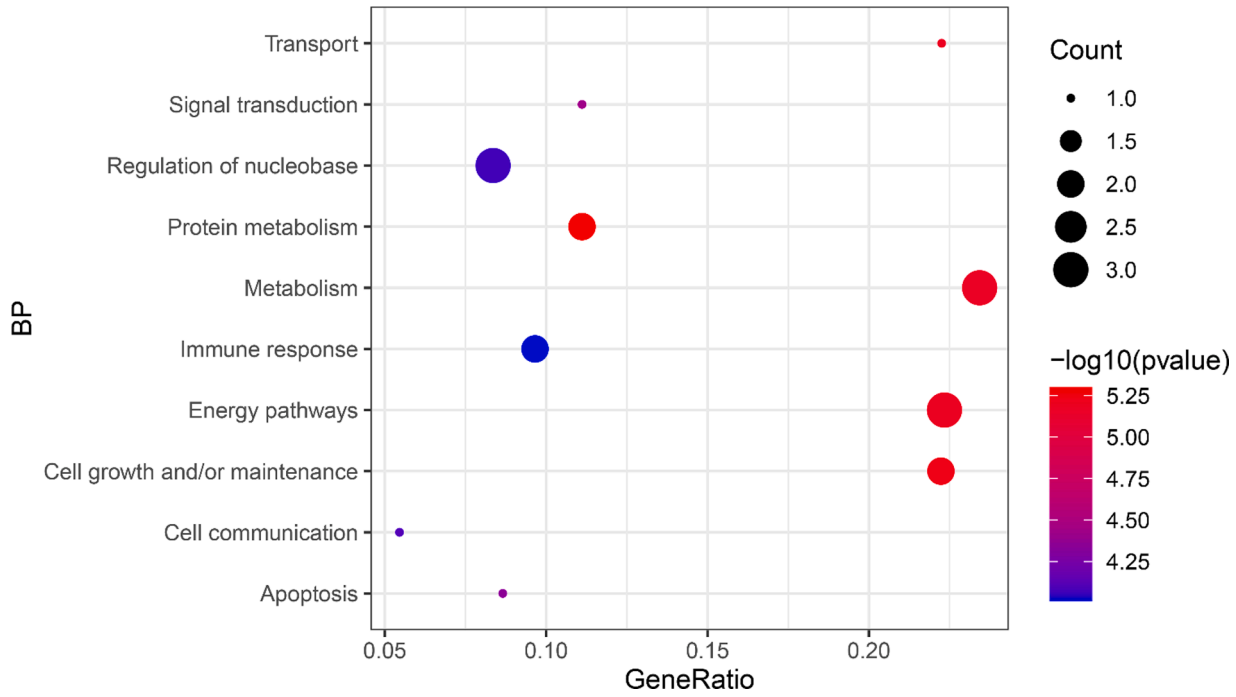

Fig. 2. GO analysis of the DEGs. Biological process (BP).

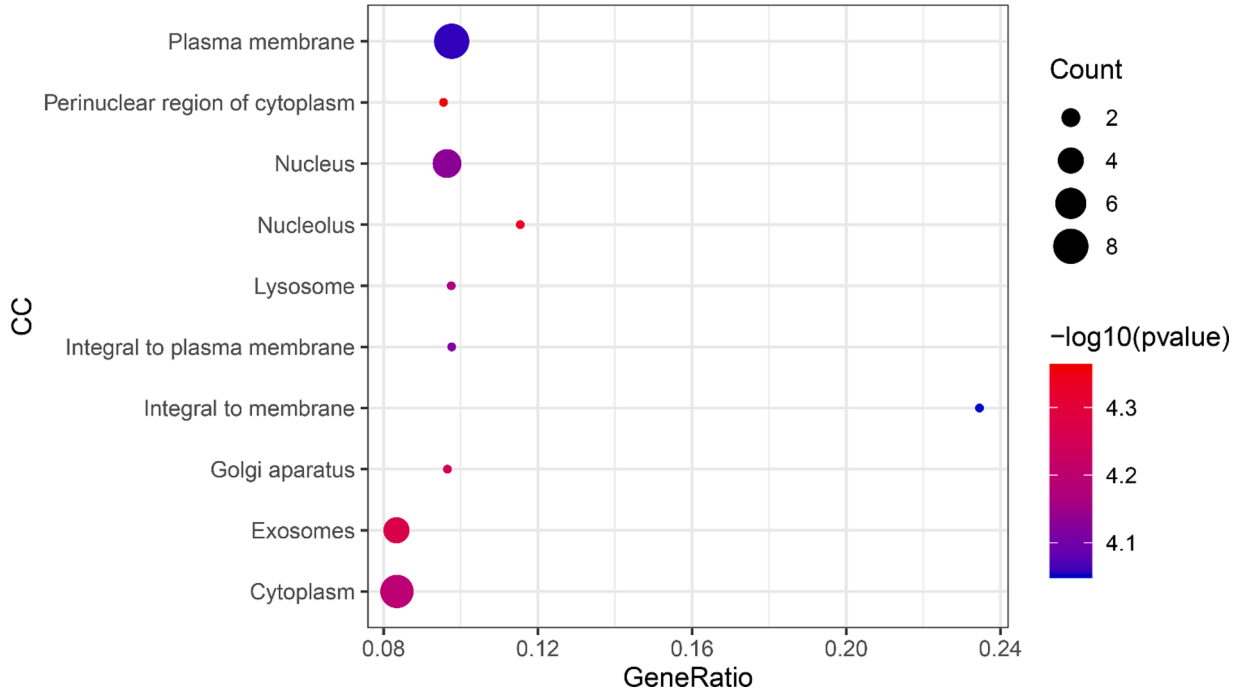

Fig. 3. GO analysis of the DEGs. Cellular component (CC).

detected MYC amplification by fluorescence *in-situ* hybridization (FISH) in six cases, all of which were secondary angiosarcoma. Furthermore, among 15 cases analyzed, MYC overexpression was detected in eight cases, consisting of seven cases of secondary angiosarcoma and one case of idiopathic angiosarcoma. Overall, MYC amplification and MYC overexpression were almost always detected in secondary angiosarcoma [37]. Among 37 patients with secondary angiosarcoma, Fraga–Guedes found that 20 patients had high levels of MYC amplification and MYC overexpression, while this pattern was not detected in any cases of primary angiosarcoma or atypical angiopathy [38]. Using the DISH and FISH detection techniques, Ko et al. reported MYC amplification in all 11 cases of secondary angiosarcoma [39]. Shon et al. identified high levels of MYC gene amplification and MYC overexpression in secondary angiosarcoma, but not in primary angiosarcoma. These results are consistent with the current research; however, some conflicting studies have also been reported. Verbeke et al. detected MYC amplification in both primary angiosarcoma and secondary angiosarcomas [40]. Therefore, further well-designed studies with a larger sample size are needed to verify our results.

SERPINE1 acts as a vital inhibitor of serine proteases that play

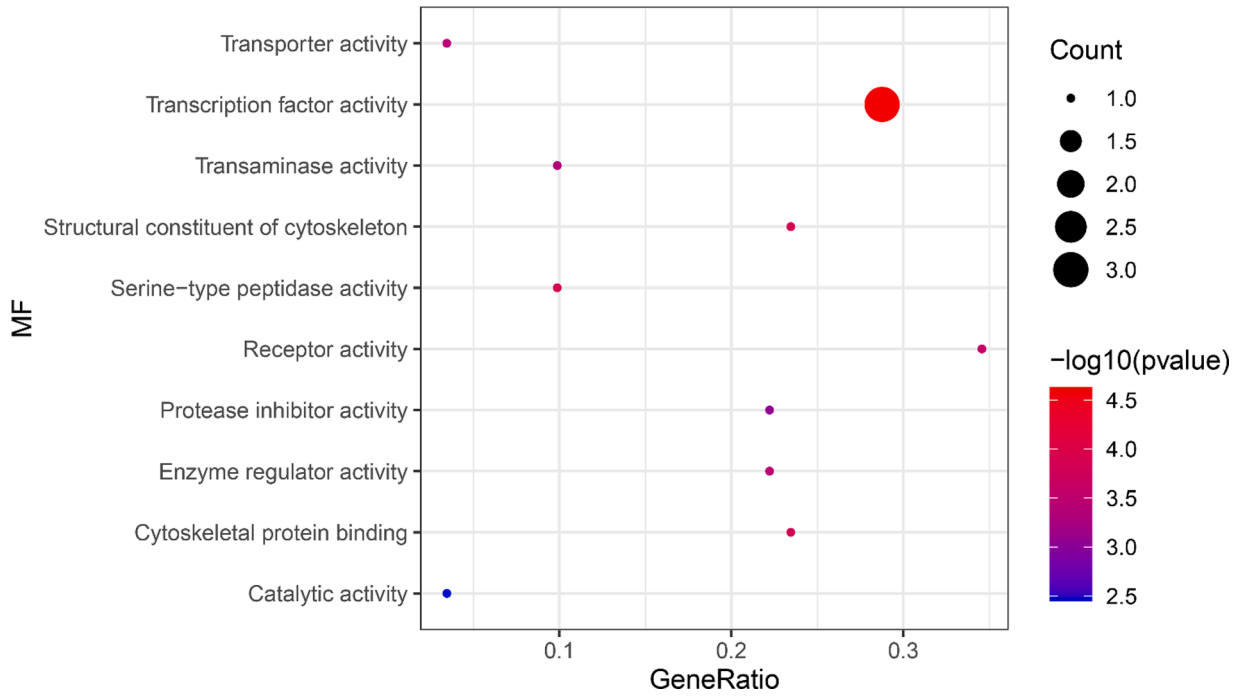

Fig. 4. GO analysis of the DEGs. Molecular function (MF).

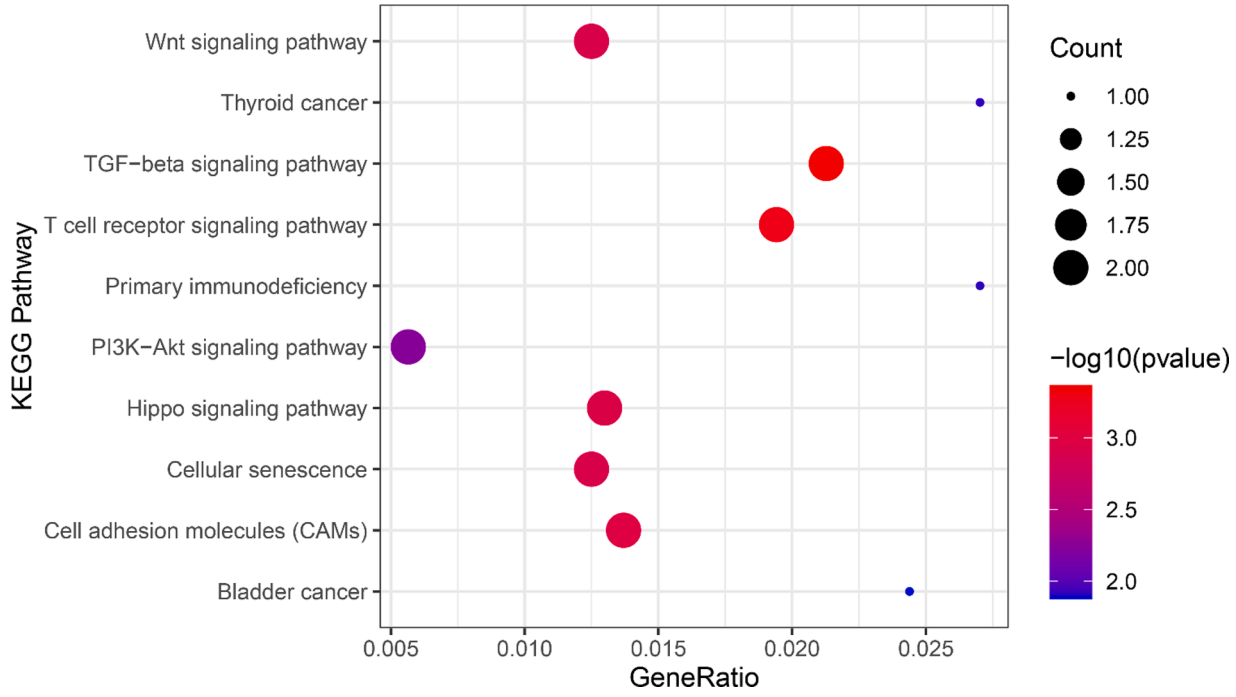

Fig. 5. KEGG pathway analysis of the DEGs.

important roles in signal transduction, cell adhesion, and cell migration in many tumors [41,42]. Hung et al. identified SERPINE1 as a useful biomarker to distinguish pseudomyogenic hemangioendothelioma from histologic mimics [43]. Bridge et al. revealed that pseudomyogenic hemangioendothelioma often harbors a rearrangement of the FOSB gene with SERPINE1. The absence or dysfunction FOXP3, which is a master switch gene for regulatory T (Treg) cells, may cause qualitative or functional deficiency of this cell type [44,45]. Gambichler et al. found that CD4 and FOXP3 expression was significantly higher in cutaneous angiosarcoma and associated with disease relapse [46]. Fujii et al. found significantly increased proportions of CD41+ FOXP31+ T cells in the peripheral blood of patients with angiosarcoma [47]. These results are consistent with our study, indicating that these hub genes play an important role in the progression of angiosarcoma. However, the roles of UNC5A, CTLA4, ISLR2, MYC, ICOS, CMBL, IQCA1, GCOM1, WASF3, RELN, PGM5, CDCA7L, CETP and other hub genes in the occurrence and development of primary and secondary angiosarcoma remain to be clarified. Thus, verification of these results is required and our findings provide an important basis for further research.

GO and KEGG pathway enrichment analysis were conducted to gain

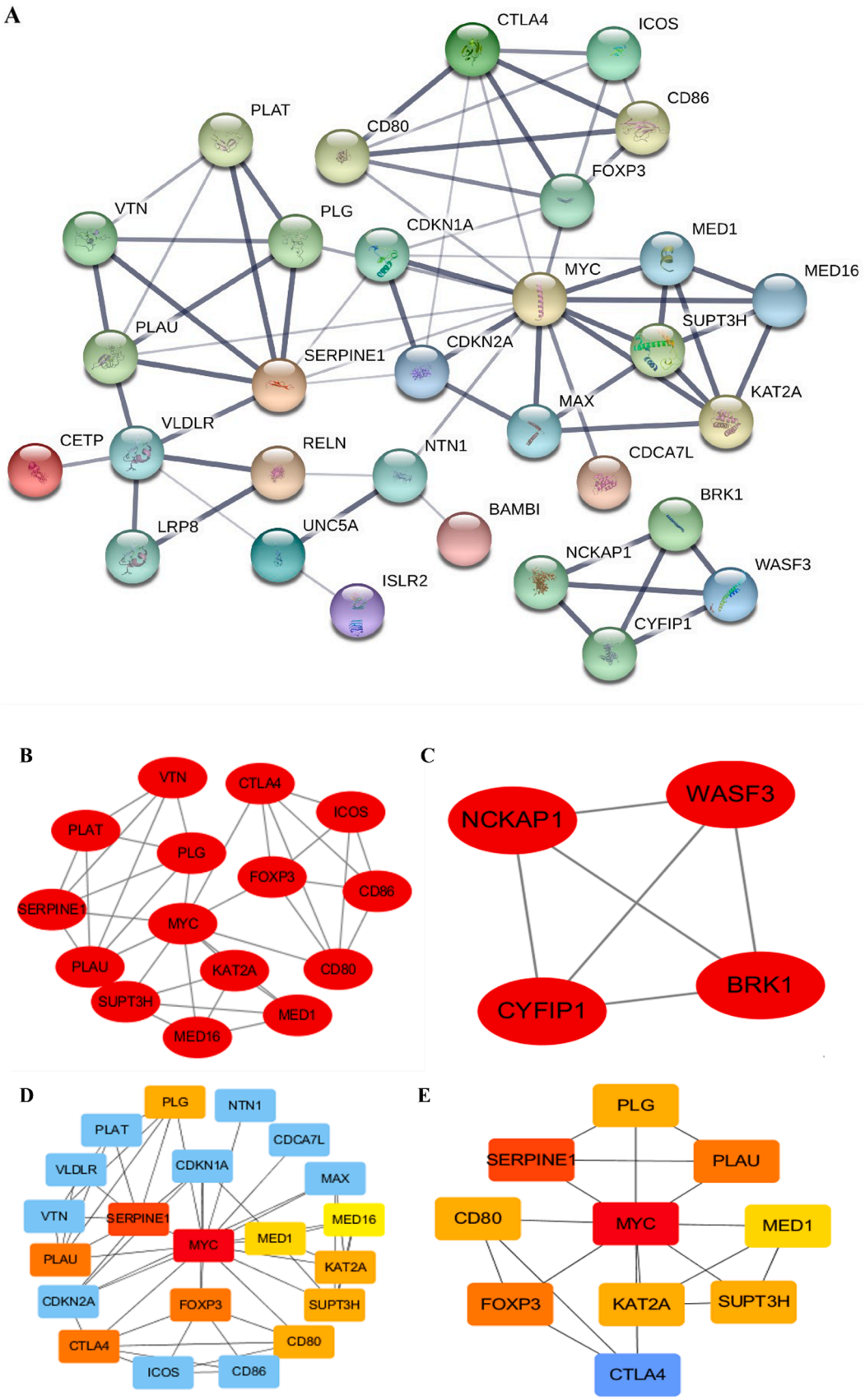

Fig. 6. PPI network, module analysis, and hub genes identification. (A) PPI network of DEGs was constructed in STRING database. (B,C) Top two modules screened using MCODE in Cytoscape software, (D) top10 hub genes with neighbors and expanded genes, (E) top 10 hub genes selected by the CytoHubba.

Table 4
The top 10 hub genes.

Gene symbol	Description	Chromosome	Map location
MYC	v-myc avian myelocytomatosis viral oncogene homolog	8	8q24.21
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	7	7q22.1
CTLA4	cytotoxic T-lymphocyte-associated protein 4	2	2q33
PLAU	plasminogen activator, urokinase	10	10q22.2
FOXP3	forkhead box P3	X	Xp11.23
KAT2A	K(lysine) acetyltransferase 2A	17	17q21
SUPT3H	suppressor of Ty 3 homolog (S. cerevisiae)	6	6p21.1-p21.3
CD80	CD80 molecule	3	3q13.3-q21
PLG	plasminogen	6	6q26
MED1	mediator complex subunit 1	17	17q12

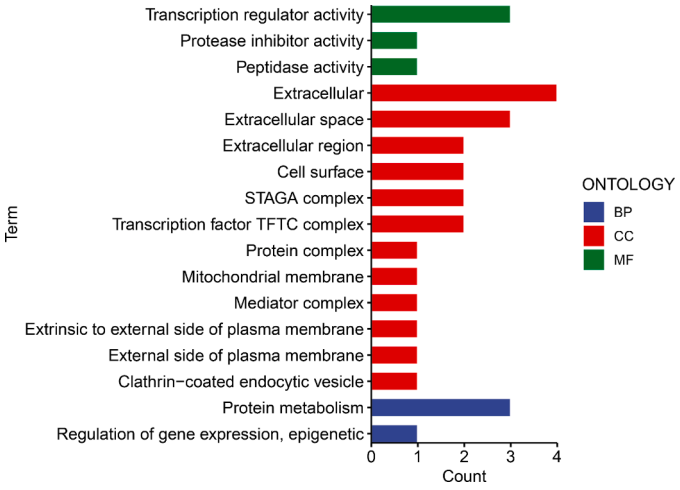

Fig. 7. GO enrichment analysis of the top 10 hub genes.

a more in-depth understanding of the overlapping DEGs and hub genes. The results showed that they were mainly related to protein metabolism and metabolism in biological process. In addition, the most enriched KEGG pathways were microRNAs in cancer, and Notch, p53, TGF-β, HIF-1, and PI3K-AKT signaling pathways. Previous studies showed that these signaling pathways were abnormally activated in angiosarcomas and contributed to malignant biological behaviors. Studies have shown that the PI3K/Akt/mTOR signaling pathway is hyperactivated in human or canine angiosarcoma [48]. Wada et al. showed that the PI3K/mTOR inhibitor, NVP-BEZ235, can inhibit the growth of angiosarcoma cells [49]. However, NVP-BEZ235 was not suitable for patient treatment due to its high toxicity [50]. Fortunately, there was another drug, homoharringtonine (HHT), approved and used in treatment of acute myeloid leukemia, which was one of the best translation inhibitors available. Meanwhile, there were a number of studies dedicated to HTT. For example, Yakhni M has performed some initial preclinical studies on HHT activity in triple negative breast cancer, showing the drug's activity in some very proliferative and invasive cell lines which have the PI3K-MYC axis overactivated [51]. So, HHT is one of interesting molecules. Perhaps it could be used in angiosarcoma animal models to find new therapeutic targets. Adachi et al. reported that PI3K/AKT/mTOR-related protein is highly expressed in canine angiosarcoma [52]. Wang et al. revealed that activation of the MAPK and PI3K pathways was closely related to the progression of angiosarcoma [53]. Beca et al. detected mutations in KDR and PIK3CA in primary breast angiosarcoma, suggesting that its pathogenesis is distinct from that of other angiosarcomas [54]. Megquier et al. disclosed that the PI3K pathway plays an important role in angiosarcoma [55]. Chadwick et al. found that the combination of mTOR and MEK inhibitors could effectively treat angiosarcoma [56]. Verbeke et al. found that both bone and soft tissue angiosarcoma patients benefitted from treatment strategies targeting the PI3K/Akt pathway [57]. These studies implicate the PI3K/AKT/mTOR pathway as a therapeutic target for angiosarcoma.

Similarly, the Notch pathway may be closely related to the progression of angiosarcoma. Aoshima et al. found that Notch signaling pathway-related proteins were upregulated in cancer stem cell (CSC)-like cells, suggesting that Notch signaling may be a key factor in maintaining canine angiosarcoma stem cell-like cells [58]. Panse et al. revealed that the expression of Notch1 and 2 in angiosarcoma is closely related to the primary site and poor clinical prognosis [59]. Dill et al. found that the disruption of Notch1 signaling in liver sinusoidal endothelial cells was closely related to the formation of angiosarcoma [60].

The TP53 gene has long been a research hotspot in the field of cancer. The deletion or mutation of TP53 is closely related to the progression of a variety of tumors and is associated with poor prognosis [61]. TP53 also plays an important role in angiosarcoma. García–Iglesias et al. found that p53 and phosphorylated p53 serine were highly expressed in angiosarcoma [62]. Kiyohara et al. suggested that serum anti-p53 antibodies maybe a potent diagnostic and prognostic biomarker of angiosarcoma [63]. Kuhn et al. detected only a few mutations in angiosarcomas, not including TP53 or TERT mutations [64]. Hung et al. showed that p53 was expressed higher levels in sporadic angiosarcomas than radiation-related angiosarcomas [65]. In contrast, Italiano et al. found that TP53 mutation and PTEN deletion were rarely involved in the pathogenesis of angiosarcoma [27]. Thus, further studies are required to verify the role of TP53 in angiosarcoma.

Our results also showed that MYC and PLAU were enriched in miRNAs in cancer. In the past few years, studies have also shown that miRNAs may be the cause of the progression of angiosarcoma. Italiano et al. showed that MYC amplification might play a key role in the angiogenic phenotype of angiosarcoma by inducing upregulation the miR-17–92 [66]. Chen et al. found that miR-497–5p inhibited the proliferation and invasion of angiosarcoma cells by targeting KCa3.1 [67]. Heishima et al. found that miRNA-214 promoted apoptosis of canine hemangiosarcoma by targeting the COP1-p53 axis [68]. Yoshikawa et al. identified miR-214 5AE as a potential novel chemotherapeutic agent of canine hemangiosarcoma [69]. Wang et al. indicated that miRNA-340 targeted SIRT7 to inhibit the growth and invasion of angiosarcoma cells [70]. Nakashima discovered that the miR-210/E2F3/ephrin A3 signaling axis could represent a new therapeutic approach against angiosarcoma [71].

The results discussed here suggest that miRNAs in cancer, the PI3K/AKT, Notch, p53 signaling pathways as well as other important signaling pathways may interact to promote the occurrence and development of angiosarcoma. However, there are some limitations in our study. Firstly, angiosarcoma is rare malignancies and most of the current information relating to angiosarcoma is provided by case reports and single-institution retrospective cohort, the further well-designed studies and a multi-center clinical study with a larger sample size need to be implemented to validate our results. Secondly, due to lack of samples, we have only conducted bioinformatics analysis and more experiments are required to validate in future.

Conclusion

The DEGs between primary and secondary breast angiosarcoma may have the potential to serve as therapeutic targets as well new biomarkers for the diagnosis and prognosis of primary and secondary angiosarcomas. Moreover, further basic research and a well-designed multi-center clinical study with a larger sample size are warranted to verify our results.

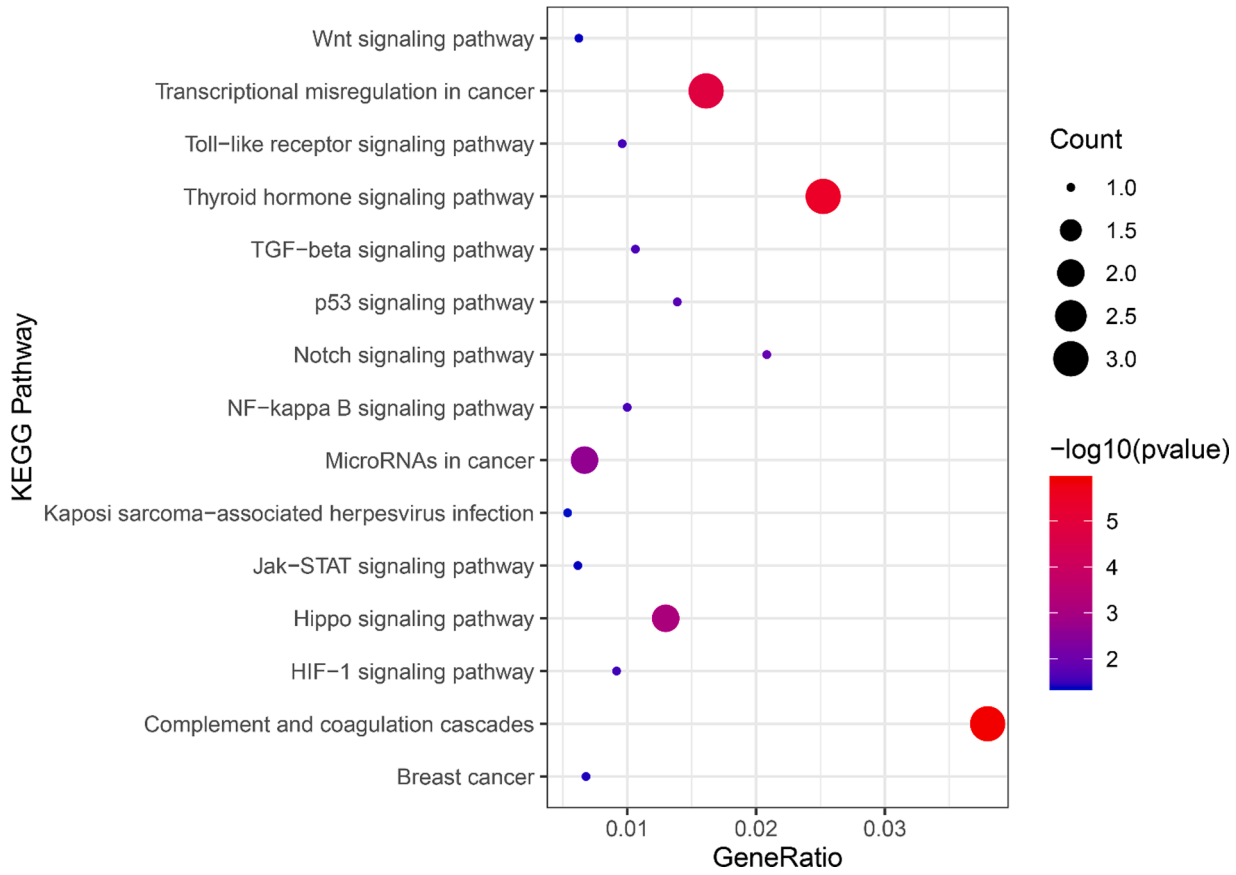

Fig. 8. KEGG pathway enrichment analysis of the top 10 hub genes.

Funding information

This research was supported by Sichuan Science and Technology Program (no, 2019YFS0109), China Postdoctoral Science Foundation (2019M663505), and Postdoctoral Interdisciplinary Innovation Foundation, Sichuan University (no, 0040204153243).

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All data is available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Yuanfeng Wei: Conceptualization, Methodology, Software, Validation, Resources, Writing – original draft. **Xi Yang:** Conceptualization, Methodology, Validation. **Limin Gao:** Validation. **Yong Xu:** Conceptualization, Validation. **Cheng Yi:** Conceptualization, Methodology, Validation.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

The authors would like to thank GEO databases for data collection. This research was supported by Sichuan Science and Technology Program (no, 2019YFS0109), China Postdoctoral Science Foundation (2019M663505), and Postdoctoral Interdisciplinary Innovation Foundation, Sichuan University (no, 0040204153243).

References

[1] R. Young, N. Brown, M. Reed, D. Hughes, Woll P. Angiosarcoma, Lancet Oncol. 11 (2010) 983–991.
[2] A. Espejo-Freire, A. Elliott, A. Rosenberg, P. Costa, P. Barreto-Coelho, E. Jonczak, et al., Genomic landscape of angiosarcoma: a targeted and immunotherapy biomarker analysis, Cancers 13 (2021) 4816 (Basel).
[3] J. Khan, R. Maki, V. Ravi, Pathologic angiogenesis of malignant vascular sarcomas: implications for treatment, J. Clin. Oncol. 36 (2018) 194–201.
[4] X. Wang, J. Jakowski, O. Tawfik, P. Thomas, F. Fan, Angiosarcoma of the breast: a clinicopathologic analysis of cases from the last 10 years, Ann. Diagn. Pathol. 13 (2009) 147–150.
[5] C. Tomasini, M. Grassi, M. Pippione, Cutaneous angiosarcoma arising in an irradiated breast. Case report and review of the literature, Dermatology 209 (2004) 208–214 (Basel).
[6] R. Siegel, K. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (2019) 7–34.
[7] R. Siegel, K. Miller, H. Fuchs, A. Jemal, Cancer statistics, 2022, CA Cancer J. Clin. 72 (2022) 7–33.
[8] M. Lehman, B. Hickey, D. Francis, A. See, Partial breast irradiation for early breast cancer, Cochrane Database Syst. Rev. 7 (2014), CD007077.
[9] D. Arthur, K. Winter, H. Kuerer, B. Haffty, L. Cuttino, D. Todor, et al., Effectiveness of breast-conserving surgery and 3-dimensional conformal partial breast reirradiation for recurrence of breast cancer in the ipsilateral breast: the NRG oncology/RTOG 1014 phase 2 clinical trial, JAMA Oncol. 6 (2020) 75–82.
[10] B. Hickey, M. Lehman, Partial breast irradiation versus whole breast radiotherapy for early breast cancer, Cochrane Database Syst. Rev. 8 (2021), CD007077.
[11] J. Hepel, D. Wazer, Partial breast irradiation is the preferred standard of care for a majority of women with early-stage breast cancer, J. Clin. Oncol. 38 (2020) 2268–2272.

[12] A. Recht, Whole-breast irradiation is the preferred standard of care for the majority of patients with early-stage breast cancer, J. Clin. Oncol. 38 (2020) 2263–2267.
[13] S. Billings, J. McKenney, A. Folpe, M. Hardacre, S. Weiss, Cutaneous angiosarcoma following breast-conserving surgery and radiation: an analysis of 27 cases, Am. J. Surg. Pathol. 28 (2004) 781–788.
[14] A. Chesebro, S. Chikarmane, E. Gombos, A. Giardino, Radiation-associated angiosarcoma of the breast: what the radiologist needs to know, AJR Am. J. Roentgenol. 207 (2016) 217–225.
[15] S. D'Angelo, C. Antonescu, D. Kuk, L. Qin, N. Moraco, R. Carvajal, et al., High-risk features in radiation-associated breast angiosarcomas, Br. J. Cancer 109 (2013) 2340–2346.
[16] P. Ginter, P. McIntire, S. Shin, Vascular tumours of the breast: a comprehensive review with focus on diagnostic challenges encountered in the core biopsy setting, Pathology 49 (2017) 197–214 (Phila).
[17] Y. Abdou, A. Elkhanany, K. Attwood, W. Ji, K. Takabe, M. Opyrchal, Primary and secondary breast angiosarcoma: single center report and a meta-analysis, Breast Cancer Res. Treat. 178 (2019) 523–533.
[18] M. Ohsawa, N. Naka, Y. Tomita, D. Kawamori, H. Kanno, K. Aozasa, Use of immunohistochemical procedures in diagnosing angiosarcoma. Evaluation of 98 cases, Cancer 75 (1995) 2867–2874.
[19] A. Nascimento, C. Raut, C. Fletcher, Primary angiosarcoma of the breast: clinicopathologic analysis of 49 cases, suggesting that grade is not prognostic, Am. J. Surg. Pathol. 32 (2008) 1896–1904.
[20] J. Mella, K. Ross, G. Li, B. Pomahac, C. Raut, D. Orgill, Cutaneous breast radiation-associated angiosarcoma: anterior chest wall reconstruction options following extra-radical resection, Plast. Reconstr. Surg. Glob. Open 6 (2018) e1938.
[21] R. Abbott, C. Palmieri, Angiosarcoma of the breast following surgery and radiotherapy for breast cancer, Nat. Clin. Pract. Oncol. 5 (2008) 727–736.
[22] M. Weidema, U. Flucke, W. van der Graaf, V. Ho, M. Hillebrandt-Roeffen, Y. Versleijen-Jonkers, et al., Prognostic factors in a large nationwide cohort of histologically confirmed primary and secondary angiosarcomas, Cancers 11 (2019) 1780 (Basel).
[23] C. Käcker, A. Marx, K. Mössinger, F. Svehla, U. Schneider, P.C. Hogendoorn, et al., High frequency of MYC gene amplification is a common feature of radiation-induced sarcomas. Further results from EORTC STBSG TL 01/01, Genes Chromosomes Cancer 52 (2013) 93–98.
[24] M. Pathan, S. Keerthikumar, C. Ang, L. Gangoda, C. Quek, N. Williamson, et al., FunRich: an open access standalone functional enrichment and interaction network analysis tool, Proteomics 15 (2015) 2597–2601.
[25] D. Bu, H. Luo, P. Huo, Z. Wang, S. Zhang, Z. He, et al., KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis, Nucleic Acids Res. 49 (2021) W317–W325.
[26] W. Tokuyama, T. Mikami, M. Masuzawa, I. Okayasu, Autocrine and paracrine roles of VEGF/VEGFR-2 and VEGF-C/VEGFR-3 signaling in angiosarcomas of the scalp and face, Hum. Pathol. 41 (2010) 407–414.
[27] A. Italiano, C. Chen, R. Thomas, M. Breen, F. Bonnet, N. Sevenet, et al., Alterations of the p53 and PIK3CA/AKT/mTOR pathways in angiosarcomas: a pattern distinct from other sarcomas with complex genomics, Cancer 118 (2012) 5878–5887.
[28] H. Lu, P. Chen, C. Yen, F. Hsiao, C. Tzeng, H. Ma, et al., Refractory cutaneous angiosarcoma successfully treated with sunitinib, Br. J. Dermatol. 169 (2013) 204–206.
[29] J. Fayette, E. Martin, S. Piperno-Neumann, A. Le Cesne, C. Robert, S. Bonvalot, et al., Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases, Ann. Oncol. 18 (2007) 2030–2036. Official Journal of the European Society for Medical Oncology.
[30] A. Ronchi, I. Cozzolino, F. Zito Marino, A. De Chiara, G. Argenziano, E. Moscarella, et al., Primary and secondary cutaneous angiosarcoma: distinctive clinical, pathological and molecular features, Ann. Diagn. Pathol. 48 (2020), 151597.
[31] J. Fodor, Z. Orosz, E. Szabó, Z. Sulyok, C. Polgár, Z. Zaka, et al., Angiosarcoma after conservation treatment for breast carcinoma: our experience and a review of the literature, J. Am. Acad. Dermatol. 54 (2006) 499–504.
[32] W. Wang, S. Hu, Y. Gu, Y. Yan, D. Stovall, D. Li, et al., Human MYC G-quadruplex: from discovery to a cancer therapeutic target, Biochim. Biophys. Acta Rev. Cancer 1874 (2020), 188410.
[33] M. Laé, A. Lebel, F. Hamel-Viard, B. Asselain, M. Trassard, X. Sastre, et al., Can c-myc amplification reliably discriminate postradiation from primary angiosarcoma of the breast? Cancer Radiother. 19 (2015) 168–174, journal de la Societe francaise de radiotherapie oncologique.
[34] E. Styring, J. Seinen, M. Dominguez-Valentin, H. Domanski, M. Jönsson, F. von Steyern, et al., Key roles for MYC, KIT and RET signaling in secondary angiosarcomas, Br. J. Cancer 111 (2014) 407–412.
[35] J. Thariat, A. Italiano, F. Collin, A. Iannessi, P. Marcy, A. Lacout, et al., Not all sarcomas developed in irradiated tissue are necessarily radiation-induced–spectrum of disease and treatment characteristics, Crit. Rev. Oncol. Hematol. 83 (2012) 393–406.
[36] J. Mito, X. Qian, V. Jo, L. Doyle, MYC expression has limited utility in the distinction of undifferentiated radiation-associated sarcomas from sporadic sarcomas and sarcomatoid carcinoma, Histopathology 77 (2020) 667–672.
[37] C. Requena, L. Rubio, J. Lavernia, I. Machado, B. Llombart, O. Sanmartín, et al., Immunohistochemical and fluorescence *in situ* hybridization analysis of MYC in a series of 17 cutaneous angiosarcomas: a single-center study, Am. J. Dermatopathol. 40 (2018) 349–354.
[38] C. Fraga-Guedes, S. André, M. Mastropasqua, E. Botteri, A. Toesca, R. Rocha, et al., Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of MYC gene amplification and protein expression, Breast Cancer Res. Treat. 151 (2015) 131–140.
[39] J. Ko, S. Billings, C. Lanigan, D. Buehler, A. Fernandez, R. Tubbs, Fully automated dual-color dual-hapten silver *in situ* hybridization staining for MYC amplification: a diagnostic tool for discriminating secondary angiosarcoma, J. Cutan. Pathol. 41 (2014) 286–292.
[40] S. Verbeke, D. de Jong, F. Bertoni, R. Sciot, C. Antonescu, K. Szuhai, et al., Array CGH analysis identifies two distinct subgroups of primary angiosarcoma of bone, Genes Chromosomes Cancer 54 (2015) 72–81.
[41] I. Azimi, R. Petersen, E. Thompson, S. Roberts-Thomson, G. Monteith, Hypoxia-induced reactive oxygen species mediate N-cadherin and SERPINE1 expression, EGFR signalling and motility in MDA-MB-468 breast cancer cells, Sci. Rep. 7 (2017) 15140.
[42] K. Yang, S. Zhang, D. Zhang, Q. Tao, T. Zhang, G. Liu, et al., Identification of SERPINE1, PLAU and ACTA1 as biomarkers of head and neck squamous cell carcinoma based on integrated bioinformatics analysis, Int. J. Clin. Oncol. 24 (2019) 1030–1041.
[43] Y. Hung, C. Fletcher, J. Hornick, FOSB is a useful diagnostic marker for pseudomyogenic hemangioendothelioma, Am. J. Surg. Pathol. 41 (2017) 596–606.
[44] J. van Loosdregt, V. Fleskens, J. Fu, A. Brenkman, C. Bekker, C. Pals, et al., Stabilization of the transcription factor Foxp3 by the deubiquitinase USP7 increases treg-cell-suppressive capacity, Immunity 39 (2013) 259–271.
[45] C. Bennett, R. Yoshioka, H. Kiyosawa, D. Barker, P. Fain, A. Shigeoka, et al., X-linked syndrome of polyendocrinopathy, immune dysfunction, and diarrhea maps to Xp11.23-Xq13.3, Am. J. Hum. Genet. 66 (2000) 461–468.
[46] T. Gambichler, S. Koim, M. Wrobel, H. Käfferlein, T. Brüning, E. Stockfleth, et al., Expression of programmed cell death proteins in kaposi sarcoma and cutaneous angiosarcoma, J. Immunother. 43 (2020) 169–174 (Hagerstown, Md: 1997).
[47] H. Fujii, A. Arakawa, D. Utsumi, S. Sumiyoshi, Y. Yamamoto, A. Kitoh, et al., $CD8^+$ tumor-infiltrating lymphocytes at primary sites as a possible prognostic factor of cutaneous angiosarcoma, Int. J. Cancer 134 (2014) 2393–2402.
[48] A. Murai, S. Asa, A. Kodama, A. Hirata, T. Yanai, H. Sakai, Constitutive phosphorylation of the mTORC2/Akt/4E-BP1 pathway in newly derived canine hemangiosarcoma cell lines, BMC Vet. Res. 8 (2012) 128.
[49] M. Wada, M. Horinaka, S. Yasuda, M. Masuzawa, T. Sakai, N. Katoh, PDK1 is a potential therapeutic target against angiosarcoma cells, J. Dermatol. Sci. 78 (2015) 44–50.
[50] H. Zhou, S. Huang, Current development of the second generation of mTOR inhibitors as anticancer agents, Chin. J. Cancer 31 (2012) 8–18.
[51] M. Yakhni, A. Briat, A. El Guerrab, L. Furtado, F. Kwiatkowski, E. Miot-Noirault, et al., Homoharringtonine, an approved anti-leukemia drug, suppresses triple negative breast cancer growth through a rapid reduction of anti-apoptotic protein abundance, Am. J. Cancer Res. 9 (2019) 1043–1060.
[52] M. Adachi, Y. Hoshino, Y. Izumi, H. Sakai, S. Takagi, Effects of inhibitors of vascular endothelial growth factor receptor 2 and downstream pathways of receptor tyrosine kinases involving phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin or mitogen-activated protein kinase in canine hemangiosarcoma cell lines, Can. J. Vet. Res. 80 (2016) 209–216.
[53] G. Wang, M. Wu, A. Durham, E. Radaelli, N. Mason, X. Xu, et al., Molecular subtypes in canine hemangiosarcoma reveal similarities with human angiosarcoma, PLoS One 15 (2020), e0229728.
[54] F. Beca, S. Lee, F. Pareja, A. Da Cruz Paula, P. Selenica, L. Ferrando, et al., Whole-exome sequencing and RNA sequencing analyses of acinic cell carcinomas of the breast, Histopathology 75 (2019) 931–937.
[55] K. Megquier, J. Turner-Maier, R. Swofford, J. Kim, A. Sarver, C. Wang, et al., Comparative genomics reveals shared mutational landscape in canine hemangiosarcoma and human angiosarcoma, Mol. Cancer Res. 17 (2019) 2410–2421.
[56] M. Chadwick, A. Lane, D. Thomas, A. Smith, A. White, D. Davidson, et al., Combined mTOR and MEK inhibition is an effective therapy in a novel mouse model for angiosarcoma, Oncotarget 9 (2018) 24750–24765.
[57] S. Verbeke, F. Bertoni, P. Bacchini, J. Oosting, R. Sciot, T. Krenács, et al., Active TGF-β signaling and decreased expression of PTEN separates angiosarcoma of bone from its soft tissue counterpart, Mod. Pathol. 26 (2013) 1211–1221, an official journal of the United States and Canadian Academy of Pathology, Inc.
[58] K. Aoshima, Y. Fukui, K. Gulay, O. Erdemsurakh, A. Morita, A. Kobayashi, et al., Notch2 signal is required for the maintenance of canine hemangiosarcoma cancer stem cell-like cells, BMC Vet. Res. 14 (2018) 301.
[59] G. Panse, J. Chrisinger, C. Leung, D. Ingram, S. Khan, K. Wani, et al., Clinicopathological analysis of ATRX, DAXX and NOTCH receptor expression in angiosarcomas, Histopathology 72 (2018) 239–247.
[60] M. Dill, S. Rothweiler, V. Djonov, R. Hlushchuk, L. Tornillo, L. Terracciano, et al., Disruption of Notch1 induces vascular remodeling, intussusceptive angiogenesis, and angiosarcomas in livers of mice, Gastroenterology 142 (2012) 967–977, e2.
[61] A. Petitjean, E. Mathe, S. Kato, C. Ishioka, S. Tavtigian, P. Hainaut, et al., Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database, Hum. Mutat. 28 (2007) 622–629.
[62] M. García-Iglesias, J. Cuevas-Higuera, A. Bastida-Sáenz, M. de Garnica-García, L. Polledo, P. Perero, et al., Immunohistochemical detection of p53 and pp53 Ser in canine hemangiomas and hemangiosarcomas located in the skin, BMC Vet. Res. 16 (2020) 239.
[63] M. Kiyohara, J. Aoi, I. Kajihara, S. Otuka, T. Kadomatsu, S. Fukushima, et al., Serum anti-p53 autoantibodies in angiosarcoma, J. Dermatol. 47 (2020) 849–854.
[64] E. Kuhn, M. Ragazzi, A. Ciarrocchi, F. Torricelli, D. de Biase, E. Zanetti, et al., Angiosarcoma and anaplastic carcinoma of the thyroid are two distinct entities: a morphologic, immunohistochemical, and genetic study, Mod. Pathol. 32 (2019)

787–798, an official journal of the United States and Canadian Academy of Pathology, Inc.
[65] J. Hung, S. Hiniker, D. Lucas, K. Griffith, J. McHugh, A. Meirovitz, et al., Sporadic versus radiation-associated angiosarcoma: a comparative clinicopathologic and molecular analysis of 48 cases, Sarcoma 2013 (2013), 798403.
[66] A. Italiano, R. Thomas, M. Breen, L. Zhang, A. Crago, S. Singer, et al., The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification, Genes Chromosomes Cancer 51 (2012) 569–578.
[67] Y. Chen, D. Kuang, X. Zhao, D. Chen, X. Wang, Q. Yang, et al., miR-497-5p inhibits cell proliferation and invasion by targeting KCa3.1 in angiosarcoma, Oncotarget 7 (2016) 58148–58161.
[68] K. Heishima, T. Mori, H. Sakai, N. Sugito, M. Murakami, N. Yamada, et al., MicroRNA-214 promotes apoptosis in canine hemangiosarcoma by targeting the COP1-p53 axis, PLoS One 10 (2015), e0137361.
[69] R. Yoshikawa, K. Heishima, Y. Ueno, M. Kawade, Y. Maeda, K. Yoshida, et al., Development of synthetic microRNA-214 showing enhanced cytotoxicity and RNase resistance for treatment of canine hemangiosarcoma, Vet. Comp. Oncol. 18 (2020) 570–579.
[70] X. Wang, Y. Song, MicroRNA-340 inhibits the growth and invasion of angiosarcoma cells by targeting SIRT7, Biomed. Pharmacother. 103 (2018) 1061–1068.
[71] S. Nakashima, M. Jinnin, H. Kanemaru, I. Kajihara, T. Igata, S. Okamoto, et al., The role of miR-210, E2F3 and ephrin A3 in angiosarcoma cell proliferation, Eur. J. Dermatol. EJD 27 (2017) 464–471.