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ORIGINAL ARTICLE

The landscape of angiogenesis subtypes for breast cancer: a comprehensive analysis based on the Cancer Genome Atlas

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Summary

Purpose: *Breast cancer is a common malignant tumor in women with a poor prognosis. This study aimed to investigate angiogenesis subtypes of breast cancer and unveil the etiology and molecular features of breast cancer.*

Methods: *Based on the angiogenesis gene set derived from AmiGO2, and breast cancer data in the Cancer Genome Atlas (TCGA), we define a novel cluster of angiogenesis subtypes for patients by consensus clustering. The gene regulation, immune landscape, molecular characteristics, and clinical features as well as enrichment pathways were explored in the angiogenesis subtypes of breast cancer.*

Results: *Two angiogenesis subtypes were established through consensus clustering, among which subtype1 included 275 patients and subtype2 included 813 patients. A total of 643 differential expressed genes and 109 miRNAs were found between the two subtypes. The gene set enrichment analysis showed that the enriched hallmark pathways in subtype2 were related to the cancer tumorigenesis and breast cancer progression, including estrogen response early estrogen response late, epithelial-mesenchymal transition (EMT), especially angiogenesis. The mutant-allele tumor heterogeneity and tumor mutation burden of non-angiogenesis subtype were significantly higher than that in the angiogenesis subtype. The stroma score, immune score and ESTIMATE score were significantly higher in angiogenesis subtype, while the tumor purity in angiogenesis subtype was considerably lower. Finally, most immune checkpoints were expressed higher in the angiogenesis subtype.*

Conclusions: *The omics analysis has established a novel angiogenesis subtype of breast cancer and identified the characteristics of the immune microenvironment and genomic alteration of breast cancer. Thus, this angiogenesis subtype might provide new evidence for inhibiting the progression and immunotherapy response in breast cancer.*

Key words: *breast cancer, angiogenesis, TCGA, subtype, landscape*

Abbreviations: *ACKR1: atypical chemokine receptor 1; ADIPOQ: adiponectin; AMPK/ ULK1: adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)/Serine/threonine-protein kinase(ULK1); CAIX: carbonic anhydrase IX; CAXII: carbonic anhydrase XII; CTLA4, cytotoxic T lymphocyte antigen-4; DEGs: differential expression genes; ESTIMATE: Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data; ECM-receptor, extracellular matrix-receptor; EMT: epithelial-mesenchymal transition; FDA: U.S. Food and Drug Administration; FGF: fibroblast growth factor; HER2: human epidermal growth factor receptor-2; HR: hormone receptor; ICIS: immune checkpoint inhibitor; GSEA: Gene Set Enrichment Analysis; GO: Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LAG3: lymphocyte-activation gene 3; MYC: proto-oncogene protein; NSCLC, non-small cell lung cancer; P13K/AKT/mTOR: phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR); PD-1: programmed cell death protein-1; PD-L1: programmed death-ligand 1; PDGF: platelet-derived growth factor; PRAME: preferentially expressed antigen in melanoma; STK11/LKB1: serine/threonine kinase 11(STK11)/liver kinase b1 (LKB1); TCGA: The Cancer Genome Atlas; TGFβ-1: transforming growth factor β-1; VEGF: vascular endothelial growth factor*

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Introduction

Breast cancer ranks first as leading cause of cancer-related death in women around the world, accounting for 10% of newly diagnosed malignancies and 22% of malignancies in females each year [1]. Since the last decade, the incidence of breast cancer in China is more than twice the average incidence in the world. It is estimated that there will be about 2.5 million breast cancer patients in China by 2021 [2]. Angiogenesis is a crucial factor in the incidence, progression and metastasis of many cancers, including breast cancer [3]. Tumor angiogenesis is closely related to angiogenic factors, such as transforming growth factor β-1(TGFβ-1) and other growth factors [4-6]. According to studies by Weidner et al [7-9], the level of angiogenic factors and the number of vascular networks formed in tumor progression are important predictive factors of breast cancer survival. Therefore, there are practical reasons to use anti-VEGF and anti-angiogenic therapies in primary, locally advanced and metastatic breast cancer [10]. Thus, it is urgent to study new angiogenesis-related markers as potential therapeutic targets.

In recent years, immune checkpoint inhibitors (ICIS) have made some progress as well as anti-angiogenic therapy. ICIS activates anti-tumor response by blocking negative regulatory immune signals [11]. Common immune checkpoints include cytotoxic T lymphocyte antigen-4 (CTLA4), lymphocyte-activation gene 3 (LAG3) as well as programmed cell death protein 1 (PD-1) [12]. These two treatments have made significant progress in the combined treatment of cancers such as hepatocellular carcinoma, as well as non-small cell lung cancer (NSCLC) [12-14]. Because these tumors have high somatic mutation rates in their progression, this will lead to neoantigen generation, thus stimulating the anti-tumor immune response [15]. For example, PD-1 inhibitors increase the activity of cytotoxic T lymphocytes by blocking the binding of PD-1 to programmed death-ligand (PD-L1), which may save patients with advanced NSCLC [16]. On the contrary, breast cancer typically exhibits low to moderate levels of immunogenicity [16,17]. Breast cancer has a lower mutation burden than other tumor types, and differs by subtype, with basal-like tumors, and ERBB2 (formerly HER2) -positive have a higher mutation burden than hormone receptor (HR) -positive tumors [18]. With the increasing study of the immune microenvironment of breast cancer, immune escape has been considered as an essential sign of breast cancer progression. Tumor cells gradually acquire the ability of immune escape in the interaction with the immune microenvironment [19,20]. These bring new challenges to immunotherapy.

In this study, we identified 474 genes related to angiogenesis and have established a novel angiogenesis subtype of breast cancer based on The Cancer Genome Atlas (TCGA) database. This study also systematically analyzed the tumor microenvironment and the mutation landscape of breast cancer. By combining omics data, we hope to supplement the immune mechanism of anti-angiogenesis therapy and provide new evidence for the targeted treatment of breast cancer.

Methods

The Cancer Genome Atlas (TCGA) dataset

The study population was based on breast cancer patients from TCGA (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) including RNAseq and miRNAseq level 3 data, genomic data, as well as clinical data. These processed data were freely available obtained from the UCSC Xena (http://xena.ucsc.edu/).

Angiogenesis genes and consensus clustering

A list of 507 angiogenesis genes was downloaded from the AmiGO database (http://amigo.geneontology.org/amigo). A total of 474 angiogenesis-related genes were obtained in the TCGA breast cancer dataset. Unsupervised consensus clustering was applied to the TCGA angiogenesis genes data for 1094 breast cancers by using ConsensusClusterPlus R package, to identify robust patient clusters with k-means clustering of 1000 rounds and maximum clusters k of 7.

Differential expression of genes and miRNAs, gene-set enrichment analysis

Differential expression genes (DEGs) and miRNAs (miRs) analysis between these two subtypes was performed using Limma R package. Genes with the absolute logFoldchange>1 and false discovery rate (FDR) <0.05 were defined as differentially expressed. We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis based on the DEGs by using clusterProfiler R package. The enrichment cutoff was set at a false discovery rate (FDR) < 0.05. Next, the hallmark gene-set was used for gene-set enrichment analysis (GSEA) on the different subtypes of breast cancer patients. The NES and NOM p value was used to define the significant pathways altered in the two subtypes of breast cancer.

The mutation landscape of angiogenesis subtypes

To analyzed the alteration of somatic mutations, the MC3 files were downloaded from the UCSC Xena. The R Maftools package were utilized to analyze the mutation landscape of the two subtypes of breast cancer, including

oncogenic driver genes, oncogenic pathway, drug-genes interactions as well as oncoplots. For each tumor sample, the tumor mutation burden and mutant-allele tumor heterogeneity (MATH) was then compared between the two subtypes.

Tumor immune microenvironment

The Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) algorithm was used to assess the immune score, stroma score, for each tumor sample based on its mRNA expression profiles. Based on the single-sample gene-set enrichment analysis (ssGSEA) score, the tumor purity was also calculated [21]. In evaluating the ratio, the original value was compared between two different angiogenesis subtypes. The expression of the fifteen immune checkpoints was analyzed between these two angiogenesis subtypes.

Statistics

All data were analyzed and visualized by R language software. The continuous variables were presented as mean ± SD, and the two-sample t-test was utilized to analyze the statistical significance. X^2 test was performed to compare the difference between two binary variables. The threshold of two-sided $p<0.05$ suggested significant difference.

Results

Angiogenesis subtypes

By performing consensus clustering of 1094 breast cancer based on the angiogenesis-related gene expression a total of 7 angiogenesis subtypes were identified, and we selected the consensus two as the most robust subtypes for angiogenesis. The subtype 1 included 243 patients, and the subtype 2 included 813 patients (Figure 1A).

Differential expression of genes and miRNAs in angiogenesis subtypes

Next, we examined gene and miRNA expression between the two angiogenesis subtypes of breast cancer. Totally, 643 differently expressed genes were found in the comparison between these two subtypes, of which 625 genes were up-regulated and 18 genes were downregulated in the subtype 2. The top ten up-regulated genes (ADH1B, SCGB2A2, ADIPOQ, CILP, TFF1, C7, OGN, FABP4, ABCA8 and DARC) and down-regulated genes (PRAME, CXorf61, MAST1, ART3, RCOR2, ROPN1, A2ML1, HORMAD1, MSLN and CA9) are

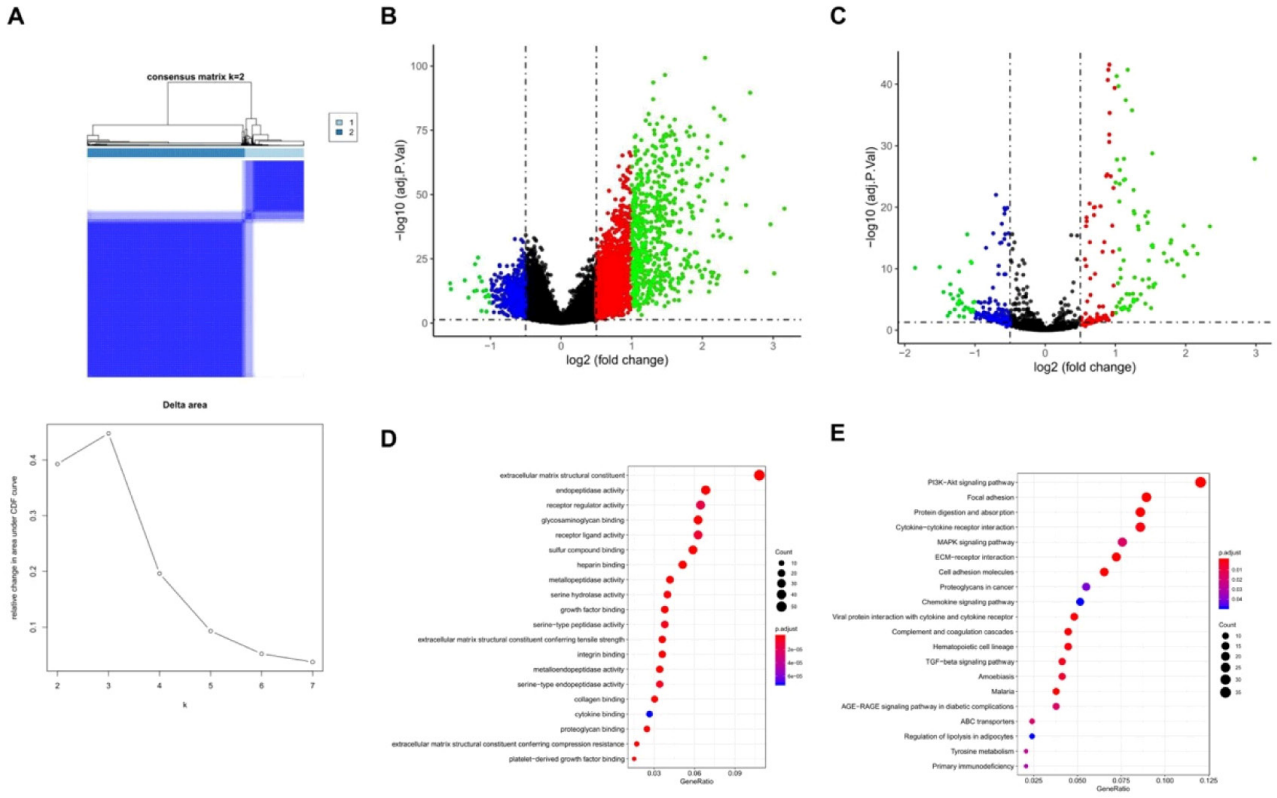

Figure 1. Consensus cluster for the angiogenesis subtype of breast cancer and the regulation network. **A:** Consensus Cluster for the angiogenesis subtype of breast cancer; **B:** Differently expressed genes for the angiogenesis subtype; **C:** Differently expressed miRNAs for the angiogenesis subtype; **D:** GO enrichment for the angiogenesis subtype; **E:** KEGG enrichment for the angiogenesis subtype.

Table 1. Differently expressed genes between angiogenesis-subtypes of BRCA

Gene	*logFC*	*Average expression*	*t*	*p value*	*FDR*	*B*
Up-regulation						
DARC	2.335	3.121	15.491	<0.0001	<0.0001	100.723
ABCA8	2.343	1.446	17.913	<0.0001	<0.0001	132.815
FABP4	2.397	3.508	12.885	<0.0001	<0.0001	69.627
OGN	2.578	3.093	18.728	<0.0001	<0.0001	144.209
C7	2.616	2.551	15.407	<0.0001	<0.0001	99.659
TFF1	2.621	4.447	9.846	<0.0001	<0.0001	38.871
CILP	2.674	6.347	22.739	<0.0001	<0.0001	203.578
ADIPOQ	2.963	2.608	13.968	<0.0001	<0.0001	82.066
SCGB2A2	3.015	5.407	9.678	<0.0001	<0.0001	37.367
ADH1B	3.159	2.889	15.148	<0.0001	<0.0001	96.413
Down-regulation						
CA9	-1.569	-0.215	-8.594	<0.0001	<0.0001	28.187
MSLN	-1.558	-0.112	-7.896	<0.0001	<0.0001	22.787
HORMAD1	-1.360	-1.410	-8.929	<0.0001	<0.0001	30.926
A2ML1	-1.309	-0.235	-6.770	<0.0001	<0.0001	14.936
ROPN1	-1.228	-0.692	-7.644	<0.0001	<0.0001	20.937
RCOR2	-1.215	1.477	-10.594	<0.0001	<0.0001	45.825
ART3	-1.193	-1.197	-7.722	<0.0001	<0.0001	21.504
MAST1	-1.173	-0.207	-11.202	<0.0001	<0.0001	51.791
CXorf61	-1.152	-1.902	-9.282	<0.0001	<0.0001	33.903
PRAME	-1.126	1.042	-4.556	<0.0001	<0.0001	2.800

Table 2. Differently expressed miRNAs between angiogenesis-subtypes of BRCA

Gene	*logFC*	*Average expression*	*t*	*p value*	*FDR*	*B*
Up-regulation						
hsa-mir-3926-2	1.805	-1.970	7.477	<0.0001	<0.0001	19.886
hsa-mir-204	1.885	-0.608	7.216	<0.0001	<0.0001	18.065
hsa-mir-543	1.972	-1.222	9.161	<0.0001	<0.0001	32.983
hsa-mir-4501	1.973	-3.533	6.548	<0.0001	<0.0001	13.679
hsa-mir-3926-1	1.994	-2.841	7.901	<0.0001	<0.0001	22.957
hsa-mir-665	2.017	-4.045	7.775	<0.0001	<0.0001	22.028
hsa-mir-541	2.107	-3.341	8.184	<0.0001	<0.0001	25.099
hsa-mir-202	2.167	-3.011	7.788	<0.0001	<0.0001	22.122
hsa-mir-184	2.344	2.483	9.126	<0.0001	<0.0001	32.683
hsa-mir-1258	2.981	-2.098	11.895	<0.0001	<0.0001	58.916
Down-regulation						
hsa-mir-4766	-1.853	-4.009	-7.021	<0.0001	<0.0001	16.749
hsa-mir-877	-1.503	-0.454	-7.062	<0.0001	<0.0001	17.018
hsa-mir-1254-1	-1.449	-3.769	-5.512	<0.0001	<0.0001	7.668
hsa-mir-1269a	-1.388	-1.614	-3.406	0.001	0.005	-1.507
hsa-mir-105-1	-1.358	-3.351	-3.769	<0.0001	0.001	-0.224
hsa-mir-548f-1	-1.351	-6.639	-6.038	<0.0001	<0.0001	10.600
hsa-mir-4665	-1.348	-5.454	-5.356	<0.0001	<0.0001	6.845
hsa-mir-7-2	-1.314	-2.422	-5.034	<0.0001	<0.0001	5.218
hsa-mir-577	-1.289	-1.136	-4.087	<0.0001	<0.0001	1.001
hsa-mir-1229	-1.248	-3.014	-4.591	<0.0001	<0.0001	3.140

shown in Table 1 and Figure 1B. Further, a total of 109 differently expressed miRNAs were found between these two subtypes, of which 75 upregulated miRNAs and 34 downregulated miRNAs were in subtype 2 (Figure 1C and Table 2). We then used the differently expressed genes to analyze the enriched GO and KEGG pathway. As a result, 53 GO enrichments were found including extracellular matrix structural constituent conferring tensile strength, glycosaminoglycan binding, extracellular matrix structural constituent, heparin-binding, sulfur compound binding, proteoglycan binding, endopeptidase activity, collagen binding, platelet-derived growth factor binding and metalloendo

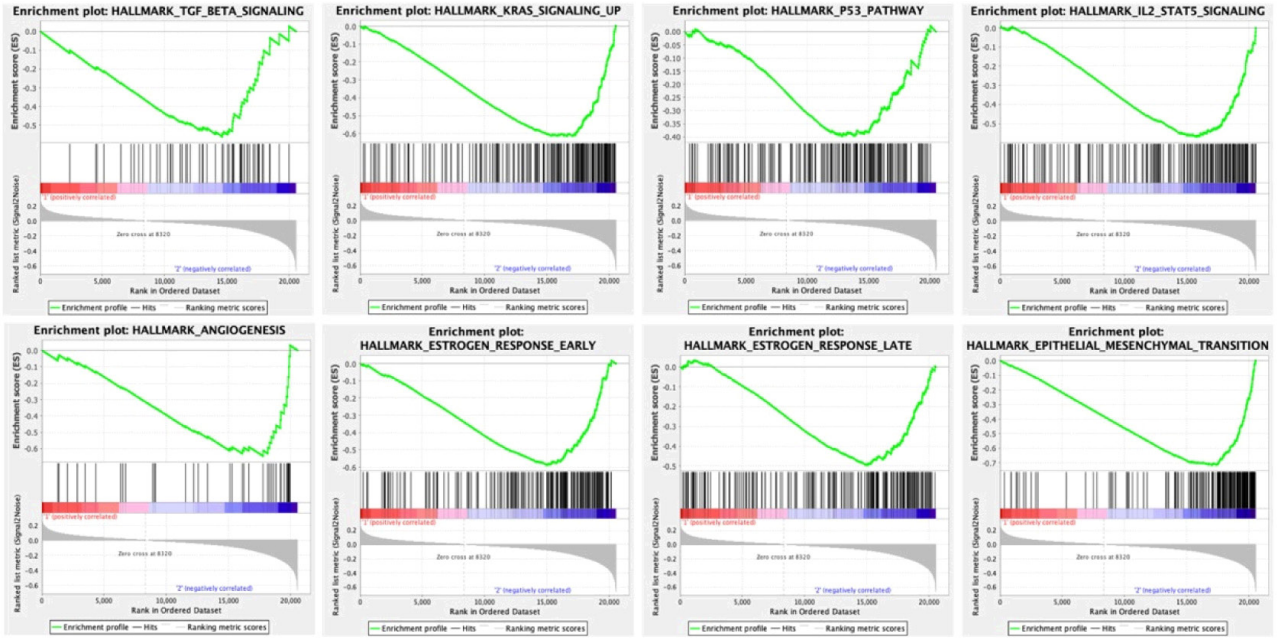

Figure 2. Gene set enrichment analysis for angiogenesis subtype of breast cancer. Top panel: Cancer-related hallmark pathway enrichment in the angiogenesis subtype; Bottom panel: Hallmark pathways enrichment-related with breast cancer progression in the angiogenesis subtype.

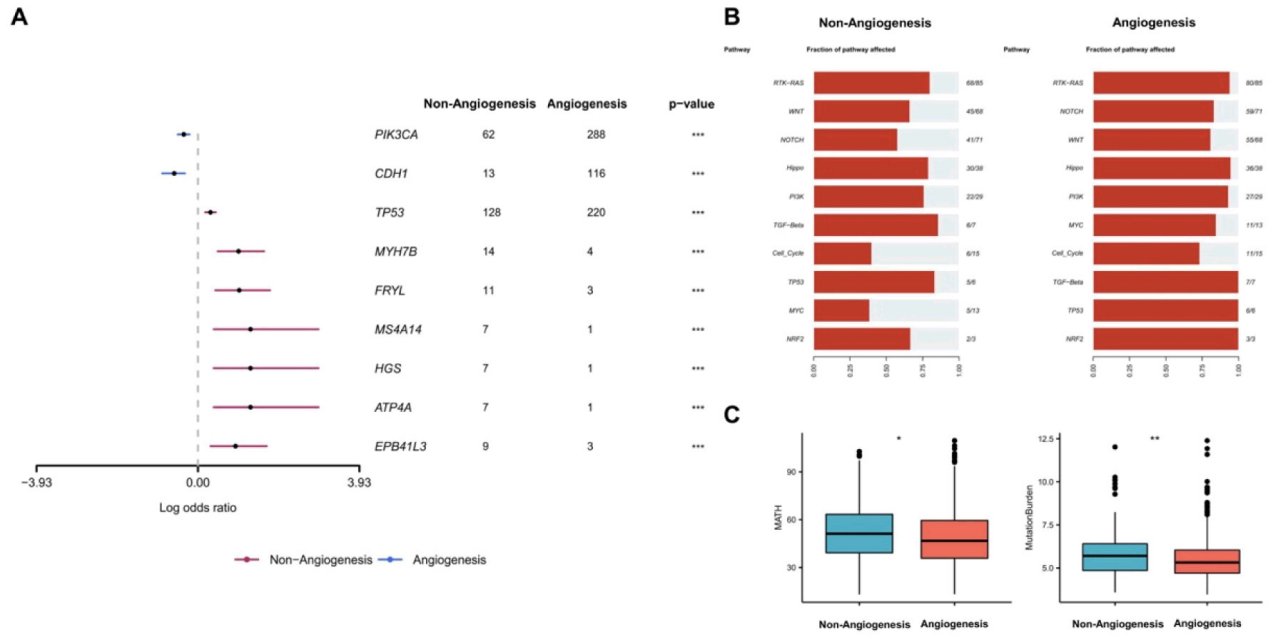

Figure 3. Somatic mutation for the angiogenesis subtype of breast cancer. **A:** The comparison of oncogenic driver genes between the non-angiogenesis subtype and angiogenesis subtype; **B:** The alteration of oncogenic pathway in the non-angiogenesis subtype and angiogenesis subtype; **C:** The heterogeneity of the mutant alleles and tumor mutation burden in the non-angiogenesis subtype and angiogenesis subtype.

peptidase activity (Figure 1D). Additionally, 20 KEEG enrichments were demonstrated to be altered by angiogenesis subtype such as PI3K-AKT signaling pathway, protein digestion and absorption, ECM-receptor interaction, focal adhesion, and malaria (Figure 1E).

GSEA for angiogenesis subtypes

Moreover, we also investigated the GSEA for these angiogenesis subtypes. Notably, 11 hallmark pathways were enriched in subtype 1 including Myc targets V1, Myc targetsv2, E2F targets, oxidative phosphorylation, G2M checkpoint, DNA repair, Mtorc1 signaling, spermatogenesis, glycolysis, mitotic spindle. Next, the most of enriched hallmark pathways in subtype 2 were related to the cancer progression such as TGF beta signaling, KRAS signaling up, p53 pathway, inflammatory response and IL2/STAT5 signaling (Figure 2 and Table 3). Notably, four pathways that were strongly associated with breast cancer progression were enriched in the subtype 2, including epithelial-mesenchymal transition, estrogen response early, estrogen response late, and especially angiogenesis. Thus, we defined subtype 2 as the angiogenesis type and subtype 1 as the non-angiogenesis type (Figure 2 and Table 3).

Molecular characteristics of the angiogenesis subtypes

As we acknowledged, the somatic mutations plays a vital role in tumor development. Firstly, we also analyzed the oncogenic driver genes in

Table 3. GSEA for Angiogenesis-subtypes of BRCA

GS details	*Size*	*ES*	*NES*	*NOM p value*	*FDR p value*	*FWER p value*	*Rank at max*	*Subtype*
Hallmark Myc Targets V1	188	0.65	2.26	0.000	0.001	0.002	6015	Non-angiogenesis
Hallmark Myc Targets V2	58	0.72	2.16	0.000	0.001	0.004	4081	Non-angiogenesis
Hallmark E2F Targets	187	0.69	1.81	0.006	0.031	0.142	3973	Non-angiogenesis
Hallmark Oxidative Phosphorylation	182	0.40	1.67	0.086	0.066	0.319	5960	Non-angiogenesis
Hallmark Unfolded Protein Response	106	0.30	1.55	0.087	0.117	0.53	4829	Non-angiogenesis
Hallmark G2M Checkpoint	184	0.57	1.53	0.078	0.106	0.554	3063	Non-angiogenesis
Hallmark DNA Repair	140	0.29	1.42	0.170	0.170	0.736	4975	Non-angiogenesis
Hallmark Mtorc1 Signaling	192	0.31	1.24	0.257	0.284	0.908	4828	Non-angiogenesis
Hallmark Spermatogenesis	132	0.27	0.92	0.596	0.620	0.988	2836	Non-angiogenesis
Hallmark Glycolysis	196	0.20	0.83	0.696	0.691	0.996	4687	Non-angiogenesis
Hallmark Mitotic Spindle	196	0.19	0.71	0.717	0.781	1	1635	Non-angiogenesis
Hallmark Apoptosis	158	-0.53	-2.09	0.000	0.040	0.017	5279	Angiogenesis
Hallmark UV Response Dn	137	-0.61	-2.04	0.000	0.026	0.021	5215	Angiogenesis
Hallmark Tgf Beta Signaling	54	-0.56	-2.04	0.000	0.018	0.022	5921	Angiogenesis
Hallmark Adipogenesis	189	-0.46	-2.02	0.000	0.016	0.027	4801	Angiogenesis
Hallmark Apical Junction	194	-0.56	-1.96	0.000	0.019	0.045	5027	Angiogenesis
Hallmark Epithelial Mesenchymal Transition	194	-0.72	-1.96	0.000	0.016	0.045	3512	Angiogenesis
Hallmark Coagulation	136	-0.61	-1.93	0.000	0.017	0.052	4201	Angiogenesis
Hallmark Androgen Response	96	-0.49	-1.85	0.006	0.030	0.092	7179	Angiogenesis
Hallmark Fatty Acid Metabolism	154	-0.45	-1.84	0.000	0.028	0.097	5294	Angiogenesis
Hallmark IL2 STAT5 Signaling	194	-0.57	-1.82	0.000	0.029	0.112	4709	Angiogenesis
Hallmark Angiogenesis	36	-0.64	-1.81	0.000	0.028	0.117	2764	Angiogenesis
Hallmark KRAS Signaling Up	193	-0.62	-1.81	0.000	0.026	0.118	3934	Angiogenesis
Hallmark Estrogen Response Early	192	-0.59	-1.81	0.004	0.024	0.124	5550	Angiogenesis
Hallmark Apical Surface	43	-0.60	-1.81	0.004	0.023	0.127	5355	Angiogenesis
Hallmark Xenobiotic Metabolism	197	-0.48	-1.73	0.002	0.042	0.233	5466	Angiogenesis
Hallmark P53 Pathway	191	-0.40	-1.69	0.017	0.049	0.286	7663	Angiogenesis
Hallmark Myogenesis	198	-0.55	-1.68	0.012	0.050	0.305	4100	Angiogenesis
Hallmark Complement	195	-0.53	-1.67	0.008	0.049	0.308	5279	Angiogenesis
Hallmark Estrogen Response Late	196	-0.50	-1.67	0.004	0.048	0.316	5572	Angiogenesis
Hallmark Inflammatory Response	197	-0.64	-1.66	0.010	0.048	0.322	4306	Angiogenesis

ES: enrichment score; FDR: false discovery rate; FWER: familywise-error rate; NES: normalized enrichment score; NOM p value: nominal p value

the angiogenesis subtype and non-angiogenesis subtype. As the results showed, six genes were revealed as the driver genes for the patients in the non-angiogenesis subtype (FANK1, PIK3CA and RGS7; FDR<0.05) and eight genes acted as the driver gene for the tumorigenesis of the patients in the angiogenesis subtype (AKT1, ATXN3, PIK3CA, C9orf3, CTSK, NAALADL1, PPP2R5B and TM9SF1; FDR<0.05, Table 4). Next, there were respectively 41 drug-genes interactions (MAP3K1, TP53, GATA3, FAT3, KMT2C, TTN, USH2A, PIK3CA, MUC16, MUC4, HMCN1 and OBSCN) in the non-angiogenesis subtype and 62 drug-genes interactions in the angiogenesis subtype (MAP3K1, TP53, GATA3, KMT2C, NCOR1, PTEN, CDH1, TTN, USH2A, DMD, PIK3CA, MUC16, HMCN1, MUC17 and RYR2, Table 5). Further, the mutation frequency of six genes (MYH7B, FRYL, MS4A14, HGS, ATP4A and EPB41L3) were significantly higher, and two genes (PIK3CA and CDH1) were lower in the non-angiogenesis subtype comparing to the angiogenesis (Figure 3A). Additionally, the ten oncogenic pathways were also analyzed based on the mutation data. This revealed that TGF-Beta pathway was the most affected in the non-angiogenesis type while TGF-Beta and TP53 pathway were found to be the most affected fraction of pathway in the angiogenesis type (Figure 3B). The results showed that MATH was significantly higher in the angiogenesis subtype and the tumor mutation burden also presented the same trend in the angiogenesis subtype (Figure 3C). The landscape of the somatic mutations is shown in Figure 4.

The immune landscape of angiogenesis subtypes

Given that the immune system exerts tumor progression activity, we next sought to explore the tumor microenvironment within the angiogenesis subtype. By using the ESTIMATE algorithm, we found that the stroma score, immune score, and ESTIMATE score were markedly lower in the angiogenesis subtype. Moreover, the tumor purity in the angiogenesis subtype was significantly lower than that in the non-angiogenesis subtype (Figure 5A). We also analyzed the expression of the 29 types of immune cells for the angiogenesis subtypes (Figure 5B). Finally, the expression of a total of 15 immune checkpoints (CTLA4, PD1, PDL1, ADORA2A, B7H3, VTCN1, BTLA, IDO, KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC7, and SIGLEC9) was compared between these two subtypes. A total of 14 of 15 immune checkpoints were expressed higher in the angiogenesis subtype except for LAG3, suggesting the immunosuppression in these patients caused by breast cancers (Figure 6).

Table 4. Oncogenic driver genes in the subtype of breast cancer

Hugo Symbol	*Frame Shift Delete*	*Frame Shift Insert*	*In Frame Delete*	*Missense Mutation*	*Nonsense Mutation*	*Splice Site*	*Translation Start Site*	*Total*	*Mutated Samples*	*Altered Samples*	*z score*	*p value*	*FDR*	*Fraction mutations in clusters*	*Cluster*
FANK1	0	0	0	5	0	0	0	5	5	5	3.557	0.000	0.007	0.800	Non-angiogenesis
PIK3CA	0	0	1	66	0	0	0	67	62	62	3.511	0.000	0.007	0.836	Non-angiogenesis
RGS7	0	0	0	4	0	1	0	5	3	3	3.060	0.001	0.023	0.600	Non-angiogenesis
AKT1	0	0	0	24	0	0	0	24	23	23	5.226	0.000	0.000	0.958	Angiogenesis
ATXN3	0	0	0	5	0	0	0	5	5	5	4.008	0.000	0.005	0.800	Angiogenesis
PIK3CA	0	0	14	312	0	0	0	326	288	288	4.042	0.000	0.005	0.929	Angiogenesis
C9orf3	0	0	0	6	0	0	0	6	4	4	2.982	0.001	0.087	0.667	Angiogenesis
CTSK	1	0	0	8	0	0	0	9	3	3	2.982	0.001	0.087	0.667	Angiogenesis
NAALADL1	4	0	0	2	0	0	0	6	4	4	2.982	0.001	0.087	0.667	Angiogenesis
PPP2R5B	0	0	0	5	1	0	0	6	4	4	2.982	0.001	0.087	0.667	Angiogenesis
TM9SF1	0	0	0	6	0	0	0	6	5	5	2.982	0.001	0.087	0.667	Angiogenesis

Clinical characteristics of the angiogenesis subtypes

As a result, the patients in the angiogenesis subtype were more likely to have lymph node metastasis than the patients in the non-angiogenesis subtype (p<0.05). Moreover, the patients in the angiogenesis subtype presented the more advanced clinical stages comparing to the patients in the non-angiogenesis subtype, suggesting the angiogenesis might increase the progression of tumors (p<0.05). Interestingly, a higher positive rate of estrogen receptor status was found in the angiogenesis subtype. Notably, progesterone receptor status and HER2 receptor status were more likely positive in the patients in the angiogenesis subtype (p<0.05, respectively; Table 6).

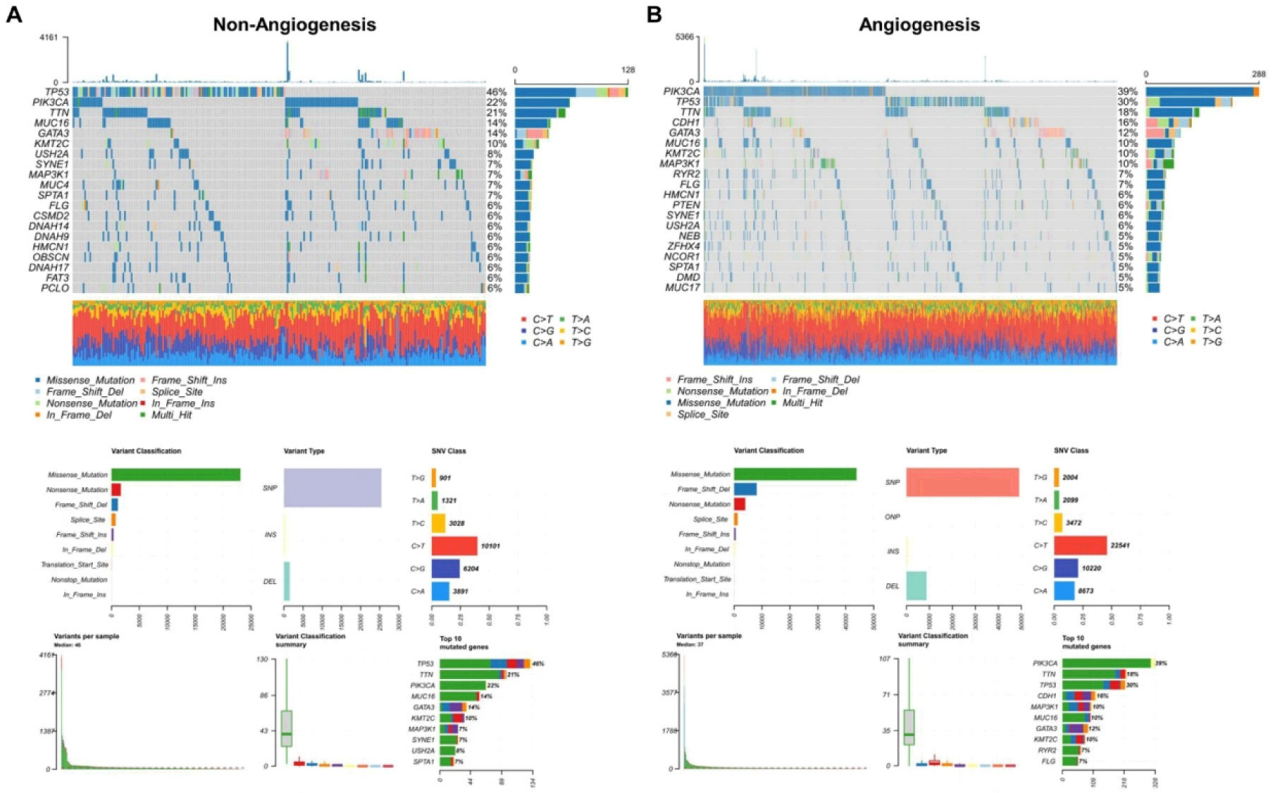

Figure 4. The mutation landscape of the angiogenesis subtype of breast cancer. **A:** Non-angiogenesis subtype; **B:** Angiogenesis subtype.

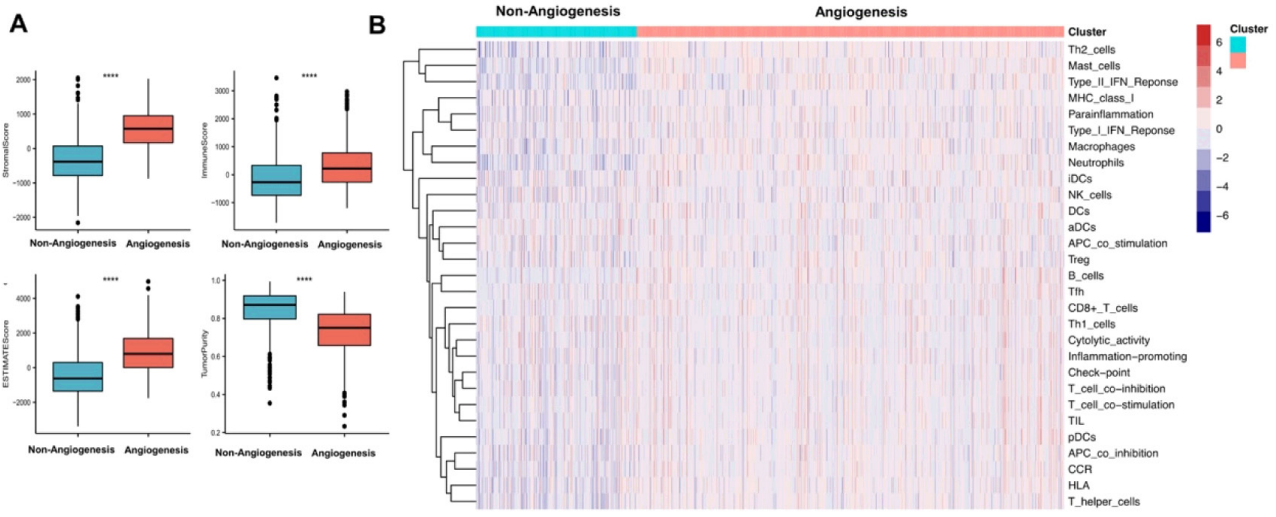

Figure 5. The tumor immune microenvironment in the angiogenesis subtype of breast cancer. **A:** The estimated score, stromal score, and immune score as well as the purity of the tumors in the two subtypes; **B:** The immune activity of different cell types in the non-angiogenesis and angiogenesis subtype of breast cancer.

Table 5. Drug-genes interactions in non-angiogenesis and angiogenesis subtype

Gene		*Category sources*	*Category*	*Subtype*
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	RussLampel, dGene, HopkinsGroom, HingoraniCasas	Druggable Genome	Non-angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	FoundationOneGenes, MskImpact	Clinically Actionable	Non-angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	dGene, GO, GuideToPharmacologyGenes	Serine Threonine Kinase	Non-angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	HopkinsGroom, GO, GuideToPharmacologyGenes	Kinase	Non-angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	GO	Tumor Suppressor	Non-angiogenesis
TP53	Tumor Protein P53	HingoraniCasas, RussLampel, HopkinsGroom	Druggable Genome	Non-angiogenesis
TP53	Tumor Protein P53	GO	Tumor Suppressor	Non-angiogenesis
TP53	Tumor Protein P53	GO	Transcription Factor Binding	Non-angiogenesis
TP53	Tumor Protein P53	GO	Rna Directed Dna Polymerase	Non-angiogenesis
TP53	Tumor Protein P53	GO	Dna Repair	Non-angiogenesis
TP53	Tumor Protein P53	GO	Histone Modification	Non-angiogenesis
TP53	Tumor Protein P53	GO	Drug Resistance	Non-angiogenesis
TP53	Tumor Protein P53	GO	Transcription Factor Complex	Non-angiogenesis
TP53	Tumor Protein P53	FoundationOneGenes, MskImpact, CarisMolecularIntelligence	Clinically Actionable	Non-angiogenesis
GATA3	Gata Binding Protein 3	FoundationOneGenes, MskImpact	Clinically Actionable	Non-angiogenesis
GATA3	Gata Binding Protein 3	GO	Tumor Suppressor	Non-angiogenesis
GATA3	Gata Binding Protein 3	GO	Transcription Factor Complex	Non-angiogenesis
GATA3	Gata Binding Protein 3	GO	Transcription Factor Binding	Non-angiogenesis
GATA3	Gata Binding Protein 3	GO	Drug Resistance	Non-angiogenesis
GATA3	Gata Binding Protein 3	GO	Histone Modification	Non-angiogenesis
FAT3	Fat Atypical Cadherin 3	HingoraniCasas	Druggable Genome	Non-angiogenesis
KMT2C	Lysine Methyltransferase 2C	GuideToPharmacologyGenes, BaderLabGenes	Methyl Transferase	Non-angiogenesis
KMT2C	Lysine Methyltransferase 2C	GO	Histone Modification	Non-angiogenesis
KMT2C	Lysine Methyltransferase 2C	MskImpact	Clinically Actionable	Non-angiogenesis
TTN	Titin	HingoraniCasas, dGene, HopkinsGroom	Druggable Genome	Non-angiogenesis
TTN	Titin	dGene, GO, GuideToPharmacologyGenes	Serine Threonine Kinase	Non-angiogenesis
TTN	Titin	GO, GuideToPharmacologyGenes, HopkinsGroom	Kinase	Non-angiogenesis
TTN	Titin	GO	Tyrosine Kinase	Non-angiogenesis
USH2A	Usherin	HingoraniCasas	Druggable Genome	Non-angiogenesis

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Gene		*Category sources*	*Category*	*Subtype*
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	RussLampel, dGene, HopkinsGroom, HingoraniCasas	Druggable Genome	Non-angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GO	Serine Threonine Kinase	Non-angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GO, GuideToPharmacologyGenes	Kinase	Non-angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	FoundationOneGenes, CarisMolecularIntelligence, MskImpact	Clinically Actionable	Non-angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GuideToPharmacologyGenes, dGene, HopkinsGroom	Phosphatidylinositol 3 Kinase	Non-angiogenesis
MUC16	Mucin 16, Cell Surface Associated	HingoraniCasas	Druggable Genome	Non-angiogenesis
MUC4	Mucin 4, Cell Surface Associated	HingoraniCasas	Druggable Genome	Non-angiogenesis
HMCN1	Hemicentin 1	HingoraniCasas	Druggable Genome	Non-angiogenesis
HMCN1	Hemicentin 1	GO	Cell Surface	Non-angiogenesis
OBSCN	Obscurin, Cytoskeletal Calmodulin And Titin-Interacting Rhogef	HopkinsGroom, GO, GuideToPharmacologyGenes	Kinase	Non-angiogenesis
OBSCN	Obscurin, Cytoskeletal Calmodulin And Titin-Interacting Rhogef	HopkinsGroom, HingoraniCasas, dGene	Druggable Genome	Non-angiogenesis
OBSCN	Obscurin, Cytoskeletal Calmodulin And Titin-Interacting Rhogef	GO, GuideToPharmacologyGenes, dGene	Serine Threonine Kinase	Non-angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	RussLampel, dGene, HopkinsGroom, HingoraniCasas	Druggable Genome	Angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	FoundationOneGenes, MskImpact	Clinically Actionable	Angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	dGene, GO, GuideToPharmacologyGenes	Serine Threonine Kinase	Angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	HopkinsGroom, GO, GuideToPharmacologyGenes	Kinase	Angiogenesis
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1	GO	Tumor Suppressor	Angiogenesis
TP53	Tumor Protein P53	HingoraniCasas, RussLampel, HopkinsGroom	Druggable Genome	Angiogenesis
TP53	Tumor Protein P53	GO	Tumor Suppressor	Angiogenesis
TP53	Tumor Protein P53	GO	Transcription Factor Binding	Angiogenesis
TP53	Tumor Protein P53	GO	Rna Directed Dna Polymerase	Angiogenesis

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Gene		*Category sources*	*Category*	*Subtype*
TP53	Tumor Protein P53	GO	Dna Repair	Angiogenesis
TP53	Tumor Protein P53	GO	Histone Modification	Angiogenesis
TP53	Tumor Protein P53	GO	Drug Resistance	Angiogenesis
TP53	Tumor Protein P53	GO	Transcription Factor Complex	Angiogenesis
TP53	Tumor Protein P53	FoundationOneGenes, MskImpact, CarisMolecularIntelligence	Clinically Actionable	Angiogenesis
GATA3	Gata Binding Protein 3	FoundationOneGenes, MskImpact	Clinically Actionable	Angiogenesis
GATA3	Gata Binding Protein 3	GO	Tumor Suppressor	Angiogenesis
GATA3	Gata Binding Protein 3	GO	Transcription Factor Complex	Angiogenesis
GATA3	Gata Binding Protein 3	GO	Transcription Factor Binding	Angiogenesis
GATA3	Gata Binding Protein 3	GO	Drug Resistance	Angiogenesis
GATA3	Gata Binding Protein 3	GO	Histone Modification	Angiogenesis
KMT2C	Lysine Methyltransferase 2C	GuideToPharmacologyGenes, BaderLabGenes	Methyl Transferase	Angiogenesis
KMT2C	Lysine Methyltransferase 2C	GO	Histone Modification	Angiogenesis
KMT2C	Lysine Methyltransferase 2C	MskImpact	Clinically Actionable	Angiogenesis
NCOR1	Nuclear Receptor Corepressor 1	MskImpact	Clinically Actionable	Angiogenesis
NCOR1	Nuclear Receptor Corepressor 1	GO	Transcription Factor Binding	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	MskImpact, FoundationOneGenes, CarisMolecularIntelligence	Clinically Actionable	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	dGene	Druggable Genome	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	dGene	Pten Family	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Tumor Suppressor	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Serine Threonine Kinase	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Drug Resistance	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Transporter	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Protein Phosphatase	Angiogenesis
PTEN	Phosphatase And Tensin Homolog	GO	Kinase	Angiogenesis
CDH1	Cadherin 1	HingoraniCasas	Druggable Genome	Angiogenesis
CDH1	Cadherin 1	FoundationOneGenes, MskImpact, CarisMolecularIntelligence	Clinically Actionable	Angiogenesis
CDH1	Cadherin 1	GO	Drug Resistance	Angiogenesis

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Gene		*Category sources*	*Category*	*Subtype*
TTN	Titin	HingoraniCasas, dGene, HopkinsGroom	Druggable Genome	Angiogenesis
TTN	Titin	dGene, GO, GuideToPharmacologyGenes	Serine Threonine Kinase	Angiogenesis
TTN	Titin	GO, GuideToPharmacologyGenes, HopkinsGroom	Kinase	Angiogenesis
TTN	Titin	GO	Tyrosine Kinase	Angiogenesis
USH2A	Usherin	HingoraniCasas	Druggable Genome	Angiogenesis
DMD	Dystrophin	GO	Transporter	Angiogenesis
DMD	Dystrophin	GO	Cell Surface	Angiogenesis
DMD	Dystrophin	GO	Ion Channel	Angiogenesis
DMD	Dystrophin	HingoraniCasas	Druggable Genome	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	RussLampel, dGene, HopkinsGroom, HingoraniCasas	Druggable Genome	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GO	Serine Threonine Kinase	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GO, GuideToPharmacologyGenes	Kinase	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	FoundationOneGenes, CarisMolecularIntelligence, MskImpact	Clinically Actionable	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	GuideToPharmacologyGenes, dGene, HopkinsGroom	Phosphatidylinositol 3 Kinase	Angiogenesis
MUC16	Mucin 16, Cell Surface Associated	HingoraniCasas	Druggable Genome	Angiogenesis
HMCN1	Hemicentin 1	HingoraniCasas	Druggable Genome	Angiogenesis
HMCN1	Hemicentin 1	GO	Cell Surface	Angiogenesis
MUC17	Mucin 17, Cell Surface Associated	GO	Cell Surface	Angiogenesis
MUC17	Mucin 17, Cell Surface Associated	GO	External Side Of Plasma Membrane	Angiogenesis
MUC17	Mucin 17, Cell Surface Associated	HingoraniCasas	Druggable Genome	Angiogenesis
RYR2	Ryanodine Receptor 2	RussLampel, HingoraniCasas, HopkinsGroom	Druggable Genome	Angiogenesis
RYR2	Ryanodine Receptor 2	BaderLabGenes, GuideToPharmacologyGenes, HopkinsGroom, GO	Ion Channel	Angiogenesis
RYR2	Ryanodine Receptor 2	GuideToPharmacologyGenes, GO	Transporter	Angiogenesis
RYR2	Ryanodine Receptor 2	GuideToPharmacologyGenes, HopkinsGroom	B30_2 Spry Domain	Angiogenesis
RYR2	Ryanodine Receptor 2	GO	Abc Transporter	Angiogenesis

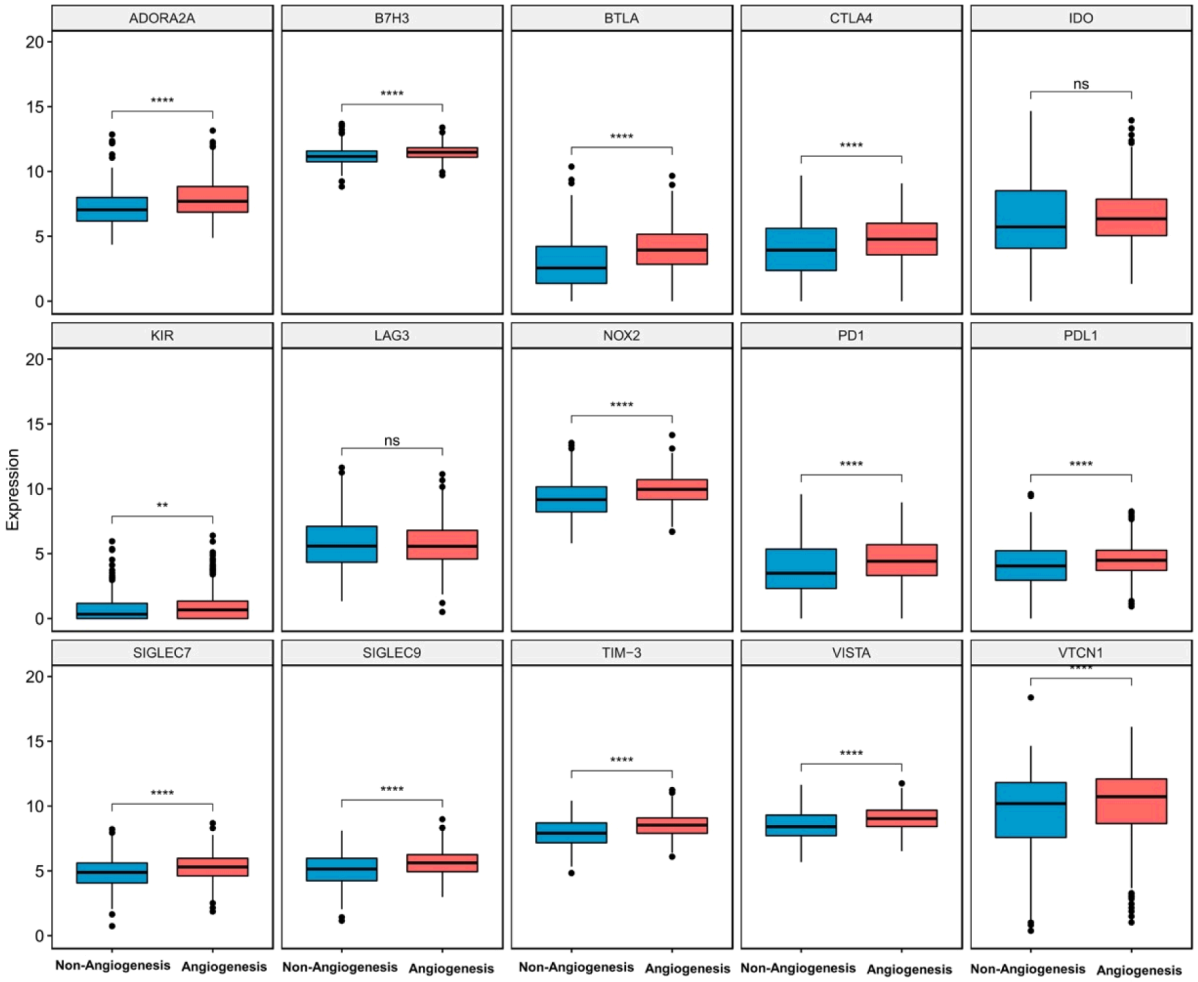

Figure 6. Expression levels for the immune checkpoints in the non-angiogenesis and angiogenesis subtype of breast cancer. Expression of immune checkpoints (CTLA4, PD1, PDL1, ADORA2A, B7H3, VTCN1, BTLA, IDO, KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC7, and SIGLEC9) was compared between these two subtypes.

Table 6. Association between Angiogenesis-subtype and clinical features of BRCA

Variables	*Group*	*Cluster 1*	*Cluster 2*	x^2	*p*
Age		59.61±13.398	57.51±13.112	2.014	0.044*
	Positive	32	152		
M	Negative	243	661	0.978	0.323
	Positive	8	14		
N	Negative	169	345	15.991	<0.001
	Positive	123	436		
T	I~II	251	662	0.204	0.651
	III~IV	46	132		
Clinical stage	I~II	242	560	12.953	<0.001
	III~IV	51	219		
Cancer status	With tumor	247	685	0.463	0.496
	Tumor free	37	89		
Estrogen receptor status	Negative	110	128	59.827	<0.001
	Positive	169	637		
Progesterone receptor status	Negative	147	197	67.428	<0.001
	Positive	131	566		
HER2 receptor status	Negative	203	504	9.637	0.002

*P value by Student's t-test

Discussion

At present, breast cancer ranks first in malignant tumors in females. Despite the remarkable progress made in early diagnosis, surgical resection, local and systemic adjuvant therapy, the mortality of breast cancer remains high. Distant metastasis is considered to be the leading cause of death in breast cancer patients [22]. What's more, angiogenesis plays a vital role in the progression of breast cancer. Therefore, targeted drugs on angiogenesis genes and pathways have become a research hotspot [23].

This study established angiogenesis-related subtypes of breast cancer, and uncovered the immune microenvironment and genomic alteration of angiogenesis-related subtypes. Using bioinformatics tools, we screened out differentially expressed genes in angiogenesis subtypes of breast cancer. Atypical chemokine receptor 1(ACKR1) is a highly promiscuous receptor, which binds plenty of chemokines from the CXC and CC subfamilies, primarily the inflammatory subgroups [24,25]. ACKR1 reduces the utilization rate of ELR and CXC chemokines through constitutive expression of venus endothelial cells, thereby promoting angiogenesis [25,26]. Adiponectin (ADIPOQ) is an endogenous bioactive peptide or protein secreted by adipocytes, which has the effects of anti-inflammation, anti-atherosclerosis and insulin sensitivity [27]. Other studies have shown that ADIPOQ induces autophagosome aggregation in breast cancer cells through STK11/LKB1-mediated AMPK-ULK1 axis [28]. Therefore, the high expression of ADIPOQ may have the effect of preventing breast cancer progression and metastasis [29]. In the study of Srishti Singh et al [30], the overexpression of transmembrane proteins CAIX and CAXII in tumor tissues was related to tumor proliferation, invasion, and increased chemotherapy resistance. Some studies have shown that PRAME antigen is widely expressed in breast cancer subtypes, which is considered to be associated with undesirable clinical outcomes. Combined with clinical parameters, it can be used as an indicator of diagnosis and prognosis of breast cancer [31,32]. The expression level of PRAME is related to negative estrogen receptor, decreased overall survival rate and increased distant metastasis rate [33]. Conversely, according to Sun et al [34], targeted knockout of the PRAME gene can reduce the expression of E-cadherin, thereby enhancing the migration and invasion of breast cancer cells. Therefore, the function of PRAME in breast cancer needs intensive study. EMT is a key driving factor for malignant tumor cells to acquire migration and invasion capabilities [35]. Epithelial cells lose their polarity, lose the connection with the basement membrane, change from epithelial phenotype to mesenchyme phenotype, and express mesenchymal markers such as FSP-1, α-SMA and vimentin, which lead to tumor metastasis [36]. It has been reported that MYC, as a transcription factor, is overexpressed in tumor tissues [37]. MYC collaborate with other transcription factors to regulate specific genes expression and controls the non-coding RNA network, which leads to tumor angiogenesis, immune escape, invasion and migration [38]. As a classic signaling pathway, PI3K/Akt/mTOR participates in inhibiting tumor cell apoptosis, regulating cell cycle, and promoting tumor angiogenesis [39]. A previous research has shown that the PI3K/AKT/mTOR signaling pathway is associated with breast cancer [40]. However, due to the problem of drug resistance, PI3K-AKT-mTOR pathway inhibitors are still an exciting research hotspot in the treatment of breast cancer. Consequently, these transcription factors (TFs) related to breast cancer angiogenesis can be used as new therapeutic targets.

It has been reported that the tumor microenvironment (TME) has an essential influence on gene expression in tumor tissues, thereby affecting clinical characteristics and prognosis [41]. The comprehension analysis of angiogenesis-related subtypes and TME may provide new insights for studying the molecular mechanisms of breast cancer and provide methods for improving patient immunotherapy [42]. Next, we analyzed the immune microenvironment of two angiogenesis-related subtypes, including immune index, interstitial index, tumor purity and immune cell distribution. Due to the low mutation burden and low immunogenicity of breast cancer, coupled with the heterogeneity of tumors, although the FDA has approved immunosuppressants for breast cancer, the proportion of patients who benefit from single-agent immunosuppressants is low, so it is urgent to combine immunosuppressants with new drugs.

Author contributions

HZ and ZM performed data analyses and helped prepare the manuscript. WC provided study materials. XP, FQ, and WC conceived the research, determined the appropriate analyses to be performed and wrote the manuscript. JZ designed this study. All the authors read and approved the final manuscript.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Wong IO, Schooling C, Cowling BJ, Leung GM. (2015) Breast cancer incidence and mortality in a transitioning Chinese population: current and future trends. Br J Cancer 2015;112:167-70.
2. Fan L, Strasser-Weippl K., Li JJ et al. Breast Cancer in China. Lancet Oncol 2014;15:e279-e289.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
4. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182-6.
5. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376:66-70.
6. Shalaby F, Rossant J, Yamaguchi TP et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995;376:62-6.
7. Horak E.R, Leek R, Klenk N et al. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer Lancet 1992; 340: 1120-4.
8. Vartanian RK, Weidner N. Correlation of intratumoral endothelial cell proliferation with microvessel density (tumor angiogenesis) and tumor cell proliferation in breast carcinoma. Am J Pathol 1994;144:1188-94.
9. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med 1991;324:s1-8.
10. Lyman GH, Burstein HJ, Buzdar AU, D'Agostino R, Ellis PA. Making genuine progress against metastatic breast cancer. J Clin Oncol 2012;;30:3448-51.
11. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. Mol Cancer 2019;18:60.
12. Hilmi M, Neuzillet C, Calderaro J et al. Angiogenesis and immune checkpoint inhibitors as therapies for hepatocellular carcinoma: current knowledge and future research directions. J Immunother Cancer 2019;7:333.
13. Schmidt EV. Developing combination strategies using PD-1 checkpoint inhibitors to treat cancer. Semin Immunopathol 2019;41:21-30.
14. Manegold C, Dingemans AM, Gray C et al. The Potential of Combined Immunotherapy and Antiangiogenesis for the Synergistic Treatment of Advanced NSCLC. J Thorac Oncol 2017;12: 194-207.
15. Lawrence M, Stojanov P, Polak P et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499:214-8.
16. Chalmers ZR, Connelly CF, Fabrizio D et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:s34.
17. Zhao Y, Pu C, Liu, Z. Exploration the Significance of a Novel Immune-Related Gene Signature in Prognosis and Immune Microenvironment of Breast Cancer. Front Oncol 2020;10:1211.
18. Luen, Virassamy B, Savas, P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. Breast 2016;29:s241-50.
19. Takada K, Kashiwagi S, Goto W et al. Significance of re-biopsy for recurrent breast cancer in the immune tumour microenvironment. Br J Cancer 2018;119:572-9.
20. Xiao Y, Ma D, Zhao S et al. Multi-Omics Profiling Reveals Distinct Microenvironment Characterization and Suggests Immune Escape Mechanisms of Triple-Negative Breast Cancer. Clin Cancer Res 2019;25:5002-14.
21. Yoshihara K, Shahmoradgoli M, Martínez E et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun 2013;4:2612.
22. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 2006;12:895-904.
23. Linderholm BK, Hellborg H, Johansson U et al. Significantly higher levels of vascular endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Ann Oncol 2009;20:1639-46.
24. Bachelerie F, Ben-Baruch A, Burkhardt AM et al. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev 2014;66:1-79.
25. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and Chemokine Receptors: New Targets for Cancer Immunotherapy. Front Immunol 2019;10:379.
26. Massara M, Bonavita O, Mantovani A, Locati M, Bonecchi R. Atypical chemokine receptors in cancer: friends or foes? J Leukoc Biol 2016; 99:927-33.
27. Dos Santos E, Benaitreau D, Dieudonne MN et al. Adiponectin mediates an antiproliferative response in human MDA-MB 231 breast cancer cells. Oncol Rep 2008;20:971-7.
28. Chung SJ, Nagaraju GP, Nagalingam A et al. ADIPOQ/adiponectin induces cytotoxic autophagy in breast cancer cells through STK11/LKB1-mediated activation of the AMPK-ULK1 axis. Autophagy 2017;13:1386-403.
29. Delort L, Jardé T, Dubois V, Vasson MP, Caldefie-Chézet F. New insights into anticarcinogenic properties of adiponectin: a potential therapeutic approach in breast cancer? Vitam Horm 2012;90:397-417.
30. Singh S, Lomelino CL, Mboge MY, Frost SC, McKenna, R. Cancer Drug Development of Carbonic Anhydrase Inhibitors beyond the Active Site Molecules 2018;23:1045.
31. Tessari A, Pilla L, Silvia D et al. Expression of NY-ESO-1, MAGE-A3, PRAME and WT1 in different subgroups of breast cancer: An indication to immunotherapy? Breast 2018;42:68-73.
32. Doolan P, Clynes M, Kennedy S, Mehta JP, Crown J, O'Driscoll L. Prevalence and prognostic and predictive relevance of PRAME in breast cancer. Breast Cancer Res Treat 2008;109:359-65.
33. Epping MT, Hart AAM, Glas, AM, Krijgsman O, Bernards R. PRAME expression and clinical outcome of breast cancer. Br J Cancer 2008;99:398-403.
34. Sun Z, Wu Z, Zhang F et al. PRAME is critical for breast cancer growth and metastasis. Gene 2016;594:160-4.

35. Ye X, Weinberg RA. Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression. Trends Cell Biol 2015;25:675-86.
36. Škovierová H, Okajčeková T, Strnádel J, Vidomanová E, Halašová E. Molecular regulation of epithelial-to-mesenchymal transition in tumorigenesis (Review). Int J Mol Med 2018;41:1187-200.
37. Schulze A, Oshi M, Endo I, Takabe K. MYC Targets Scores Are Associated with Cancer Aggressiveness and Poor Survival in ER-Positive Primary and Metastatic Breast Cancer. Int J Mol Sci 2020;S21:8127.
38. Meškytė EM, Keskas S, Ciribilli Y. MYC as a Multifaceted Regulator of Tumor Microenvironment Leading to Metastasis. Int J Mol Sci 2020;21:7710.
39. Garcia-Echeverria CS, Sellers WR. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. Oncogene 2008;27:5511-26.
40. McKenna M, McGarrigle S, Pidgeon GP. The next generation of PI3K-Akt-mTOR pathway inhibitors in breast cancer cohorts. Biochim Biophys Acta Rev Cancer 2018;1870:185-97.
41. Şenbabaoğlu Y, Gejman RS, Winer AG et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. Genome Biol 2016;17:231.
42. Wang S, Zhang Q, Yu C, Cao Y, Zuo Y, Yang L. Immune cell infiltration-based signature for prognosis and immunogenomic analysis in breast cancer. Brief Bioinform 2021;22:22.