Original Article

Angiogenesis related gene expression significantly associated with the prognostic role of an urothelial bladder carcinoma

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Background: Bladder urothelial carcinoma (BLCA) is still one of the most malignant diseases and has a dismal outcome. Angiogenesis has confirmed its critical role in the development of malignant neoplasms. In this study, we uncovered the prognostic implications of the angiogenesis-related gene panel in urothelial tumors.

Methods: The RNA-seq data and clinical records of 402 patients with BLCA were collected from the TCGA database. The panel, including 145 genes involved in angiogenesis, was retrieved from the Uniprot database and the published work. The patients with similar expressed profiles were clustered, and the differences in gene expression were compared. The correlation of gene expression and BLCA outcomes or clinical features were analyzed.

Results: There were two clusters of BLCA patients identified on the expressed basis of angiogenesis-related genes. A significant difference was detected in the tumor stages between the two clusters (P<0.001) and a striking advantaged prognosis shown in cluster_1 (86.83 *vs.* 27.06 months, P=0.001). According to statistics, 115 genes showed a discrepancy in expression between the two clusters, and 16 genes positively correlated to tumor stage progression. Separately analyzed the correlation of those stage-related genes and overall survivals (OS) revealed that high expression of 8 genes, including ECM1 (HR =1.72, P<0.001), FN1 (HR =1.564, P=0.004), FGF1 (HR =1.519, P=0.005), FAP (HR =1.449, P=0.020), JAM3 (HR =1.396, P=0.026), THBS1 (HR =1.402, P=0.028), MFGE8 (HR =1.394, P=0.028) and COL8A2 (HR =1.388, P=0.035), were showed worse prognosis of BLCA, respectively.

Conclusions: This study showed an integrated profile of angiogenesis-related genes and identified the different BLCA subgroups with favorable prognosis and poor prognosis depended on the expression pattern of angiogenesis-related genes. Furthermore, this work revealed the single gene expressions of ECM1, FN1, FGF1, FAP, JAM3, THBS1, MFGE8 and COL8A2 involved in angiogenesis associated the prognosis remarkably.

Keywords: Bladder urothelial carcinoma (BLCA); angiogenesis; gene expression profile; prognosis

Submitted Aug 07, 2020. Accepted for publication Oct 09, 2020.
doi: 10.21037/tau-20-1291
View this article at: http://dx.doi.org/10.21037/tau-20-1291

Introduction

Bladder cancer has been identified as the most common genitourinary malignancy with high morbidity and mortality worldwide (1). Radical cystectomy with bilateral pelvic lymph node dissection is the gold-standard treatment for early bladder cancer (2). The cisplatin-based chemotherapy established from the 1970s as well as combined treatment of surgery, chemotherapy, and radiotherapy notably prolonged the bladder cancer survival in decades (3,4). However, the overall 5-year survival rate of bladder cancer remains dismal and less than 20% (5). To understand the molecular mechanisms of bladder carcinoma, previous research had identified multiple gene variations in TP53, MLL2, KRT14, KRT5, KRT6A, PPARG, E2F3, EGFR, FGFR3 and so on (6).Few molecularly targeted anticancer agents have been available to treat complex diseases (2). The major pathological types of bladder cancers are bladder urothelial carcinoma (BLAC), of which muscle-invasive type shows an aggressive manifestation and dismal prognosis compared to non-muscle invasive type (3). In muscle-invasive bladder cancer, it has been reported that KLK6, TNS1, TRIM56, IL1B, S100A8, S100A9 and EGFR were capable to predict the progression (7,8). BLCA is a highly heterogeneous disease caused by a genome harboring various genetic variations involving in somatic mutations and substantial chromosomal abnormalities or discrepancies in the functional gene expression (9,10). Hence, identification of effective subtypes with molecular features and developing available target drugs in BLCA is still a challenge.

Angiogenesis has known as clinicopathological factors as well as characteristics of biological aggressiveness in bladder cancer (11). It has well known that angiogenesis promoted the tumor extracellular milieu according to provided oxygen, nutrients and growth factors to the carcinoma cells (12). Molecules involving angiogenesis are commonly detected and the gene expression of VEGF, bFGF and TSP-1 and MVDCD31 were correlated to urothelial cancer prognosis (10). Previous work showed that the angiogenesis related genes expression tremendously varied in BLCA (13).In an earlier study, thrombospondin 1 and primary fibroblast growth factors were reported as an independent prognostic factor of BLCA (10). Multiple agents targeting the VEGF (vascular endothelial growth factor) pathway have been studied in several clinical trials in advanced urothelial cancer to suppress aggressiveness (14-16). The earliest evidence to indicate angiogenesis related genes as promising therapeutic targets approved from a randomized phase 2 trial revealing combination of ramucirumab and docetaxel could prolong PFS in patients with advanced disease (17). However, the clinical benefit has been limited to only some patients (14). Attributed to a complex biological process and multimolecular involved hallmark, the comprehensive observation of angiogenesis-related genes changing is crucial for BLCA management.

There have been many studies on how angiogenesis-related markers expression associated with prognosis in BLCA patients (10,11,18,19). A previous article studied the expression level of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), and indicated that bFGF and IL-8 expression is crucial in the regulation of angiogenesis at the early stage of tumor growth, progressing and subsequent metastases of bladder cancer (20). However, global analysis of angiogenesis-related gene profiles has not yet been performed for BLCA. Tumor angiogenesis plays a vital role in the multiple biological processes including formation of new blood vessel in the primary and distant metastatic tumor, promoting tumor growth according to supplying sufficient energy sources to cancer cells and facilitating tumor cells metastasize to distant sites (21,22). To systematically target tumor angiogenesis, genes coding particular proteins involved in angiogenesis, the sprouting or splitting of capillaries from pre-existing vasculature, should be taken into consideration (23). Keywords of angiogenesis on the Uniprot database provided a superior gene set to search the completed genes related to angiogenesis (24-26). Therefore, we focused on the expression profile of angiogenesis that involves genes in the RNA-seq data of BLCAs, of which hubs are potentially explored to develop agents targeting tumor angiogenesis as an efficient approach for BLCA therapy in combination with the conventional treatment .

In this study, transcriptome sequencing data in 402 BLCAs were assessed, and the association of expression of angiogenesis involved genes and clinical outcomes was investigated. We uncovered an expression profile of angiogenesis involving genes and the correlation of the gene set and the individuals to clinical outcomes in BLCA.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/tau-20-1291).

Methods

Samples

The RNA sequencing data and the corresponding clinical record of 402 patients with BLCA from cbioportal were downloaded (cbioportal.org, a TCGA data platform) (27,28). We filter the data with whether the mRNA expression values histological diagnosis results and survival data are comprehensive. All those patients were pathologically diagnosed as muscle-invasive urothelial carcinoma. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene set construction

Angiogenesis related genes in the assessed panel were collected and arranged from the previous articles and Uniport-keyword database (KW0037, uniport.org/keywords), which has published work mentioned (24,29). One hundred forty-six genes were integrated into a gene set, and the corresponding expression levels were shown as mRNA z-score data. Then the genes were listed; among those data, transcriptome information of gene APELA was missing in the studied population. One hundred forty-five genes involved in angiogenesis were finally included. The interrelated gene expression and the single gene expression of distinct groups were compared to detect the correlation for clinical features of BLCA with TCGA data.

Bioinformatics

A cluster of the 145 genes expressional value in patients with muscle-invasive type BLCA was assessed to distinguish samples with gene expression patterns. The subjects with different gene expression patterns were identified from the entire data set. The transcriptional levels of contained genes were shown as mRNA z-scores and grouped using the hierarchical clustering algorithm by the Gene Cluster 3.0 program (30). The heat maps and cluster overview according to the genes variations were generated with the Java Treeview program (31).

Prognostic implication analysis

To assess the prognostic role of the cancer angiogenesis-related genes, we used GraphPad Prism version 7.0 (GraphPad Software, Inc., CA, USA) to compare the survival between the different clusters. The correlation of gene expression and outcomes were investigated to detect the difference in median survival between the subgroups with low and high gene expressional levels.

Statistical analysis

Survival curves were plotted according to the Kaplan-Meier method and compared using the log-rank test in GraphPad Prism version 7.0. The correlations of clinical characteristics and the variables to determine the clusters of patients were evaluated using Fisher's exact test and the Pearson correlation analysis. The different gene expression between clusters were assessed using ANOVA. The regression analysis determines correlations between variables. All tests were performed with SPSS version 24.0 (IBM, Inc., New York, USA). A P value less than 0.05 was considered statistically significant.

Results

The expression profile of genes involved in angiogenesis dramatically heterogeneous in BLCA

To investigate angiogenesis-related gene variation in BLCA, we grouped the patients with similar transcriptional angiogenesis genes. One hundred forty-five protein-coding genes involved in the sprouting or splitting of capillaries from pre-existing vasculature were included in the array in total online: https://cdn.amegroups.cn/static/application/791eea1171cf823cc20cd235ca04dc77/tau-20-1291-1.pdf. Differences in the gene expression sorted the patients with muscle invasion type BLCA according to the mRNA expression level. According to filtration, 402 patients with survival records were recruited. There were 150 patients in the first group defined as cluster_1 and the 252 patients in another group defined as cluster_2 primary divided from the entire population (*Figure 1A*). A comparison of the clinical characters regarding the two clusters revealed there was no difference in terms of age and sex (P>0.05, *Table 1*). However, a considerable discrepancy was shown in the AJCC stage (P<0.001). After analyzing the detailed pathological stage, we detected that cluster_1 tended to be with an earlier stage in M stage (P<0.001), N stage (P<0.05), and T stage (P<0.001) compared to cluster_2 (*Table 1*).

Angiogenesis related gene variation associated with prognosis in BLCA

To compare the survival between the two clusters, we

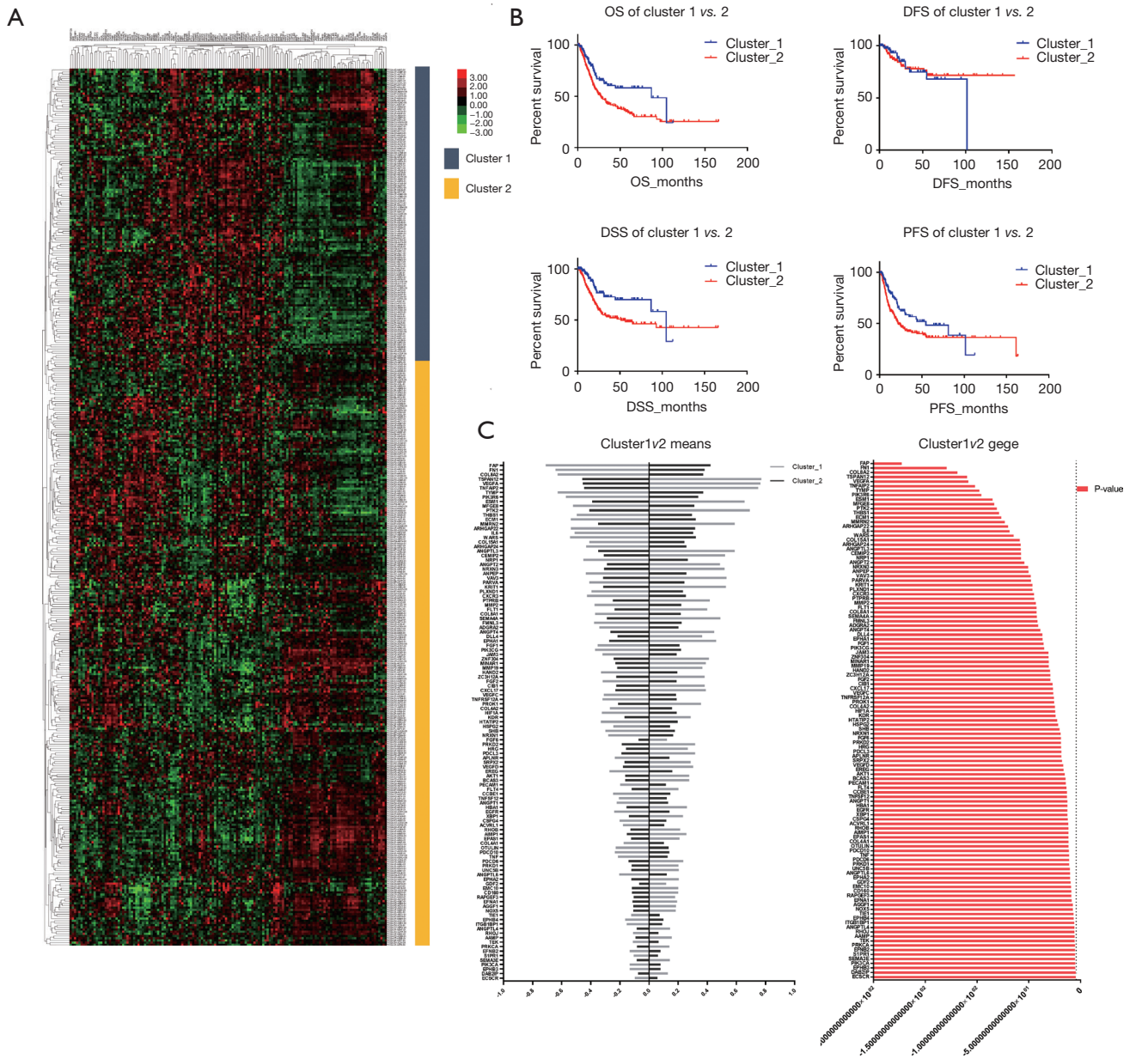

Figure 1 There were two clusters identified in the bladder urothelial carcinoma (BLCA) according to angiogenesis related gene profile. (A) the entire cohort divided into cluster_1 and the cluster_2 basing on genes expression; (B) the patients in cluster_1 showed a favorable prognosis in the terms of overall survival (OS), progression-free survival (PFS), disease-free survival (DFS) and disease-specific survival (DSS); (C) angiogenesis related genes were differently expressed between the two clusters.

assess overall survival (OS), progression-free survival (PFS), disease-free survival (DFS) and disease-specific survival (DSS) of each cluster (*Table 2*). The statistic showed cluster_1 was with a prolonged prognosis than the patients in cluster_2 regarding OS (86.83 *vs.* 27.06 months, P=0.001), DSS (104.6 *vs.* 51.16 months, P=0.001) and PFS (54.74 *vs.* 21.86 months, P=0.006) (*Table 2*, *Figure 1B*). Compared to cluster_2, patients with the expression pattern as cluster_1 tend to have a significantly better outcome (HR =0.5756, 0.5007, and 0.6304 in the OS, DSS, and PFS, respectively). We compared the expression of angiogenesis-related genes between the two clusters and detected the

Table 1 The clinical features of each cluster

Clinical features	Cluster_1 (n=150)	Cluster_2 (n=251)	P value
Age	67.16667	68.72112	0.208864
Sex			0.241233
Male	116	180	
Female	34	71	
AJCC stage			<0.001***
Stage I	2	0	
Stage II	72	56	
Stage III	39	99	
Stage IV	35	96	
NA[&]	2	0	
Path M stage			<0.001***
M0	93	98	
M1	2	9	
MX	54	143	
NA[&]	1	1	
Path N stage			0.045*
N0	97	136	
N1	8	37	
N2	24	50	
N3	2	5	
N4	16	20	
NA[&]	3	3	
Path T stage			<0.001***
T0	1	0	
T1	2	1	
T2	63	54	
T3	51	140	
T4	16	41	
TX	1	0	
NA[&]	16	15	

[&], represented data deficient; *, P<0.05; ***, P<0.001.

notable difference in the expression levels (P<0.05) of 115 genes (*Figure 1C*). Among those distinguishing genes, FAP, FN1, and COL8A2 showed the most significant differences (*Table S1*).

Variations in angiogenesis-related genes implicate tumor stage progression in BLCA

In the earlier data, we have detected that the clusters with a different distribution of angiogenesis are shown significantly in the tumor stage. To further investigate the implication of gene expression and tumor stage progression, we conducted regression analysis (*Figure 2*). There are 16 genes correlated to tumor stages in the BLCA with tremendously statistical significance (*Table 3*). Gene FN1 was with the strongest correlation to the BLCA tumor stage (r=0.34, P<0.001). Its expression was available to predict stage progression (AUC =0.720, 95% CI: 0.666–0.775, P<0.001) (*Figure 3A*). Sixteen genes contained a model set up to optimize the predictive performance, and a superior efficacy was shown in the stage's prediction progression (AUC =0.834, 95% CI: 0.793–0.875, P<0.001) (*Figure 3B*).

The prognostic role of the angiogenesis-related gene in BLCA

The comparison of the two clusters revealed the expression profile of angiogenesis-related genes was strictly correlated to the prognosis of BLCAs. To investigate the prognostic role of the individual gene, we divided the patients into two subgroups according to the different gene expression: high-expressed group and low-expressed group. Comparison of the OS in the paired groups showed that elevated expression of 8 genes, ECM1, FN1, FGF1, FAP, JAM3, THBS1, MFGE8, and COL8A2, were significantly shortened the OS in BLCA (P<0.05, *Figure 4*). Among those genes, the expression of ECM1 revealed the most detected negative correlation to OS (56.48 *vs.* 23.21 months, P<0.001; HR =1.72, 95% CI: 1.275–2.320) (*Table 4*).

Discussion

Although multiple agents were developed to target specific proteins, bladder cancer still is an insensitive disease for clinical management. Platinum-based chemotherapy is an acceptable front-line regiment for patients with advanced or metastatic BLCA, while the outcomes are unsatisfactory (4). Prognosis in chemotherapy resistance patients remains poor, with a median overall survival with monotherapy of approximately seven months (32). Compared with chemotherapy in second-line treatment, a superior survival benefit has been showed in immune-checkpoint inhibitors therapy However, the survival benefit from immunotherapy

Table 2 The comparison of survival corresponds to each cluster

Prognosis	Cluster_1	Cluster_2	P value	Hazard ratio	95% CI of the ratio
OS	86.83	27.06	0.001**	0.5756	0.4241 to 0.7812
DFS	101.5	Undefined	0.978	0.9899	0.4853 to 2.019
DSS	104.6	51.16	0.001**	0.5007	0.3458 to 0.7249
PFS	54.74	21.86	0.006**	0.6304	0.4636 to 0.8573

**, P<0.01.

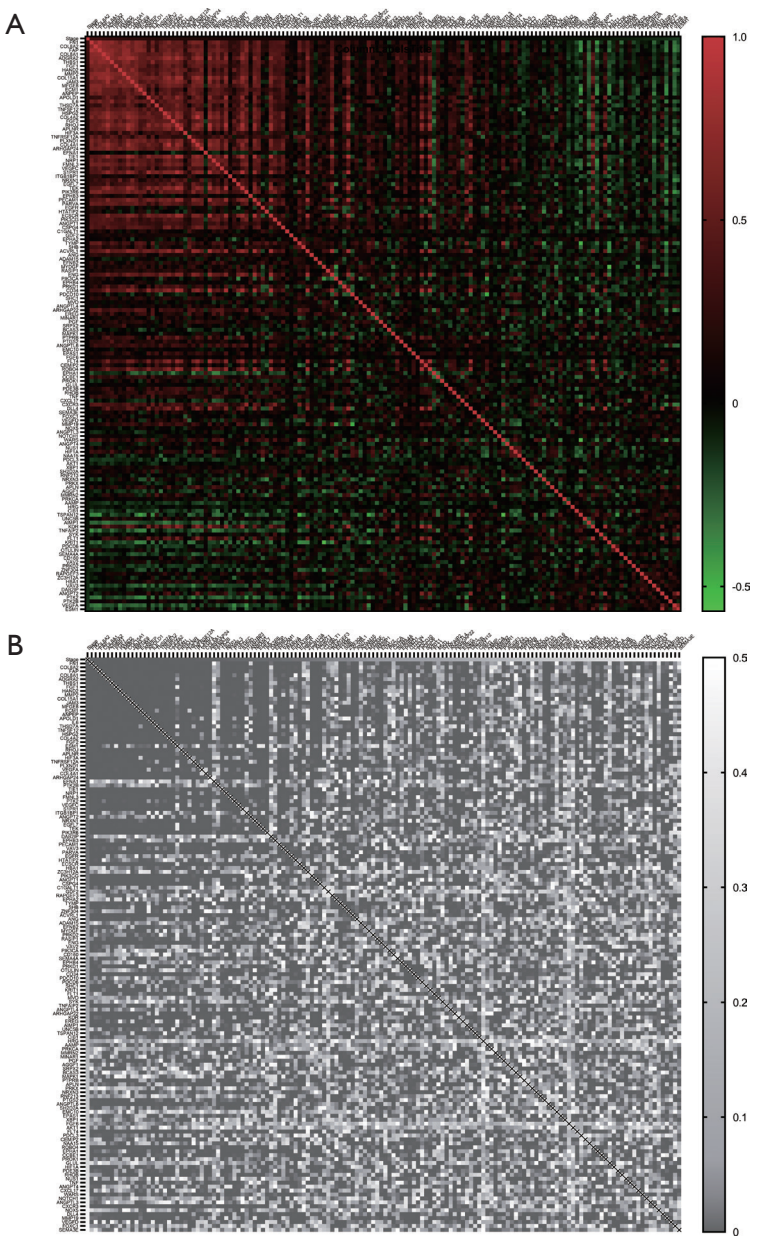

Figure 2 Regression analysis revealed the genes expression correlated with stage progression. (A) the Pearson correlation value (r) of stage and genes; (B) the statistical significance (P value) of corresponding correlation values.

Table 3 The angiogenesis-related gene correlated with tumor stages

Genes	Pearson correlation	P value
FN1	0.34	1.6E-12
COL8A2	0.33	7.67E-12
FAP	0.33	7.71E-12
COL8A1	0.29	9.26E-10
ADGRA2	0.29	2.35E-09
THBS1	0.27	2.14E-08
FGF1	0.26	7.65E-08
HAND2	0.26	1.11E-07
MMP2	0.25	2.55E-07
COL15A1	0.23	1.06E-06
JAM3	0.23	1.28E-06
MFGE8	0.23	2.5E-06
ECM1	0.22	3.28E-06
ANPEP	0.21	7.83E-06
APOLD1	0.21	8.36E-06
IL6	0.21	1.02E-05

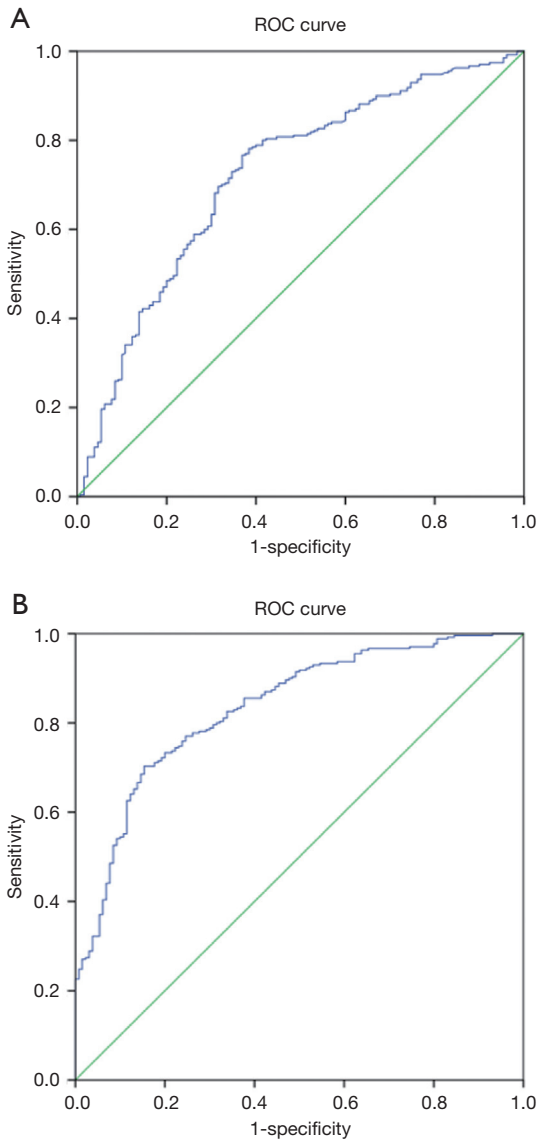

Figure 3 The angiogenesis related gene signatures were capable to predicate the stage in bladder urothelial carcinoma (BLCA). (A) the expression of single gene FN1 was available to predicted the stage progression; (B) the genes set combining 16 genes was with superior efficacy in the prediction of the stage progression.

was reported in only 20% of patients (33). Recently, an angiogenesis targeted study revealed ramucirumab combined docetaxel is the first regiment in a phase 3 study to show improved PFS in patients with platinum-insensitive advanced urothelial carcinoma (2). These data prompted that inhibition of angiogenesis-related signaling offered a potentially effective treatment possibility for patients with urothelial carcinoma. Therefore, a new targeted gene involved in the angiogenetic process must be investigated.

In the current study, we found that the profile of angiogenesis-related gene expression was significantly associated with the tumor stage and outcomes. The cluster_1, represented by depression of FAP, FN1, and COL8A2, shows a significant survival advantage. The elevated expression of those three genes is respectively correlated with the advanced tumor stage in the BLCA. The FN1 gene coding a fibronectin 1 protein was previously reported as a survival predictor and associated with the pathologic stage, which detection was inconsistent with our finding (34). Cytoplasmic immunostaining of FAP (fibroblast activation protein-coding gene) in cancer-associated fibroblasts showed worse DSS and were associated with tumor staging, which data supported our results (35). The COL8A2 gene coding a collagen alpha-2 (VIII) chain, a component of the endothelia of blood vessels, was rarely studied in the bladder cancer. However, the gene was consistently increased in the tumor content of prostate cancer (36). Our data suggested that high expression of COL8A2 was not only associated with advanced tumor

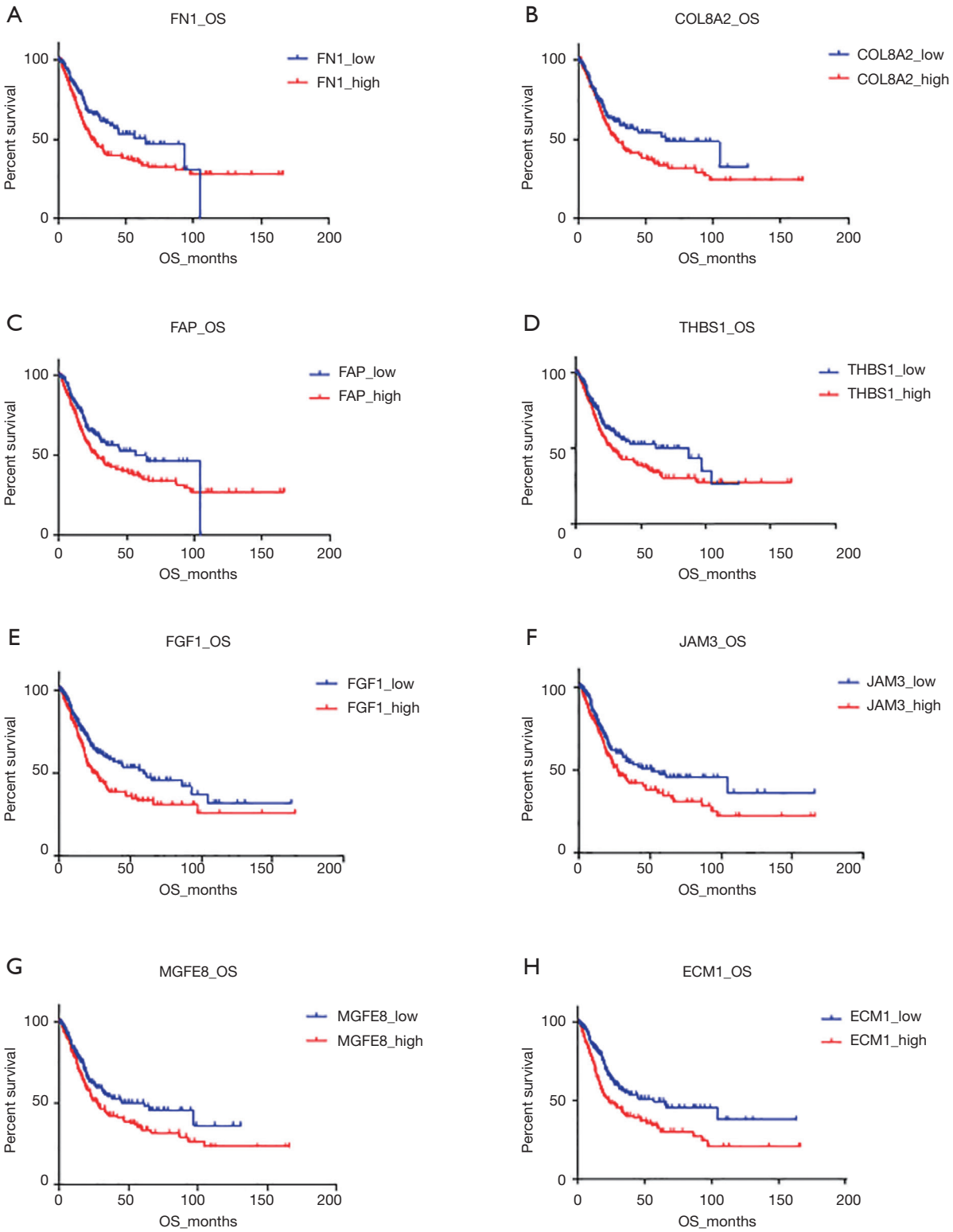

Figure 4 The elevated expression of 8 genes were significantly associated with worse prognosis in BLCA (bladder urothelial carcinoma). (A,B,C,D,E,F,G,H) The different OS (overall survivals) regarding to high expression population and low expression population of ECM1, FN1, FGF1, FAP, JAM3, THBS1, MFGE8 and COL8A2.

Table 4 The correlation of single gene expression with survival rate

Genes	Median survival in low expression (Month)	Median survival in high expression (Month)	P value	Hazard Ratio (log-rank)	95% CI of the ratio
ECM1	56.48	23.21	<0.001***	1.72	1.275 to 2.320
FN1	64.8	26.14	0.004**	1.564	1.162 to 2.105
FGF1	59.31	23.64	0.005**	1.519	1.129 to 2.044
FAP	56.48	27.45	0.020*	1.449	1.073 to 1.959
JAM3	51.16	27.06	0.026*	1.396	1.037 to 1.879
THBS1	86.83	27.45	0.028*	1.402	1.043 to 1.885
MFGE8	61.45	27.45	0.028*	1.394	1.037 to 1.873
COL8A2	64.8	28.24	0.035*	1.388	1.031 to 1.868
COL8A1	61.45	28.41	0.074	1.314	0.9739 to 1.773
ADGRA2	44.32	28.24	0.124	1.263	0.9387 to 1.696
APOLD1	41.75	28.41	0.132	1.255	0.9332 to 1.687
HAND2	61.45	28.41	0.158	1.237	0.9204 to 1.662
ANPEP	41.75	33.01	0.216	1.205	0.8941 to 1.624
COL15A1	56.48	32.02	0.323	1.162	0.8639 to 1.564
MMP2	41.75	33.01	0.432	1.127	0.837 to 1.518
IL6	31.2	34.98	0.872	1.025	0.7625 to 1.377

*, P<0.05; **, P<0.01; ***, P<0.001.

stage but also showed a worse prognosis in BLCA. Hence, this gene deserves further study in BLCA.

The statistics showed that the different genes associated with the different prognosis were also correlated to the stage progression. This result showed that the prognostic role of angiogenesis-related genes might implicate by the effect to promote tumor progression. Therefore, we discovered the tumor stage-correlated genes and detected their performances to distinguish the early or advanced tumor stage, according to the ROC curve. We found no matter the single gene, FN1, or the combined 16 gene model that would capably show the tumor stage of the BLCA. To assess the prognostic effect of individual genes, we perform multiple survival comparisons. The data revealed that besides FAP, FN1, and COL8A2, the genes ECM1, FGF1, JAM3, THBS3, MFGE8, and COL8A2 also showed significant effects for indicating BLCA outcomes. Of which, the expression of ECM1 (Extracellular matrix protein 1 coding gene) was reported to display a significant increase compared with noncancerous counterparts and associated with a poor prognosis in bladder cancer. Depressing EMC1 affected not only cell proliferation and migration but also cell invasion ability and apoptosis potential (37). Downregulating the ECM1 in BLCA would significantly suppress the expression of GLUT1, LDHA and HIF-1α and reduced the lactate production in ECM1 suppressed cells, indicating that ECM1 potentially regulated the cancerous metabolism in BLCA (37). Consistent with our finding, ECM1 is with the potential to be developed a targeting for inhibiting BLCA.

In conclusion, we found that the expression of angiogenesis-related genes had significant differences in tumor stage, and the profile could predict the prognosis in BLCA. The results supply potential targets for the management of the BLCA.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/

tau-20-1291

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tau-20-1291). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: J. Chapnick)

Cite this article as: Wang J, Guo M, Zhou X, Ding Z, Chen X, Jiao Y, Ying W, Wu S, Zhang X, Geng N. Angiogenesis related gene expression significantly associated with the prognostic role of an urothelial bladder carcinoma. Transl Androl Urol 2020;9(5):2200-2210. doi:10.21037/tau-20-1291

Supplementary

Table S1 Differently expressed genes between the two clusters

Genes	P values	Mean expression value in Cluster_1	Mean expression value in Cluster_2
FAP	1.22E-52	−0.71219	0.42392
FN1	1.47E-39	−0.64355	0.383066
COL8A2	1.86E-36	−0.62956	0.374739
TSPAN12	1.09E-33	0.774641	−0.4611
VEGFA	2.49E-33	0.767452	−0.45682
TNFAIP2	2.64E-31	0.751268	−0.44718
TYMP	4.53E-30	−0.62808	0.373854
PIK3R6	1.61E-29	−0.57319	0.341186
ESM1	3.09E-26	0.658365	−0.39188
MFGE8	1.02E-25	−0.52427	0.312067
PTK2	5.48E-25	0.69457	−0.41343
THBS1	2.35E-24	−0.49913	0.297104
ECM1	1.07E-23	−0.53988	0.321357
MMRN2	1.21E-22	0.590577	−0.35153
ARHGAP22	1.07E-21	−0.53779	0.320115
IL6	2.72E-21	−0.51258	0.305104
WARS	3.85E-20	−0.54455	0.324137
COL15A1	2.52E-18	−0.41217	0.245338
ARHGAP24	4.2E-18	−0.43441	0.258575
ANGPTL3	4.82E-18	0.590072	−0.35123
CEMIP2	5.83E-18	0.524714	−0.31233
NRP1	6.06E-18	−0.45278	0.269511
ANGPT2	5.49E-17	0.489635	−0.29145
NRXN3	7.54E-16	0.52137	−0.31034
ANPEP	8.2E-16	−0.43563	0.259303
VAV3	3.55E-15	0.534683	−0.31826
PARVA	4.8E-15	−0.41303	0.245851
KRIT1	1.14E-14	0.530007	−0.31548
PLXND1	1.27E-14	−0.39681	0.236198
CXCR3	3.75E-14	−0.42852	0.255069
PTPRB	4.31E-14	0.420305	−0.25018
MMP2	1.21E-13	−0.37564	0.223598
FLT1	1.81E-13	0.401674	−0.23909
COL8A1	2.02E-13	−0.37136	0.22105
SEMA4A	2.2E-13	0.491908	−0.2928
FMNL3	3.48E-13	−0.37989	0.226123
ADGRA2	5.61E-13	−0.34422	0.204892
ANGPT4	2.62E-12	0.448416	−0.26691
DLL4	9.71E-12	0.366835	−0.21835
EPHA1	1.14E-11	0.463117	−0.27567
FGF1	2.43E-11	−0.35539	0.211541
PIK3CG	3.08E-11	−0.37258	0.221775
JAM3	5.28E-10	−0.32311	0.192326
ZNF304	6.59E-10	0.413028	−0.24585
MINAR1	7.22E-10	0.392291	−0.23351
MMP19	7.84E-10	0.367841	−0.21895
HAND2	1.74E-09	−0.33064	0.196807
ZC3H12A	1.99E-09	0.38305	−0.22801
FGF2	2.19E-09	−0.32073	0.19091
CIB1	9.77E-09	0.384205	−0.22869
CXCL17	1.22E-08	0.391253	−0.23289
VEGFC	1.58E-08	−0.31405	0.186933
TNFRSF12A	2.34E-08	−0.32205	0.191699
PROK1	2.72E-08	0.359058	−0.21372
COL4A2	4.02E-08	−0.26925	0.160269
HIF1A	5.42E-08	−0.32646	0.194324
KDR	6.94E-08	0.286836	−0.17074
HTATIP2	1.95E-07	−0.33507	0.199448
HSPG2	4.22E-07	−0.24745	0.147291
SHB	9.83E-07	−0.30112	0.179239
NRXN1	1.98E-06	−0.28212	0.167926
FGF6	2.21E-06	0.126084	−0.07505
PRKD2	2.37E-06	0.31899	−0.18987
HRG	2.76E-06	0.268654	−0.15991
PDCL3	3.42E-06	0.319955	−0.19045
APLNR	3.52E-06	−0.23848	0.141951
SRPX2	4.98E-06	0.287026	−0.17085
VEGFD	9.95E-06	0.305397	−0.18178
EREG	1.37E-05	−0.2746	0.163454
AKT1	3.49E-05	0.278346	−0.16568
BCAS3	5.34E-05	0.27783	−0.16538
PECAM1	6.94E-05	−0.1998	0.11893
FLT4	0.000115	0.204357	−0.12164
CCBE1	0.000138	−0.249	0.148212
TNFSF12	0.000141	−0.20855	0.124138
ANGPT1	0.000177	−0.22422	0.133465
HBA1	0.000193	0.262918	−0.1565
EGFR	0.000198	−0.24009	0.142914
XBP1	0.000208	0.235631	−0.14026
CSPG4	0.000265	−0.20411	0.121496
ACVRL1	0.000321	−0.18018	0.107249
RHOB	0.000362	0.216142	−0.12866
AIMP1	0.000379	0.258381	−0.1538
EPAS1	0.000383	0.212453	−0.12646
COL4A1	0.000417	−0.17544	0.104431
OTULIN	0.000423	−0.23162	0.137869
PDCD10	0.000471	−0.23597	0.140461
TNF	0.000591	−0.21641	0.128817
PDCD6	0.000597	0.23889	−0.1422
PRKD1	0.000702	0.204308	−0.12161
UNC5B	0.000942	0.206711	−0.12304
ANGPTL6	0.001225	−0.20781	0.123699
EPHA2	0.001262	0.207137	−0.1233
GDF2	0.001483	0.119507	−0.07114
EMC10	0.002135	0.203182	−0.12094
CD160	0.002441	0.201907	−0.12018
RAPGEF3	0.002685	0.182248	−0.10848
EFNA1	0.003462	0.195042	−0.1161
AGGF1	0.007101	0.186722	−0.11114
NOX5	0.007547	0.18537	−0.11034
TIE1	0.01076	−0.12543	0.07466
EPHB4	0.011818	−0.16719	0.099516
ITGB1BP1	0.018531	−0.15539	0.092496
ANGPTL4	0.019834	0.146825	−0.0874
RHOJ	0.02072	−0.11266	0.067058
AAMP	0.022248	0.159327	−0.09484
TEK	0.02284	−0.1134	0.067498
PRKCA	0.029006	0.141675	−0.08433
EFNB2	0.029184	−0.13632	0.081143
S1PR1	0.031866	−0.10726	0.063843
SEMA3E	0.033715	0.145457	−0.08658
PIK3CA	0.036101	−0.13583	0.080854
EPHB3	0.038304	−0.13581	0.080837
DAB2IP	0.047238	0.130468	−0.07766
ECSCR	0.049793	−0.09987	0.059445