DOI: 10.4081/aiua.2025.13516

ORIGINAL PAPER - SUPPLEMENTARY MATERIAL

Predictive role of lactylation-related gene signature in the prognosis and immunotherapy response in bladder cancer

Guoyuan Liu [1*], Ting Hong [2*], Xinyu Liu [2*], Xuanhao Lin [3], Peixiu Yao [3], Xifeng Chen [3], Yonghai Zhang [1], Kemal Sarica [4, 5], Xuwei Hong [1]

[1] Department of Urology, Shantou Central Hospital, Shantou, China;
[2] Clinical Medical Research Center, Shantou Central Hospital, Shantou, China;
[3] Department of Biobank, Shantou Central Hospital, Shantou, China;
[4] Department of Urology, Health Sciences University, Prof. Dr. Ilhan Varank Education and Training Hospital, Istanbul, Turkey;
[5] Department of Urology, Biruni University, Medical School, Istanbul, Turkey.

** These authors contributed equally to this work.*

SUPPLEMENTARY TABLES

Table S1.
Clinical characteristics and survival outcomes of BLCA patients from TCGA-BLCA and GSE13507 cohorts.

	TCGA-BLCA	GSE13507
Cases (n)	412	165
Gender		
Male, n (%)	304 (73.8)	135 (81.8)
Female, n (%)	108 (26.2)	30 (18.2)
Age		
> 60, n (%)	304 (73.8)	119 (72.1)
≤ 60, n (%)	108 (26.2)	46 (27.9)
T stage		
T1T2, n (%)	123 (29.9)	111 (67.3)
T3T4, n (%)	255 (61.9)	30 (18.2)
NA, n (%)	34 (8.2)	24 (14.5)
N stage		
N0, n (%)	239 (58.0)	149 (90.3)
N1, n (%)	47 (11.4)	8 (4.9)
N2, n (%)	76 (18.4)	6 (3.6)
N3, n (%)	8 (2.0)	0 (0)
NA, n (%)	42 (10.2)	2 (1.2)
M stage		
M0, n (%)	196 (47.5)	158 (95.8)
M1, n (%)	11 (2.7)	7 (4.2)
NA, n (%)	205 (49.8)	0 (0)
Grade		
high, n (%)	388 (94.2)	60 (36.4)
low, n (%)	21 (5.1)	105 (63.6)
NA, n (%)	3 (0.7)	0 (0)
State		
alive, n (%)	203 (49.3)	96 (58.2)
dead, n (%)	209 (50.7)	69 (41.8)
Survial time (month)		
midian	17.7	36.6
interval of quartile	10.9~31.5	17.1~75.1

Table S2.
Univariate Cox regression and Kaplan-Meier analysis of lactylation genes.

Gene ID	HR	HR.95%CI Low	HR.95%CI High	P-value of Cox	P-value of K-M
HDAC8	0.599	0.424	0.845	0.004	< 0.001
HAGH	0.744	0.568	0.975	0.032	< 0.001
HDAC3	0.873	0.659	1.157	0.345	0.012
SIRT3	0.847	0.568	1.264	0.416	0.014
HDAC1	0.865	0.679	1.103	0.243	0.021
SIRT1	0.891	0.712	1.116	0.315	0.026
EP300	0.980	0.794	1.209	0.849	0.036
GLO1	1.052	0.901	1.230	0.521	0.052
SIRT2	1.158	0.903	1.485	0.248	0.055
HDAC2	0.983	0.788	1.227	0.881	0.101
CREBBP	1.040	0.809	1.338	0.758	0.249

HR: Hazard ratio; CI: Confidence interval; Cox: Cox regression analysis; K-M: Kaplan-Meier analysis.

Table S3.
Univariate Cox regression analysis of 275 DEGs screened between cluster A and cluster B.

Gene ID	HR	HR.95%CI Low	HR.95%CI High	P-value
EP300	0.980	0.794	1.209	0.849
SIRT1	0.891	0.712	1.116	0.315
BPTF	0.982	0.760	1.268	0.888
HECTD1	1.179	0.950	1.464	0.135
AFF4	1.019	0.848	1.226	0.838
ANKRD17	0.914	0.721	1.160	0.460
KDM3B	0.914	0.722	1.156	0.452
KMT2E	0.908	0.726	1.135	0.395
USP34	1.111	0.919	1.342	0.277
JMJD1C	1.034	0.806	1.326	0.792
TCF12	1.118	0.882	1.417	0.357
AKAP11	0.931	0.742	1.167	0.533
HMG20A	0.913	0.706	1.180	0.485
MANEA	0.874	0.681	1.123	0.293
ZNF770	1.099	0.932	1.297	0.262
UFL1	0.940	0.764	1.157	0.559
HBP1	1.054	0.851	1.304	0.631
ARID2	0.858	0.666	1.105	0.236
C5orf24	1.096	0.863	1.392	0.451
PIK3C2A	1.017	0.795	1.301	0.894
SCAF8	0.876	0.682	1.124	0.298
SMARCAD1	0.797	0.636	0.999	0.049
ADNP	0.918	0.747	1.128	0.416
RBL2	0.914	0.770	1.086	0.306
PHF3	0.877	0.696	1.105	0.266
SMAD5	0.897	0.719	1.120	0.338
APAF1	0.890	0.720	1.100	0.281
GTF2I	0.991	0.830	1.183	0.917
CEP350	1.067	0.829	1.373	0.615
SON	1.100	0.875	1.384	0.414
SMG1	0.930	0.771	1.123	0.450
MIB1	1.086	0.871	1.356	0.463
SP3	1.012	0.801	1.280	0.919
INTS2	1.067	0.845	1.349	0.585
ROCK1	1.030	0.829	1.280	0.791
GOLGA4	1.117	0.909	1.373	0.293
AP3M1	1.094	0.882	1.358	0.414
MFAP3	1.115	0.941	1.322	0.207
CASD1	0.792	0.630	0.995	0.045
SDE2	1.089	0.870	1.363	0.458
KIDINS220	1.061	0.875	1.288	0.547
PREPL	0.944	0.757	1.177	0.609
TMF1	1.012	0.810	1.265	0.913
PPP1R12A	0.929	0.753	1.146	0.490
STAG1	1.251	1.009	1.552	0.041
BCOR	0.818	0.671	0.999	0.048
G3BP2	0.921	0.745	1.138	0.446
ELF1	0.950	0.812	1.111	0.521
CSNK1A1	1.004	0.829	1.217	0.965
ZBTB41	1.019	0.809	1.283	0.873
TBC1D14	1.031	0.844	1.258	0.768
EIF3L	1.030	0.860	1.233	0.748
NCOA4	0.994	0.808	1.223	0.956
EIF4G2	1.058	0.848	1.319	0.620
BMPR2	1.006	0.824	1.228	0.955
CLINT1	0.957	0.794	1.152	0.640
NAA10	0.890	0.728	1.089	0.258
SPOPL	0.815	0.646	1.029	0.085
PRRC1	1.012	0.817	1.253	0.913
GNA13	1.054	0.881	1.261	0.566
PPP1R15B	1.081	0.863	1.353	0.499
LRBA	0.993	0.806	1.224	0.946
LANCL1	1.090	0.905	1.313	0.364
OSBPL8	1.139	0.944	1.374	0.173
MORC3	0.995	0.803	1.233	0.963
UBE4A	0.974	0.785	1.207	0.808
DDX21	1.190	1.020	1.390	0.027
PRRC2C	1.052	0.880	1.258	0.579
ARID1A	0.923	0.744	1.144	0.465
SURF2	0.935	0.767	1.141	0.510
SMC3	1.122	0.940	1.339	0.202
TOR1AIP1	1.062	0.854	1.322	0.587
NUDT21	1.034	0.859	1.243	0.726
TJP1	1.118	0.904	1.383	0.303
REL	0.949	0.791	1.139	0.577
PRKAR1A	1.029	0.832	1.272	0.794
BTAF1	0.900	0.747	1.085	0.269
ZNF217	0.978	0.836	1.143	0.778
PSMG3	1.057	0.858	1.300	0.604
ZNF106	1.150	0.941	1.406	0.172
SLK	0.958	0.805	1.140	0.626
CHD6	0.972	0.787	1.201	0.794
ACAP2	1.172	0.946	1.453	0.147
TAF2	1.124	0.928	1.361	0.233
MEX3C	1.082	0.877	1.335	0.460
NT5C2	1.006	0.869	1.164	0.940
RASA1	1.253	1.025	1.531	0.028
EIF3A	1.126	0.922	1.375	0.244
ATP2B1	0.814	0.666	0.996	0.046
LMBRD1	0.889	0.751	1.053	0.173
ATP11B	1.071	0.902	1.273	0.432
DYNC1LI2	1.089	0.907	1.308	0.359
CNOT1	1.182	0.965	1.449	0.106
MAP4K3	0.955	0.803	1.136	0.601
NBN	0.923	0.758	1.124	0.426
NUP153	1.162	0.978	1.380	0.087
LONRF1	0.986	0.814	1.195	0.889
KLHL24	0.810	0.689	0.951	0.010
FAM50A	1.068	0.893	1.277	0.470
MRPL41	0.881	0.764	1.015	0.080
SERINC1	1.171	0.959	1.431	0.121
DLG5	1.000	0.852	1.174	1.000
ANO6	0.970	0.833	1.129	0.692
TSPYL1	0.969	0.815	1.151	0.717
INAFM1	1.038	0.856	1.260	0.702
CLTC	1.262	1.031	1.544	0.024
SEC24C	1.267	1.057	1.518	0.010
CPNE3	0.988	0.819	1.193	0.902
IQGAP1	1.200	0.980	1.469	0.078
PJA2	1.121	0.927	1.356	0.239

ATP2A2	1.107	0.915	1.339	0.294
SLC38A1	0.888	0.763	1.033	0.124
CLCN3	0.818	0.690	0.969	0.020
SMARCC2	0.972	0.802	1.179	0.775
MTPN	1.195	0.994	1.436	0.058
FAM13B	0.730	0.602	0.885	0.001
TMED7	1.045	0.873	1.251	0.629
PGAP1	0.841	0.696	1.017	0.075
FAM214A	0.851	0.721	1.004	0.056
ARID5B	1.082	0.930	1.259	0.306
FNDC3A	0.945	0.795	1.124	0.525
LCOR	0.770	0.639	0.928	0.006
ADAM17	1.296	1.099	1.527	0.002
EHF	0.943	0.857	1.038	0.233
SLC39A6	1.031	0.893	1.189	0.678
PCMTD2	0.882	0.742	1.048	0.153
TMTC2	0.818	0.676	0.990	0.039
CCDC85B	1.082	0.940	1.245	0.271
ZNF664	0.869	0.736	1.025	0.095
NFAT5	0.983	0.856	1.129	0.811
SASH1	1.028	0.871	1.215	0.743
ARRDC3	1.079	0.910	1.280	0.380
ZDHHC20	0.829	0.696	0.987	0.035
RB1	1.034	0.873	1.226	0.697
NFE2L2	0.942	0.796	1.116	0.490
NRIP1	1.080	0.941	1.241	0.274
BTBD3	0.905	0.764	1.071	0.244
S100A13	0.934	0.799	1.091	0.389
ABCC4	0.828	0.728	0.942	0.004
TOP2B	0.968	0.844	1.110	0.643
EVA1B	1.179	1.001	1.389	0.048
PGM2	0.980	0.832	1.156	0.814
TMEM160	0.906	0.765	1.072	0.251
MAPK6	1.135	0.961	1.341	0.136
BCKDHB	0.817	0.690	0.969	0.020
ENTPD7	0.990	0.848	1.156	0.901
DCK	0.960	0.808	1.141	0.642
FAT1	0.938	0.828	1.062	0.313
UBL3	1.010	0.865	1.179	0.902
BNIP3L	1.159	0.985	1.363	0.076
ISOC1	0.841	0.717	0.987	0.033
WEE1	1.014	0.855	1.203	0.873
ADAM10	0.962	0.822	1.127	0.633
ANKRD50	0.890	0.779	1.017	0.087
STAG2	1.012	0.868	1.180	0.881
CCNG1	0.854	0.730	0.999	0.049
PTPRK	0.963	0.818	1.134	0.651
SESN3	1.018	0.887	1.168	0.799
COMTD1	1.116	0.953	1.307	0.173
CCNG2	0.807	0.693	0.940	0.006
ACADM	0.962	0.828	1.119	0.619
ASAP2	1.247	1.071	1.452	0.004
PTPN13	0.907	0.813	1.012	0.082
TP53INP1	0.762	0.651	0.892	0.001
PLS1	0.882	0.784	0.993	0.038
FRRS1	1.097	0.956	1.259	0.187
CD2AP	0.842	0.730	0.972	0.019

NAPRT	0.869	0.763	0.989	0.033
DPM3	0.863	0.747	0.996	0.044
UPP1	1.094	0.958	1.248	0.184
SMC4	1.137	0.975	1.325	0.103
TCIRG1	0.786	0.682	0.907	0.001
RAB11FIP1	1.034	0.913	1.170	0.599
LRIG3	1.171	1.024	1.338	0.021
SEMA3C	0.935	0.838	1.043	0.227
EIF4EBP1	1.141	1.002	1.300	0.046
SPRY2	1.005	0.879	1.148	0.943
EPS8	0.942	0.829	1.070	0.356
LGR4	0.986	0.852	1.143	0.855
C4orf48	0.916	0.821	1.022	0.115
ITGB1	1.173	1.008	1.364	0.039
HES4	0.921	0.839	1.010	0.081
ROBO1	1.090	0.963	1.233	0.172
NBL1	1.024	0.918	1.142	0.674
ISG15	1.031	0.948	1.121	0.480
TMEM123	0.966	0.843	1.108	0.625
GJA1	1.146	1.052	1.250	0.002
TSPAN12	0.948	0.853	1.054	0.322
MYLIP	0.845	0.749	0.954	0.007
TIMP1	1.023	0.925	1.131	0.661
ACER2	0.943	0.874	1.019	0.136
ELOVL5	1.173	1.025	1.342	0.020
MBOAT1	0.995	0.888	1.115	0.927
IRS1	0.918	0.809	1.041	0.181
PHLDA2	1.110	0.985	1.251	0.087
PLA2G4A	0.881	0.780	0.995	0.042
KIT	0.974	0.877	1.082	0.623
SLC40A1	0.972	0.870	1.086	0.618
ATP8B1	0.856	0.764	0.958	0.007
C19orf33	0.939	0.870	1.013	0.106
FXYD5	1.026	0.926	1.138	0.621
SORL1	0.870	0.787	0.961	0.006
GRHL1	0.976	0.874	1.091	0.671
HCST	0.980	0.879	1.093	0.717
RCN3	1.193	1.086	1.310	< 0.001
IL32	1.011	0.928	1.102	0.803
MAN1A1	1.050	0.936	1.179	0.405
RRAS	1.058	0.942	1.189	0.338
DSG2	1.084	0.965	1.219	0.173
GNLY	0.926	0.831	1.031	0.160
BATF	0.854	0.769	0.949	0.003
APOC1	1.001	0.914	1.098	0.977
CLEC11A	1.140	1.019	1.274	0.021
RHPN1	0.899	0.809	0.999	0.048
SFN	1.112	1.022	1.211	0.014
GZMB	0.955	0.873	1.045	0.317
IDH1	0.913	0.825	1.009	0.075
IFITM3	1.090	1.000	1.189	0.050
FOSL1	1.168	1.060	1.287	0.002
BTG2	0.893	0.814	0.980	0.017
CITED4	1.026	0.929	1.132	0.614
SPHK1	1.199	1.091	1.317	< 0.001
NNMT	1.083	1.011	1.162	0.024
RGS5	1.038	0.942	1.143	0.454

LGALS1	1.170	1.071	1.279	< 0.001
FSTL3	1.143	1.044	1.253	0.004
EMP3	1.137	1.039	1.245	0.005
THY1	1.176	1.067	1.297	0.001
CDA	1.172	1.074	1.279	< 0.001
RARRES2	1.015	0.936	1.100	0.722
IFI6	1.091	1.018	1.170	0.014
TPM2	1.095	1.013	1.183	0.022
MT2A	1.083	1.016	1.154	0.015
TMEM47	0.973	0.887	1.066	0.553
IFITM1	1.060	0.984	1.142	0.126
SLITRK6	0.913	0.854	0.976	0.008
CST6	1.078	1.009	1.152	0.027
WNT5A	1.041	0.960	1.129	0.334
CLCA4	0.968	0.914	1.025	0.267
MAOA	0.880	0.814	0.952	0.001
CCL5	1.008	0.938	1.083	0.826
BGN	1.144	1.056	1.239	0.001
TOX3	0.885	0.815	0.960	0.003
APOE	1.052	0.980	1.130	0.159
LY6E	1.081	0.995	1.175	0.066
IGFBP6	1.024	0.948	1.106	0.547
FOXA1	0.914	0.852	0.980	0.012
PTGES	0.955	0.881	1.035	0.262
C1QA	1.047	0.978	1.120	0.184
CDKN2A	0.971	0.908	1.038	0.393
UGT1A10	0.987	0.921	1.056	0.697
TBX3	0.880	0.819	0.946	0.001
PPP1R3C	0.896	0.831	0.967	0.005
PCP4L1	0.974	0.909	1.045	0.467
SLC14A1	0.947	0.892	1.006	0.077
GRHL3	0.960	0.896	1.028	0.245
MMP11	1.109	1.040	1.183	0.002
TP63	0.936	0.871	1.006	0.074
AEBP1	1.124	1.052	1.201	0.001
SAA1	1.013	0.951	1.079	0.692
PDZK1IP1	1.046	0.982	1.115	0.161
CLIC3	1.138	1.068	1.212	< 0.001
IFI27	1.051	0.990	1.117	0.105
IGFBP5	1.075	1.008	1.146	0.027
KRT17	1.009	0.957	1.064	0.744
CRABP2	0.992	0.931	1.057	0.806
TNNI2	0.957	0.905	1.012	0.120
CLCA2	1.020	0.966	1.078	0.479
LCN2	1.035	0.987	1.086	0.158
S100A8	1.082	1.041	1.125	< 0.001
RPS4Y1	0.947	0.907	0.989	0.014
PI3	1.058	1.020	1.097	0.002
KRT16	1.080	1.032	1.131	0.001
KRT14	1.060	1.022	1.100	0.002
KRT20	1.000	0.959	1.041	0.983

DEGs: Differentitally expressed genes; HR: Hazard ratio; CI: Confidence interval.

Supplementary figures

Figure S1.
The different pathways between the two subtypes.
The score of pathways in two clusters, including HALLMARK, KEGG, and Reactome pathways.

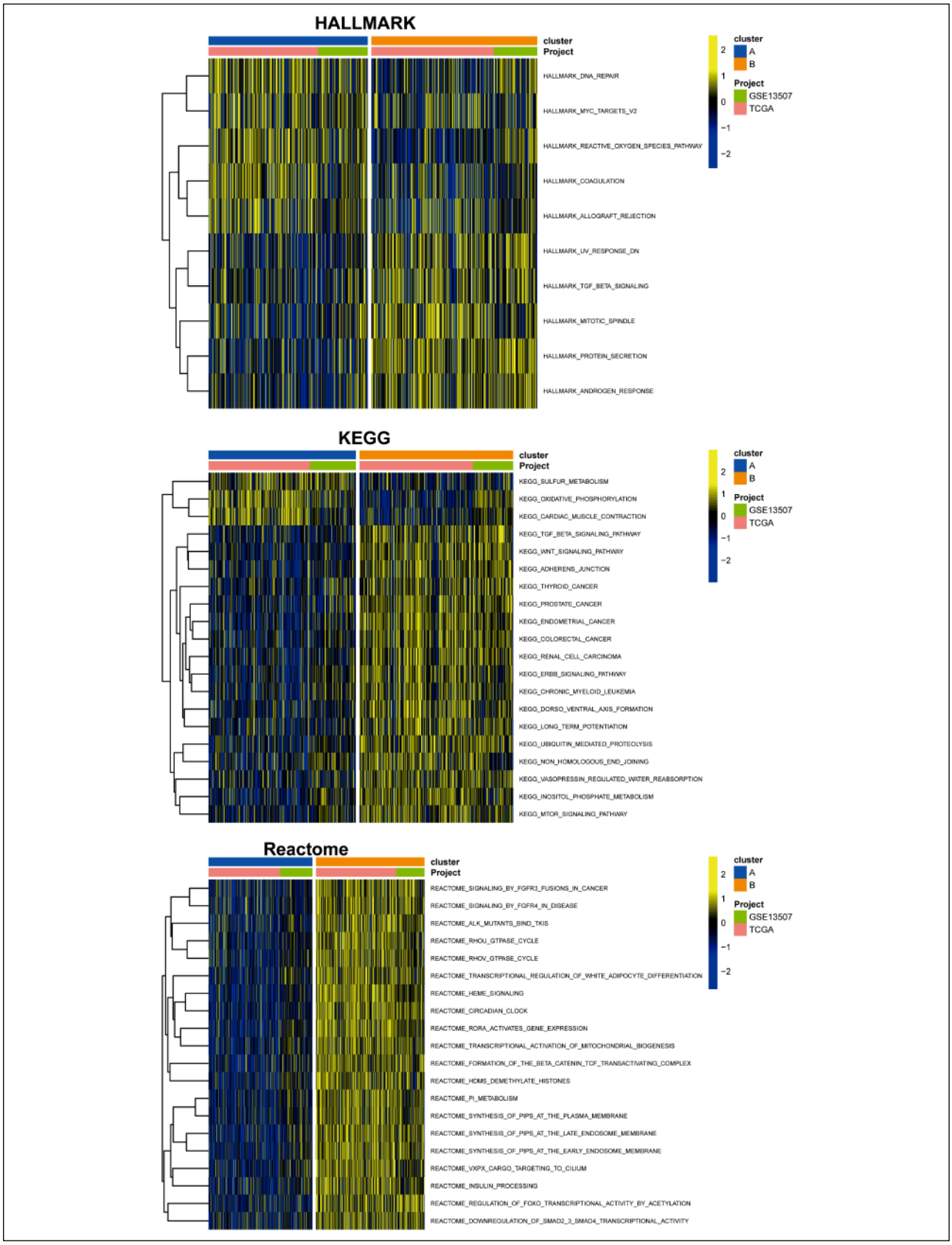

Figure S2.
The SNV of prognostic DEGs
A. The frequency of deleterious mutations of lactylation genes in BLCA.
B. The mutations waterfall plot of indicated genes in BLCA.

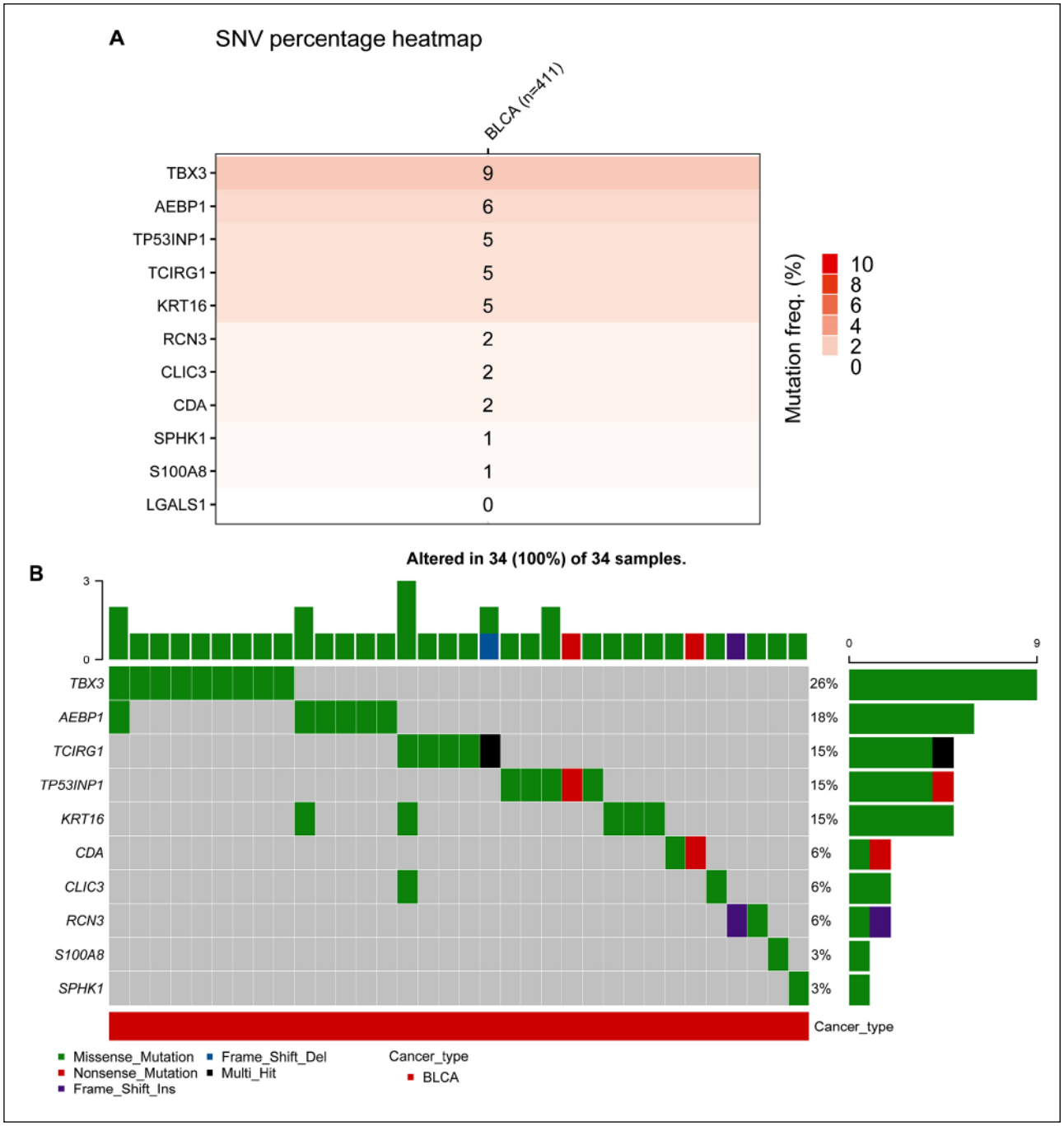

Figure S3.
The CNV and methylation of DEGs
A. The CNA percentage of DEGs in BLCA. B. The homozygous and heterozygous CNV of DEGs in BLCA.
C. The correlation of CNV with mRNA expression in BLCA. D. The correlation of methylation level with mRNA expression.

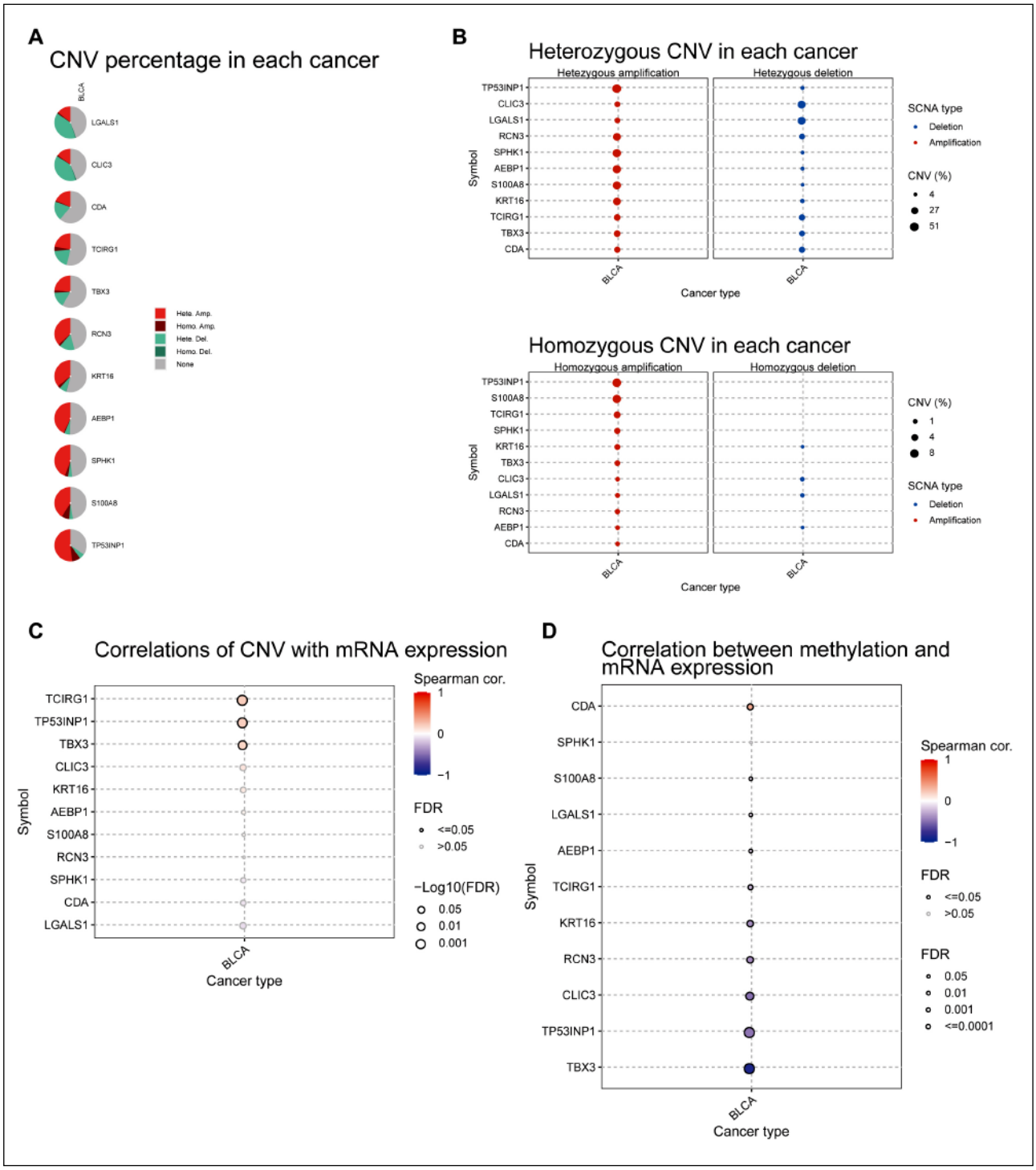

Figure S4.
The gene mutation differences in high and low lactylation score group
A. The gene mutation frequency in high lactylation score group in BLCA. B. The gene mutation frequency in low lactylation score group in BLCA. C. The gene mutation differences in high and low lactylation score group.

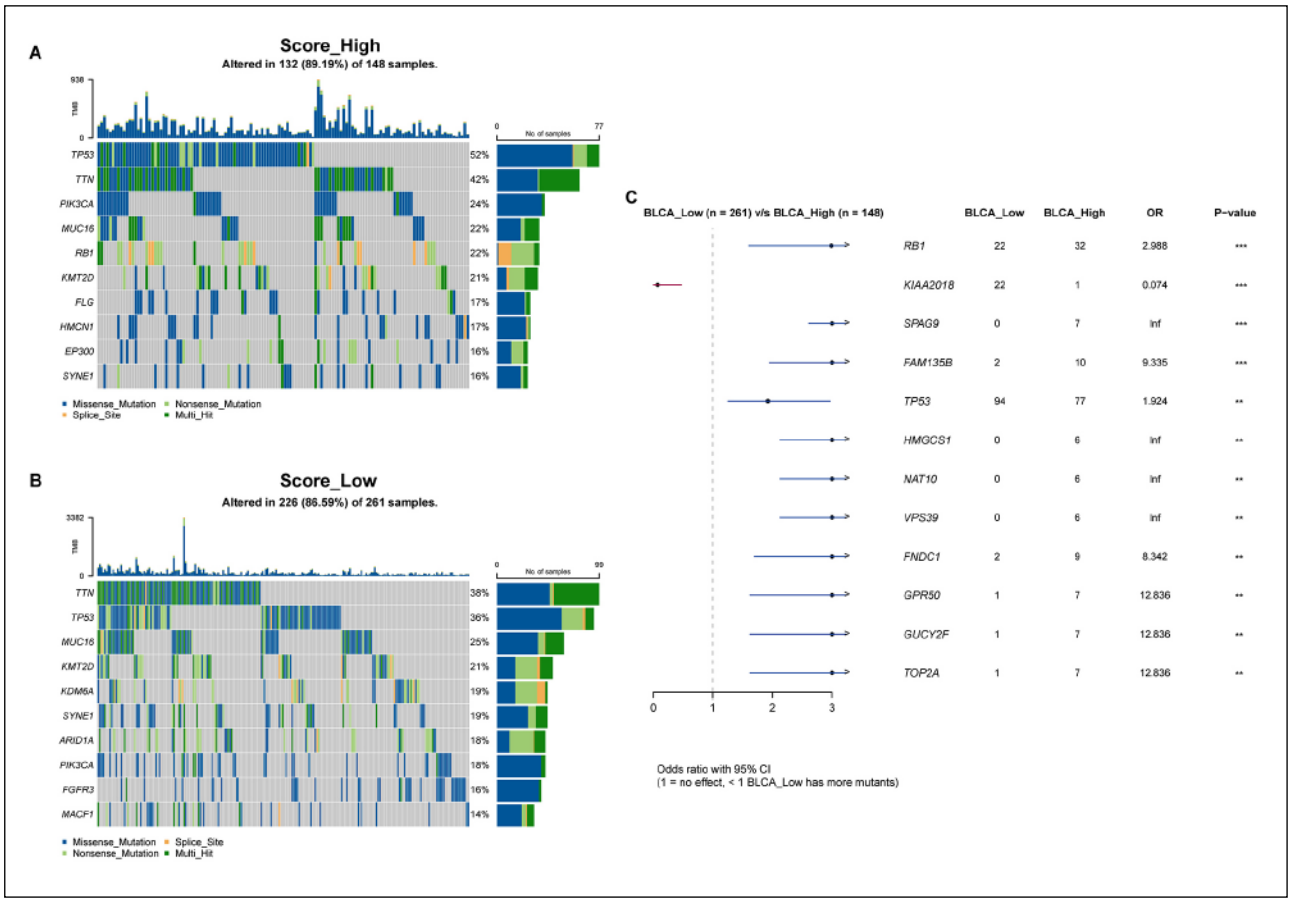

Figure S5.
The analysis of curative effect of anti-tumor drugs.
The IC50 of indicated anti-tumor drugs in high and low lactylation score groups.