

AHSA1 is a Prognostic Biomarker that Correlates with Macrophage Infiltration in Pan-Cancer

Fulei Li (✉ 15890109636@163.com)

Zhengzhou University First Affiliated Hospital <https://orcid.org/0000-0002-3019-397X>

Chengdong Liu

Southern Medical University Nanfang Hospital

Yanling Chen

Zhengzhou University First Affiliated Hospital

Shasha Li

Zhengzhou University First Affiliated Hospital

Lu Bai

Zhengzhou University First Affiliated Hospital

Research Article

Keywords: AHSA1, pan-cancer, tumor associated macrophages, TCGA

Posted Date: August 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-746415/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Activator of heat shock 90 kDa protein ATPase homolog 1 (AHSA1) is differentially expressed in several tumor types. However, its association with immune cell infiltration remains elusive.

Methods: AHSA1 expression was analyzed using The Cancer Genome Atlas (TCGA) pan-cancer data and normal tissue expression data from Genotype-Tissue Expression (GTEx). The clinical prognostic role of AHSA1 in pan-cancer was investigated, and an enrichment analysis of AHSA1 was performed using the R package “clusterProfiler.” We downloaded data regarding the immune cell infiltration level of TCGA pan-cancer tissues and analyzed the association between immune cell infiltration and AHSA1 expression.

Results: The results of TCGA pan-cancer data analysis revealed that AHSA1 was overexpressed and associated with poor survival in patients with cancer. Furthermore, the infiltration levels of tumor-associated macrophages (TAMs) were higher, while those of CD8+ T cells were lower, in the high AHSA1 expression group.

Conclusions: Our study suggests that AHSA1 is an oncogene and a risk factor for patient survival in cancer. AHSA1 may contribute to high infiltration levels of TAMs and low infiltration levels of CD8+ T cells, thus indicating that high AHSA1 expression may be associated with the tumor immunosuppressive microenvironment.

Background

The activator of heat shock 90 kDa protein ATPase homolog 1 (AHSA1) is a chaperone of heat shock 90 kDa (HSP90) and stimulates the ATPase activity of HSP90[1]. Notably, AHSA1 reportedly plays additional roles apart from regulating the HSP90 ATP hydrolysis rate[2]. Some researchers have revealed the critical role of AHSA1 in tumor progression. For example, knockdown of AHSA1 could inhibit the proliferation and invasion of osteosarcoma cells. However, research on AHSA1 in tumors, especially in the tumor immune microenvironment, remains scarce.

Several studies have reported that the tumor immune microenvironment has clinicopathological significance in predicting the therapeutic effect and prognosis of patients presenting tumors [3]. Tumor-associated macrophages (TAMs) and CD8 + T cells play a key role in tumor progression and immunotherapy[4]. Moreover, it has been confirmed that solid tumors are composed of malignant, non-malignant, hematopoietic, and mesenchymal cells. Among the non-malignant cells, TAMs play an essential role in promoting tumor angiogenesis. The extensive heterogeneity of TAMs enables these cells to adapt or alter their phenotypes to conform to the tumor microenvironment, playing a role in cancer progression and metastasis[5].

In this study, we evaluated the expression of AHSA1 and its association with the prognosis in patients with cancer. We observed that AHSA1 was overexpressed in most tumor types. AHSA1 is predicted to be involved cell cycle-related pathways. We further examined the correlation between AHSA1 expression and

the immune cell infiltration score and found that TAM infiltration increased and CD8 + T cell infiltration decreased in tissues with high AHSA1 expression. Our results present novel insights into the functional role of AHSA1 in pan-cancer, highlighting a potential mechanistic basis, whereby AHSA1 influences TAM and CD8 + T-cell infiltration, as well as the tumor immunosuppressive microenvironment.

Methods

Data Collection and Analysis

The expression and clinical data of The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) were downloaded from the UCSC Xena database (<https://xenabrowser.net/datapages/>). For AHSA1, the DNA copy number and methylation data were downloaded from the cBioPortal database (<https://www.cbioportal.org/>). The protein level of AHSA1 was downloaded from the Ualcan database (<http://ualcan.path.uab.edu/index.html>).

Correlation and Enrichment Analyses

Correlation analysis between AHSA1 and other mRNAs was performed using TCGA pan-cancer data, and Pearson's correlation coefficient was calculated. The top 300 genes most positively correlated with AHSA1 expression were selected for enrichment analysis to reflect the AHSA1 function. Gene set enrichment analysis (GSEA) was conducted using the R package "clusterProfiler."

Immune Cell Infiltration

We downloaded the immune cell infiltration score of TCGA from a previously published study, "The Immune Landscape of Cancer" [6]. For each TCGA tumor type, patients were divided into two groups (high and low AHSA1 expression groups), based on the median AHSA1 expression to compare levels of immune cell infiltration.

Results

Pan-Cancer Analysis of AHSA1 Expression

We first evaluated AHSA1 expression using RNA sequence data combined with TCGA and GTEx expression profilers. The results revealed that AHSA1 was overexpressed in 27 cancer types, including ACC (Adrenocortical carcinoma), BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma), CHOL (Cholangiocarcinoma), COAD (Colon adenocarcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), ESCA (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIRP (Kidney renal papillary cell carcinoma), LGG (Brain Lower Grade Glioma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), OV (Ovarian serous cystadenocarcinoma), PAAD (Pancreatic adenocarcinoma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SKCM (Skin

Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumor), THCA (Thyroid carcinoma), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma), and UCS (Uterine Carcinosarcoma). In comparison, low AHSA1 expression was observed in only 2 cancer types, KIRC (Kidney renal clear cell carcinoma) and LAML (Acute Myeloid Leukemia) (Fig. 1a). In tumor tissues derived from TCGA, AHSA1 expression was highest in TGCT and lowest in KIRC (Fig. 1b). In normal tissues from GTEx, the highest AHSA1 expression was detected in the testis, with the lowest observed in the pancreas (Fig. 1c).

For paired tumors and normal tissues from TCGA cohort, AHSA1 was highly expressed in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, PAAD, READ, and STAD, while low expression was observed in KIRC (Fig. 2a–m). We further assessed AHSA1 expression in different WHO tumor classifications. We observed that AHSA1 expression was higher in markedly malignant stages of BLCA, ESCA, HNSC, LIHC, LUAD, LUSC, KIRC, and KIRP (Fig. 3a–h). Furthermore, we evaluated the AHSA1 protein levels using the Ualcan database. The results revealed that AHSA1 level was higher in breast cancer, colon cancer, ovarian cancer, and UCEC, but lower in KIRC (Fig. 3i).

Genetic Alteration of AHSA1

Genetic and epigenetic alterations induce changes in gene expression. We explored genetic alterations in AHSA1 using cBioPortal and observed that patients with high AHSA1 expression presented gene alterations in uterine and cervical cancers, as well as DLBC (Fig. 4a). Copy number values were positively correlated with AHSA1 expression (Fig. 4b). In addition, the methylation level of the AHSA1 promoter was negatively correlated with AHSA1 expression (Fig. 4c). These results suggest that high copy number values and low methylation levels contribute to the high expression of AHSA1 observed in pan-cancer analysis.

Prognostic Significance of AHSA1

To evaluate the significance of AHSA1 in predicting the prognosis of patients with tumors, we performed univariate Cox regression analysis and Kaplan-Meier survival analysis using TCGA pan-cancer data. Results of the univariate Cox regression analysis indicate that AHSA1 is a risk factor for patients with ACC, HNSC, KIRP, LIHC, LUAD, MESO, PRAD, and UVM and a protective factor in LGG and OV (Fig. 5a). Kaplan-Meier survival analysis revealed that high AHSA1 expression predicted worse overall survival of patients with ACC, HNSC, KIRP, LIHC, LUAD, and UVM (Fig. 5b). We further assessed the significance of AHSA1 in predicting the disease-free interval (DFI), progression-free interval (PFI), and disease-specific survival (DSS) in patients with tumors, using univariate Cox regression analysis. DFI analysis revealed that AHSA1 acts as a risk factor for ACC, KIRP, LIHC, LUAD, and MESO and a protective factor for OV (Fig. 6a). PFI analysis revealed that AHSA1 acts as a risk factor in ACC, BLCA, ESCA, HNSC, KIRP, LIHC, LUAD, PRAD, and UVM, while serving as a protective factor in LGG, OV, and STAD (Fig. 6b). Finally, the DSS analysis showed that AHSA1 acts as a risk factor in ACC, ESCA, HNSC, KIRP, LIHC, LUAD, LUSC, MESO, and UVM and a protective factor in LGG and OV (Fig. 6c).

Enrichment Analysis of AHSA1

To predict the functions of AHSA1, we performed GSEA using TCGA pan-cancer data. The results suggested that AHSA1 was significantly associated with cell cycle-related and immune regulation-related pathways in ACC, HNSC, KIRP, LIHC, LUAD, and UVM (Fig. 7a–f). For example, AHSA1 was predicted to be involved in pathways such as “Cell Cycle,” “Cell Cycle, Mitotic,” “Adaptive Immune System,” and “Innate Immune System” (Fig. 7d). These results suggest that AHSA1 is strongly associated with tumor cell cycle arrest and tumor immune microenvironment regulation.

Tumor Immune Microenvironment Analysis

We further downloaded the immune cell infiltration score of TCGA pan-cancer from a previously published article [6]. We divided each tumor into two groups according to the median expression of AHSA1 to compare possible differences in immune cell infiltration. We observed that macrophage infiltration levels were significantly higher in the high AHSA1 expression groups of BRCA, CESC, HNSC, KIRC, LIHC, SKCM, and STAD. Simultaneously, the number of CD8 + T cells were lower in the high AHSA1 expression groups of these tumor types (Fig. 8a–g). These results suggest that high AHSA1 expression is associated with the tumor suppressor microenvironment.

Discussion

Immune cells in the tumor microenvironment play a vital role in restricting tumor progression. However, tumor cells can eventually escape immune surveillance and inhibit the cytotoxicity of antitumor immune cells through various mechanisms. The remodeling of immune cells by tumor cells can lead to immune escape, a known cancer marker [7].

Macrophages are multifunctional components of myeloid cells. These cells can phagocytize invading microorganisms or cell debris in injured parts, release immune-regulatory cytokines, and activate the adaptive immune system [8]. Previously, activated TAMs were suspected of demonstrating cytotoxic activity in tumor cells; however, several studies have confirmed the pre-tumor function of TAMs[9]. In recent decades, human samples with CD68 as a macrophage marker and CD163/CD204 as an M2 macrophage marker have been extensively investigated. Several immunohistochemical studies using tumor samples of different histological types and various locations have revealed that the greater the total number of TAMs, the worse the clinical prognosis of patients with tumors[10, 11] .

In human cancer, cell infiltration is a regulator of natural disease progression. As a primary effector cell of anticancer immunity, CD8 + T cells are usually in a state of dysfunction or are reduced in number when infiltrating cancer tissues[12]. Depleted CD8 + T cells are characterized by impaired activity and proliferation, increased apoptotic rates, and decreased production of effector cytokines. The increase in TAM infiltration and decrease in CD8 + T-cell infiltration, observed in the tumor microenvironment, frequently indicates that the tumor is in a state of immunosuppression, which is unfavorable for

implementing immunotherapy. Screening markers that can signal the immunosuppressive status of tumors help identify patients with tumors sensitive to immunotherapy.

In this study, we identified AHSA1 as an oncogene in most tumor types. We observed that AHSA1 was overexpressed in 27 cancer types, including ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS, while low expression was noted in only 2 cancer types (KIRC and LAML). The univariate Cox regression analysis suggested that AHSA1 was a risk factor in patients with ACC, HNSC, KIRP, LIHC, LUAD, MESO, PRAD, and UVM, whereas in LGG and OV, AHSA1 was deemed a protective factor. We further divided each tumor into two groups according to the median expression of AHSA1 to compare the differences in immune cell infiltration. We observed that TAM infiltration levels were significantly elevated and the number of CD8 + T cells were reduced in the high AHSA1 expression groups in BRCA, CESC, HNSC, KIRC, LIHC, SKCM, and STAD. These results suggest that high AHSA1 expression indicates the tumor immunosuppressive status.

Conclusion

In summary, we conducted a comprehensive assessment on AHSA1, revealing the potential cancer-promoting effect of AHSA1 in and its role as an indicator of patient prognosis. Importantly, high AHSA1 expression often indicates that the tumor is immunosuppressed, which may render immune checkpoint inhibitors unsuitable for treatment. Targeting AHSA1 may increase the sensitivity to immune checkpoint inhibitors, providing a potential direction for tumor immune therapy.

Abbreviations

AHSA1

Activator of heat shock 90 kDa protein ATPase homolog 1; TCGA:The Cancer Genome Atlas; GTEX:Genotype-Tissue Expression; TAMs:tumor-associated macrophages; HSP90:heat shock 90 kDa; GSEA:Gene set enrichment analysis; ACC:Adrenocortical carcinoma; BLCA:Bladder Urothelial Carcinoma; BRCA:Breast invasive carcinoma; CESC:Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL:Cholangiocarcinoma; COAD:Colon adenocarcinoma; DLBC:Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA:Esophageal carcinoma; GBM:Glioblastoma multiforme; HNSC:Head and Neck squamous cell carcinoma; KICH:Kidney Chromophobe; KIRP:Kidney renal papillary cell carcinoma; LGG:Brain Lower Grade Glioma; LIHC:Liver hepatocellular carcinoma; LUAD:Lung adenocarcinoma; LUSC:Lung squamous cell carcinoma; OV:Ovarian serous cystadenocarcinoma; PAAD:Pancreatic adenocarcinoma; PRAD:Prostate adenocarcinoma; READ:Rectum adenocarcinoma; SKCM:Skin Cutaneous Melanoma; STAD:Stomach adenocarcinoma; TGCT:Testicular Germ Cell Tumor; THCA:Thyroid carcinoma; THYM:Thymoma; UCEC:Uterine Corpus Endometrial Carcinoma; UCS:Uterine Carcinosarcoma; DFI:disease-free interval; PFI:progression-free interval; DSS:disease-specific survival.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All the data will be provided on reasonable request from the corresponding author.

Competing interests

No conflict or financial interests.

Funding

National Natural Science Foundation of China (2018ZX10301201-008)

Authors' contributions

Fulei Li, Chengdong Liu, Yanling Chen designed the study. Fulei Li and Shasha Li processed the data analysis; Fulei Li and Chengdong Liu wrote the manuscript. Yanling Chen and Lu Bai helped with the validation. All authors contributed to the article and approved the submitted version.

Acknowledgments

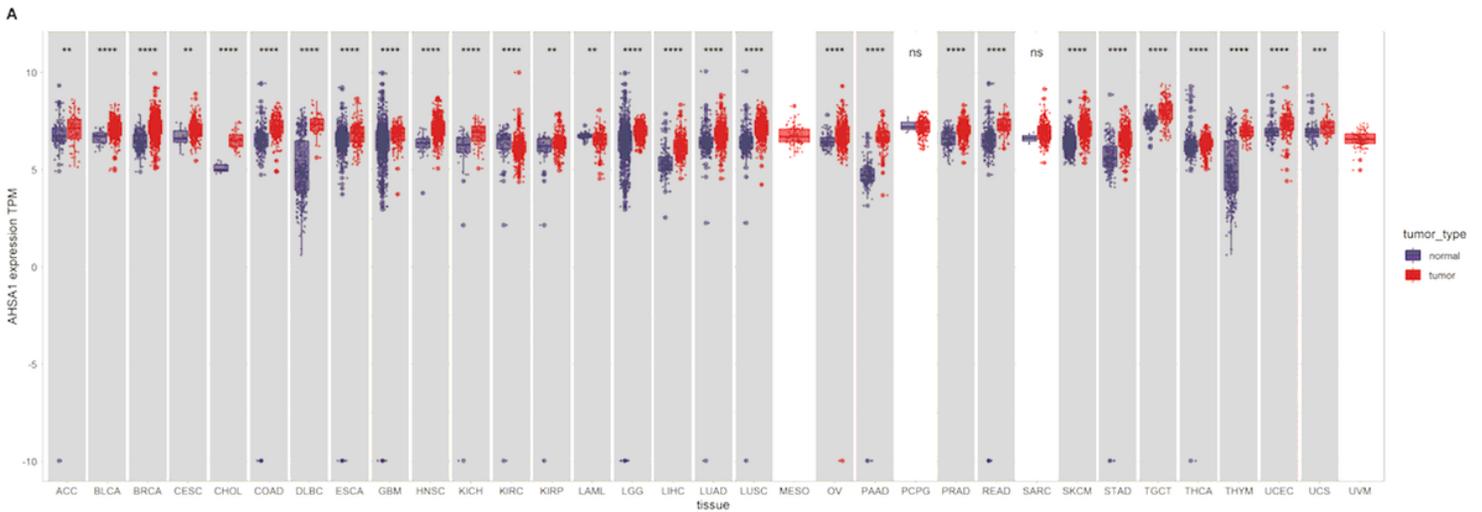
We thank Wechat public account “Shengxinxiaoketang” for providing technical advice on our bioinformatics analysis.

References

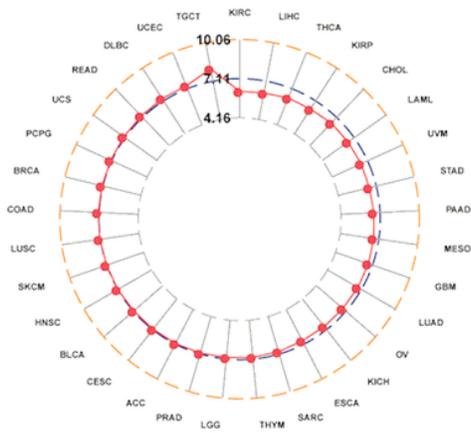
1. Shelton LB, Baker JD, Zheng D, Sullivan LE, Solanki PK, Webster JM, Sun Z, Sabbagh JJ, Nordhues BA, Koren JR, et al. Hsp90 activator Aha1 drives production of pathological tau aggregates. *Proc Natl Acad Sci U S A*. 2017;114:9707–12.
2. Tripathi V, Darnauer S, Hartwig NR, Obermann WM. Aha1 can act as an autonomous chaperone to prevent aggregation of stressed proteins. *J Biol Chem*. 2014;289:36220–8.
3. Jiang W, He Y, He W, Wu G, Zhou X, Sheng Q, Zhong W, Lu Y, Ding Y, Lu Q, et al. Exhausted CD8 + T Cells in the Tumor Immune Microenvironment: New Pathways to Therapy. *Front Immunol*. 2020;11:622509.
4. Shen L, Zhou Y, He H, Chen W, Lenahan C, Li X, Deng Y, Shao A, Huang J. Crosstalk between Macrophages, T Cells, and Iron Metabolism in Tumor Microenvironment. *Oxid Med Cell Longev*. 2021;2021:8865791.

5. Kim J, Bae JS. Tumor-Associated Macrophages and Neutrophils in Tumor Microenvironment. *Mediators Inflamm.* 2016;2016:6058147.
6. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou YT, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, et al. The Immune Landscape of Cancer. *Immunity.* 2018;48:812–30.
7. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. *Ann Oncol.* 2016;27:1482–92.
8. Wang J, Li D, Cang H, Guo B. Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Med.* 2019;8:4709–21.
9. Laviron M, Boissonnas A. Ontogeny of Tumor-Associated Macrophages. *Front Immunol.* 2019;10:1799.
10. Lyssiotis CA, Kimmelman AC. Metabolic Interactions in the Tumor Microenvironment. *Trends Cell Biol.* 2017;27:863–75.
11. Anfray C, Ummarino A, Andón FT, Allavena P. **Current Strategies to Target Tumor-Associated-Macrophages to Improve Anti-Tumor Immune Responses.** *Cells-Basel* 2019, 9.
12. Maimela NR, Liu S, Zhang Y. Fates of CD8 + T cells in Tumor Microenvironment. *Comput Struct Biotechnol J.* 2019;17:1–13.

Figures



B Mean expression of AHSA1 in TCGA



C Mean expression of AHSA1 in GTEx

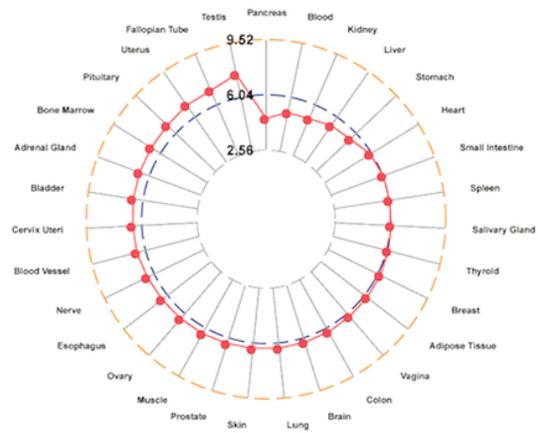


Figure 1

Pan-cancer AHSA1 expression analysis. (A), Pan-cancer differential expression of AHSA1 between tumor tissues from TCGA and normal tissues from TCGA and GTEx database. (B), AHSA1 expression in tumor tissues from TCGA database. (C), AHSA1 expression in normal tissues from GTEx database.

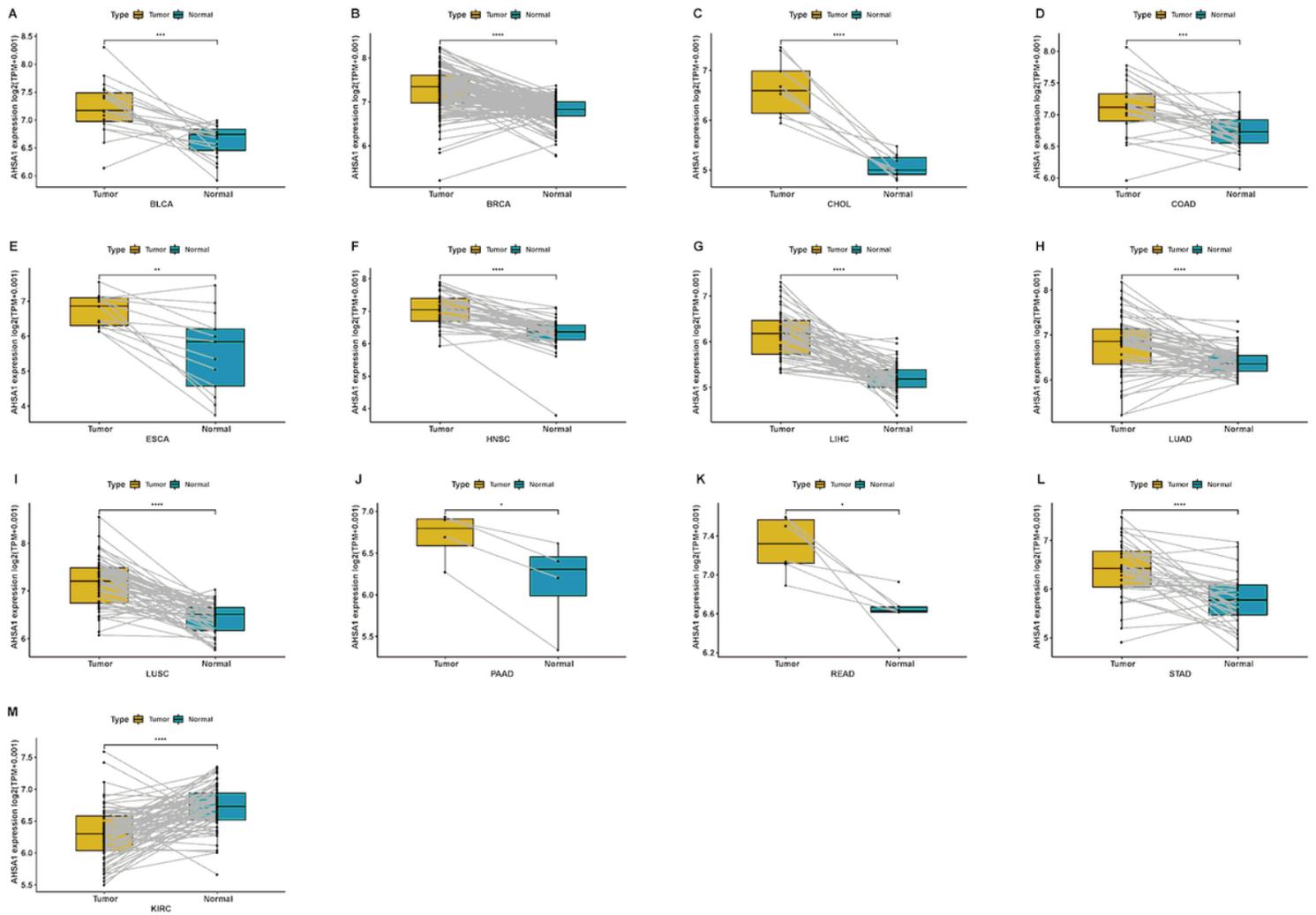


Figure 2

Pan-cancer AHS1 expression in paired tumor and adjacent normal tissues. (A-M), Pan-cancer differential expression of AHS1 in paired tumor and adjacent normal tissues in indicated tumor types from TCGA database.

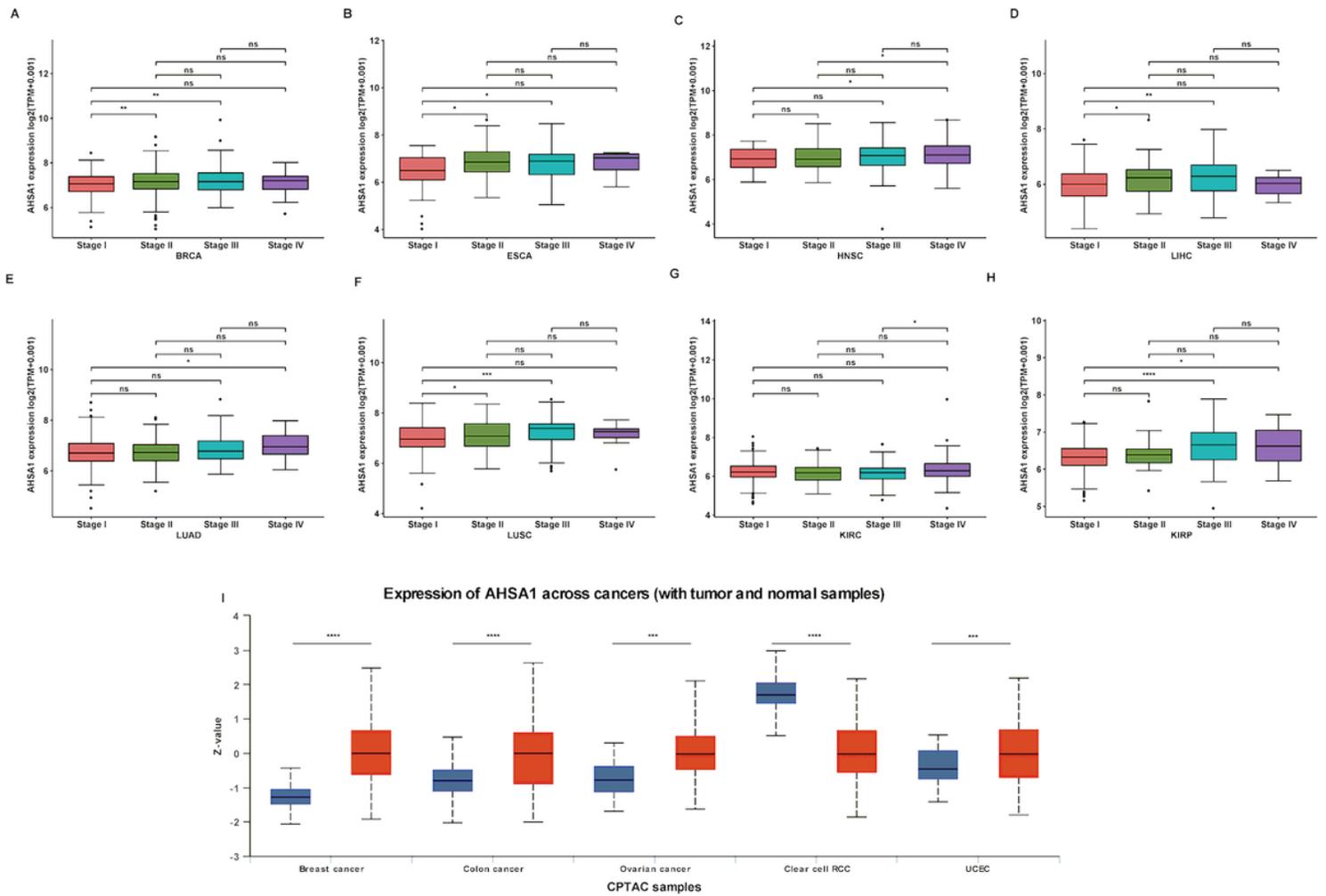


Figure 3

Pan-cancer AHSA1 expression in WHO stages. (A-M), Pan-cancer differential expression of AHSA1 in WHO stages in indicated tumor types from TCGA database (Figure 3A-H). (I), The protein expression of AHSA1 in indicated tumor types from Clinical Proteomic Tumor Analysis Consortium (CPTAC).

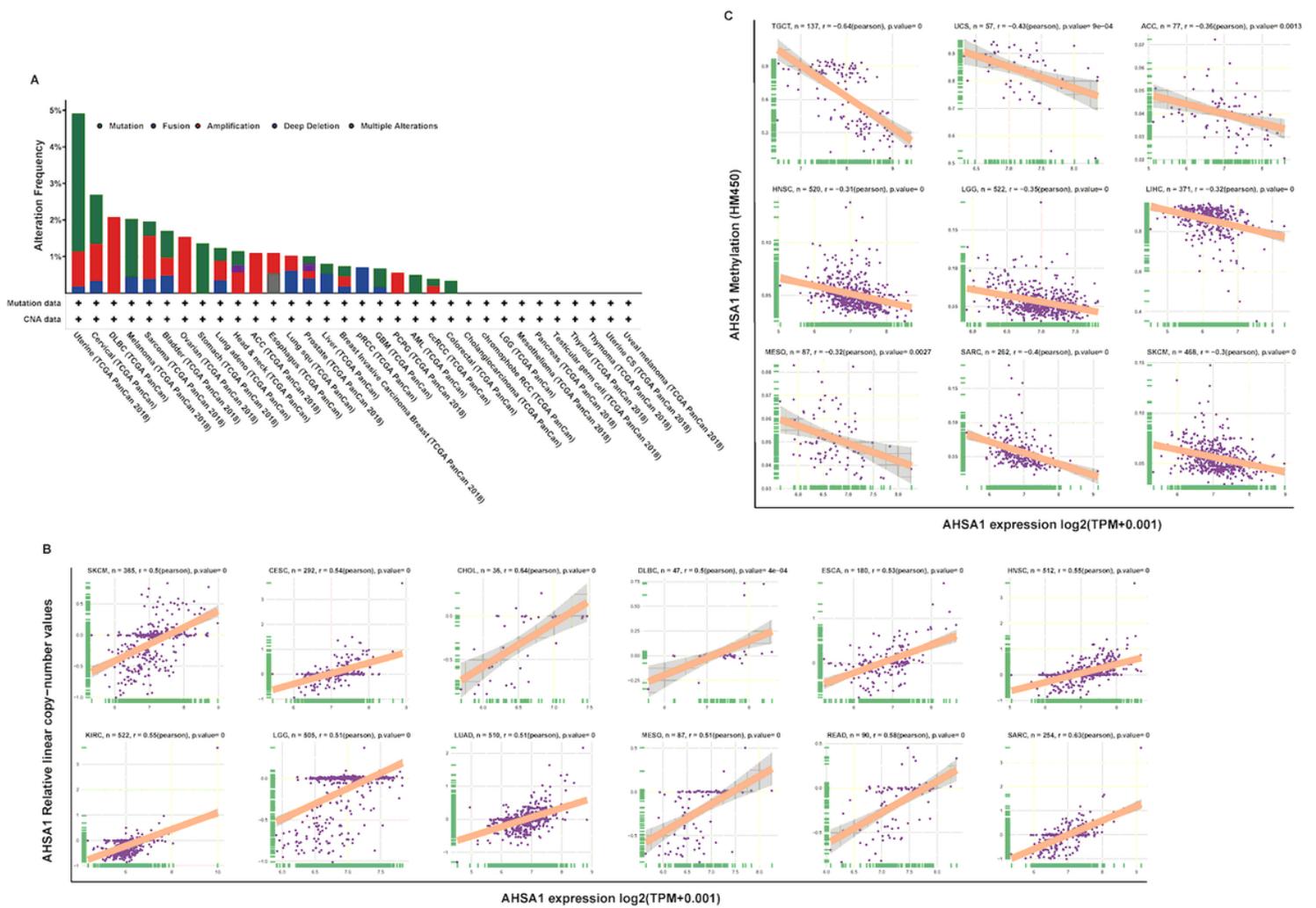


Figure 4

The gene alteration of AHSA1 in pan-cancer. (A), CNA and mutation frequency of AHSA1 in TCGA pan-cancer were accessed from cBioPortal database. (B), The correlation of AHSA1 mRNA expression and linear copy-number value in indicated tumor types from TCGA. (C), The correlation of AHSA1 mRNA expression and promoter methylation level in indicated tumor types from TCGA.

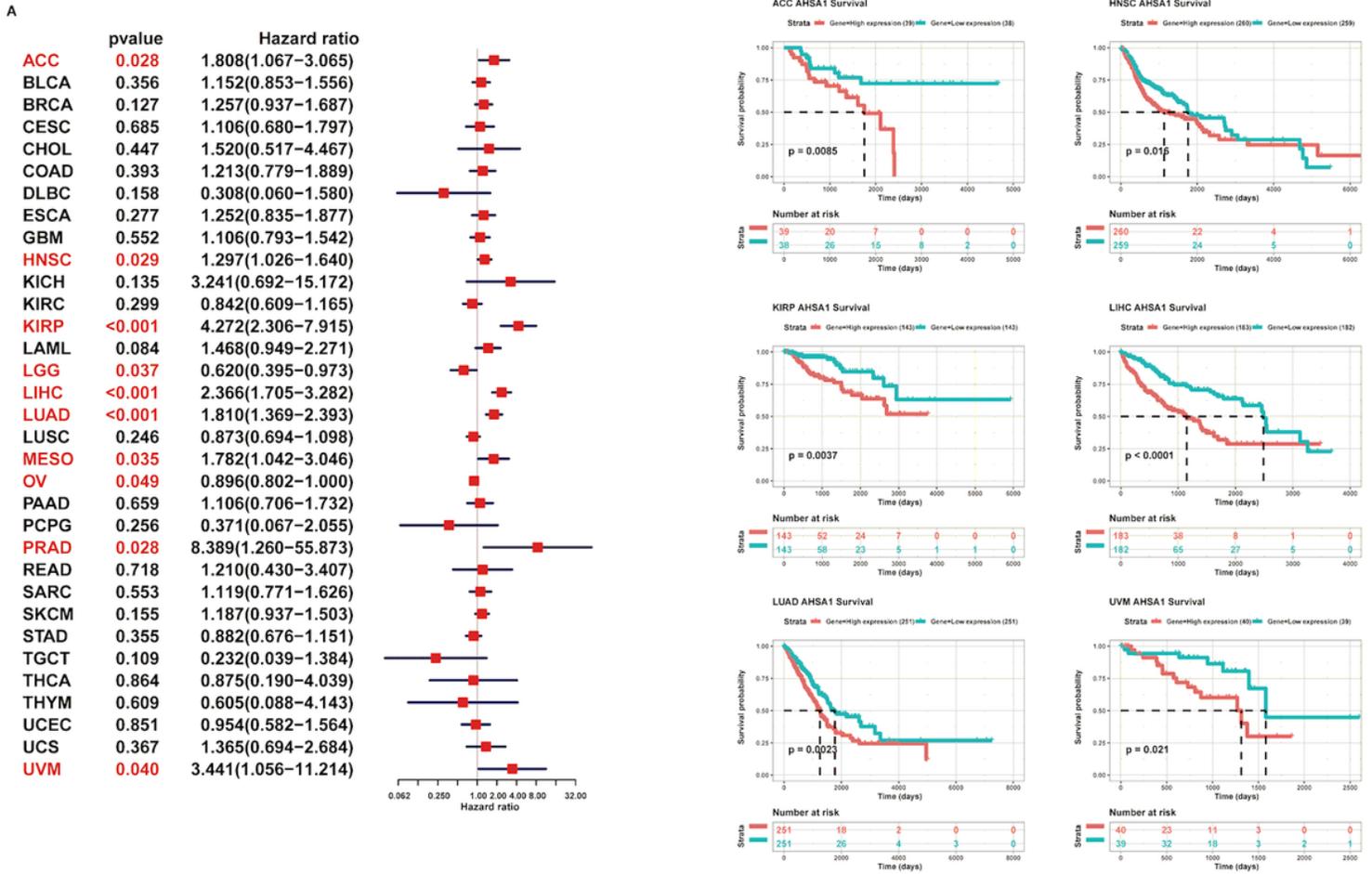


Figure 5

Overall survival analysis of AHSA1 in pan-cancer. (A), Forest map shows the univariate cox regression analysis results of AHSA1 in TCGA pan-cancer. Red color represents significant results. (B), Kaplan-Meier survival analysis results of AHSA1 in indicated tumor types from TCGA pan-cancer.

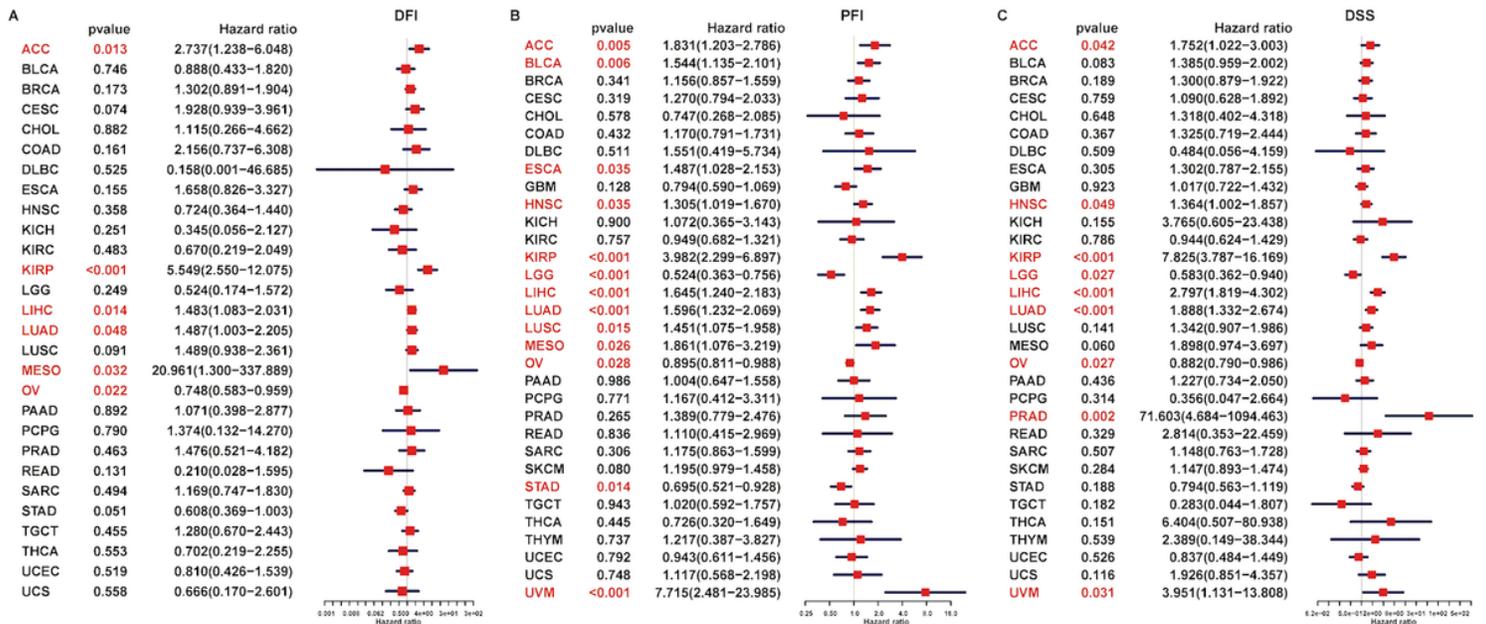


Figure 6

DFI, PFI, and DSS analysis of AHSA1 in pan-cancer. (A), Forest map shows the univariate cox regression analysis results of AHSA1 for DFI in TCGA pan-cancer. (B), Forest map shows the univariate cox regression analysis results of AHSA1 for PFI in TCGA pan-cancer. (C), Forest map shows the univariate cox regression analysis results of AHSA1 for DSS in TCGA pan-cancer. Red color represents significant results.

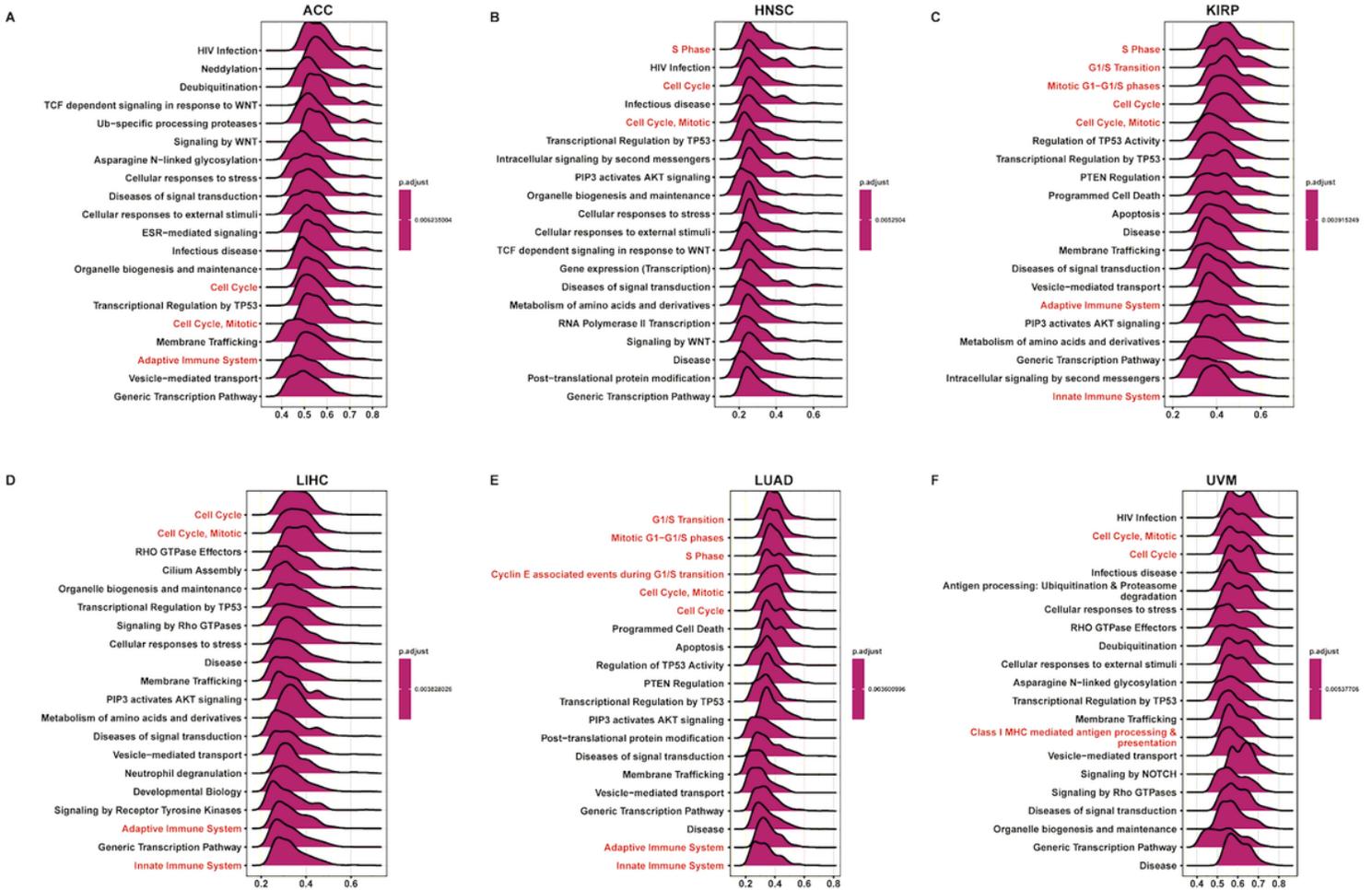


Figure 7

GSEA of AHSA1 in pan-cancer. (A-F), TOP20 GSEA terms in indicated tumor types (NES ≥ 1.5 , p.adjust < 0.05). Red color represents cell cycle related or immune regulation related terms.

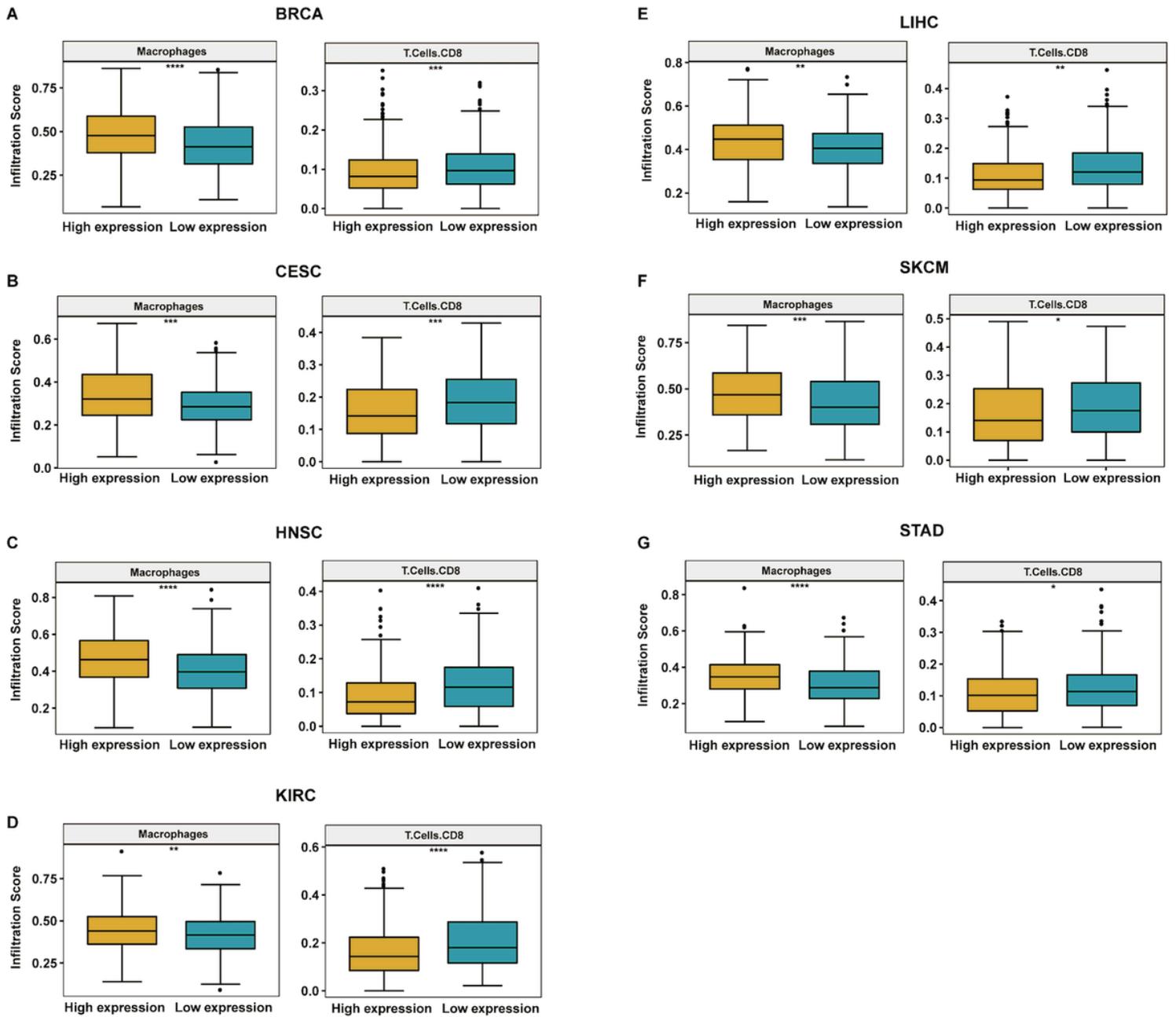


Figure 8

Immune cell infiltration analysis. (A-G), The infiltration level difference of TAM and CD8 positive T cells in indicated tumor types from TCGA pan-cancer.