Comprehensive Bioinformatics Analysis of Toll-like Receptors (TLRs) in Pan-Cancer

Wei Ping
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Senyuan Hong
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Yang Xun
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Cong Li (licongtjm@163.com)
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
https://orcid.org/0000-0001-6986-629X

Research

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Abstract

Background: To conduct a comprehensive bioinformatics analysis on the transcriptome signatures of Toll-like receptors (TLRs) in pan-cancer.

Materials and methods: A total of 11,057 tissues consisted of 33 types carcinoma in the cancer genome atlas (TCGA) were retrieved and then we further explored the correlation between TLRs' expression with tumorigenesis, immune infiltration, and drug sensitivity. We conducted a comprehensive bioinformatics analysis on TLR1 to 10 in pan-cancer, including differential expression analysis between normal and tumor tissues, differential immune subtype correlation, survival analysis, tumor immune infiltration estimating, stemness indices correlation, and drug responses correlation.

Results: TLR2 was high expressed in most type of tumors. TLR9 was hardly expressed compared to other TLR genes, which lead to TLR9 showed less correlation with both immune-estimate scores and stromal-estimate scores. All the TLRs were related with immune subtype of tumor samples that all of them were differentially expressed in differential immune subtype samples. Expression of TLRs were positively related with immune-estimate scores and stromal-estimate scores in almost all types of tumor. Expression of TLRs were negatively correlated with mRNA expression based stemness scores (RNAss) in nearly almost type of tumors except kidney renal clear cell carcinoma (KIRC), and also negatively correlated with DNA methylation based stemness scores (DNAss) in many types of tumors except Adrenocortical carcinoma (ACC), cholangiocarcinoma (CHOL), KIRC, acute myeloid leukemia (LAML), low-grade glioma (LGG), Testicular Germ Cell Tumors (TGCT), Thyroid carcinoma (THCA), Thymoma (THYM), and Uveal Melanoma (UVM). The expression of TLR9 was significant positively correlated with the drug sensitivity of Fluphenazine, Alectinib, Carmustine, and 7–Hydroxystaurosporine. TLR7 was significant positively correlated with the drug sensitivity of Alectinib.

Conclusions: Our study reveals the significant role of TLRs family in pan-cancer and provides potential therapeutic strategies of cancer.

Background

Toll-like receptors (TLRs) represent a family of transmembrane pattern recognition receptors that plays an essential role in the detection and defense against microbial pathogens in innate immunity [1]. TLRs are first line protective immune sentries that can distinguish pathogen-associated molecular patterns (PAMPs), which usually included unmethylated double-stranded DNA (CpG), single-stranded RNA (ssRNA), lipoproteins, lipopolysaccharide (LPS), and flagellin [2]. On this basis, TLRs have been widely studied as the main medium of innate immunity in animals from insects to humans [3–5]. The discovery of TLRs as components to recognize conservative structure of pathogens has greatly promoted understanding of how the body perceives pathogen invasion, triggers innate immune responses and initiates antigen-specific adaptive immunity [6].

It has been reported that Drosophila mutants in the Toll gene are highly susceptible to fungal infection, which identified the innate immune function of TLR [7]. Then, a human Toll homologue, now called TLR4, was identified [8]. Nowadays, A total of 10 TLR family members were identified in humans, and at least 13 have been discovered in mice, which usually expressed on various immune cells, such as dendritic cells (DCs), macrophages, T-cell subsets, and B cells. Besides, also expressed in non-immune cells (e.g. epithelial cells and fibroblasts) in humans [9]. In addition, all components of TLRs include an N-terminal domain (characterized by multiple leucine-rich repeats) and a carboxyl-terminal TIR domain that interacts with TIR-containing adapters. Among them, nucleic acid-sensing TLRs (TLR3, TLR7, TLR8 and TLR9) are located in the endoplasmic chamber, while the left TLRs are present on the plasma membrane. [10, 11].

In recent years, because of the role of TLR in tumor progression, TLR has aroused great interest in tumor research, and many therapeutic interventions for TLR are being developed or studied. Some studies have explored in detail the role of TLR regulation in cancer development [12–14]. Compared with normal patients, the expression of TLR1, 2, 4 and 8 mRNA increased in patients with colorectal cancer [15]. TLRs are also associated with prostate cancer. By promoting malignant transformation of epithelial cells and tumor growth, or on the contrary, inducing apoptosis and inhibiting tumor progression, it may be a double-edged sword of prostate cancer tumorigenesis [16]. The regulation of TLR not only increases the susceptibility of some microorganisms to infection, but also contribute to the development of cancer by altering microbiota and resultant inflammation [17]. On the one hand, TLRs play a essential role in tumor immunity, by which they can activate a variety of cells such as DCs, T-cell subsets, and even tumor cells, on the other hand, the activation of TLRs can also lead to inflammation that result in tumor promotion [18].

However, the characteristics of TLRs are different, and different homologous types may have different effects on different tumor types. In addition, so far, no bioinformatics study has systematically studied the transcriptional level of each TLRs in pan-cancer. Therefore, it is of great significance to study the expression pattern of TLRs in pan-cancer tissues and to develop the potential of TLRs targeted drugs in the treatment of TLRs differentially expressed tumors. In our study, we analyzed the expression characteristics of TLR1 to 10 in pan-cancer by a variety of bioinformatics methods, and comprehensively analyzed TLRs, and found that the transcriptional level of TLRs was associated with stemness, tumor purity and drug sensitivity in TCGA cancers.

Results

Differential expression analysis of TLRs between tumor and normal adjacent tumor tissues

The flowchart of the study was summarized in Figure 1 and the abbreviations of the 33 tumor types in TCGA was shown in Table 1. The gene expression of TLR1 to TLR10 in pan-cancer was displayed (Figure 2A), and it seems the TLR9 was hardly expressed compared to other TLR genes. Also, differential expression analysis with the Wilcox test were performed on 10 TLR family genes between tumor and

normal adjacent tumor tissues. Meanwhile, as the 5 highest expression genes, TLR1 to TLR5 were selected to show the differential expression statu. For TLR1, it was significant low expression in most type of tumors except CHOL, GBM, and KIRC (Figure 2B). TLR2 was significant high expression in most type of tumors except BRCA, LIHC, LUAD, LUSC, and PRAD (Figure 2C). TLR3 was significant low expression in most type of tumors except GBM and KIRC (Figure 2D). TLR4 was ignificant low expression in most type of tumors except GBM and KIRC (Figure 2E). TLR5 was significant low expression in most type of tumors except CHOL, GBM and LIHC (Figure 2F).

Table 1
Abbreviations of the 33 tumor types in TCGA

Abbreviation	Tumor type
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
KIRC	Kidney renal clear cell carcinoma
KIRP	kidney renal papillary cell carcinoma
LAML	Acute Myeloid Leukemi
LGG	Brain Lower Grade Glioma
LIHC	liver hepatocellular carcinoma
LUAD	lung adenocarcinoma
LUSC	lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadeno carcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach adenocarcinoma

Abbreviation	Tumor type
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma

Co-expression analysis of TLRs in pan-cancer and log-rank survival analysis

More detailed information about the differential expression status such as the log_2FC (Fold change) was shown in Figure 3A, it was obvious that TLR2 was high expressed in most type of tumors and TLRs family were low expressed in LUSC and LUAD. In addition, subsequently co-expression analysis of TLRs suggested that all the TLRs were positively correlated with each other except TLR3 that was negatively correlated with

TLR9 (Figure 3B). Then we employed Kaplan-Meier methods to plot the survival curves and performed log-rank analysis to investigate the prognostic value of TLRs in 33 TCGA tumors, and the prognostic TLRs with tumor type and p-value was shown in Table 2, and here we selected KIRC to plot the survival curves, and the four TLRs genes with prognostic value in KIRC were TLR1 (Figure 3C), TLR3 (Figure 3D), TLR4 (Figure 3E), and TLR9 (Figure 3F). Among of them, low expression of TLR1, TLR3, and TLR4 were significantly associated with poor overall survival of KIRC, and high expression of TLR9 was significantly associated with poor overall survival of KIRC.

Table 2
Detailed information of the survival analysis of the TLRs family in pan-cancer with significant p-value,

gene	CancerType	pValue
TLR1	KIRC	0.034688969
TLR1	LGG	0.000296933
TLR1	SARC	0.026721888
TLR1	SKCM	0.000697683
TLR1	UVM	0.002732005
TLR2	LGG	1.65E-05
TLR2	LUAD	0.008433019
TLR2	MESO	0.017243009
TLR2	SKCM	2.17E-06
TLR2	TGCT	0.018921076
TLR2	THYM	0.009423895
TLR3	KIRC	2.94E-07
TLR3	KIRP	0.004130991
TLR3	LGG	0.000104245
TLR3	MESO	0.002692585
TLR3	PAAD	0.024365165
TLR3	SARC	0.009139017
TLR3	SKCM	0.000167396
TLR3	TGCT	0.042432124
TLR3	UCEC	0.031991718
TLR4	ACC	0.007177616
TLR4	KIRC	0.007164204
TLR4	LAML	0.044171562
TLR4	LUAD	0.028500171
TLR4	SKCM	7.46E-05
TLR4	TGCT	0.021867869

gene	CancerType	pValue
TLR4	THYM	0.020130819
TLR4	UCEC	0.00545563
TLR5	ACC	0.01419818
TLR5	ESCA	0.040248748
TLR5	LGG	0.014075805
TLR5	OV	0.036628212
TLR5	SKCM	0.022197327
TLR5	STAD	0.008021708
TLR5	THYM	0.005545537
TLR6	BLCA	0.036456726
TLR6	ESCA	0.01912187
TLR6	KIRP	0.008085447
TLR6	LGG	0.003399361
TLR6	SKCM	0.003736075
TLR7	DLBC	0.032371891
TLR7	LAML	0.01921935
TLR7	LGG	0.00593611
TLR7	LUAD	0.000486804
TLR7	SARC	0.016297397
TLR7	SKCM	0.001124964
TLR7	UVM	0.03400155
TLR8	LAML	0.032090719
TLR8	LGG	0.003214408
TLR8	SKCM	1.26E-06
TLR8	THYM	0.020533224
TLR8	UVM	0.004248326
TLR9	KIRC	0.020215421
TLR9	LAML	0.038214202

gene	CancerType	pValue
TLR9	UCEC	2.97E-05
TLR10	CESC	0.02579765
TLR10	COAD	0.041270017
TLR10	HNSC	0.013737092
TLR10	LGG	0.003769908
TLR10	LUAD	0.000369848
TLR10	READ	0.028148853
TLR10	SARC	0.000659968
TLR10	SKCM	1.31E-05
TLR10	UCEC	0.003987076

Cox regression and immune subtype analysis

Univariate Cox proportional hazard regression was performed to explore the prognostic values of TLRs separately in 33 types of tumor. Gene was considered as a risk factor if hazard ration (HR) >1, in contrast, a protective factor if HR <1. According to the forest plot (Figure 4A), we found that TLRs play a complex role in cancer prognosis that were risky in some types of tumor, and protective in the left types of tumor. In addition, we performed Kruskal test on the expression of TLRs in six immune subtypes across 33 TCGA tumor types (Figure 4B). Interestingly, all the TLRs were related with immune subtype of tumor samples that all of them were differentially expressed in differential immune subtype samples. Among of them, TLR1, TLR2, TLR3, TLR5, TLR6, TLR7, and TLR8 were highest expression in C6 immune subtype samples, TLR4 and TLR10 were highest expression in C5 immune subtype samples.

TLRs and tumor microenvironment in pan-cancer

Immune-estimate scores and Stromal-estimate scores of samples were calculated by the R package 'ESTIMATE' [19], Spearman correlation test was applied to explore the correlation between TLRs' expression and tumor microenvironment. For the Immune score, expression of TLRs were positively related with Immune scores in almost all types of tumors except TLR1 in UVM, TLR3,4, 5 in THYM, and TLR10 in DLBC (Figure 5A). Also, for the Stromal scores, expression of TLRs were positively related with Stromal scores in almost all types of tumors except TLR1 in UVM, and TLR3 in ACC, LAML, MESO, and READ (Figure 5B). It needs to be pointed that TLR9 showed less correlation with both Immune scores and Stromal scores, which may because of the low expression of TLR9 in all tumor samples.

TLRs and Stemness indices in pan-cancer

We downloaded the stemness indices of all samples from UCSC Xena database, which were calculated by using a one-class logistic regression (OCLR) in Malta's research [20]. Meanwhile, two types of stemness index were accessed including DNA methylation based stemness scores (DNAss) and mRNA expression based stemness scores (RNAss). Interestingly, it seems the expression of TLRs were negatively correlated with RNAss in nearly almost type of tumors except KIRC (Figure 5C), and also negatively correlated with DNAss in many types of tumors except ACC, CHOL, KIRC, LAML, LGG, TGCT, THCA, THYM, and UVM (Figure 5D). Among of the DNAss, nearly all TLRs were positively correlated with the DNAss in THYM samples except TLR7 and TLR9.

TLRs and drug respones in pan-cancer.

The expression profile of NCI-60 cancer cell lines and their drug sensitivity were downloaded from the CellMiner database, then Pearson correlation test was carried to further analysis the correlation between the expression of TLRs and drug response of 263 antineoplastic drugs. All the results with significant correlation between TLRs and drug sensitivity were displayed in supplementary Table, and the 25 most significant results with the smallest p-value were shown as scatter plot ranked by p-value (Figure 5E). Among them, the 5 most significant correlation were as follow, the expression of TLR9 was significant positively correlated with the drug response of Fluphenazine (co-efficient =0.680, p<0.001), Alectinib (co-efficient =0.637, p<0.001), Carmustine (co-efficient =0.598, p<0.001), and 7−Hydroxystaurosporine (co-efficient =0.550, p<0.001). TLR7 was significant positively correlated with the drug response of Alectinib (co-efficient =0.595, p<0.001).

TLRs in KIRC

Finally, we explored the TLRs in KIRC by comparing the transcriptional expression of TLRs in different Stage of KIRC, comparing the differential expression of TLRs in different immune subtype, and investigating the correlation between TLRs and stemness indices or tumor purity in KIRC. TLR2, TLR3, TLR4, TLR10 were significantly differentially expressed between Stage I to Stage IV with p <0.05 (Figure 6A), and TLR1, TLR3, TLR4, TLR7, TLR8, TLR10 were significantly differential expressed between C1 to C6 immune subtype with p <0.001 (Figure 6B). For the RNAss in KIRC samples, TLR5 and TLR9 was significantly negatively correlated with it (correlation co-efficient =-0.12, p =0.042 and correlation co-efficient =-0.23, p <0.001), TLR1, TLR2, and TLR3 were significantly positively correlated with it (correlation co-efficient =0.11, p =0.048, correlation co-efficient =0.14, p =0.014 and correlation co-efficient =0.14, p =0.013).

For the DNAss in KIRC samples, it was interesting that all the TLRs were negatively correlated with DNAss in KIRC patients, among of which TLR1, TLR2, TLR6, TLR7, TLR8, TLR10 were significant with p <0.05. Also, all the TLRs were significantly positively related with the Immune scores, Stromal scores, and Estimate scores. Among them, TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR10 were significantly positively correlated with Stromal scores in KIRC patients with p <0.05, TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and TLR10 were significantly positively related with Immune scores in KIRC patients

with p <0.05, TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR10 were significantly positively related with Estimate scores in KIRC patients with p <0.05.

Discussion

Many studies have demonstrated that several cellular and molecular mechanisms can help tumors escape the body's own natural immune response [21, 22]. The importance of immune regulation to cancer progression can be explained by the increase in the number of immunosuppressive factors and cells and the lack of immune-system–activating signals in tumor microenvironment. TLRs is one of the important receptors that activate immune cells on the surface of immune cells. Hence, it is worthwhile to explore TLRs role in tumor development. TLRs can up-regulate the expression of costimulatory molecules such as CD40, CD80, CD86 and cytokines such as IL-12, thus stimulating other immune cells such as T lymphocytes. [23, 24]. Meanwhile, TLR expression can lead to tumor growth by triggering on other cells including cancer cells [25]. In this study, we are committed to explore the relationship between TLRs' transcriptional expression and TCGA tumor characteristics, including TME, clinical significance, immune subtypes, stem cells and drug response. TLRs isotype has a significant effect on tumorigenesis. First, we analyzed the differential expression of 33 TCGA tumor types in 11057 samples (including 10327 tumor samples and 730 paracancerous samples). Through multidimensional analysis, we found that there were significant differences in TLRs expression levels among different tumor types. Moreover, survival analysis and Cox proportional hazard regression were also performed. In some types of cancers, we found that there was a statistically significant difference in survival between patients with high and low expression of TLRs, suggesting that TLRs may be a potential prognostic indicator for clinical application. Furthermore, we carried out the drug response analysis to explore the relationship between drug sensitivity and TLRs. This is expected to provide some help for anticancer therapies.

In our study, it was obvious that TLR2 was high expressed in most type of tumors. This result is similar to that of most previous studies [26–28]. Gergen et al. [29] reported TLR2 activation induced proliferation of lung adenocarcinoma cells by activating NF-κB. As a special medium between lung cancer cells and mesenchymal stem cells in tumor microenvironment, TLR2 promotes crosstalk and ultimately promotes the change of tumor supporting phenotype of mesenchymal cells [30]. Furthermore, the expression of TLR2 protein has been confirmed to be up regulated in colon cancer, so the high expression of TLR2 is significantly correlated with the low overall survival rate of patients with colon cancer [31, 32]. This means the TLR2 signaling pathway may be an important potential therapeutic target in tumors. In addition, we found that TLR9 was hardly expressed compared to other TLR genes, which lead to TLR9 showed less correlation with both immune scores and stromal scores. But several studies have reported TLR9 were associated with the development of cancer, such as gynecologic cancer [33, 34]. The activation of TLR9 on DC and pDC promotes the secretion of a large amount of type I IFN, which has direct (tumor cell inhibitory effect) and indirect (antitumor immune responses) effects on cancer cells, and is most obvious in the early stage of anti-tumor immune response [35].

Thorsson et al. [36] identified the immune landscape of cancer into C1-C6 immune subtypes. In our study, we classified tumor samples by representative immune signatures and detected the RNA-seq level of TLR 1-10 from C1 to C6. Interestingly, all the TLRs were related with immune subtype of tumor samples that all of them were differentially expressed in differential immune subtype samples. Tumor microenvironment (TME) is closely related to immune functions such as extracellular matrix, tumor vascular system and tumor cells, and has an important impact on treatment response and clinical prognosis [37]. TLRs are expressed in TME [38]. Our study further confirmed this point, we extracted the fractions of stromal cells and immune cells in tumor samples of 33 TCGA cancer types by calculating stromal scores, immune scores, and ESTIMATE scores. Expression of TLRs were positively related with immune scores and stromal scores in almost all types of tumor. On the one hand, TLRs is expressed in programmed cell death induced by TME, on the other hand, it releases cytokines and chemokines in the tumor environment, and recruits immune cells to further release pro-inflammatory cytokines, angiogenic factors and growth factors, such as TGF β, IL-8, CXCR4, ICAM-1 and VEGF,. It can repair the anti-tumor function and apoptosis response of antigen-presenting cells (APCs) and effector T cells [39, 40]. TLRs signaling pathway plays an essential role in controlling tumor progression, metastasis, recurrence and chemotherapy tolerance by inappropriate immune enhancement and anti-tumor immunity [41].

Stemness was applied to distinguish the stem cell-like characteristics of the tumor, such as self-renewal and dedifferentiation [42]. Two types of stemness indices were accessed including DNAss and RNAss [43]. We found that expression of TLRs were negatively correlated with RNAss in nearly almost type of tumors except KIRC, and also negatively correlated with DNAss in many types of tumors except ACC, CHOL, KIRC, LAML, LGG, TGCT, THCA, THYM, and UVM. TLR3 activation facilitated the expression of stemness-associated genes, including OCT3/4, NANOG, and SOX2 [44]. TLR4 expression in HCC were associated with increased stem-like properties [45]. NF-κB, activated by TLR signaling, was closely bound up with the proliferation, invasion and tumorigenesis, invasion, and tumorigenesis of tumor stem cells [46]. Meanwhile, our study also found that the transcriptional expression level of TLR7 and TLR9 was associated with drug responses. Among them, the expression of TLR9 was significant positively correlated with the drug sensitivity of Fluphenazine, Alectinib, Carmustine, and 7−Hydroxystaurosporine. There was a significant positive correlation between TLR7 and the drug sensitivity of Alectinib. These results have clinical relevance for guiding selection of antitumor therapies.

Finally, we explored the relationship between TLRs and KIRC. TLR2, TLR3, TLR4, TLR10 were significantly differentially expressed between Stage I to Stage IV. TLR1, TLR3, TLR4, TLR7, TLR8, TLR10 were significantly differential expressed between C1 to C6 immune subtype. All the TLRs were positively correlated with the immune scores, stromal scores, and estimate scores. Morikawa et al. [47] reported that TLR3 is overexpressed in KIRC, which suggested TLR3 pathway may be a novel therapeutic target in KIRC. Moreover, the expression of TLR9 is an independent prognostic marker of KIRC, and the loss of TLR9 expression is related to the poor prognosis of KIRC [48]. Our results provide guidance for further exploration of the role of TLR in KIRC.

Although this is the first study to multidimensionally analyze TLRs in pan-cancer, it still has some limitations. First, our results have not been verified by other independent databases, and thus, it is necessary to validate the conclusions by our own data and other public database in the future. Second, we have not explored the underlying mechanisms behind bioinformatics analysis through molecular and animal experiments. Finally, we studied the relationship between the TLRs family and a variety of combinatorial data. However, biometric correlation may not directly clarify the mechanisms of interaction and regulation Thus, further studies need to be carried to verify these potential mechanisms via laboratory molecular experiments. Besides, in order to find the potential of TLRs and its coactivators as tumor targets, further investigation is needed.

Conclusions

TLRs expressed differently in both different tumor types and different immune subtype tissues. We reveal the significant role of the TLRs family in pan-cancer and provides potential therapeutic strategies of cancer. However, more laboratory studies are needed to confirm our results.

Materials And Methods

Data sources

The transcriptome profile, clinical phenotype information, survival information, immune subtype profile, DNAss and RNAss profiles of these 33 types of tumor were all downloaded from GDC TCGA sets or TCGA Pan-cancer sets in UCSC-Xena database (http://xena.ucsc.edu/), and our latest search was on Nov 15th, 2020. The transcriptome profiles containing both tumor tissues and normal adjacent tumor (NAT) tissues with a total of 11057 samples were in formats of Fragments Per Kilobase per Million (FPKM).

Expression status of TLRs in pan-cancer

We firstly extract the expression of TLRs in pan-cancer, then visualized it to determine the expression of TLRs in pan-cancer. Then we selected the 5 highest expressed TLRs to do the further differential expression analysis. Also, here we sort the expression profiles that only retained those tumor types with expression profile of normal adjacent tumor tissues, and they were BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Then we extracted the expression of the 5 highest expression TLRs in these tumor types and did the differential expression analysis between tumor and NAT. Also, for all the TLRs, we calculated the $\log_2$Fold Change (logFC) of each TLR in these tumor types and presented them in a heatmap. Subsequently, we applied the correlation test to explore the co-expression of these 10 TLRs according to the expression profile.

Prognostic value of TLRs in pan-cancer

For each TLR gene and each tumor type, we performed log-rank survival analysis (grouped by the medium expression of the TLR in each tumor type) and univariate cox regression to explore the prognostic value of TLRs in pan-cancer.

Immune subtype correlation, Stemness indices correlation, and tumor microenvironment estimating

According to the immune subtype profile of these samples, we explored the differential expression status of TLRs in differential immune subtype. Besides, we further probed the correlation between the expression of TLRs and the Stemness index of samples containing DNAss and RNAss in pan-cancer. Also, we applied ESTIMATE methods to analyze the Immune-estimate score and Stromal-estimate score for each sample, then performed the correlation test to examine the correlation between the expression of TLRs and these two scores.

Drug sensitivity analysis of TLRs in pan-cancer

Data including both expression of TLRs and drug sensitivity was retrived from the CellMiner database ((https://discover.nci.nih.gov/cellminer/), which collected genomic and pharmacologic information for investigators to determine the correlation between gene expression and drug sensitivity in the NCI-60 cell line sets. Thus, we conducted Pearson correlation test between the expression of TLRs and drug sensitivity.

TLRs in KIRC

Finally, as TLRs showed a great performance in predicting the overall survival of KIRC, we did the same analysis flow as before in KIRC instead of pan-cancer, containing immune subtype correlation, Stemness indices correlation, and tumor microenvironment correlation. In addition, we explored the differential expression status of TLRs between Stage I to Stage IV to determine whether TLRs could serve as a biomarker of survival and progression.

Statistical analysis

All the statistical analyses were conducted by the R software (version 4.0.2). $P < 0.05$ was regarded as statistically significant.

Abbreviations

TLRs Toll-like receptors

RNAss Mrna Expression Based Stemness Scores

KIRC Kidney Renal Clear Cell Carcinoma

DNAss DNA Methylation Based Stemness Scores

ACC Adrenocortical Carcinoma

CHOL Cholangiocarcinoma

LAML Acute Myeloid Leukemia

LGG Low-Grade Glioma

TGCT Testicular Germ Cell Tumors

THCA Thyroid Carcinoma

THYM Thymoma

UVM Uveal Melanoma

PAMPs Pathogen-Associated Molecular Patterns

ssRNA Single-Stranded RNA

LPS Lipopolysaccharide

DCs Dendritic Cells

TCGA The Cancer Genome Atlas

HR Hazard Ration

OCLR One-Class Logistic Regression

Declarations

Ethics approval and consent to participate

This study was not applicable for ethical approval, source data of this study were derived from the public repositories.

Consent for publication

Not applicable

Availability of data and materials

Source data of this study were derived from the public repositories, as indicated in the section of “Materials and Methods” of the manuscript. And all data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

Funding Statement

None

Author's contributions

PW and LC: design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript; PW and HSY: statistical analysis; PW, XY, HSY and LC: methodology; LC: project administration; PW and XY: Writing (original draft); PW, HSY, XY, and LC: Writing (review& editing).

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Figures

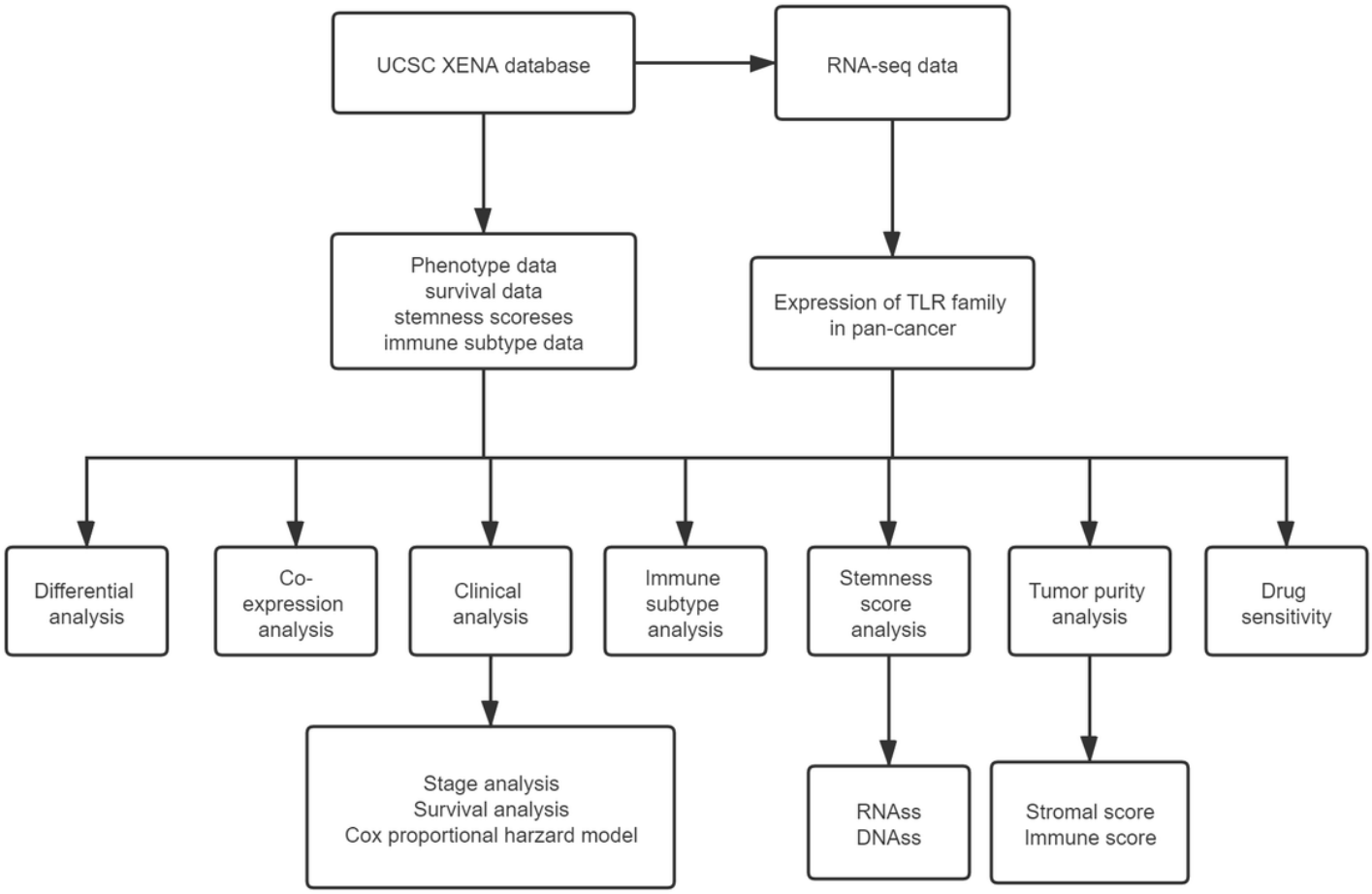

Figure 1

The study flow chart

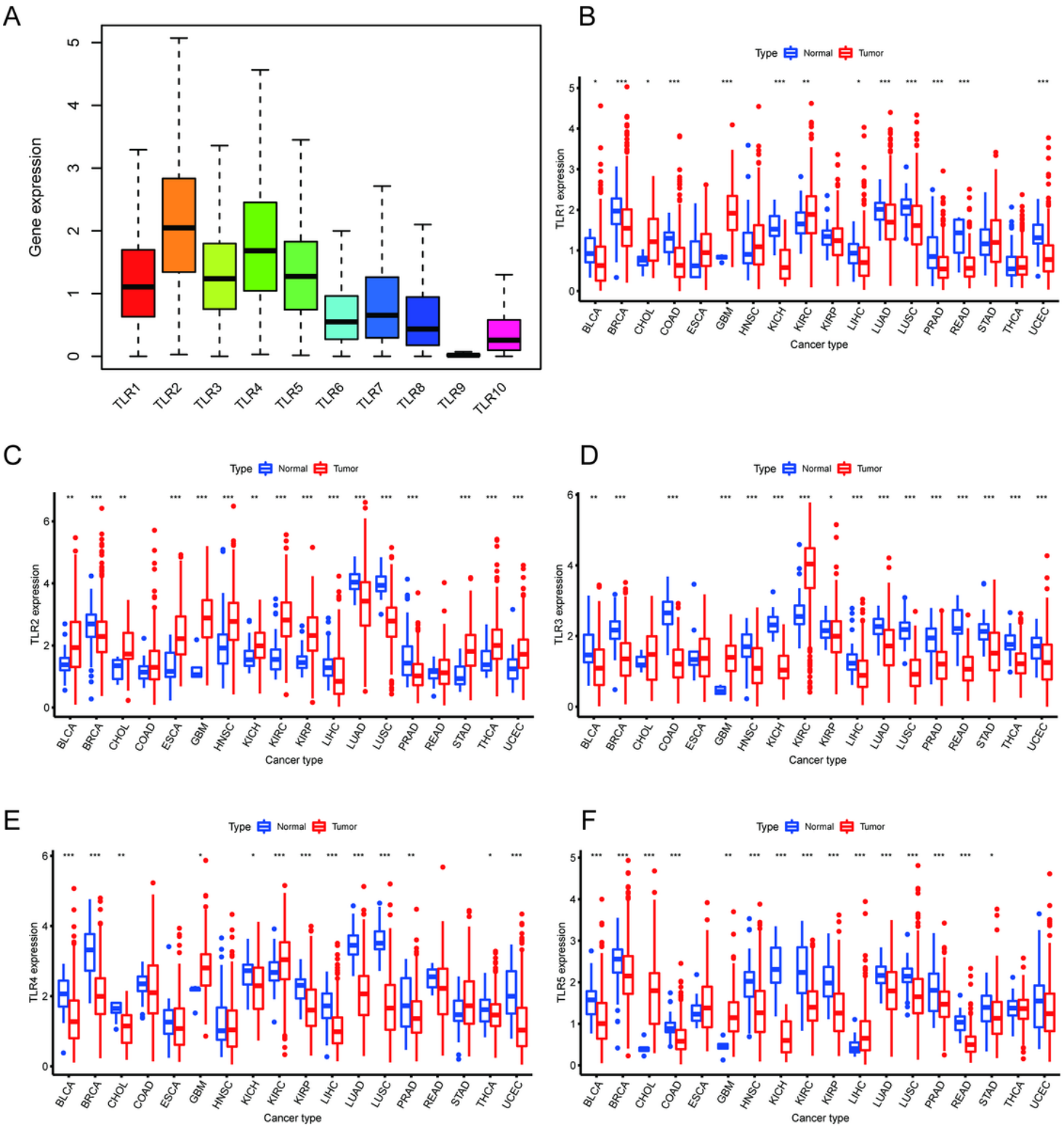

Figure 2

Expression status of TLRs. (A) Expression of TLRs in pan-cancer. (B) Differential expression of TLR1 in pan-cancer. (C) Differential expression of TLR2 in pan-cancer. (D) Differential expression of TLR3 in pan-cancer. (E) Differential expression of TLR4 in pan-cancer. (F) Differential expression of TLR5 in pan-cancer.

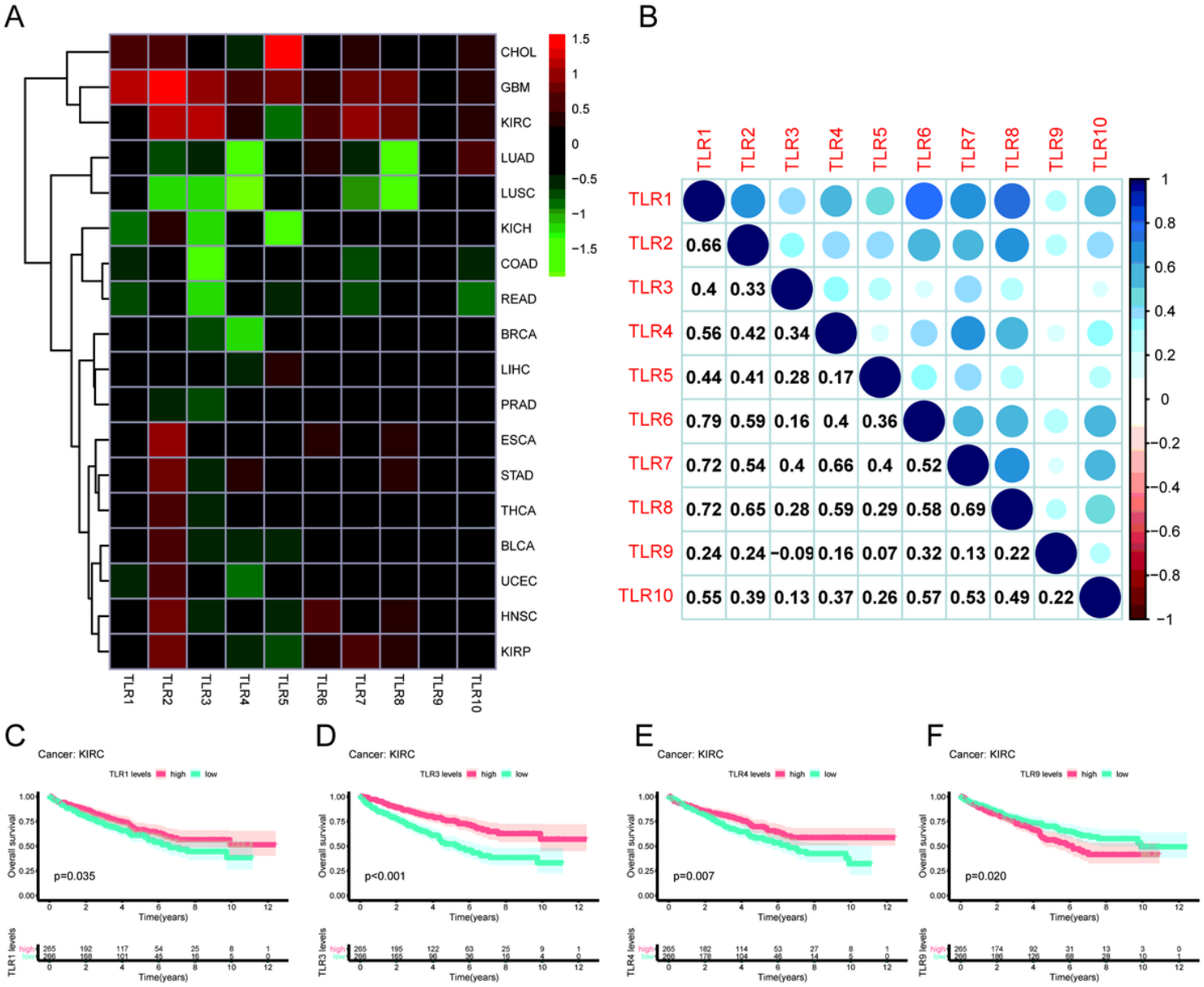

Figure 3

Co-expression of TLRs and survival curves in KIRC. (A) Differential expression status of TLR1 to TLR10 in pan-cancer. (B) Co-expression of TLRs in pan-cancer. (C) TLR1 as a candidate prognostic factor in KIRC. (D) TLR3 as a candidate prognostic factor in KIRC. (E) TLR4 as a candidate prognostic factor in KIRC. (F) TLR9 as a candidate prognostic factor in KIRC.

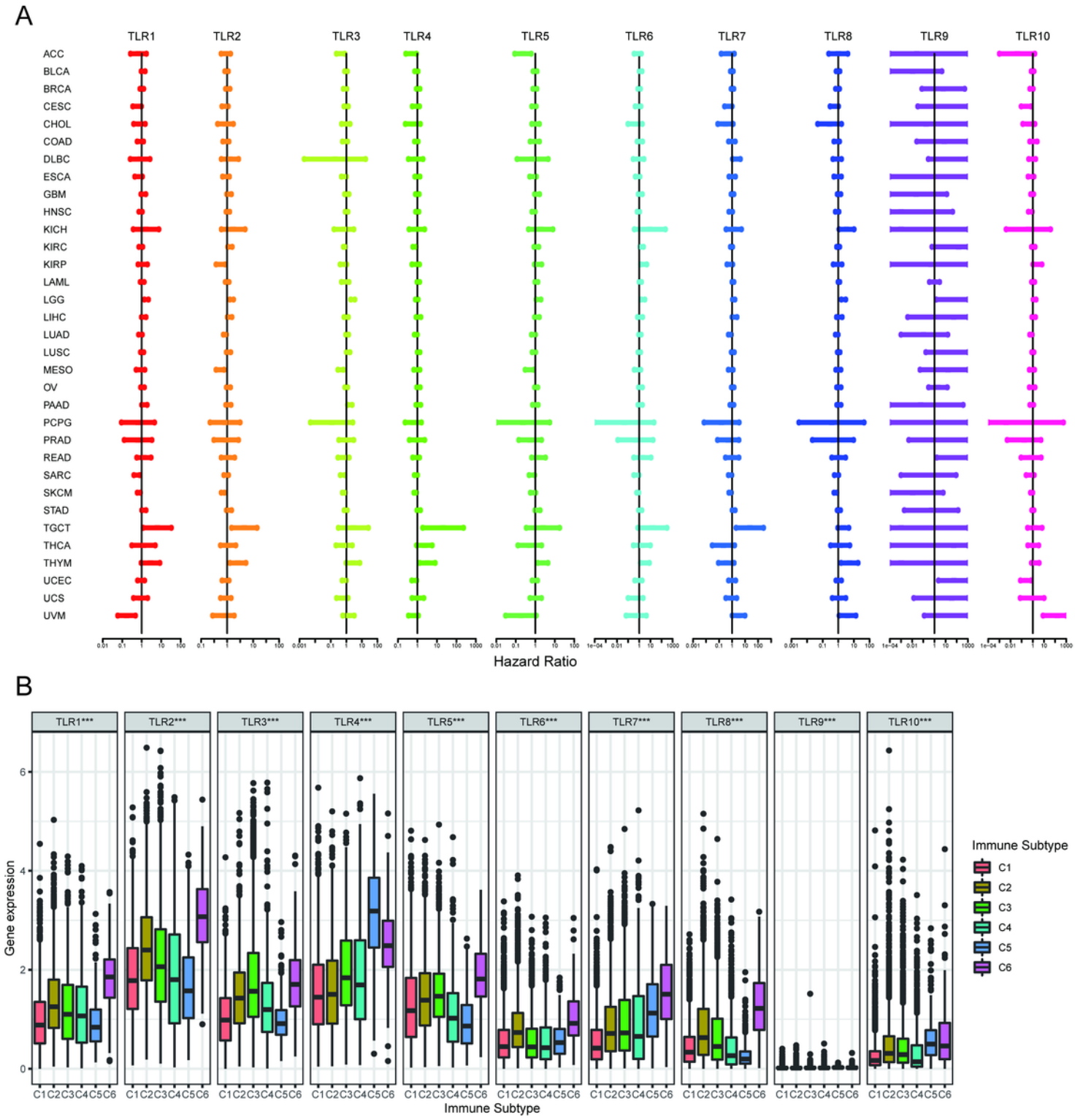

Figure 4

Cox regression and immune subtype analysis in pan-cancer. (A) univariate cox regression for each TLR gene in pan-cancer. (B) Differential expression of TLRs in differential immune subtype.

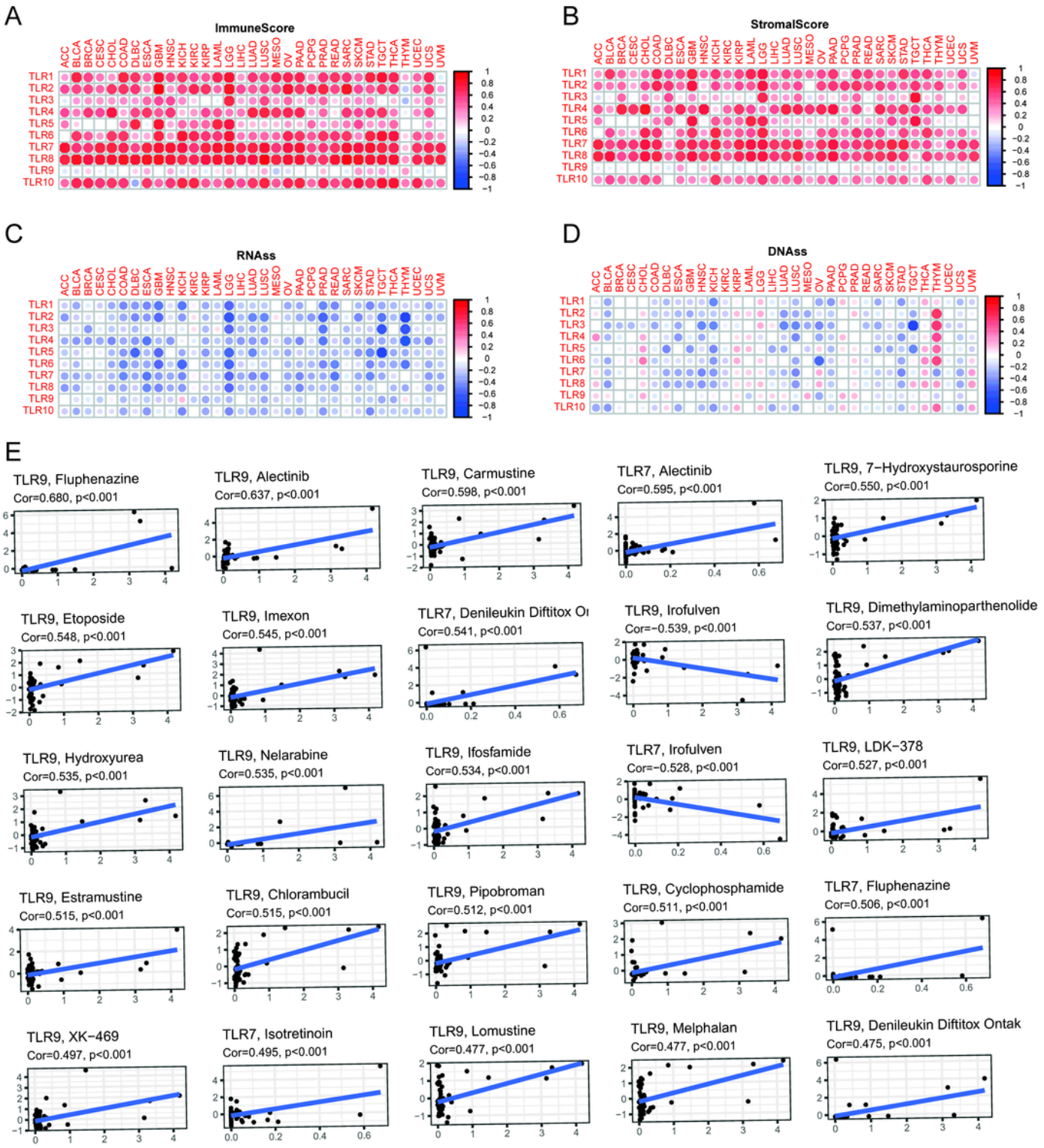

Figure 5

Stemness indices analysis , tumor microenvironment analysis and drug sensitivity analysis in pan-cancer. (A) The correlation between Immune score and expression of TLRs. (B) The correlation between Stromal score and expression of TLRs. (C) The correlation between RNAss and expression of TLRs. (D) The correlation between DNAss and expression of TLRs. (E) Drug sensitivity of TLRs.

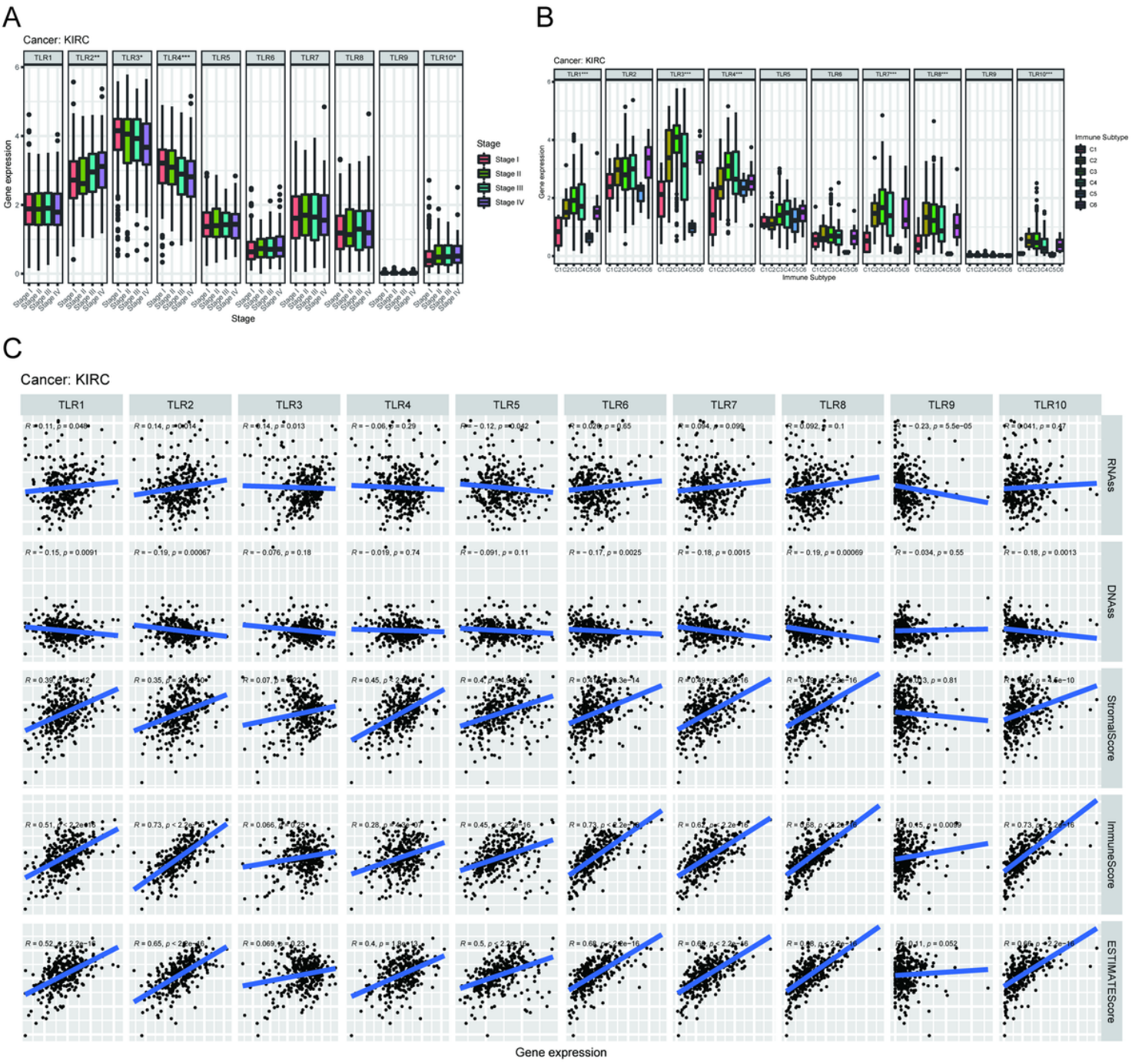

Figure 6

TLRs in KIRC. (A) Differential expression of TLRs between Stage I to Stage IV in KIRC. (B) Differential expression of TLRs in different immune subtype in KIRC. (C) Correlation between the expression of TLRs and Stemness indices, tumor microenvironment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable.pdf