In-Silico CLEC5A mRNA expression analysis to predict Dengue susceptibility in cancer patients

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ABSTRACT

Dengue fever is the fastest-growing infectious disease in the world. It is the leading vector-borne viral neglected tropical disease. The most acute immune response to dengue virus infection is dengue shock syndrome and hemorrhagic fever, which is due to the activation of CLEC5A C-type lectin domain family 5, member A (CLEC5A). It is a cell surface receptor, and its well-known ligand is the dengue virus. It gets activated by the attachment of dengue virion, which, as a result, phosphorylates its adaptor protein DAP12 leading to the induction of various pro-inflammatory cytokines. Clinical data suggested that the kidneys and lungs are among the major hit organs in the case of severe dengue infection. Here we predict kidney and lung cancer patients are vulnerable to dengue virus infection as CLEC5A mRNA expression in tumor samples using publicly available software such as TIMER and GEPIA database. We also identified the immunomodulatory role CLEC5A gene therefore targeting it could be a vital tool to cure dengue.

1. Introduction

Dengue fever is among the most prevalent vector-borne viral neglected tropical diseases, affecting nearly 2.5 billion people at risk globally. The causing agent of the disease is dengue virus (DENV), a flavivirus carried by mosquitoes Aedes sp. as vectors [1]. The condition presents multiple clinical complications, from a mild flu-like illness to severe hemorrhagic fever and dengue shock syndrome. Host immunity is vital for a patient in a critical stage [2]. The most acute immune responses to dengue virus infection are dengue shock syndrome and hemorrhagic fever due to activation of CLEC5A C-type lectin domain family 5, member A (CLEC5A). It is a cell surface receptor, and its well-known ligand is the dengue virus. It gets activated by the attachment of dengue virion, which as a result, phosphorylates its adaptor protein DAP12 leading to the induction of various pro-inflammatory cytokines [3].

Despite ample data in the literature which established CLEC5A is a critical potential prognostic biomarker in diverse types of cancers and can serve as a target for anti-tumor therapy [4–6]; however, still, there is no significant association reported to date between dengue and cancer risk [7]. Chien et al., reported that Dengue virus infection is associated with an increased risk of leukemia, thus underlining the overall significance of further research to establish clear evidence of the involvement of dengue infection in human malignancies [7]. As per various reports, peripheral organs such as the lungs and kidneys are severely affected in case fatal dengue cases, and virus-specific triggers of the pro-inflammatory response are observed in these tissues [8]. Therefore, it is fascinating to analyze how immunocompromised cancer patients responds to DENV. Here we investigated whether the risk of Dengue severity was increased in Lung cancer and kidney cancer patients by comparing CLEC5A mRNA expression in tumor and normal tissues by using publicly available software such as TIMER (Tumour Immune Estimation Resources) and UCSC XENA (https://xenabrowser.net/datapages/). This study's results will contribute to narrowing the gaps in dengue immuno-pathogenesis, especially in cancer patients.

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2. Materials and methods

2.1. TIMER: mRNA expression and tumor infiltrates and correlation analysis

We investigated the expression level in different tumor types compared to its normal tissue using the TIMER (Tumor Immune Estimation Resources) web tool (https://cistrome.shinyapps.io/timer/), which gives an analytical view of the RNA-seq data of malignant tumors in TCGA database [9,10]. The statistical significance computed by differential analysis (edgeR) on RNA-Seq raw counts is annotated by the number of stars (*: P-value <0.05; **: P-value <0.01; ***: P-value <0.001). The CLEC5A gene was analyzed in the gene module to explore the correlation of CLEC5A expression with the immune infiltrating abundance. TISIDB (http://cis.hku.hk/TISIDB) is an online analysis website that contains a variety of immunological data that can be used to analyze the interaction between tumors and the immune system [11]. We analyzed the correlation between CLEC5A and tumor-infiltrating cells using the TISIDB database. In addition, the TIMER database (http://timer.cistrome.org) was also applied to determine the correlations of CLEC5A expression with gene marker sets of different immune infiltrating cells, including B cells, T-helper cells, cytotoxic- T cells, macrophages, neutrophils, and DCs. used to confirm the correlation [9, 10,12]. The expression database was further validated using the GEPIA

A)

B)
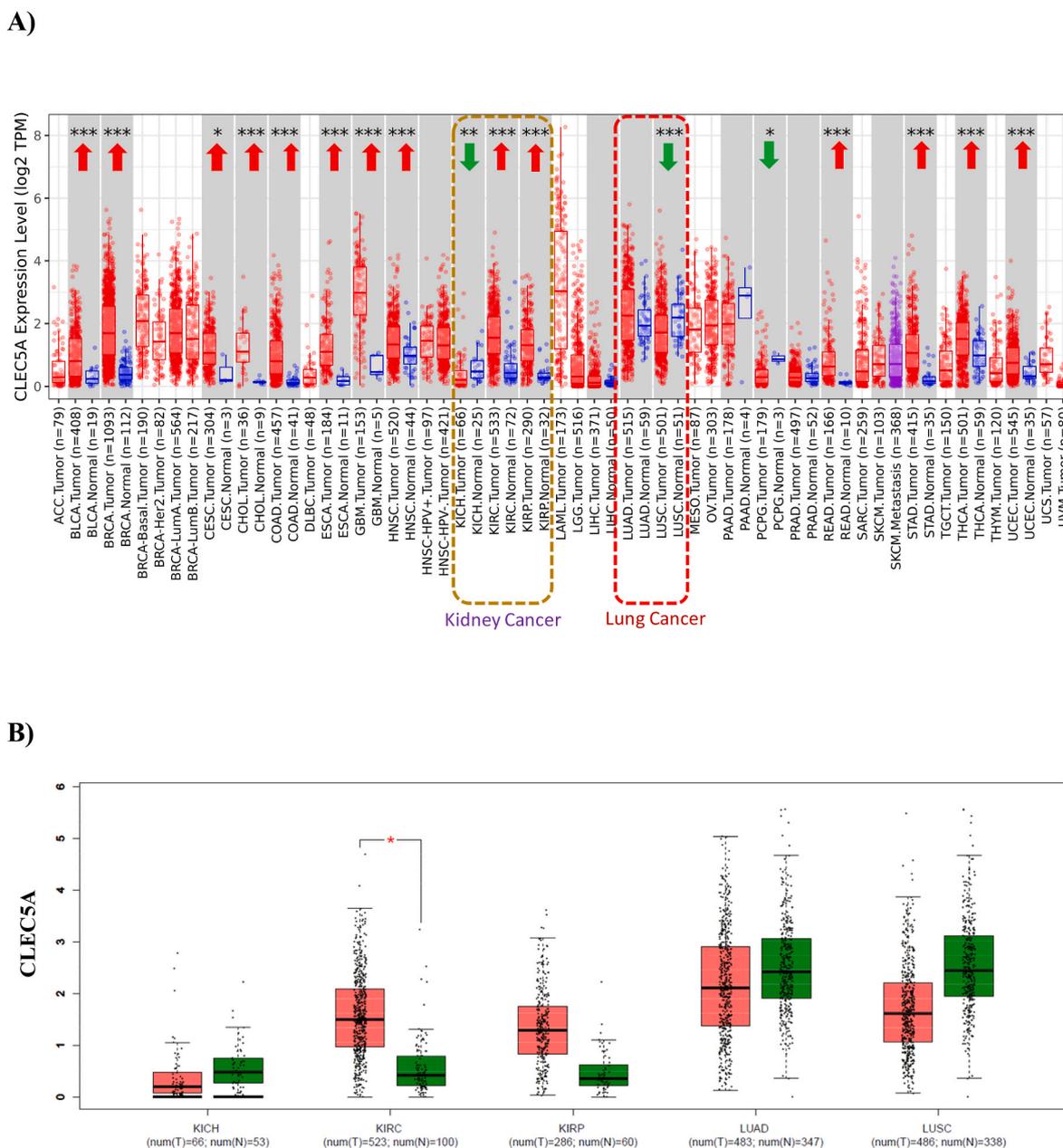
Fig. 1. A) CLEC5A expression levels in diverse tumor and normal tissues were analyzed in the TIMER database (*: P-value <0.05; **: P-value <0.01; ***: P-value <0.001). **B) GEPIA** analysis revealed that CLEC5A are significantly overexpressed in kidney renal clear cell carcinoma (KIRC, 523 tumor, 100 normal). The RNA-seq data are expressed as relative gene expression using transformed log2 (TPM+1) value (Y-axis) of tumor (red) and normal (grey) samples from different cancer types and displayed as a whisker plot. The whisker plot solid horizontal black line is the median, the box represents the upper and lower quartiles and the two lines (whiskers) outside the box extend to the highest and lowest observations of the sample population. The difference in CLEC5A expression in tumors compared to normal tissue control is significant based on one-way ANOVA (*P < 0.01). TPM, transcript per million. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

web too [13].

2.2. CLEC5A expression transcripts: UCSC Xena

UCSC Xena Browser(https://xenabrowser.net/transcripts/) [14], is used for exploring the range of CLEC5A transcripts in LUAD and KIRP. The Transcript View shows the percentage of transcript-specific expression or isoform for tumor TCGA data and normal GTEX data. The tool allows for comparisons of the distribution of values for two groups of samples.

3. Results

3.1. Pan-cancer analysis of CLEC5A expression and its co-relation with immune infiltrates

RNA-sequencing data from TCGA data sets were used to analyze the CLEC5A mRNA expression profile compared with normal tissues by using the TIMER web server (P<0.001) in a pan-cancer context. As shown in (Fig. 1A), the mRNA expression levels of CLEC5A were significantly over-expressed and under-expressed in a variety of cancers. The analysis results revealed the higher CLEC5A mRNA expression in fourteen tumors, including Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangiocarcinoma (CHOL), Esophageal carcinoma (ESCA), Glioblastoma multiforme (GBM), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Colon adenocarcinoma (COAD), Rectum adenocarcinoma (READ), Stomach adenocarcinoma (STAD), Thyroid carcinoma (THCA), Head and Neck squamous cell carcinoma (HNSC) and Uterine Corpus Endometrial Carcinoma (UCEC). In contrast, lower expression was observed in Kidney Chromophobe (KICH), Lung squamous cell carcinoma (LUSC), and Pheochromocytoma and Paraganglioma (PCPG). Since the kidney and Liver are the organs severely affected by Dengue, we further analyze m-RNA expression data using the GEPIA web server, taking normal tissue of the GTEx dataset as controls (Fig. 1B). The
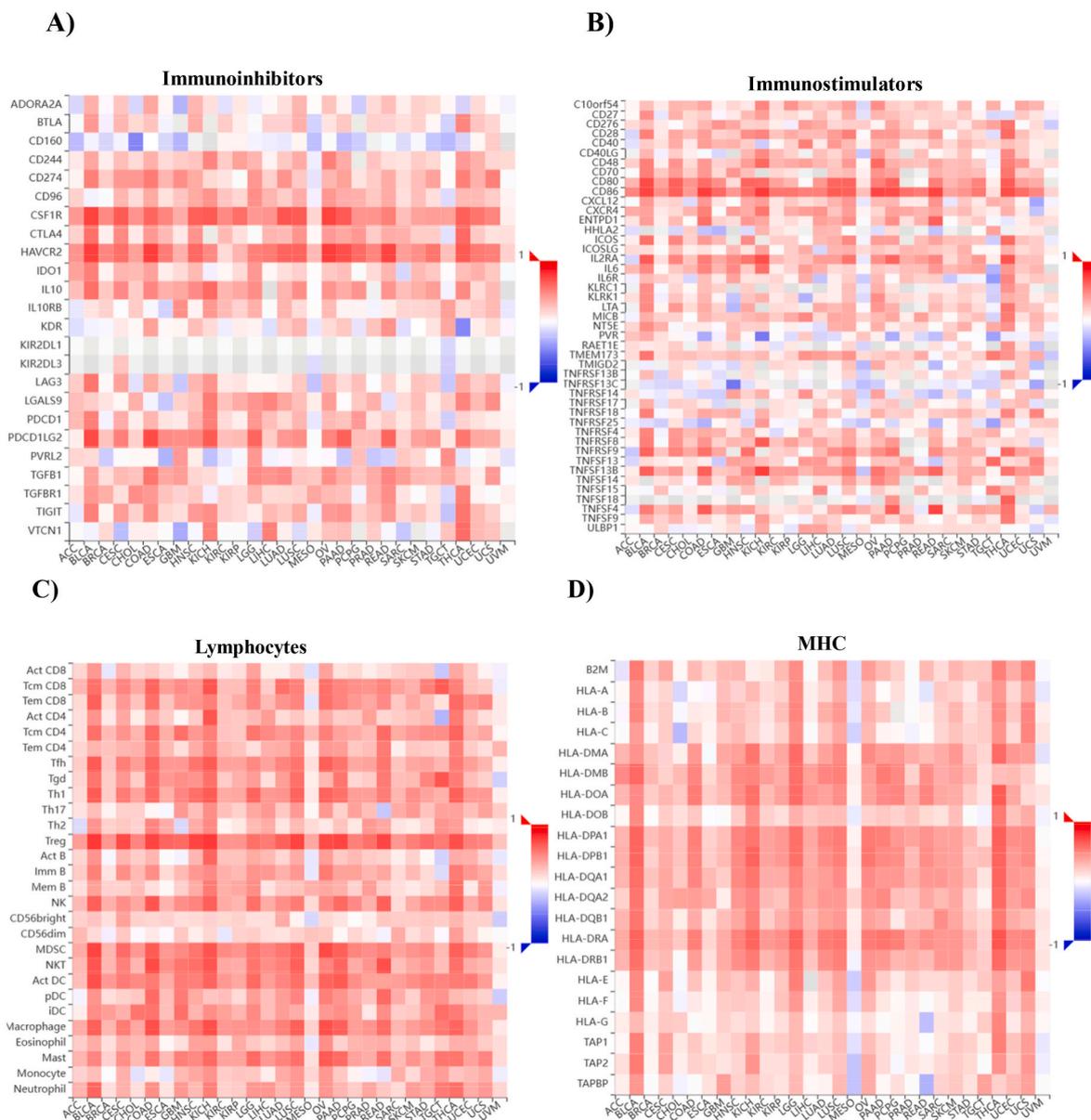

Fig. 2. Transcriptional level expression correlation between CLEC5A and immunomodulators, chemokines, and lymphocytes analyzed by TISIDB. (A) Relationship between CLEC5A and immunostimulators. **(B)** Relationship between CLEC5A and immunoinhibitors. **(C)** Relationship between CLEC5A and MHC. **(D)** Relationship between CLEC5A and Lymphocytes.

expression of CLEC5A mRNA was significantly upregulated in one cancer type, i.e., KIRC (tumor 523 and normal 100). The immune system plays an important role in providing viral defense. To evaluate how CLEC5A mRNA expression and immune infiltrate are correlated, we analyzed the TISDB database. Obtained result identified a negative correlation between immunoinhibitors such as CSF1R, HAVCR2, and PDCD1LG1 (Fig. 2A) in most tumor types. We also found a positive correlation between CLEC5A expressions and immunostimulators of CD86 and CD80 (Fig. 2B), lymphocytes Treg and MDSC (Fig. 2C), MHC

molecules of HLA-DRA and HLA-DMB (Fig. 2D) in most tumor types.

3.2. CLEC5A transcript expression in the TCGA-KIRC & TCGA- KIRP dataset and GTEx kidney dataset

To evaluate the distribution of CLEC5A transcript expression in normal kidneys, KIRC and KIR, we further validated the visualization of CLEC5A transcript-specific expression, log2 transcripts per million in TCGA-KIRC & KIRP dataset and GTEx Kidney dataset [15]. The Toil

A)
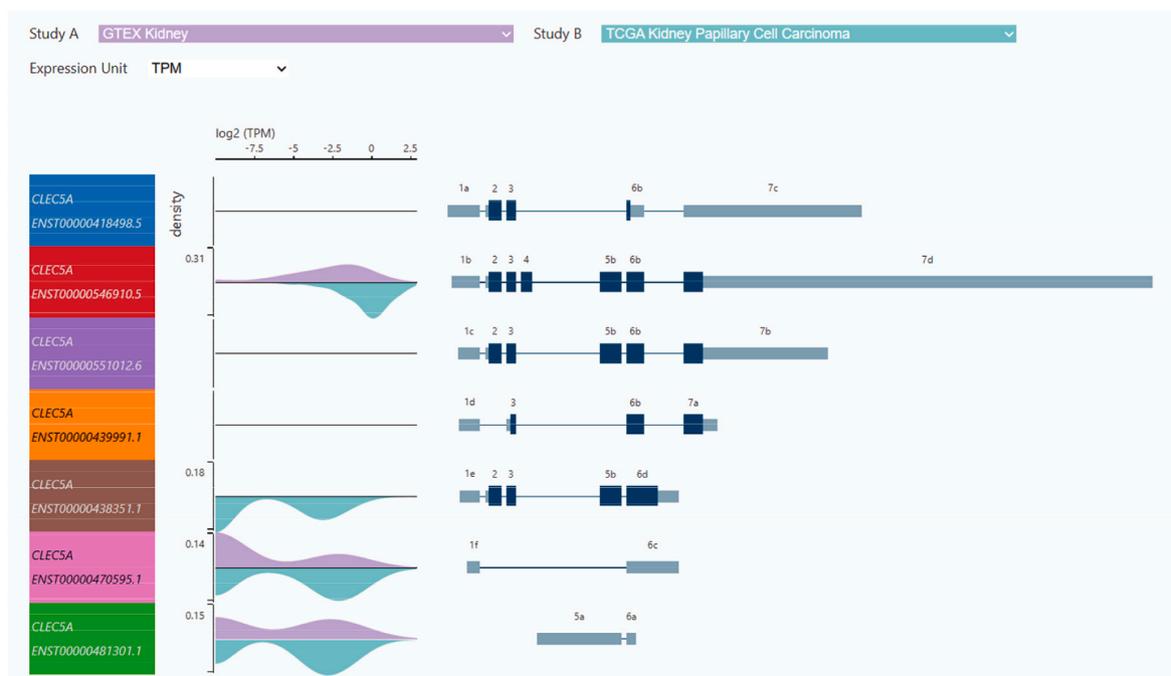
B)
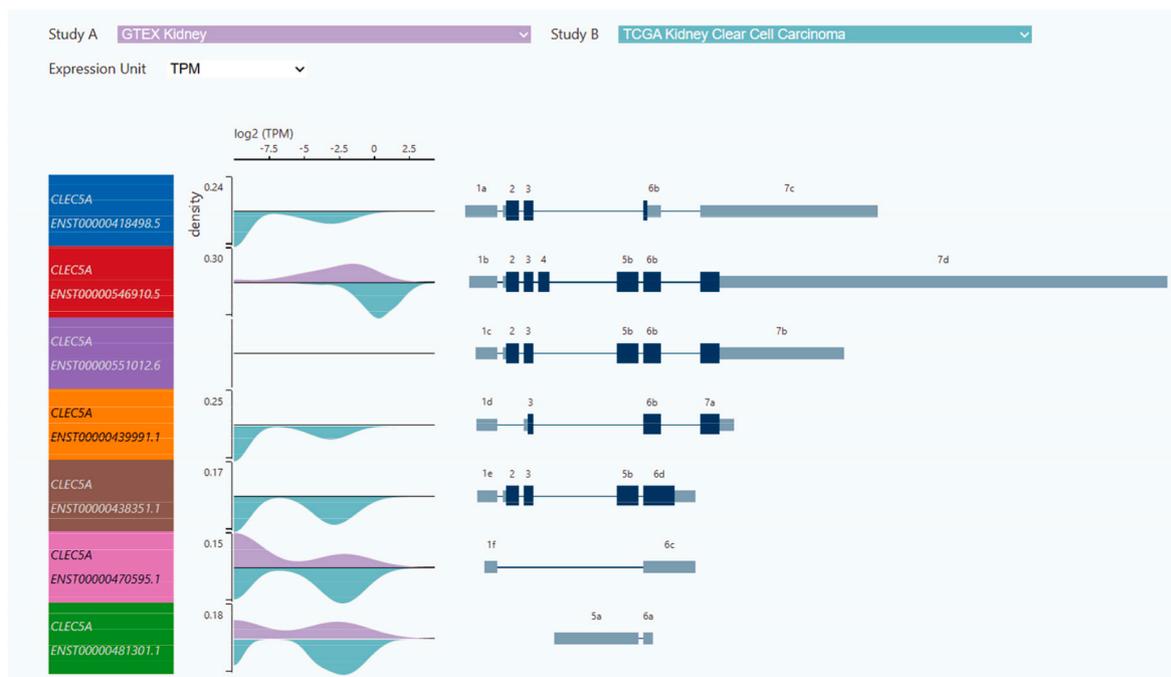
Fig. 3. Range of CLEC5A expression transcripts in Kidney Renal Papillary Cell Carcinoma (KIRP) and Lung Adenocarcinoma (LUAD): The Transcript View showed transcript-specific expression for seven variants of CLEC5A for 'tumor' TCGA data and 'normal' GTEX data in KIRP and LUAD. TPM: Transcript per million.

pipeline generated all RNAseq data recomputing by the UCSC Computational Core using the RSEM package [14]. We found that the expression of CLEC5A transcripts was different in the TCGA-KIRC & TCGA-KIRP dataset compared with the GTEx dataset (Fig. 3).
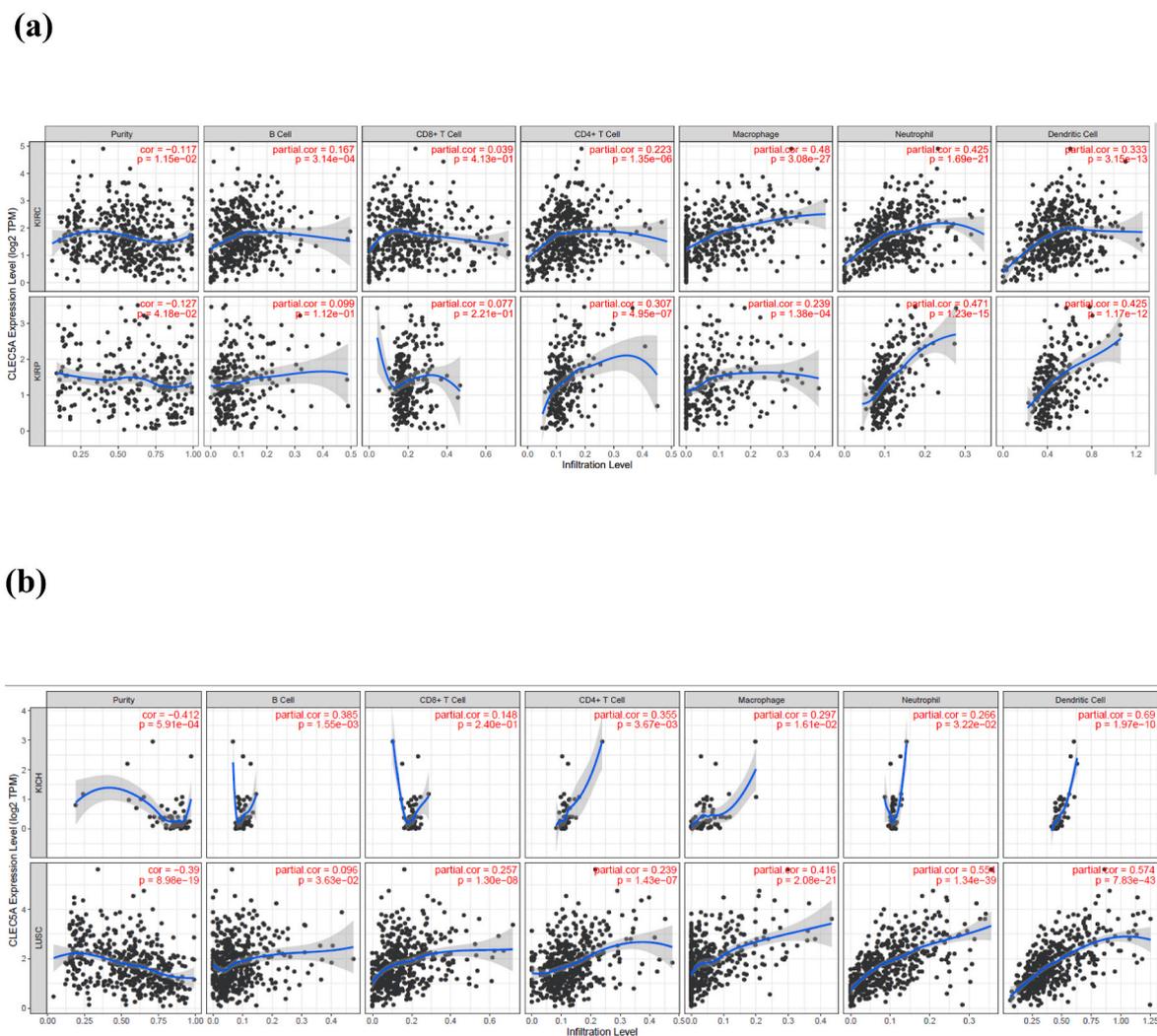
3.3. CLEC5A expression is correlated with immune infiltration levels in lung, and kidney cancers

To understand CLEC5A and the tumor immune microenvironment, we analyzed TIMER database to explore the association between CLEC5A mRNA expression level and tumor purity [12]. Understanding tumor purity is very important as it tells percentage of tumor cells in particular tumor tissue. Studies have identified the paradoxical role of tumor-infiltrating immune cells play crucial role in tumor microenvironment. There are infiltrations of immune cells in lung cancer and kidney which through their functional interaction either promote or suppress tumor progression. Immune-infiltrating cells play important role in tumor tissues by perturbing the cytokine signal in tumor microenvironment thereby determining therapeutic outcome. Moreover, Tumor infiltrating lymphocytes (TILs) are key predictors for the status of sentinel lymph node (Cancer cells are most likely to spread from this lymph node from a primary tumor) and prognosis of cancer patients [16]. Tumor purity means the percentage of tumor cells present in the tumor tissue. We came across that expression of CLEC5A is significant and positively co-linked with the level of tumor immune infiltrate i.e., macrophages and Dendritic cell (DC), neutrophils and CD4$^+$ T cell, in KIRP (Kidney Renal Papillary Cell Carcinoma). While, not significantly correlated with CD8$^+$ T cell and B cells. Similarly, the correlation between all the major tumor infiltrates and CLEC5A expression level in LUAD is positively significantly correlated with B cells, macrophages, Dendritic cell (DC), CD8$^+$ T cell, CD4$^+$ T cell and neutrophils (Fig. 4a and b). These findings strongly suggest that CLEC5A plays a specific role in immune infiltration in lung and kidney cancers, especially those of neutrophil, macrophages, CD4$^+$ T cells and DCs.

4. Discussion

Dengue is a leading and rapidly spreading mosquito-borne infectious disease [17]. In recent years, dengue has become a global challenge for health sectors as it spread to new geographical areas due to multiple factors, including rapid urbanization and global climate change [18]. Successful viral infection requires its efficient attachment to the host cell surface receptor to enter the host cell [19]. The C-type lectin-like protein CLEC5A has shown to be a macrophage receptor (macrophages are common host cell) for dengue virus; the interaction of the virus and CLEC5A activate macrophage for the release of proinflammatory

(a)

(b)

Fig. 4. Correlation between immune infiltration and CLEC5A expression in Kidney Renal Papillary Cell Carcinoma (KIRP) and Lung Adenocarcinoma (LUAD) using TIMER: CLEC5A expressions were significantly positively correlated with (**A**) B cell, CD4 + T cell, CD8$^+$ T cell immune infiltration levels of LUAD; the level of immune infiltration of CD4+T cell in KIRP. (**B**) the level of immune infiltration of macrophage, neutrophil, and dendritic cells in KIRP and LUAD.

cytokine. Thus, Inhibition of virus attachment by blocking CLEC5A becomes a novel therapeutic strategy in case of dengue virus disease, thereby improving survival [20]. Very recently, studies have identified the association of dengue virus with the diagnosis of leukemia years after acute infection [7]. In this context, it is essential to understand how other cancers are vulnerable to being affected by dengue.

Here, we provide the first study to uncover the m-RNA expression status of CLEC5A in human cancers. Our results demonstrate that in Kidney renal cell carcinoma patients overexpressed CLEC5A as evidenced by TIMER and GEPIA data sets. This finding is important because, in the current understanding, Dengue infection has been associated with various renal diseases [21]. Moreover, after acute dengue infection, the observance of renal failure, proteinuria, hematuria, and glomerulonephritis is widespread [21]. Previous studies have also reported involvement of chronic kidney disease (CKD) led to late diagnosis of dengue shock syndrome, thereby narrowing the therapeutic modalities [22]. Furthermore, patients with renal transplantation were found to be more prone to Dengue and high mortality [23], indicating the involvement of immune cells in driving this disease.

Recent studies have also shown that CLEC5A is essential for the immune-inflammatory response, which includes neutrophil extracellular trap (NET), the release of pro-inflammatory cytokines, and macrophage activation. CLEC5A high expression is seen in a variety of illnesses, including tumors. In conclusion, we demonstrated that CLEC5A expression is connected to immune infiltration and influences immunotherapy sensitivity patient prognosis in pan-cancer. This suggests that CLEC5A can function as a biomarker for tumor immunity and prognosis of a possible promising anti-tumor therapeutic target. To understand immune association, the [11] atabase was applied to explore the relationship between CLEC5A and immunoinhibitory, immunostimulatory, lymphocytes, and MHC. Various therapeutic approach is to hinder the CLEC5A interaction with the DENV by blocking its entry. As CLEC5A is one of the known ligands of DENV, which results in phosphorylating its adaptor protein DAP12 leading to the induction of various pro-inflammatory cytokines [24], hindering the interaction using small molecules or CLEC5A receptor blocker can be a better approach in KIRP. Another strategy is creating immune modulation materials that stimulate or suppress the immune response. These materials might be used to develop vaccines for patients with dengue cancer or to prevent cytokine storms, improving patients' therapeutic relief [25].

Dengue is the leading vector-borne viral neglected tropical disease which progresses to severe stages and thousands of deaths annually. Various clinical investigations regarding the immunopathogenic process in dengue revealed its severe forms leading to imbalanced cellular immunity [26]. Atypical clinical manifestations of dengue have been reported in various organs, including Kidneys and lungs [27], Therefore we analyze the expression of CLEC5A (a critical receptor that interacts directly with the dengue virion) in Kidney and lung cancer. In this context, our analysis found which cancer would be more prone to dengue as in cancer already, the system is immunocompromised, so further dengue virus infection will be more lethal for life [28]. We assessed whether the CLEC5A gene, a known dengue virus receptor, was expressed with the pattern of local cytokine response and cell infiltrates. The overall scenario of the study predicts that in both cancers, i.e., KIRP and LUAD, the expression of CLEC5A is high as it is better for survival which makes it more vulnerable to the dengue virus (DENV) as we already know CLEC5A is one of the known legends of DENV which in results phosphorylate its adaptor protein DAP12 leading to induction of various pro-inflammatory cytokines [29].

Utilizing TIMER, the relationship between immune infiltration and CLEC5A expression in KIRP and LUAD was examined. Immune infiltrates B cells, CD4$^+$ T cells, CD8$^+$ T cells, macrophages, neutrophils, and dendritic cells were significantly positively linked with CLEC5A expression levels in LUAD. While CLEC5A mRNA expression in KIRP was strongly positively linked with immunological infiltrates CD4+T cell,

macrophage, neutrophil, and dendritic cell. This significantly supports the association between immune infiltration and CLEC5A expression in KIRP and LUAD [9]. Various therapeutic approach is to hinder the CLEC5A interaction with the DENV by blocking its entry as CLEC5A is one of the known ligands of DENV. As a result, it phosphorylates its adaptor protein DAP12 leading to the induction of various pro-inflammatory cytokines [24]; hindering the interaction using small molecules or CLEC5A receptor blocker can work better for therapeutics in KIRC. Another strategy is creating immune modulation materials that stimulate or suppress the immune response. These materials might be used to develop vaccines for patients with dengue cancer or to prevent cytokine storms, improving patients' therapeutic relief [25]. Early and accurate disease detection is more important to save the patient from fatal consequences, for that immune diagnostic-based approach is among the most promising areas that could be important in precise detection and therapy. However, clinical validation of our prediction must be validated in clinics before use however this kind of approach.

5. Conclusion

We designed a computational approach to study the dengue virus susceptibility in cancer patients based on gene expression data using various web tools. These approaches will help us understand gene-based immune correlations, thereby helping us to understand the underlying molecular mechanism of tumor progression.

Declaration of competing interest

No potential conflicts of interest were disclosed.

Data availability

Data will be made available on request.

Acknowledgement

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