Short Communication

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T Cell Gene Expression Showed Tighter Spatial Compactness and Less Diversity in IgA Nephropathy than Healthy Controls

Aibing Rao*

Shenzhen Luwei (Biomanifold) Biotechnology Limited, 10th Floor, Clou Building B, Baoshen Road, Nanshan District, Shenzhen, ZIP: 518057, PR China

***Corresponding author:** Aibing Rao

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ABSTRACT

Background: Immunoglobulin A nephropathy (IgAN) is the most common primary glomeru- lonephritis in the world and occurs mainly among young people. Its pathogenesis is not fully understood but evidence shows that T cells play important roles. This study considered T cell signature gene expression as multi-dimensional characteristic vectors of samples, and the spatial density was formulated and compared between IgAN group and healthy controls (HC) from a geometric point of view.

Methods: Three peripheral blood mononuclear cells (PBMC) gene expression data sets from Gene Expression Omnibus (GEO) database, GSE14795, GSE58539, and GSE73953, were com- bined to contribute 35 IgAN and 19 healthy control (HC) liquid biopsy samples, and in parallel, two kidney biopsy GEO data sets, GSE37460 and GSE93798, were also combined to contribute 47 IgAN and 49 healthy control tissue samples. Five types of T cells with pre-selected signature gene sets were investigated on both data set respectively. The gene sets consist of driver genes from the transition from Th0 to Th1, Th2, Th17, Treg, and Tfh (T follicular helper cell) respectively: (1) Th1: IL2, IFNG, STAT4, STAT1, TBR1, CXCR3, TNF, etc.; (2) Th2: IL4, IL10, STAT6, GATA3, CCR3, CCR4, CCR8, IL5, IL6, IL13, etc.; (3) Th17: IL17A, IL17B, STAT3, RORA, RORB, RORC, CCL20, etc.; (4) Treg: IL23A, TGFB1, STAT5A, STAT5B, etc.; (5) Tfh: IL21R, BCL6, PDCD1, ICOS, CD40LG, CD40, CXCR5, etc. For a given T cell type with signature set of N genes, samples were mapped to points in a N -dimensional space. Spatial density analysis was then applied to IgAN and HC groups in each data set. Spatial density (compactness) of a sample group was defined in two ways: (1) Radius: the Euclidean distance from a sample to the group centroid; (2) Pairwise distance: the Euclidean distance between any sample pair in the group.

Spatial density was then summarized as the group average of radius or median pairwise distance. Finally statistical t-tests were applied between IgAN group and HC for PBMC and tissue independently.

Results: For both PBMC and tissue samples respectively, the average radius or median pairwise distance of IgAN group was universally smaller than HC for all 5 T cell types, most of which the difference is statistically significant ($p < 0.05$) with two exceptions. For Th17 in PBMC and Tfh in tissue, both distances showed $p > 0.05$.

Conclusion: T cell gene expression for the genes driving and participating the transition from Th0 to Th1, Th2, Th17, Treg and Tfh were analyzed for IgAN and HC, with PBMC and kidney biopsy tissue independently. In both PBMC and biopsy tissue, all T cell types showed more compact spatial distribution in IgAN group than HC, indicating a much lower dynamic ranges among patients in a concert of Th1, Th2, Th17, Tfh and Treg in the disease state. T cell immunity may have become more homogeneous and with less diversity in IgA nephropathy than healthy controls.

Keywords: IgA Nephropathy; T Cell Immunity; Tfh; Th1; Th17; Th2; Treg; Gene Expression; IgA Pathogenesis; Spatial Density

Abbreviations: IgAN: IgA Nephropathy; HC: Healthy Control; PBMC: Peripheral Blood Mononuclear Cell; GEO: Gene Expression Omnibus

Introduction

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis in the world. Among kidney biopsy diagnosed patients, about 40% will develop end-stage kidney disease eventually. IgAN is characterized by the dysfunctional immune system pertaining to IgA immunity. IgAN onset starts with a respiratory tract infection or an intestinal infection which triggers the innate IgA immune pathways. A set of intermediate molecules, called IgA immune complex, such as IgA-IgG, IgA-IgM, etc. are generated and cleared. Due to the dysfunction of the immune system of IgAN, these IgA immune complex cannot be cleared and is deposited in the mesangial region of the kidney, invoking a local immune reaction, and then leading to glomerulonephritis, at last causing irreversible kidney injury within several years after the onset. The roles of T cells play in IgAN pathogenesis has been shown and have clinical significance from the T cell population point of views [1]. In this research we explore pre-selected signature gene sets of T cell types and their corresponding expression vectors. Spatial density analysis is performed and compared between IgAN group and HC.

Materials and Methods

Microarray Data

The microarray datasets downloaded from Gene Expression Omnibus (GEO) databases are summarized in Table 1. Firstly, the expression data matrix was downloaded. Then the data from different data sets of the same sample source (PBMC or Tissue) was normalized by firstly shifting the median expression of each sample to zero level and then applying inter-quantile (25% and 75% percentiles) normalization with respect to gene or sample respectively. Lastly, the normalized data was stacked together to construct two data sets, one PBMC, the other Tissue.

Table 1: GEO Data Set Summary.

GEO ID	Platform	Sample Source	Sample Type (Count)	References
GSE14795	GPL96/571	PBMC	HC(8), IgAN(12)	[2]
GSE58539	GPL10558	PBMC	HC(9), IgAN(8)	[3]
GSE73953	GPL4133	PBMC	HC(2), IgAN(15)	[5]
GSE93798	GPL22945	Kidney Tissue	HC(22), IgAN(20)	[4]
GSE37460	GPL11670/14663	Kidney Tissue	HC(27), IgAN(27)	[1]

Spatial Density Analysis

For T cell type Th1, Th2, Th17, Treg and Tfh, signature gene sets were pre-selected, and the corresponding gene expression visualization was plotted. Spatial density was then introduced and analyzed. AT last statistical tests were applied to compare IgAN patient group and HC.

Pre-Selected Signature Gene Sets: Based on the review of T lymphocytes in IgAN pathogenesis [1], T cell differentiation pathways from Th0 to 5 T cell type Th1, Th2, Th17, Treg and Tfh, with different driving genes and other related genes are listed in Table 2. For a given T cell type, the pre-selected gene set (say, with N genes in the fixed order) spans a N -dimensional vector space, and a sample is represented as a point with its expression vector. Next the spatial distribution of IgAN and HC samples is explored, analyzed, visualized and compared.

Table 2: Pre-selected Signature Genes for Five Types of T Cells.

T Cell Type	Pre-selected Signature Genes
Tfh	BCL6, CCR6, CD3D, CD3E, CD3G, CD4, CD40, CD40LG, CD80, CD86, CD8A, CXCR5, ICOS, IL21R, PDCD1, PDCD1LG2
Th1	CCL4, CCR2, CCR5, CXCR3, IFNG, IFNGR1, IFNGR2, IL2, IL20RA, JAK2, JAK3, STAT1, STAT4, TBR1, TNF
Th17	CCL20, IL17A, IL17B, IL17RA, IL17RB, IL6, IL6R, IL6ST, NOTCH1, NOTCH2, NOTCH3, NOTCH4, RORA, RORB, RORC, STAT3, VEGFA, VEGFB, VEGFC
Th2	BCL2, BCL2L1, CCR3, CCR4, CCR8, CD28, CXCL11, CXCL12, GATA3, IL10, IL13, IL4, IL4R, IL5, NFAT-C2IP, STAT6
Treg	GZMA, GZMH, HDAC7, IKZF4, IL23A, IL2RA, IL2RB, IL2RG, KAT5, KITLG, NFATC1, NFATC3, NFATC4, RELA, STAT5A, STAT5B, TGFB1, TNFRSF11A

T Cell Gene Expression Visualization: Two types of visualization were plotted. One type was to visualize the expression values versus genes colored by sample types, the other was to visualize the 3-D distribution using the top 3 genes with the largest variation among all samples. For this purpose, for a given gene set, genes were sorted decreasingly based on the sample standard variation calculated from all samples, including both HC and IgAN.

Spatial Density and Compactness Analysis: For a given sample group, spatial density is defined as sample-to-centroid distance, also called radius, or as sample pairwise distance within a given group. Statistical t-tests were applied to each single gene expression or spatial density measurments in order to find the T cell differences between IgAN group and HC. PBMC and tissue samples were analyzed independently.

Results/Observations

2D and 3D Visualization Plots

For a given T cell type, the pre-selected genes were sorted decreasingly based on the standard variation of a gene across all samples from the sources of either PBMC or tissue. 2D plots were generated with each curve representing a sample, colored BLACK for HC and RED for IgAN (Figure 1). Almost all T cell types showed a wider dynamic range for HC than for IgAN. On the other hand, in order to plot a 3D visual plot, top 3 genes with the largest standard deviations were selected as axises, the spatial distribution of IgAN group was shown to be tighter and more compact than HC (Figure 2).

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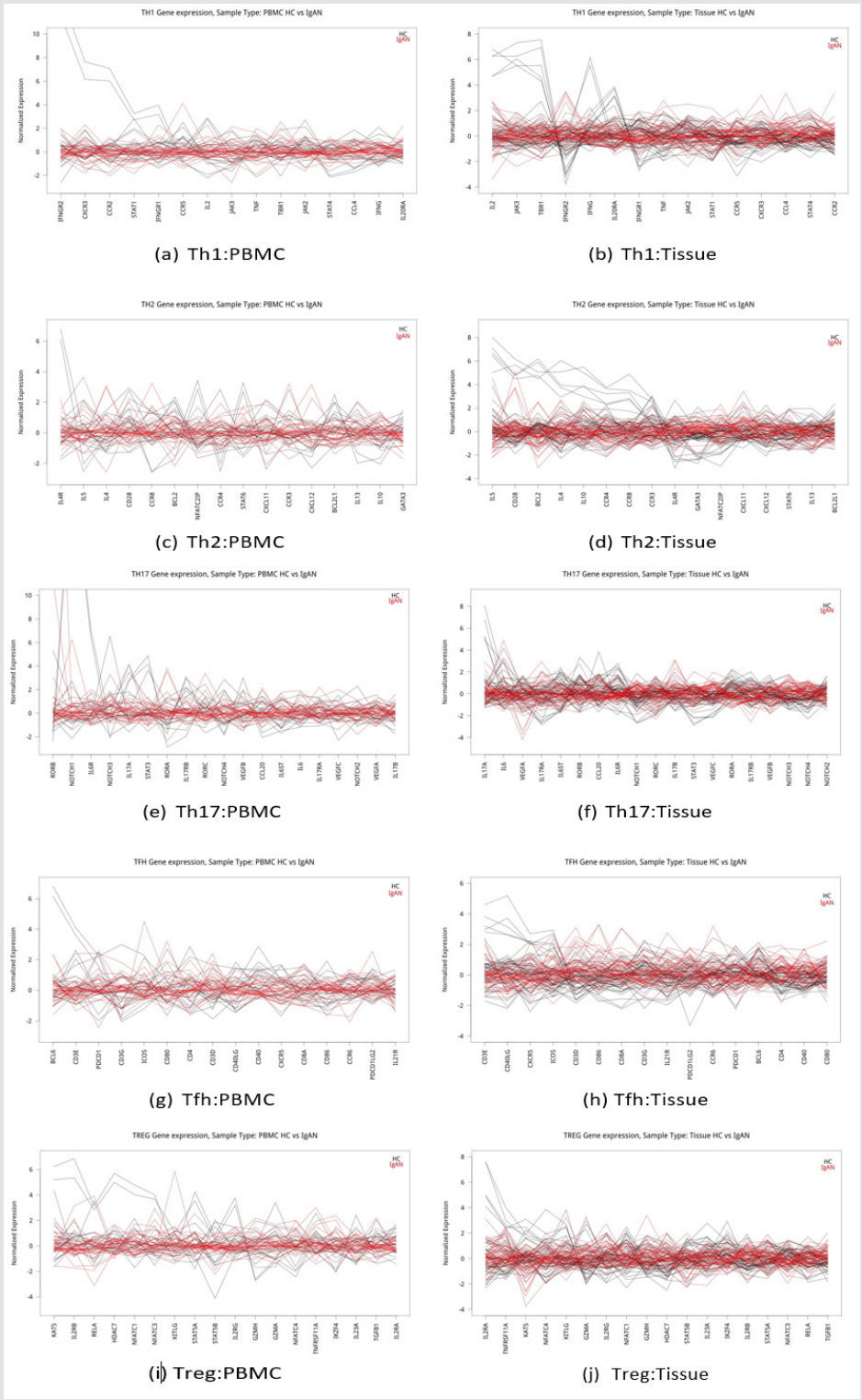

Figure 1: Gene expression visualization of 5 types T cells for HC and IgAN with PBMC or tissue sample sources shows that HC(BLACK) has wider dynamic ranges than IgAN(RED) in general.

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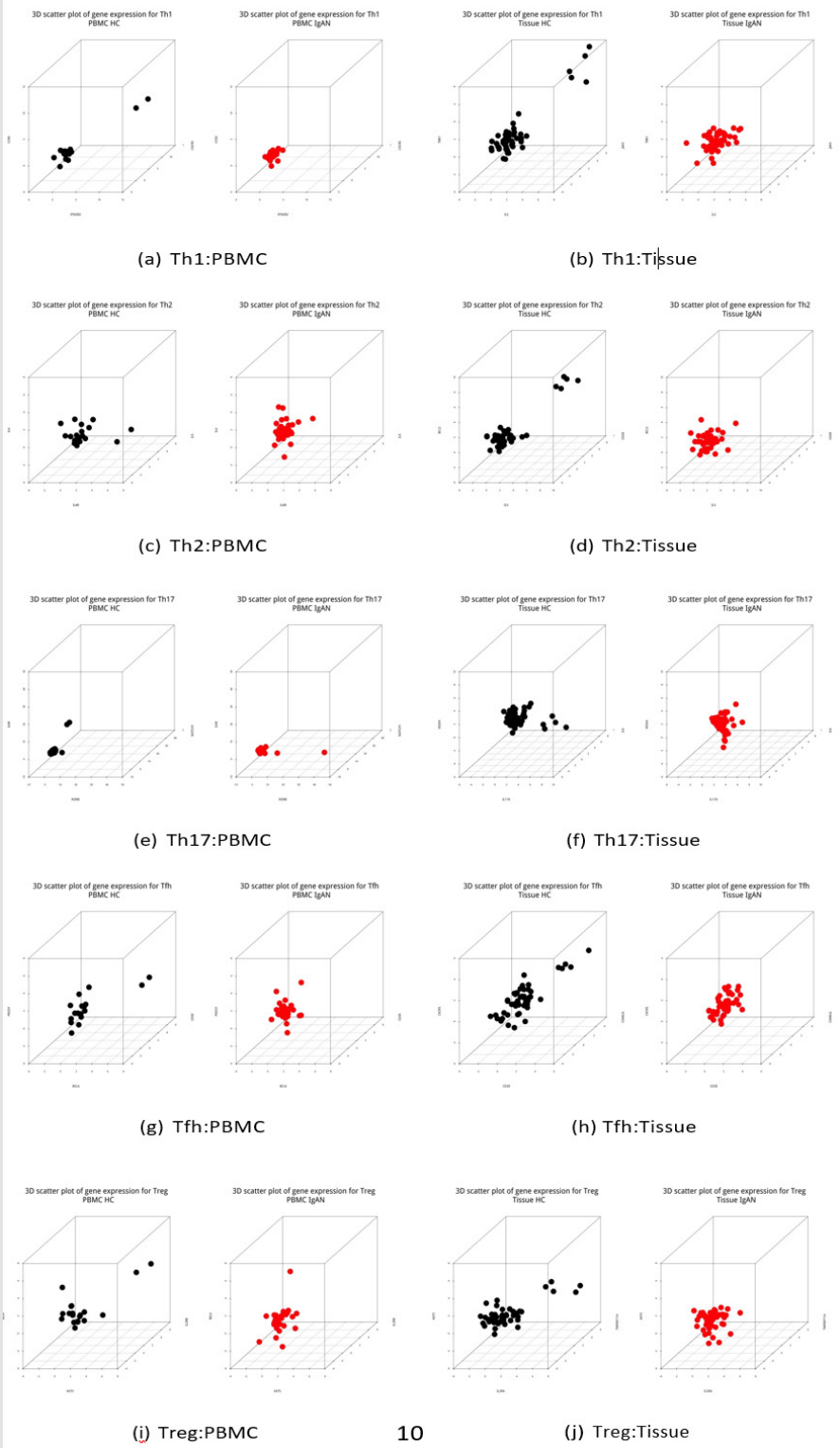


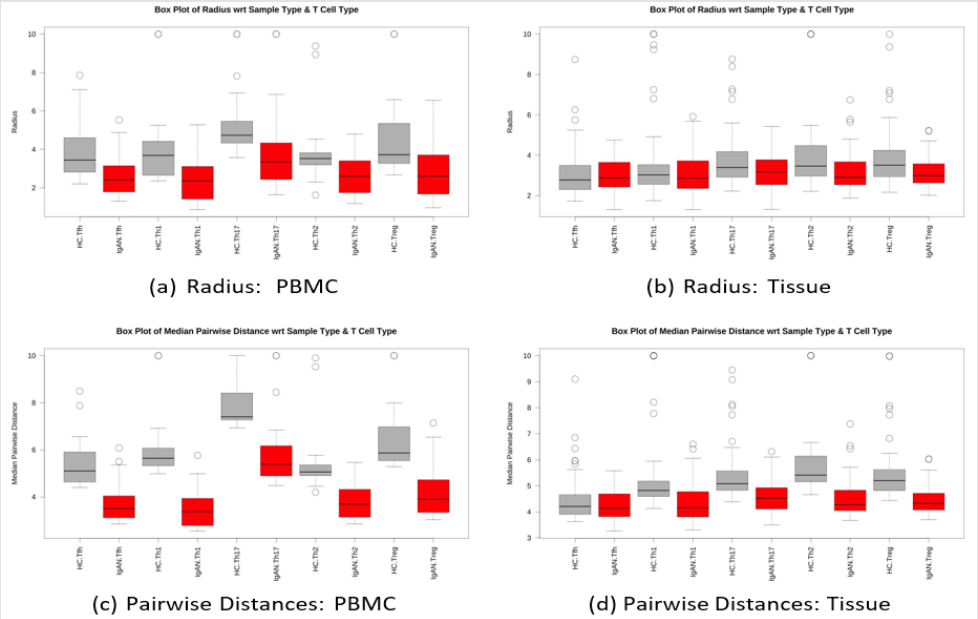
Figure 2: 3D plots of top 3 most variable genes in each of the 5 types T cells for HC and IgAN with PBMC or tissue sample sources shows that HC(BLACK) has wider dynamic ranges than IgAN(RED) in general.

Spatial Density Statistics

The spatial density (compactness) of a given sample group was calculated as radius and as pair- wise distance within the group. Summary statistics such as mean, standard deviation was then summarized, and t-test was applied to compare IgAN and HC foreach T cell type respectively. PBMC and tissue samples were analyzed independently. The summary statistics and t-test results are in Table 3 for PBMC, and in Table 4 for tissue. In addition, box plots are shown side by side for HC and IgAN and from left to right in order of Tfh, Th1, Th17, Th2 and Treg (Figure 3). The left column is for PBMC with radius (Figure 3(a) and pairwise distance 3(c)); the right column is for tissue with radius (Figure 3(b) and pairwise distance 3(d)). The spatial distribution of IgAN group was shown tighter and more compact than HC, t-tests indicated the difference is significant for most of the cases except Th17 in PBMC and Tfh in tissue samples.

Table 3: Spatial compactness summary and t-test statistics for PBMC samples. IgAN group turned to be more compact with smaller mean radius and pairwise distance than HC with pv < 0.05 (p-value) for all T cell types except Th17 of which p > 0.05.

PBMC Sample		Radius				Pairwise Distance			
		Stats		t-test		Stats		t-test	
T Cell	Type	Mean	Std	t	pv	Mean	Std	t	pv
Tfh	HC	3.91	1.56	3.28	0.0029	5.45	1.56	5.91	0
	IgAN	2.6	1.05			3.7	1.05		
Th1	HC	4.78	3.77	2.7	0.0139	6.72	3.77	4.42	3e-04
	IgAN	2.39	1.1			3.45	1.1		
Th17	HC	6.63	5.25	1.25	0.2164	9.2	5.25	1.72	0.0928
	IgAN	4.61	6.31			6.74	6.31		
Th2	HC	3.93	1.95	2.65	0.0142	5.5	1.95	4.57	1e-04
	IgAN	2.66	1.04			3.8	1.04		
Treg	HC	4.84	2.72	2.9	0.008	6.78	2.72	4.83	1e-04
	IgAN	2.9	1.42			4.22	1.42		



(a) Radius: PBMC (b) Radius: Tissue

(c) Pairwise Distances: PBMC (d) Pairwise Distances: Tissue

Figure 3: Box plots of radius and pairwise distance with respect to T cell type and sample type. IgAN group is smaller than HC for all T cell types. The compactness reduction from HC to IgAN is significant (p < 0.05) for almost all cases except Th17 for PBMC and Tfh for tissue.

Table 4: Spatial compactness summary and t-test statistics for tissue samples. IgAN group turned to be more compact with smaller mean radius and pairwise distance than HC with pv < 0.05 (p-value) for all T cell types except Tfh of which p > 0.05.

Tissue Sample		Radius				Pairwise Distance			
		Stats		t-test		Stats		t-test	
T Cell	Type	Mean	Std	t	pv	Mean	Std	t	pv
Tfh	HC	3.21	1.35	0.76	0.4507	4.56	1.35	1.71	0.092
	IgAN	3.04	0.79			4.28	0.79		
Th1	HC	3.92	2.58	2.17	0.0334	5.61	2.58	3.96	2e-04
	IgAN	3.05	1.07			4.33	1.07		
Th17	HC	3.88	1.52	2.29	0.0248	5.48	1.52	4.31	0
	IgAN	3.29	0.96			4.64	0.96		
Th2	HC	4.37	2.74	2.79	0.007	6.2	2.74	4.88	0
	IgAN	3.2	1.02			4.53	1.02		
Treg	HC	3.92	1.69	2.76	0.0073	5.55	1.69	5.3	0
	IgAN	3.18	0.78			4.47	0.78		

Discussion

IgAN pathogenesis onset is typically due to mucosal infection which stimulates the immune system. As par of the immune reaction, naive T cells go through transition to various types of mature T cells such as Tfh, Th2, Th17, Th1, Treg and so on. For IgAN, T cells of type Tfh, Th2 and Th17 have higher proportions while Th1 and Treg seem to have lower proportions [1]. Higher proportion of circulatory Tfh enhances more IL-21 production, higher Th2 enhances more IL-4 and Il-6, while higher Th17 enhances more IL-17. In the downstream of the cascading process, more IL-21 and IL-4 enhances more IgA1 production, and more IL-4, IL-6 and IL-17 enhance more IgA1 glycosylation alteration. On the other hand, less Th1 proportion leads to less cellular cytotoxicity and reduced macrophage activation, while lower Treg proportion leads to more excessive immune response, hence less protection of the body from autoimmune responses. T cell immunity imbalance may lead to IgA immunity abnormality. The analysis from the view point of taking the expression vector of T cell transitional driver genes showed that IgAN vectors displayed more homogeneous and tighter spatial distribution than HC. It is the activation which conduct the concert of more compact gene expression in various T cell types. In a study conducted with NGS to compare IgAN and HC on complementary determining region 3 (CDR3) of the B cell receptors including IgA, IgG, IgM, IgD and IgE [2], IgAN group was also showed with increased portion of IgA, IgG and decreased IgE, shortened CDR3 length and less diversity of CDR3. Hence, the IgAN group showed less diversity in both T cells and B cells than HC. In conclusion, both T cells and B cells showed similar imbalance trend by promoting a majority of subtypes while reducing other subtypes [3-7]. Diversity was also reduced for driver gene expression in the case of T cells or antibody in the case of B cells, therefore, the role of the immune system imbalance should be further investigated in the process of IgAN pathogenesis.

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Compliance with Ethical Standards

Conflict of Interest

The authors have declared that no conflict of interest exists.

Ethical Standards

This article does not contain any studies with human participants or animals performed by the author.

Informed Consent

Informed consent was not involved.

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