Values of OAS Gene Family in the Expression Signature, Immune Cell Infiltration and Prognosis of Human Bladder Cancer

Ji-Min Cao (caojimin@sxmu.edu.cn)
Shanxi Medical University

Lijuan Gao
Shanxi Medical University

Ruimin Ren
Shanxi Bethune Hospital (Third Hospital of Shanxi Medical University)

Jing Shen
Shanxi Medical University

Jiayi Hou
Shanxi Provincial Academy of Traditional Chinese Medicine

Junya Ning
Shanxi Medical University

Yanlin Feng
Shanxi Medical University

Meiyue Wang
Shanxi Medical University

Lifei Wu
Shanxi Medical University

Yaojun Sun
Shanxi Medical University

Huang Wang
Shanxi Bethune Hospital (Third Hospital of Shanxi Medical University)

De-Ping Wang
Shanxi Medical University

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Abstract

Background: BLCA is one of the most common genitourinary malignancies in the world, but its pathogenic genes have not been fully identified and the treatment outcomes are still unsatisfactory. Although the members of 2', 5'-oligoadenylate synthetase (OAS) gene family are known involved in some tumorous biological processes, the roles of the OAS gene family in BLCA are still undetermined.

Methods: By combining the vast bioinformatic datasets analyses of BLCA and the experimental verification on clinical BLCA specimen, we identified the expressions and biological functions of OAS gene family members in BLCA with comparison to normal bladder tissues.

Results: We showed that the expression levels of OAS gene family members were higher in BLCA than in normal bladder tissues. The expression levels of most OAS genes had correlations with prognosis, genomic mutation, methylation and infiltration levels of CD4+T cells, CD8+T cells, neutrophils, and dendritic cells in the microenvironment of BLCA. In addition, high expression levels of OAS1, OAS2, OAS3, and OASL predicted better overall survival in BLCA patients.

Conclusions: We conclude that the expression levels of OAS genes may be considered as a signature of BLCA and can reflect immune cell infiltration in BLCA microenvironment and predict the overall survival of BLCA. The study may provide new insights into the diagnosis, treatment, and prognosis of BLCA.

1. Introduction

Bladder cancer (BLCA) is a major malignant tumor in the urinary system, and is one of the most common malignancies worldwide [1]. Many BLCA patients are diagnosed at middle or late stage, missing the optimal opportunity for intervention and treatment. Some treatments for BLCA have been developed, including transurethral resection of the tumor, radical cystectomy, chemotherapy, and bacillus Calmette Guerin treatment. These treatments show great effects in short terms, but the recurrence and metastasis rates are relatively high, and the five-year survival rates are still low. Therefore, exploring the mechanisms of infiltration and metastasis of BLCA and identifying potential therapeutic targets have substantial clinical values.

The 2', 5'-oligoadenylate synthetase (OAS) family members are IFN-induced antiviral enzymes. OASs and their downstream effector RNase L play vital roles in host defense against virus infection. The OAS family is composed of four members, including OAS1, OAS2, OAS3, and OASL. These four members differ in the numbers of OAS domain, types of synthesized 2–5A, and oligomerization level [2, 3, 4]. We have shown in a recent study focusing on psoriasis that the OAS family is closely related to BLCA as indicated by the pathway enrichment data [5]. This finding reminded us that OASs may be prognostic indicators for BLCA. In addition, OAS family is related to the diagnosis and prognosis of a variety of cancers, such as breast cancer [6], pancreatic adenocarcinoma [7], and prostate cancer [8], but the relationship between OAS family and BLCA has not yet been studied. Therefore, it is necessary to fully characterize the pathological value of OAS family in BLCA.

In the present study, we comprehensively analysed the expression characteristics of OAS family in BLCA using multiple bioinformatics methods, and experimentally verified the results of bioinformatics. Our study suggests potential value and molecular mechanism of OAS family in the progression and development of BLCA, which may contribute to the improvements of therapeutic strategy and prognosis accuracy for BLCA.

2. Materials And Methods

Oncomine dataset analysis

Oncomine gene expression array dataset [9] (https://www.oncomine.org/resource/login.html), an online cancer microarray database, were used to analyze the transcription levels of OAS family in different cancers. The mRNA expressions of OAS gene family in clinical BLCA specimens were compared with normal bladder specimens, and the cutoffs of P value and fold change were defined as 0.0001 and 2, respectively.

GEPIA dataset analysis

Gene Expression Profiling Interactive Analysis (GEPIA) [10] (http://gepia.cancer-pku.cn/) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors from the Cancer Genome Atlas (TCGA) and 8,587 normal samples from the Genotype-Tissue Expression (GTEx) projects, using a standard processing approach. In the present study, GEPIA is mainly used to verify the gene expression level of the OAS family, the relationship between the expression of OAS family with the clinical stages, their co-expressed genes of OAS family, and the prognostic value of OAS family in BLCA.

TIMER analysis

Tumor Immune Estimation Resource (TIMER) [11] (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types based on 32 cancer types and 10897 samples from TCGA. In this database, six immune infiltrates (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells) were selected to evaluate the correlation between OASs and the infiltration of immune cells based on TIMER algorithm. $P < 0.05$ was considered statistically significant.

Patients and bladder tissue sampling

The study included seven inpatients diagnosed with BLCA in Shanxi Bethune Hospital, Taiyuan, China. Patient information were showed in Table S1. The experiments on bladder specimens from these patients were mainly used to verify the reliability of bioinformatic analyses. All the patients were in advanced stage and underwent radical tumor excision surgery. The BLCA tissues and paired adjacent normal bladder tissues were harvested during surgeries, and were frozen in liquid nitrogen and stored at an ultra-low-temperature freezer for experiments of RT-qPCR, Western blotting, and immunohistochemistry.

RNA isolation and Real-Time quantitative PCR (RT-qPCR)

RT-qPCR was performed to examine the mRNA levels of OASs in BLCA and adjacent tissues. Total RNA was extracted from tissues using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. PrimeScript™ RT reagent Kit (TaKaRa, Osaka, Japan) was used to reversely transcript the RNA into cDNA. RT-qPCR was performed according to the instructions of TaKaRa TB Green Premix Ex Taq II (TaKaRa, Osaka, Japan), primer sets for selected genes were designed by Sangon Biotech Co., Ltd (Shanghai, China). The expression data were normalized to the reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences for RT-qPCR are shown as following: GAPDH forward: 5'-CTGGGCTACACTGAGCACC-3', GAPDH reverse: 5'-AAGTGGTCGTTGAGGGCAATG-3'. OAS1 forward: 5'-AGTTGACTGGCGGCTATAAAC-3', OAS1 reverse: 5'-GTGCTTGACTAGGCGGATGAG-3'. OAS2 forward: 5'-AGGTGGCTCCTATGGACGG-3', OAS2 reverse: 5'-TTTATCGAGGATGTCACGTTGG-3'. OAS3 forward: 5'- GAAGGAGTTCGTAGAGAAGGCG – 3', OAS3 reverse: 5'-CCCTTGACAGTTTTCAGCACC-3'. OASL forward: 5'-CCCTTGACAGTTTTCAGCACC-3', OASL reverse: 5'-CTTCAGCTTAGTTGGCCGATG-3'.

Western blotting

Total proteins for western blotting were extracted from bladder cancer tissues and adjacent normal bladder tissues, respectively. The protein concentration in all samples was determined using the bicinchoninic acid (BCA) assay (Solarbio Co., Ltd, Beijing, China). A total amount of 40μg extracted protein of each sample were separated by 10% SDS-PAGE. Then, proteins from the SDS-PAGE gel were transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Membranes were blocked with 5% nonfat milk for 1–2 h at 20–25°C. The membranes were then incubated with the primary antibodies overnight at 4°C respectively. The membrane was washed with TBST and then incubated with the secondary antibody conjugated with horseradish peroxidase for 2 h at 20–25°C. The ECL reagent (Millipore, Billerica, MA, USA) was added and the blots were scanned using ChemiDoc™ XRS (Bio-Rad Laboratories, Hercules, CA, USA). The gray value of protein bands was determined using Image Lab 2.0 (Genmall Biotechnology Co.,Ltd, Wuhan, China) and β-actin (ZSGB-Bio, China) was used for normalization. The primary antibodies (anti-OAS1 – 3) were purchased from Peprotech (New Jersey, USA), anti-OASL was purchased from Abcam (Cambridge, MA, USA), the secondary antibodies were purchased from Zhongshan Golden bridge Biotechnology (Beijing, China).

Immunohistochemistry

To perform immunohistochemical staining of OAS1, OAS2, OAS3, and OASL, bladder cancer tissues and adjacent normal bladder tissues were fixed in 10% formalin, embedded in paraffin, sectioned (3 μm) and attached to slides. Tissue sections were incubated with commercial rabbit polyclonal antibodies against OAS1 (dilution 1:300), OAS2 (dilution 1:300), OAS3 (dilution 1:100), and OASL (dilution 1:250) overnight at 4 °C. Then, the sections were conjugated with a horseradish peroxidase (HRP) antibody (dilution 1:500) at room temperature for 2 h, washed, reacted with 3,3-diaminobenzidine (DAB), washed with water,

counterstained, and then coverslipped. Positive staining signals of all fields were observed under a light microscopy and images were taken.

Kaplan-Meier plotter and Oncolnc analysis

The Kaplan-Meier (KM) plotter [12] (https://kmplot.com/analysis/) is capable to analyze the survival biomarkers across 21 cancer types, based on sources including Gene Expression Omnibus database (GEO), European Genome-phenome Archive (EGA), and the Cancer Genome Atlas (TCGA). The prognostic values of the OASs signature for overall survival (OS) were calculated using the KM plotter.

OncoLnc [13] (http://www.oncolnc.org/) is a newly available tool for interactively exploring survival correlations, and for downloading clinical data coupled to expression data for genes. It contains survival data for 8,647 patients from 21 cancer studies performed by TCGA, which create high quality OS plots for further analyses in this study.

cBioPortal analysis

The cBio Cancer Genomics Portal (cBioPortal) [14] is a comprehensive open-access web resource which can help to visualize and explore multidimensional cancer genomics data (www.cbioportal.org). Genetic alterations of OAS gene family in BLCA, including mutation and methylation, were analyzed using cBioPortal database.

GeneMANIA analysis

GeneMANIA [15] (http://www.genemania.org) is a user-friendly website that provides information for protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity. Top 20 closely related neighbor genes were isolated from GeneMANIA in this study.

Gene Ontology (GO) enrichment analysis

DAVID 6.8 (https://david.ncifcrf.gov/home.jsp) is a comprehensive, functional annotation website that clarify the biological function of submitted genes. Gene Ontology (GO) enrichment analysis of 20 closely related neighbor genes with OAS family were analyzed by DAVID 6.8. In GO enrichment analysis, biological processes (BP), cellular components (CC), and molecular functions (MF) were included. Enrichment results were visualized with R project (v3.5.3) using a “ggplot2” package. $P < 0.05$ was considered significant.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

KEGG (https://www.kegg.jp/) is a database for systematic analysis of gene function and genomic information, which integrate information from genomics, biochemistry, and functional omics. KEGG pathway is one of 16 sub-databases that contains different types of information, including molecular interactions and relationship networks related to metabolism, regulation, pathways, biochemistry, disease, and drugs. KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/anno_iden.php) online software was used to analyze

the KEGG pathway and enrichment results were visualized with R project (v3.5.3) using a “ggplot2” package. P < 0.05 was considered significant.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0 software. Statistical significance was set at P < 0.05. Data are represented as the mean ± standard deviation (SD); two-tail t-test was used to compare the means of two groups of samples.

3. Results

Transcriptional levels of OAS family in different types of human cancers

To determine the difference of OAS gene family expression levels between tumor tissues and normal tissues, the mRNA levels of OASs among various cancers were identified based on Oncomine database, TIMER database, and GEPIA database. Analysis of Oncomine showed that the OAS family was highly expressed in different types of cancer compared with the normal tissues, including breast cancer, liver cancer, pancreatic cancer, and bladder cancer (Fig. 1-A). Similarly, analyses of TIMER and GEPIA databases showed that OASs were highly expressed in more than ten kinds of cancers including bladder cancer compared with the respective normal tissues (Fig. 1-B,C,D).

Detailed mRNA expressions of OASs in BLCA and normal bladder tissues and verified results by RT-qPCR, western blotting, and immunohistochemistry

Analyses of Oncomine and GEPIA databases and RT-qPCR experiments were performed to demonstrate the detailed mRNA expression levels of OASs in BLCA and normal bladder tissues. Oncomine analysis showed that all the four OAS members, including OAS1, OAS2, OAS3, and OASL, were significantly upregulated in BLCA tissues compared with the normal tissues. The mRNA levels of OASs in Oncomine were higher than the normal tissues mainly in three datasets with |logFC| > 1 (FC, fold change) and $P < 0.05$, including Sanchez-Carbayo Bladder 2 [16], Dyrskjot Bladder 3 [17], and Lee Bladder [18] dataset (Table 1).

Table 1
The mRNA levels of OAS family in different types of BLCA tissues and normal bladder tissues at transcriptome level (ONCOMINE)

Gene	Types of Bladder Cancer vs. Normal	Fold Change	*P*value	tTest	References
OAS1	Superficial Bladder Cancer (28) vs. Normal (48)	8.816	6.52E-18	11.253	Sanchez-Carbayo Bladder 2
	Infiltrating Bladder Urothelial Carcinoma (81) vs. Normal (48)	2.755	1.59E-9	6.427	Sanchez-Carbayo Bladder 2
	Superficial Bladder Cancer (28) vs. Normal (14)	2.344	3.08E-4	3.889	Dyrskjot Bladder 3
OAS2	Infiltrating Bladder Urothelial Carcinoma (81) vs. Normal (48)	2.470	1.32E-7	5.621	Sanchez-Carbayo Bladder 2
	Superficial Bladder Cancer (126) vs. Normal (68)	1.435	8.25E-4	3.200	Lee Bladder
OAS3	Infiltrating Bladder Urothelial Carcinoma (81) vs. Normal (48)	2.284	6.01E-10	6.667	Sanchez-Carbayo Bladder 2
	Superficial Bladder Cancer (28) vs. Normal (48)	3.675	2.96E-15	9.908	Sanchez-Carbayo Bladder 2
	Infiltrating Bladder Urothelial Carcinoma (13) vs.Normal (14)	1.567	0.003	3.185	Dyrskjot Bladder 3
	Superficial Bladder Cancer (28) vs. Normal (14)	1.380	6.78E-4	3.461	Dyrskjot Bladder 3
OASL	Infiltrating Bladder Urothelial Carcinoma (81) vs. Normal (48)	1.455	4.31E-7	5.204	Sanchez-Carbayo Bladder 2

OAS1 was overexpressed with a fold change of 8.816 in superficial bladder cancer, and with a fold change of 2.755 in infiltrating bladder urothelial carcinoma, in the Sanchez-Carbayo bladder 2 dataset. OAS1 was also highly expressed in superficial bladder cancer (fold change = 2.344) in the Dyrskjot bladder 3 dataset (Table 1).

OAS2 was 2.470 times higher in infiltrating bladder urothelial carcinoma in the Sanchez-Carbayo bladder 2 dataset, and was 1.435 times higher in superficial bladder cancer in the Lee Bladder dataset, compared with the respective normal tissues (Table 1).

OAS3 was also upregulated in infiltrating bladder urothelial carcinoma and superficial bladder cancer with a fold change respectively of 2.284 and 3.675 in the Sanchez-Carbayo bladder 2 dataset. In the Dyrskjot bladder 3 dataset, OAS3 was 1.567 times higher in infiltrating bladder urothelial carcinoma and was 1.380 times higher in superficial bladder cancer compared to the respective normal tissues (Table 1).

OASL was only reported in the Sanchez-Carbayo bladder 2 dataset and was 1.455 times higher in infiltrating bladder urothelial carcinoma compared with normal samples (Table 1).

To better characterize the transcription levels of OAS gene family in BLCA, we selected some representative results of Sanchez-Carbayo Bladder 2 dataset analysis and showed in Fig. 2A, which showed that all the mRNA levels of the four OAS genes were upregulated in BLCA compared with the normal tissues. Figure 2B showed the mRNA levels of the four OAS genes obtained from the GEPIA database analysis, the mRNA expressions of OAS1, OAS2, OAS3, and OASL were all higher in BLCA tissues than in the normal tissues, especially OAS3 and OASL.

To further verify the bioinformatic results shown in Fig. 1, 2-A, B and Table 1, we performed RT-qPCR, Western blotting, and immunohistochemistry on human BLCA tissues and paired adjacent normal bladder tissues. RT-qPCR results were consistent with bioinformatic results (Fig. 2-C). Western blotting results showed that the protein expression levels of OAS1, OAS2, OAS3, and OASL in BLCA were all significantly elevated in BLCA compared with the normal tissues (Fig. 2-D). Immunohistochemistry results were consistent with the results of bioinformatics, RT-qPCR, and Western blotting (Fig. 2-E).

Relationship between gene expression and tumor stage, and the prognostic values of OAS family for BLCA

For further validation, we analyzed the differential expressions of OAS1, OAS2, OAS3, and OASL in BLCA of various clinical stages. Results indicated that the OAS1 expression levels were significantly different in various clinical stages, with the highest in stage II and the lowest in stage IV, whereas OAS2, OAS3, and OASL did not show significant difference in various stages (Fig. 3-A).

The potential prognosis values of OAS1, OAS2, OAS3, and OASL in BLCA were investigated using Kaplan-Meier Plotter, OncoLnc and GEPIA. Results indicated that the increased levels of OAS1, OAS2, OAS3, and OASL were associated with better overall survival (OS) in BLCA, especially, higher mRNA levels of OAS1, OAS2, and OASL were significantly related to greater OS in Kaplan-Meier Plotter (Fig. 3-B) and higher mRNA levels of OAS1, OAS3, and OASL were significantly related to greater OS in OncoLnc (Fig. 3-C). Nevertheless, prognostic analyses of GEPIA database showed that only increased OAS1 mRNA was associated with favorable OS in BLCA (Fig. 3-D). Thus, OAS1 may be a prognostic factor and even a novel therapeutic target in BLCA.

Relation between OAS family expression and immune cells infiltration

The tumor microenvironment (TME) [19] is a complex milieu of immune cells, connective tissue cells, and vascular components that are essential to cancer progression and metastasis. Previous studies have shown that the prognosis and therapeutic response of cancer was closely related to TME, especially tumor-infiltrating immune cells. We investigated whether OASs expression was associated with the level of immune cell infiltration in BLCA using TIMER database (Fig. 4). Results showed that the expressions of OAS2, OAS3, and OASL had significant negative correlations with tumor purity in BLCA (Fig. 4-A). OAS1 expression showed positive correlation with the infiltration level of B cells (partial.cor = 0.171), and other three OAS genes did not have significant correlation with B cells (Fig. 4-B). OAS2, OAS3, and OASL had positive correlations with the infiltration levels of CD8 + T cells, with the partial correlation (partial.cor) respectively of 0.225, 0.307, and 0.25 (Fig. 4-C). All the four OASs had significant correlations with CD4 + T cells infiltration (partial.cor = 0.117, 0.269, 0.189, 0.198) (Fig. 4-D). OAS1, OAS2, and OASL had negative correlations with macrophage infiltration (partial.cor = − 0.133, − 0.114, − 0.113, respectively) (Fig. 4-E). All the four OASs had significant correlations with the infiltration of neutrophils and dendritic cells. The partial correlations of OAS1, OAS2, OAS3, and OASL with neutrophils were respectively 0.25, 0.53, 0.506, and 0.462 (Fig. 4-F). The partial correlations of OAS1, OAS2, OAS3, and OASL with dendritic cells were respectively 0.093, 0.407, 0.375, and 0.387 (Fig. 4-G).

Genetic and epigenetic changes of OAS gene family in BLCA

To determine the frequency and type of OAS gene family alterations in BLCA, we applied cBioPortal to analyze the mutation and methylation of OAS genes in the BLCA dataset based on 2365 patients/2410 samples of 12 studies. Results demonstrated that patient numbers showing mutations of OAS1, OAS2, OAS3, and OASL were respectively 50 (2.5%), 74 (4%), 61 (3%), and 39 (2%) (Fig. 5-A). In addition, the mutation frequencies of OAS gene family were 5.56% and 7.97% respectively in the bladder/urinary tract subtype and bladder urothelial carcinoma subtype (Fig. 5-B). Genetic mutations of OAS gene family mainly showed missense, truncating and splicing (Fig. 5-C). Furthermore, the degrees of DNA methylation of OAS gene family were negatively correlated with the expression level of OAS family (Fig. 5-D), suggesting that DNA methylation may suppress the transcription of the OAS genes.

Relationships among OAS family and co-expressed genes of OAS family in BLCA

The OAS family members may cooperate in the development of BLCA. Therefore, we investigated the potential correlations among OAS1, OAS2, OAS3, and OASL. OAS1 expression level was positively correlated with OAS2, OAS3, OASL (R = 0.64, 0.75, and 0.47, respectively) in the GEPIA database. The expression of OAS2 was positively correlated with OAS3 and OASL (R = 0.89 and 0.69, respectively), and OAS3 expression was positively correlated with OASL (R = 0.57) in GEPIA database analysis (Fig. 6-A). Similar results were obtained from a Kaplan-Meier Plotter analysis (Fig. 6-B). These correlation analyses indicated that the four OAS genes were positively correlated with each other which may reflect the collaboration of the four genes in BLCA.

The co-expressed genes of OAS family in BLCA were examined using the Oncomine and GeneMANIA databases. The genes co-expressed with OAS1, OAS2, OAS3, and OASL in Sanchez-Carbayo Bladder 2 dataset were identified using Oncomine in 48 normal samples, 81 filtrating bladder urothelial carcinoma tissues, and 28 superficial bladder cancer tissues. Results showed that OAS1 was positively correlated with OAS3, MX2, USP18, F12, GAALNT14, UNC58, DEF6, SH3BP1, NOL12, PLCH2, DGKA, THS4, ITGB4, and SEMA3F. OAS2 was positively correlated with USP18, OAS3, OAS1, MX2, CDK18, F12, GALNT14, UNC5B, DEF6, SH3BP1, NOL12, PLCH12, DGKA, TNS4, ITGB4, and SEMA3F. OAS3 was positively correlated with OAS1, MX2, USP18, OAS2, CDK18, F12, GALNT14, UNC5B, DEF6, SH3BP1, NOL12, PLCH2, DGKA, TNS4, ITGB4, and SEMA3F. OASL was positively associated with OAS2, MX1, IF16, ISG15, IF127, LY6E, BST2, CXCL16, IRF1, TRIM69, IRF9, STAT1, RARP9, PARP14, HLA-E, PSME2, PAME1, PR1C285, and FBXO6 (Fig. 7-A). Analysis of GeneMANIA showed that the top 20 co-expressed genes of OAS family included ISG15, IFI4L, MX1, IFI44, RSAD2, IFIT3, MX2, IFIT1, IFI35, STAT1, IFI27, IFIT5, IRF7, IRF9, BST2, EIF2AK2, CHMP1A, XAF1, LY6E, and UBE2L6 (Fig. 7-B). These top 20 co-expression genes were chosen to perform further biological function analysis (shown below).

Biological functions and pathways of OAS family in BLCA

GO enrichment analysis predicted the functional roles of target host genes based on three aspects, including biological processes (BP), cellular components (CC), and molecular functions (MF). We found that GO:0060337 (type I interferon signaling pathway), GO:0051607 (defense response to virus), GO:0009615 (response to virus), GO:0045071 (negative regulation of viral genome replication), GO:0060333 (interferon-gamma-mediated signaling pathway), GO:0001730 (2'-5'-oligoadenylate synthetase activity), GO:0003725 (double-stranded RNA binding), and GO:0005829 (cytosol) played critical roles in the development and progression of BLCA. Most of these functions were related to the process of virus infection and immune response, indicating the correlation between immune cell infiltration and tumorigenesis (Fig. 8A-C; Tables S 2–4).

By analyzing the KEGG pathway, we identified 21 pathways related to the functions of OAS family and neighbor genes in the pathogenesis of BLCA, including hsa05160 (Hepatitis C), hsa05164 (Influenza A), hsa05162 (Measles), hsa05169 (Epstein-Barr virus infection), hsa05168 (Herpes simplex virus 1 infection), hsa04621 (NOD-like receptor signaling pathway), hsa05165 (Human papillomavirus infection), hsa04217 (Necroptosis), and hsa05167 (Kaposi sarcoma-associated herpesvirus infection) (Fig. 8D, Table S5). The top five pathways were shown in Fig. 8E-I.

4. Discussion

BLCA is the second most common urinary tumor with an increasing incidence all around the world [20]. According to the pathological characteristics, BLCA is classified to non-muscle invasive BLCA (NMIBC) and muscle invasive BLCA (MIBC) of ratio approximately 70% and 30%, respectively [21]. NMIBC is associated with a longer survival period compared with MIBC, although NMIBC is prone to local recurrence and can develop to invasive disease. NMIBC (stage Ta or T1) has a good prognosis with 90% of 5-year overall survival rate, whereas the 5-year overall survival rate of MIBC (stage T2-T4) decreases to

60% or worse [22, 23]. The incidence of BLCA in men is three to four times higher than in women [24]. Cigarette smoking is the most common exposure contributing to the increased incidence of BLCA in western countries, and the degree of smoking may relate to the aggressiveness of BLCA [25]. With the rapid improvement of medical technology, the diagnosis and treatment of the disease have been obviously advanced but are still unsatisfied. Therefore, it is of great clinical significance to clarify the molecular mechanism of BLCA for the discovery of new therapeutic strategies.

The highlight of the present study was the comprehensive analysis of OAS gene family potentially related to BLCA, including OAS1, OAS2, OAS3, and OASL. The OASs are associated with the occurrence and development of many diseases and pathologies, including chronic infections, autoimmune diseases, cancers [26], apoptosis [27], autophagy [28], and the progression of coronavirus disease 2019 (COVID-19). Some studies have shown that the OAS gene family may provide a potential therapeutic target for COVID-19 if the gene is mutated on chromosome 12q24.13 (a gene cluster that encodes OAS1, OAS2, and OAS3 antiviral restriction enzyme activators) [29] [30]. Previous studies of OAS family mainly focus on their anti-viral function, while the relationship of OAS gene family with cancer, especially bladder cancer, has rarely been studied.

Na et al. [31] observed that OAS1 was one of core genes related to the prognosis of bladder urothelial carcinoma by integrated bioinformatics analysis. Luo et al. [32] identified that OAS1 was one of the differentially expressed immune-related genes reflecting the microenvironment of BLCA based on TCGA and ImmPort databases. Here, we found that the expression of OAS1 in BLCA tissues was significantly higher than that in normal bladder tissues, and the expression level was significantly different in different BLCA stages. Using multivariable Cox regression and subgroup analyses, we also found that OAS1 was a clinically independent prognostic factor for BLCA patients. In addition, researchers have developed some drugs targeting OAS1 to treat some diseases, such as 5-azacytidine (AZA). As a DNA methyltransferase inhibitor, AZA can lead to tumor cell death through the 2’- 5’ oligoadenylate synthetase (OAS)-RNase L pathway. AZA has already been used as an approved drug in the treatment of myelodysplastic syndromes and acute myeloid leukemia. OAS1 expression is related to AZA sensitivity in the NCI-60 set of tumor cell lines, suggesting that the level of OAS1 can be a biomarker for predicting AZA sensitivity of tumor cells [33]. According to related studies, the expression level of OAS1 in anti-viral infection is higher than that of OAS2 and OAS3 [34], and there is a high correlation between the expression of OAS1 and the other three OAS genes, which may indicate the important role of OAS1.

OAS2 is one of antiviral interferon-stimulated gene and plays an important role in resisting virus infection [35] and innate immune response in COVID-19 [36]. OAS2 is related to many diseases, including inflammation [37], autoimmune, malignant diseases, breast cancer [38], and colorectal cancer [39]. However, the biological functions of OAS2, including that in BLCA, remain to be clarified [40]. We showed here that the expression level of OAS2 in BLCA was higher than that in the normal bladder tissues. To our knowledge, this is the first study to demonstrate the relationship between OAS2 and BLCA. OAS2 has outstanding performance in tumor immune cell infiltration, but the specific mechanism remains to be identified.

OAS3 may also play a potential role in BLCA. The expression of OAS3 and other two OAS genes are closely related to each other, and their functions are similar. The 2',5'-oligoadenylate synthetase (OAS)-RNase L system is an antiviral signaling pathway induced by IFN, OAS3 displays a higher affinity for dsRNA in intact cells than either OAS1 or OAS2 in the antiviral process, which is consistent with its dominant role in RNase L activation [41, 42, 43]. At present, only a few studies showed that OAS3 affected the occurrence and development of chronic lymphocytic leukemia [44, 45]. Unfortunately, till now, no study has been performed to show the function of OAS3 in BLCA. We showed here that the expression of OAS3 in BLCA was significantly higher than in normal bladder tissues, suggesting the important role of OAS3 in BLCA. Not like the other three OAS genes that express in all species, OAS3 is only expressed in mice and humans. In addition, OAS3 does not harbor the catalytic activity required for synthesizing 2-5As and differs from the other human OAS family members by having two C-terminal ubiquitin-like domains. The detailed functional mechanisms of OAS3 in BLCA need further investigation.

Despite its lack of enzymatic activity, human OASL plays an important role in the antiviral process [46]. The relationship between OASL and cancer has been reported [47], such as breast cancer, cervical cancer, kidney cancer, and lung cancer. However, there is no report at present to show the role of OASL in BLCA. Here, we demonstrated that the expression of OASL was significantly higher in BLCA and had a beneficial effect on the overall survival based on analyses of Oncomine, TIMER, and GEPIA databases. Other studies have shown that OASL is closely related to the drug sensitivity of cervical cancer. Different expressions of OASL represent different drug-sensitivity to cisplatin in HPV + and HPV − cervical cancers. Patients with higher OASL expression exhibit stronger resistance to cisplatin than those with lower OASL expression [48]. OASL may also serve as prognostic biomarker predicting the overall survival for Kidney Renal Clear Cell Carcinoma [49, 50]. Besides, OASL is closely related to the occurrence of lung cancer. So far, there have been studies on the important role of OASL gene in the treatment of lung cancer. Lv et al [51] reported that OASL can be one of the decisive regulators to maintain lung cancer cell susceptibility to actinidia chinensis planch root extract and may be associated with the development of drug resistance. The regulation of OASL may be an alternative strategy to improve drug efficacy during cancer therapies.

The present study identified high expression of OAS gene family in BLCA and its association with some important biological processes of BLCA including genetic and epigenetic alterations and immune cell infiltration, and confirmed its impact on BLCA prognosis. These findings suggest that OAS gene family is important in the pathogenesis and development of BLCA and may become biomarkers of this tumor. Targeting OAS gene family may have clinical perspectives in the treatment and prevention of BLCA.

Limitations. Because of the molecular complexity of BLCA, we could not provide sufficient information on the systematic functions of OAS gene family in the pathogenesis of BLCA. This issue

In summary, the present study demonstrated the relationship between the OAS gene family and bladder cancer. The role of OAS1 in BLCA is particularly prominent among the four OAS genes, as OAS1 is not only highly expressed in BLCA tissues, but also is closely related to the prognosis of BLCA. The findings may contribute to better understanding on the molecular mechanisms of BLCA.

Declarations

Author contributions

GL: wrote the main manuscript text, RR: collected patient data and pathological tissue. SJ, HJ, NJ, FY, WM, WL, SY, WH: helped deal with clinical samples, analysis of data and draw the figures1-8. CJ and WD: supervised the study and revised the manuscript. All authors reviewed the manuscript.

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Ethics approval and informed consent

This study was approved by the Shanxi Bethune Hospital (Approval no.: YXLL-2021-066), all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and conducted according to the principles expressed in the Helsinki Declaration of 1964 and later versions. All patients have signed informed consent.

Consent for publication

Not applicable

Availability of Data and Materials

Oncomine (https://www.oncomine.org/resource/login.html), Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/), Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/), The Kaplan-Meier plotter (KM plotter, https://kmplot.com/analysis/), OncoLnc (http://www.oncolnc.org/), The cBio Cancer Genomics Portal (cBioPortal, www.cbioportal.org), GeneMANIA (http://www.genemania.org), DAVID 6.8 (https://david.ncifcrf.gov/home.jsp), and KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/anno_iden.php)

Competing interests

The authors declare that they have no competing interests

References

1. Wong MCS, Fung FDH, Leung C et al. The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. Scientific reports 2018, 8(1):1129.
2. Kakuta S, Shibata S, Iwakura Y. Genomic structure of the mouse 2',5'-oligoadenylate synthetase gene family. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 2002, 22(9):981-993.
3. Lin RJ, Yu HP, Chang BL et al. Distinct antiviral roles for human 2',5'-oligoadenylate synthetase family members against dengue virus infection. Journal of immunology (Baltimore, Md : 1950) 2009, 183(12):8035-8043.
4. Hu J, Wang X, Xing Y et al. Origin and development of oligoadenylate synthetase immune system. BMC evolutionary biology 2018, 18(1):201.
5. Gao LJ, Shen J, Ren YN et al. Discovering novel hub genes and pathways associated with the pathogenesis of psoriasis. Dermatologic therapy 2020, 33(6):e13993.
6. Zhang Y, Yu C. Prognostic characterization of OAS1/OAS2/OAS3/OASL in breast cancer. BMC cancer 2020, 20(1):575.
7. Wen X, Shao Z, Chen S et al. Construction of an RNA-Binding Protein-Related Prognostic Model for Pancreatic Adenocarcinoma Based on TCGA and GTEx Databases. Frontiers in genetics 2020, 11:610350.
8. Molinaro RJ, Jha BK, Malathi K et al. Selection and cloning of poly(rC)-binding protein 2 and Raf kinase inhibitor protein RNA activators of 2',5'-oligoadenylate synthetase from prostate cancer cells. Nucleic Acids Res 2006, 34(22):6684-6695.
9. Rhodes DR, Yu J, Shanker K et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia (New York, NY) 2004, 6(1):1-6.
10. Tang Z, Li C, Kang B et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017, 45(W1):W98-W102.
11. Li T, Fan J, Wang B et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer research 2017, 77(21):e108-e110.
12. Győrffy B, Lanczky A, Eklund AC et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast cancer research and treatment 2010, 123(3):725-731.
13. Anaya J. OncoLnc: Linking TCGA survival data to mRNAs, miRNAs, and lncRNAs. PeerJ Computer Science 2016, 2(2):e67.
14. Cerami E, Gao J, Dogrusoz U et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012, 2(5):401-404.
15. Warde-Farley D, Donaldson SL, Comes O et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010, 38(suppl

2):W214-W220.

16. Sanchez-Carbayo M, Socci ND, Lozano J et al. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. J Clin Oncol 2006, 24(5):778-789.
17. Dyrskjøt L, Kruhøffer M, Thykjaer T et al. Gene expression in the urinary bladder: a common carcinoma in situ gene expression signature exists disregarding histopathological classification. Cancer Res 2004, 64(11):4040-4048.
18. Lee JS, Leem SH, Lee SY et al. Expression signature of E2F1 and its associated genes predict superficial to invasive progression of bladder tumors. J Clin Oncol 2010, 28(16):2660-2667.
19. Vitale I, Manic G, Coussens LM et al. Macrophages and Metabolism in the Tumor Microenvironment. Cell Metab 2019, 30(1):36-50.
20. Antoni S, Ferlay J, Soerjomataram I et al. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. European urology 2017, 71(1):96-108.
21. Wang S, Jin S, Shu Q et al. Strategies to Get Drugs across Bladder Penetrating Barriers for Improving Bladder Cancer Therapy. Pharmaceutics 2021, 13(2):166.
22. Patel VG, Oh WK, Galsky MD. Treatment of muscle-invasive and advanced bladder cancer in 2020. CA: a cancer journal for clinicians 2020, 70(5):404-423.
23. Ghandour R, Singla N, Lotan Y. Treatment Options and Outcomes in Nonmetastatic Muscle Invasive Bladder Cancer. Trends in cancer 2019, 5(7):426-439.
24. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians 2019, 69(1):7-34.
25. Cumberbatch MG, Rota M, Catto JW et al. The Role of Tobacco Smoke in Bladder and Kidney Carcinogenesis: A Comparison of Exposures and Meta-analysis of Incidence and Mortality Risks. European urology 2016, 70(3):458-466.
26. Choi UY, Kang JS, Hwang YS et al. Oligoadenylate synthase-like (OASL) proteins: dual functions and associations with diseases. Experimental & molecular medicine 2015, 47(3):e144.
27. Domingo-Gil E, Esteban M. Role of mitochondria in apoptosis induced by the 2-5A system and mechanisms involved. Apoptosis 2006, 11(5):725-738.
28. Leisching G, Wiid I, Baker B. OAS1, 2, and 3: Significance During Active Tuberculosis? The Journal of infectious diseases 2018, 217(10):1517-1521.
29. Pairo-Castineira E, Clohisey S, Klaric L et al. Genetic mechanisms of critical illness in COVID-19. Nature 2021, 591(7848):92-98.
30. Shaath H, Vishnubalaji R, Elkord E et al. Single-Cell Transcriptome Analysis Highlights a Role for Neutrophils and Inflammatory Macrophages in the Pathogenesis of Severe COVID-19. Cells 2020, 9(11).
31. Na L, Bai Y, Sun Y et al. Identification of 9-Core Immune-Related Genes in Bladder Urothelial Carcinoma Prognosis. Frontiers in oncology 2020, 10:1142.

32. Luo Y, Chen L, Zhou Q et al. Identification of a prognostic gene signature based on an immunogenomic landscape analysis of bladder cancer. Journal of cellular and molecular medicine 2020, 24(22):13370-13382.
33. Banerjee S, Gusho E, Gaughan C et al. OAS-RNase L innate immune pathway mediates the cytotoxicity of a DNA-demethylating drug. Proceedings of the National Academy of Sciences of the United States of America 2019, 116(11):5071-5076.
34. Hancks DC, Hartley MK, Hagan C et al. Overlapping Patterns of Rapid Evolution in the Nucleic Acid Sensors cGAS and OAS1 Suggest a Common Mechanism of Pathogen Antagonism and Escape. PLoS Genet 2015, 11(5):e1005203.
35. Liao X, Xie H, Li S et al. 2', 5'-Oligoadenylate Synthetase 2 (OAS2) Inhibits Zika Virus Replication through Activation of Type I IFN Signaling Pathway. Viruses 2020, 12(4):418.
36. Prasad K, Khatoon F, Rashid S et al. Targeting hub genes and pathways of innate immune response in COVID-19: A network biology perspective. International journal of biological macromolecules 2020, 163:1-8.
37. Manning G, Tichý A, Sirák I et al. Radiotherapy-Associated Long-term Modification of Expression of the Inflammatory Biomarker Genes ARG1, BCL2L1, and MYC. Frontiers in immunology 2017, 8:412.
38. Fite BZ, Wang J, Kare AJ et al. Immune modulation resulting from MR-guided high intensity focused ultrasound in a model of murine breast cancer. Scientific reports 2021, 11(1):927.
39. Kim JC, Ha YJ, Tak KH et al. Opposite functions of GSN and OAS2 on colorectal cancer metastasis, mediating perineural and lymphovascular invasion, respectively. PloS one 2018, 13(8):e0202856.
40. Dar AA, Pradhan TN, Kulkarni DP et al. Extracellular 2'5'-oligoadenylate synthetase 2 mediates T-cell receptor CD3-ζ chain down-regulation via caspase-3 activation in oral cancer. Immunology 2016, 147(2):251-264.
41. Li Y, Banerjee S, Wang Y et al. Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. Proceedings of the National Academy of Sciences 2016, 113(8):2241-2246.
42. Li Y, Dong B, Wei Z et al. Activation of RNase L in Egyptian Rousette Bat-Derived RoNi/7 Cells Is Dependent Primarily on OAS3 and Independent of MAVS Signaling. mBio 2019, 10(6).
43. Ibsen MS, Gad HH, Thavachelvam K et al. The 2'-5'-oligoadenylate synthetase 3 enzyme potently synthesizes the 2'-5'-oligoadenylates required for RNase L activation. Journal of virology 2014, 88(24):14222-14231.
44. Sava GP, Speedy HE, Di Bernardo MC et al. Common variation at 12q24.13 (OAS3) influences chronic lymphocytic leukemia risk. Leukemia 2015, 29(3):748-751.
45. Herishanu Y, Pérez-Galán P, Liu D et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood 2011, 117(2):563-574.
46. Ibsen MS, Gad HH, Andersen LL et al. Structural and functional analysis reveals that human OASL binds dsRNA to enhance RIG-I signaling. Nucleic Acids Res 2015, 43(10):5236-5248.

47. Muhammad SA, Guo J, Nguyen TM et al. Simulation Study of cDNA Dataset to Investigate Possible Association of Differentially Expressed Genes of Human THP1-Monocytic Cells in Cancer Progression Affected by Bacterial Shiga Toxins. Frontiers in Microbiology 2018, 9(380).
48. Zhang L, Jiang Y, Lu X et al. Genomic characterization of cervical cancer based on human papillomavirus status. Gynecologic oncology 2019, 152(3):629-637.
49. Xiang Y, Zhou S, Hao J et al. Development and validation of a prognostic model for kidney renal clear cell carcinoma based on RNA binding protein expression. Aging 2020, 12(24):25356-25372.
50. Zhong W, Huang C, Lin J et al. Development and Validation of Nine-RNA Binding Protein Signature Predicting Overall Survival for Kidney Renal Clear Cell Carcinoma. Frontiers in genetics 2020, 11:568192.
51. Lv J, Wang L, Shen H et al. Regulatory roles of OASL in lung cancer cell sensitivity to Actinidia chinensis Planch root extract (acRoots). Cell Biol Toxicol 2018, 34(3):207-218.

Figures

Figure 1

The mRNA expression patterns of OAS1, OAS2, OAS3, and OASL in different types of human cancer. (A) Results of Oncomine database analysis (Fold change > 2, $P < 0.0001$). (B) Human OAS gene family expression levels in different tumor types from TCGA database determined by TIMER (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (C) Expression of four genes in various cancers. (D) Results of GEPIA database analysis (Black font imply OAS gene family were undifferentiated expressed in those cancers. Red font represents significantly high expression, green font meaning low expression).

Abbreviations of cancer types: ACC, adrenocortical carcinoma. BLCA, bladder urothelial carcinoma. BRCA, breast invasive carcinoma. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. CHOL, cholangio carcinoma. 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 leukemia. LGG, brain lower grade glioma. LIHC, liver hepatocellular carcinoma. LUAD, lung adenocarcinoma. LUSC, lung squamous cell carcinoma. OV, ovarian serous cystadenocarcinoma. PAAD, pancreatic adenocarcinoma. PCPG, pheochromocytoma and paraganglioma. PRAD, prostate adenocarcinoma. READ, rectum adenocarcinoma. SARC, sarcoma. 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. UVM, uveal melanoma.

Figure 2

Detailed expression levels of OAS gene family in bladder cancer. (A) Box plot of OAS1, OAS2, OAS3, and OASL expression levels in Sanchez-Carbayo Bladder 2 dataset from Oncomine. (B) Expression characterization of OAS1, OAS2, OAS3, and OASL in GEPIA database. (C) Results of RT-QPCR, mRNA levels of OASs in BLCA tissues and normal tissues. (D) Results of western blotting, representative electrophoresis bands of western blots for OASs expression in BLCA tissues and normal tissues, respectively. (E) Results of immunohistochemistry, immunohistochemical stains of OASs in BLCA tissues and normal tissues. ** $P < 0.01$, *** $P < 0.001$.

Figure 3

(A) The relationship between gene expression and clinical stage. (B-D) Prognostic value of OAS gene family for BLCA from Kaplan-Meier Plotter, OncoLnc, and GEPIA database.

Figure 4

Correlation of OAS gene family with tumor immune cells infiltration level in BLCA via Tumor Immune Estimation Resource (TIMER). (A) OAS1 expression has no significant correlation with tumor purity and has significant positive correlations with infiltrating levels of B cells, CD4+ T cells, neutrophils, and dendritic cells, other than macrophage in BLCA. (B) OAS2 expression has positive correlations with tumor purity and infiltrating levels of CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells, while has negative correlations with macrophages in BLCA. (C) OAS3 expression is significantly negatively related to tumor purity and has significant positive correlations with infiltrating levels of CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells in BLCA. (D) OASL expression is significantly negatively related to tumor purity and has significant positive correlations with infiltrating levels of CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells, but has negative positive correlations with macrophages in BLCA.

Figure 5

Visual summary of OAS gene family alterations in BLCA. (A) OAS gene family expression and mutation analysis in BLCA. (B) Frequency of gene alterations in OAS1, OAS2, OAS3, and OASL in different types of BLCA. (C) The mutational situation of OAS1, OAS2, OAS3, and OASL. (D) The effect of DNA methylation on mRNA expression.

Figure 6

The relationship among OAS gene family. (A) Pearson correction analysis of GEPIA database. (B) Spearman and Pearson correction analysis among OAS gene family from Kaplan-Meier Plotter.

Figure 7

Co-expressed genes with OAS1, OSA2, OAS3, and OASL. (A) The genes relevant to the expression of OAS1, OSA2, OAS3, and OASL, respectively. (B) The common network for OAS gene family and their neighbor top 20 co-expression genes.

Figure 8

GO and KEGG pathway enrichment analysis of OAS gene family ($P < 0.05$). (A-C) GO enrichment analysis predicted the functional roles based on three aspects, including Biological processes, Cellular components, Molecular functions. (D) KEGG pathway analysis. (E-I) Top 5 KEGG pathways of OAS gene family and top 20 co-expression genes. (E) Hepatitis C, (F) Influenza A, (G) Measles. (H) Epstein-Barr virus infection. (I) Herpes simplex virus 1 infection.

Supplementary Files

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