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Analysis of the Expression of Exostosins and Clinicopathological Features in Membranous Lupus Nephritis in a Chinese Cohort

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INTRODUCTION

Exostosin 1/Exostosin 2 (EXT1/EXT2) were recently described by Sethi *et al.*[1] as 2 new proteins and putative antigens in secondary membranous nephropathy, which were frequently associated with autoimmune findings of an underlying autoimmune disease, such as systemic lupus erythematosus (SLE) or mixed connective tissue disease. To date, limited studies have been published to validate and explore their clinical and pathological significance. In this study we aimed to evaluate the expression of EXTs, their clinicopathologic features, and the prognosis for EXT1/2–positive membranous lupus nephritis (MLN).

RESULTS

EXT1/2 Expression in MLN

A total of 86 cases with available tissues for immunofluorescence staining were included in this study. All patients had a diagnosis of SLE and the kidney biopsy specimens revealed the characteristics of MLN. Of all patients, 25 (29.1%) were positive for glomerular EXT1/2 staining along capillary walls, of which 12 (48%) biopsy specimens showed pure class 5 MLN (Supplementary Figure S1). In this analysis, no single case showed isolated EXT1 or EXT2 staining positivity. A total of 20 PLA2R-related membranous nephropathy and 20 proliferative lupus nephritis patients (class 3: $n = 8$, class 4: $n = 12$) were selected as controls and were negative for EXT1/EXT2 staining. Representative EXT1 and EXT2 staining of positive and negative cases are shown in Figure 1 and Supplementary Figure S2.

Clinical and Pathologic Characteristics of EXT1/EXT2 Positive and Negative Patients

Of the 25 patients who were EXT1/EXT2 positive, the mean age at the time of kidney biopsy was 35.8±12.6 years and the female proportion was 96%. The mean esitmated glomerular filtration rate and serum albumin levels were 108.0±43.5 ml/min and 23.5±5.2 g/l, respectively. According to the SLEDAI-2k scores, the disease activities of 17 (68%) patients were mild to moderate. In this group, pure class 5 MLN patients showed higher levels of estimated glomerular filtration rate and complement C3 and C4, whereas lower anti-dsDNA antibody titers compared to patients with proliferative features (class 3/4) (Supplementary Table S1).

Compared with EXT1/EXT2-negative patients, EXT1/EXT2-positive patients had significantly lower serum albumin levels (23.5±5.2 vs. 27.0±7.6 g/l, $P = 0.042$). EXT1/EXT2-positive patients tended to have higher levels of proteinuria, C3, C4, and a lower proportion of severe disease activity according to SLEDAI scores, although the differences were not significant ($P > 0.05$) (Supplementary Table S2).

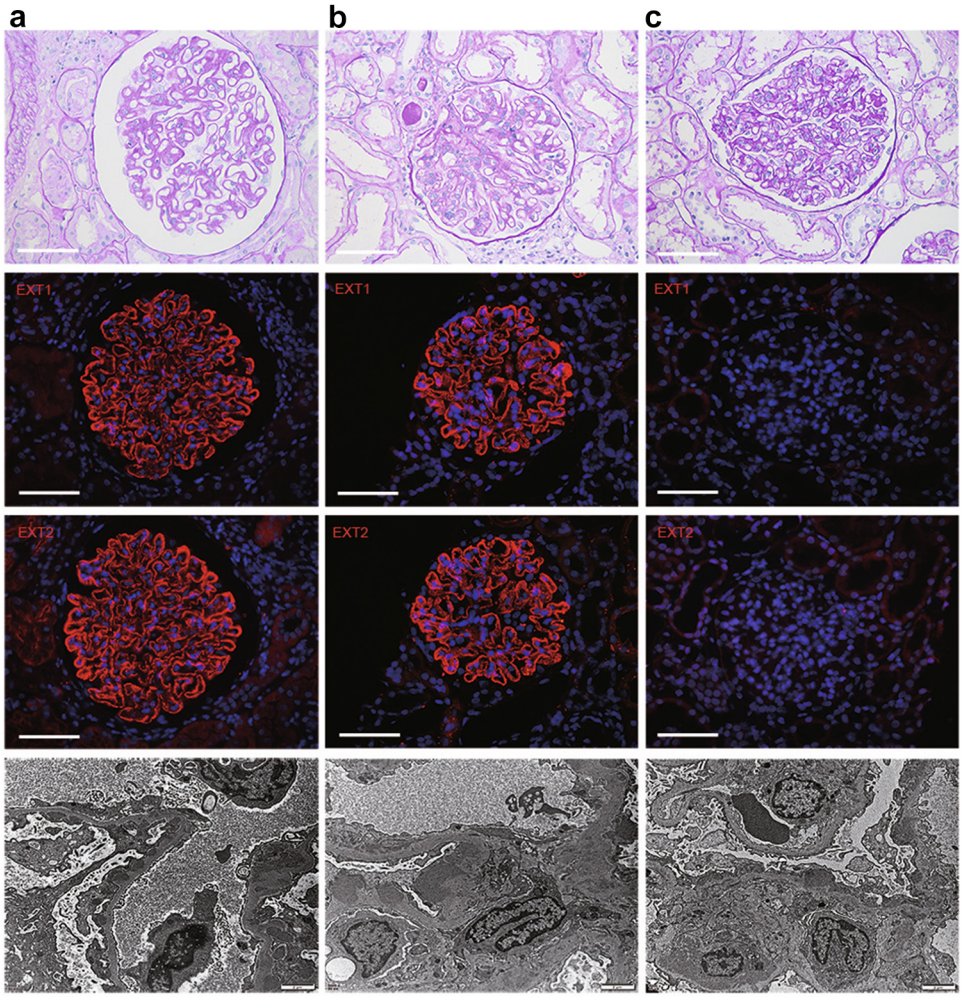

Figure 1. Light microscopy, immunofluorescence and electron microscopy of EXT1/EXT2 positive and negative MLN. (a) Example of renal PAS staining from a patient with pure class 5 MLN was shown in top left panel, immunofluorescence staining for EXT1 and EXT2 were shown in the middle left panels, respectively. Transmission electron microscope image of the renal tissue from the same pure class 5 MLN patient was shown in the bottom left panel. (b) Example of PAS staining, EXT1 and EXT2 expression and electron microscopy in the renal biopsy tissue from a patient with class 5 MLN with class 3 proliferative lupus nephritis. (c) Negative staining for EXT1/EXT2 in class 5 MLN with class 4 proliferative lupus nephritis. White scale bar: 100 μm for PAS staining and EXT1/EXT2 IF images. Black scale bar: 2μm for electron microscope images. EXT1, exostosin 1; EXT2, exostosin 2.

Pure Class 5 MLN Without Class 3/4 Lupus Nephritis

Of the 39 patients with pure class 5 MLN, 12 (30.8%) were EXT1/EXT2-positive and 27 (69.2%) were EXT1/EXT2-negative. In accordance with what was observed in the combined cohort, the EXT1/EXT2 positive patients presented with lower levels of serum albumin (22.8±5.7 vs. 29.1±7.7 g/l, $P = 0.016$). EXT1/EXT2 positive patients showed a tendency to have higher levels of proteinuria than patients who were negative (Supplementary Table S3).

Class 5 MLN with Class 3/4 Lupus Nephritis

Thirteen of 25 (52%) EXT1/EXT2-positive cases and 34 of 61 (55.7%) EXT1/EXT2-negative cases were class 5 MLN with proliferative features (class 3 or 4). There were no statistically significant differences in the mean age, serum creatinine, albumin, hematuria, proteinuria, and complement levels between the 2 groups (Supplementary Table S4).

Clinical Follow-Up of EXT1/EXT2-Positive and EXT-Negative MLN

Follow-up data was available for 69 of 86 patients; 19 (27.5%) were EXT1/EXT2-positive MLN and 50 (72.5%) were EXT1/EXT2-negative MLN. At the time of biopsy, EXT1/EXT2-positive patients exhibited a significantly lower baseline serum albumin level (23.0±5.8 vs. 27.6±7.8 g/l, $P = 0.02$), and tended to have increased proteinuria (3.17±1.90 vs. 2.36±1.39

Table 1. Clinical characteristics, follow-up, and outcomes of EXT 1/EXT2-positive and EXT1/EXT2-negative MLN (with or without class 3/4 lupus nephritis)

Variable	EXT1/EXT2-Positive, n = 19/25 (76%)	EXT1/EXT2-Negative, n = 50/61 (82.0%)	P value
Age (yr)	37.4±12.7	35.1±11.2	0.468
Female, n (%)	18 (94.7)	42 (84.0)	0.237
SCr (μmol/l)	48.0 (38.0, 60.0)	54.0 (45.0, 75.0)	0.207
eGFR (ml/min per 1.73 m^2)	113.3±41.9	110.1±34.1	0.747
Albumin (g/l)	23.0±5.8	27.6±7.8	0.02
Proteinuria (g/24 h)	3.17±1.90	2.36±1.39	0.054
Nephrotic syndrome, n (%)	10 (52.6)	21 (42.0)	0.428
Hemaurine, n (%)	9 (47.4)	25 (50.0)	0.845
C3 (g/l)	0.72±0.31	0.58±0.24	0.052
C4 (g/l)	0.15±0.10	0.12±0.06	0.137
dsDNA (IU/ml)	18.3 (2.8, 86.9)	55.0 (8.3, 100)	0.115
ESR (mm/h)	60.5±37.8	41.8±27.9	0.046
SLEDAI group			0.355
Mild	6 (31.6)	8 (16.0)	
Moderate	7 (36.8)	22 (44.0)	
Severe	6 (31.6)	20 (40.0)	
Proliferative features, n (%)	8 (42.1)	28 (56.0)	0.302
Sclerosed glomeruli >10%, n (%)	6 (31.6)	8 (15.7)	0.139
Full house staining, n (%)	16 (84.2)	33 (66.0)	0.136
Extraglomerular staining, n (%)	5 (26.3)	17 (34.0)	0.541
At end of follow-up			
SCr (μmol/l)	50.5 (44.8, 69.5)	57.0 (49.0, 73.0)	0.305
eGFR (ml/min per 1.73 m^2)	106.8±34.5	108.3±27.5	0.928
Albumin (g/l)	37.9±6.4	37.7±6.8	0.928
Proteinuria (g/24 h)	0.35 (0.07, 0.73)	0.45 (0.1, 1.64)	0.323
Clinical remission			
Complete	13 (68.4)	30 (60.0)	0.519
Partial	4 (20.8)	4 (8.0)	
No remission	2 (10.8)	16 (32.0)	
Composite remission	17 (89.2)	34 (68.0)	0.07
Time of follow-up (mo)	18.4±13.2	17.1±12.9	0.717

eGFR, estimated glomerular filteration rate; ESR, erythrocyte sedimentation rate; SCr, serum creatinine; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

g/24h, $P = 0.054$) and higher levels of serum complement C3 (0.72±0.31 vs. 0.58±0.24, $P = 0.052$).

There were no differences between the 2 groups on either median serum creatinine or proteinuria at the end of the follow-up. Clinical remission, either complete or partial, seemed more frequent in the EXT-positive group (89.2% vs. 68.0%, $P = 0.07$) (Table 1). In further subgroup analysis, EXT-positive patients were more likely to achieve clinical remission in MLN groups with or without concurrent class 3 or class 4 lupus nephritis, although the differences were not statistically significant (Supplementary Table S5 and S6).

DISCUSSION

Recently, the deposition of EXT1/EXT2 in MLN glomeruli have been validated in a few studies.[2-6] We confirmed that a subset of MLN patients was positive for EXT1/EXT2 in a Chinese cohort and the ratios of EXT-positive among combined and pure MLN were similar to studies by Ravindran *et al.*[4] and Saïdi *et al.*,[2] whereas another recently published Chinese study showed higher percentage as 46% of patients with MLN and lower percentage as 9% patients with class 5 +3/4 MLN were EXT-positive.[6] Previous studies consistently demonstrated that EXT-positive patients had better renal function, greater daily proteinuria, and less chronicity on biopsy specimens at the time of biopsy. We observed that EXT1/EXT2-positive patients had lower serum albumin levels than EXT1/EXT2-negative patients in the whole cohort and the difference was also noted in the pure MLN group. Regarding proteinuria, we observed EXT1/EXT2-positive patients tended to have greater proteinuria, though not statistically significant. Nevertheless, in clinical practice, low serum albumin was mainly due to proteinuria, and serum albumin might be more reliable and stable than proteinuria. Therefore, these findings were consistent with previous studies. In addition, regarding the immunoserological markers representative of SLE disease activity, EXT1/EXT2-positive patients were more likely to have higher C3 level, higher proportion of less severe patients, and lower dsDNA titers than EXT1/EXT2-negative patients, similar to a previous Japanese study.[3]

After a similar follow-up time, clinical outcomes in EXT1/EXT2 positive group seemed to be better in terms of proteinuria, which was very similar to the study by Saïdi *et al.*[2] Ravindran *et al.*[4] reported that EXT1/EXT2-positive patients were less likely to progress to ESKD compared to EXT1/EXT2-negative cases in a large series of MLN patients after a 10-year follow-up. Taken together, EXT1/EXT2-positive MLN patients showed favorable renal outcomes despite greater proteinuria at diagnosis.

To our knowledge, the potential protective mechanism of EXT1/EXT2 in MLN still remains elusive. EXTs are glycosyltransferases that are crucial for the glycosylation of the heparan-sulfate which can facilitate removal of the immune complexes and proteins involved in the immune response.[7-9] Ravindran *et al.*[4] speculated that the increased secretion of catalytic domain EXT1/EXT2 into the glomerular basement membrane results in increased synthesis of the heparan sulfates that may offer protection from damaging events. Nevertheless, studies were required to confirm this hypothesis and elucidate the trigger for overproduction of EXT1/EXT2. In addition, it is notable that circulating autoantibodies to EXT1/EXT2 have not been identified. The possible reasons may be that the serum antibody may not recognize the epitopes on recombinant EXT1/EXT2 proteins or that they exist at a low titer. Therefore, whether EXT1/EXT2 is an antigen or a biomarker remains unclear.

Our study has some limitations. First, this is a single-center study with a limited sample size, which might make the statistical analyses deviated and failed to determine the difference between groups. Second, in this retrospective study, some patients had received immunosuppressive therapy at baseline, which may have affected the serum and urine detection results. In addition, due to short follow-up time, we could not further elucidate the association between EXT1/EXT2 status and long-term renal prognosis.

In summary, we explored the prevalence, clinical characteristics and outcomes of EXT1/2-positive versus negative MLN in northwestern Chinese patients and it revealed 29.1% MLN patients were positive for EXT1/EXT2. EXT1/EXT2-positive patients presented with lower serum albumin levels at diagnosis and tended to have better renal prognosis compared with EXT1/EXT2-negative cases. In view of these findings, glomerular EXT1/EXT2 staining could help identify distinct phenotypes in MLN patients, and should be recommended to be involved in clinical practice.

DISCLOSURE

All the authors declared no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Flowchart of the study cohort. Membranous lupus nephritis is categorized into EXT1/EXT2-positive and EXT1/EXT-2 negative cohorts, which are further subcategorized with and without proliferative features.

Figure S2. Low power immunofluorescence (IF, 200×) of EXT1/EXT2 positive and negative MLN. (A) Example of low power IF staining for EXT1 and EXT2 from a patient with pure class 5 MLN were shown in the top and bottom left panels, respectively. (B) EXT1 and EXT2 expression in the renal biopsy tissue from a patient with class 5 MLN with class 3 proliferative lupus nephritis. (C) Negative staining for EXT1/EXT2 in class 5 MLN with class 4 proliferative lupus nephritis. White scale bar: 100μm for EXT1/EXT2 IF images.

Table S1. Clinicopathologic features and follow-up of EXT1/EXT2-positive MLN patients with and without proliferative features.

Table S2. Clinicopathologic and pathologic features of EXT1/EXT2-positive and EXT1/EXT2-negative MLN.

Table S3. Clinical characteristics of EXT1/EXT2-positive and EXT1/EXT2-negative MLN without proliferative features (pure class 5).

Table S4. Clinical characteristics of EXT1/EXT2-positive and EXT1/EXT2-negative MLN with proliferative features (combined class 5 MLN with class 3/4 lupus nephritis).

Table S5. Clinical characteristics of EXT1/EXT2-positive and EXT1/EXT2-negative MLN without proliferative features (pure class 5) at last follow-up.

Table S6. Clinical characteristics of EXT1/EXT2-positive and EXT1/EXT2-negative MLN with proliferative features (combined class 5 MLN with class 3/4 lupus nephritis) at last follow-up.

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