Item S1

Detailed Methods

We prepared kidney serial sections for SBF-SEM examination from 7 Japanese patients with biopsy-proven IgA nephropathy, as described previously.[1] Following fixation with buffered 2.5% glutaraldehyde, the tissues were treated with 2% (wt/vol) osmium tetroxide reduced with 1.5% (wt/vol) potassium ferrocyanide in 0.1 M cacodylate buffer for 60 min on ice, 1% (wt/vol) thiocarbohydrazide for 20 min at room temperature, and 2% (wt/vol) osmium tetroxide for 30 min at room temperature. Every treatment was followed by washing with PBS. The tissues were then stained *en bloc* with 2% uranyl acetate solution at 4°C or 37°C overnight, and lead aspartate (pH 5.5) at 60°C for 30 min. The tissues were dehydrated in ethanol and embedded in carbon-based conductive resin. The embedded specimens were mounted on aluminum pins, trimmed, coated with gold, and then imaged in field emission–SEM (Merlin or Sigma, Carl Zeiss) equipped with 3View (Gatan). The obtained serial images were processed with the Fiji image processing platform (http://fiji.sc/wiki/index.php/Fiji). We examined 321–920 serial images from two–three regions of interest per patient for a total of 11,301 images sized 22.9–57.3 × 23.0–57.5 μm. We assessed the continuity or

disruption of the GBM by tracing each serial section and evaluating the morphological changes in the glomerular components if disrupted areas were detected. Segmentation and image analyses were performed in the Microscopy Image Browser (http://mib.helsinki.fi)[2,3] and Amira (FEI Visualization Science Group, Hillsboro, OR, USA).

All procedures that involved human participants were approved by the institutional ethical committee and performed in accordance with the ethical standards of the institution at which the study was conducted (institutional review board approval number 2015-1-537, Tohoku University School of Medicine) according to the 1964 Helsinki declaration and its later amendments or with comparable ethical standards. Written informed consent was obtained from all study participants.

References

1. Takaki T, Ohno N, Saitoh S, et al. Podocyte penetration of the glomerular basement membrane to contact on the mesangial cell at the lesion of mesangial interposition in lupus nephritis: a three-dimensional analysis by serial block-face scanning electron microscopy. Clin Exp Nephrol.

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2. Belevich I, Joensuu M, Kumar D, et al. Microscopy image browser: a platform for segmentation and analysis of multidimensional datasets. PLoS Biol. 2016;14(1):e1002340.
3. Cocks E, Taggart M, Rind FC, et al. A guide to analysis and reconstruction of serial block face scanning electron microscopy data. J Microsc. 2018;270(2):217-234.

An overview of sample preparation for SBF-SEM

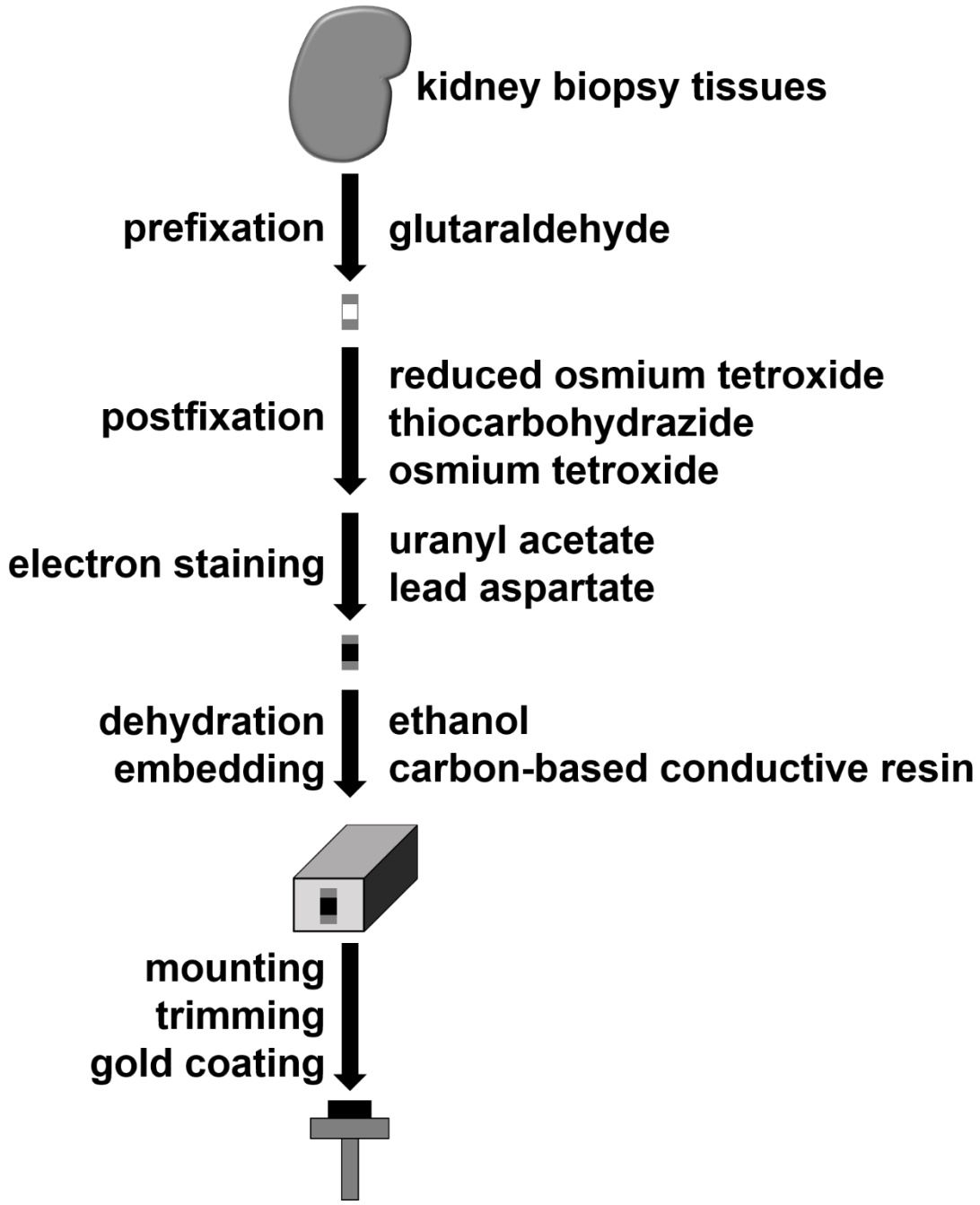

Table S1. Clinical and histological findings from the study participants.

patient	age	sex	serum creatinine (mg/dL)	urinary red blood cell (/high-power field)	urinary protein/creatinine ratio (g/gCr)	Oxford classification
1	20s	M	1.21	>100	4.11	M1 E1 S1 T0 C1
2	30s	F	1.16	30-50	4.17	M1 E1 S1 T0 C1
3	30s	M	0.97	30-50	0.75	M0 E1 S1 T0 C1
4	30s	M	0.85	10-20	1.41	M1 E0 S1 T0 C1
5	30s	F	0.59	>100	2.27	M0 E0 S1 T0 C1
6	20s	F	0.50	50-100	0.08	M0 E0 S0 T0 C0
7	20s	F	0.45	10-20	0.13	M0 E0 S1 T0 C1