Charged nanoparticles for MRI of the basement membrane

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Introduction: The basement membrane (BM) is a major functional component of the extracellular matrix. Cells reside in the three-dimensional matrix of proteins and proteoglycans comprising and surrounding the BM, and the BM plays a role in cell function and motility throughout the body (1-2). In the kidney, the glomerular basement membrane is exposed to the circulation, and maintains a barrier to large and anionic proteins in the blood (3). The breakdown of this charge barrier is associated with a number of renal diseases. Small cationic particles are known to bind to anionic proteoglycans in the BM of the kidney, pancreas, and other organs, and have been used to detect ultra-structure with EM (4-6). Anionic agents are used to detect cartilage breakdown with MRI (7). The goal of this work was to develop an MRI-visible cationic contrast agent to target the BM, based on the binding to sites of opposite charge. Here we show that the accumulation of cationic nanoparticles (ferritin) in the glomerular basement membrane (GBM) of the kidney can be detected with MRI, and a change in this accumulation can be used to detect renal glomerular disease *in vivo*.

Methods: In Vitro Preparation and Imaging: Rats were given an injection of 3.3mg/100 g of native horse spleen ferritin (NF, Sigma, St. Lous) or cationized horse spleen ferritin (CF), in a bolus repeated one to four times in 1.5 hour intervals. Animals were sacrificed by perfusion, and kidneys were placed in 2% gluteraldehyde buffer in a syringe. Kidneys were imaged in a Bruker 11.7T scanner with a 3D GRE sequence with TE/TR = 12/30 ms, and resolution of 50 x 50 x 50 µm. For *in vivo* studies, rats were imaged rather than sacrificed after the final CF injection. Rats were anesthetized, intubated, and ventilated, and a 30 mm surface coil was placed over the left kidney. Respiratory gated multi-slice images were acquired with 30^0 flip-angle and TE/TR = 6/30 ms at 100 x100 x500 µm. Disease Model: To model focal segmental glomerulosclerosis (FSGS, ref. 5), causing GBM charge breakdown, rats were given IP injections (100 mg/kg) of puromycin aminonucleoside (PAN), and additional doses (20 mg/kg) every 2 weeks. Animals presented intensive proteinuria, hypoalbumia, and hyperlipidemia 7 days after PAN injection. Imunohistochemistry: Anti-horse spleen ferritin (Sigma-Aldrich, St. Louis, MO) was applied to sections in DPSS with 1% BSA. Alexa594 (Invitrogen, Carlsbad, CA) secondary IgG was applied. Images were acquired on a Zeiss LSM 510 confocal microscope. Electron microscopy: Sections of kidney cortex were embedded in expoxy and imaged with a JEM-1200CX. Ferritin was detected without counterstain because of its electron dense iron core.

Results and Conclusions: Hypointense spots, consistent with single glomeruli, were detected in *ex vivo* images of CF injected rats, but not in normal or NF-injected controls (Fig.1). In FSGS, CF was detected outside the glomerulus in tubules, consistent with breakdown of the GBM charge barrier (Fig.2, top images). CF accumulation in normal and FSGS rats was detected *in vivo*, and CF caused 75% decrease in signal intensity over controls, as measured by line profiles and their spatial power spectra (Fig 2, bottom). Accumulation of CF in the basement membrane was confirmed by immunofluorescence (Fig. 3). Electron microscopy showed that CF accumulation was specific to the basement membrane, and NF did not accumulate. In FSGS rats, CF immunofluorescence was reduced, and EM showed a diffuse distribution of CF in the podocyte cell body. We conclude that cationic nanoparticles can be used as highly specific contrast agents to detect basement membrane, and the distribution of these agents is sensitive to the breakdown of the basmement membrane with disease. Because of their affinity to charged proteoglycans, cationic contrast agents could potentially be used to detect molecular alterations of the BM and other extracellular matrix components throughout the body.

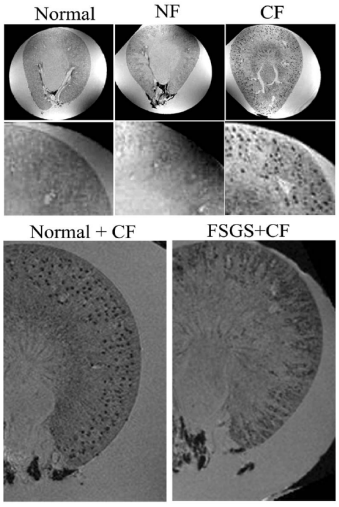

Figure 1: (Top panel) Gradient echo MRI of fixed rat kidneys (with magnifications in second row) showing hypointense spots where cationic ferritin (CF) accumulated, consistent with labeling of individual glomeruli. No spots were present in native-ferritin inoculated rats or in normal kidneys. CF accumulation with FSGS was diffuse (bottom images).

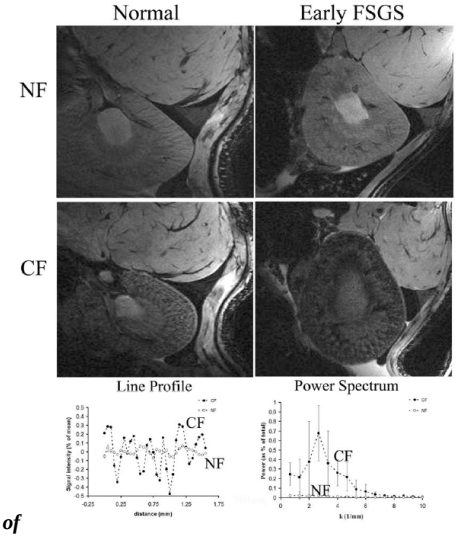

Figure 2: (Top panel) In vivo MRI of kidneys of normal and diseased (FSGS) rats, showing accumulation of CF in cortex that became diffuse with FSGS. (Bottom panel) Line profiles through cortex and spatial power spectra confirmed a 75% decrease in intensity in cortex of CF rats compared to controls, and inter-glomerular spacing of 2.5/mm.

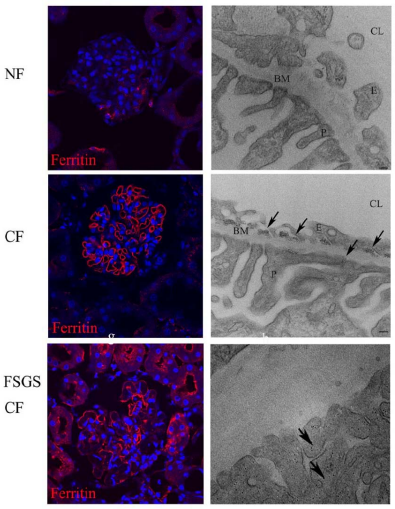

Figure 2: (Left)Ferritin (red) confocal immunofluorescence with DAPI (blue), showing CF, but no NF, accumulation in the ribbon of GBM. In disease (FSGS) CF accumulation was partially disrupted, and CF was present in the surrounding proximal tubule. (Right) TEM confirmed CF, but no NF, accumulation in the BM (arrows). In FSGS, CF was found in the podocyte cell bodies.

References (1) Gupta GP et al. Cell. 127(4), 2006. (2) Sherwood DR. Trends Cell Biol. 16(5), 2006. (3) Deen WM et al. Am J Physiol. 281(4); 2001. (4) Danon et al. J Ultrastr Res. 38(5), 1972. (5) Abrahamson et al. Anat Rec. 216(4), 1986. (6)Alcorn D. Pathology. 13(1), 1981. (7) Lesperance et al. J Orthop Res. 11(4), 1992.