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A role for spleen tyrosine kinase in renal fibrosis in the mouse obstructed kidney

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ABSTRACT

Aims: Spleen tyrosine kinase (Syk) is a non-receptor tyrosine kinase involved in the signalling pathways of the B cell receptor, Fcγ-receptor and some leukocyte integrins. However, Syk can also be expressed by some non-haematopoietic cell types, although whether Syk signalling in these cells contributes to the pathogenesis of kidney disease is unknown. To address this question, we examined the function of Syk in antibody-independent renal interstitial fibrosis in the unilateral ureteric obstruction (UUO) model.
Main methods: Groups of C57BL/6J mice were treated with a selective Syk inhibitor (CC0417, 30 mg/kg/bid), vehicle, or no treatment, from the time of surgery until being killed 7 days later.
Key findings: A substantial accumulation of interstitial Syk^+ cells was seen in the UUO kidney. Double staining identified Syk expression by infiltrating macrophages and by a subset of α-SMA^+ myofibroblasts. CC0417 treatment substantially reduced the Syk^+ cell population in conjunction with a reduction in both myofibroblast and macrophage accumulation. This was associated with a substantial reduction in collagen IV deposition and mRNA levels of pro-fibrotic (collagen I, collagen IV, fibronectin, α-SMA, TGF-β1 and PAI-1) and pro-inflammatory molecules (MCP-1, TNF-α and NOS2). CC0417 treatment reduced both PDGF-B mRNA levels and $Ki67^+$ proliferating interstitial cells in the UUO kidney. Furthermore, CC0417 inhibited PDGF-AB induced ERK activation and cell proliferation of cultured primary kidney fibroblasts.
Significance: This study has identified a pathologic role for Syk in renal interstitial fibrosis. Syk inhibitors may have therapeutic potential in chronic fibrotic kidney disease.

1. Introduction

Fibrosis is a common pathologic feature in chronic kidney disease. In particular, fibrosis of the tubulointerstitial compartment is a strong predictor of loss of renal function [1,2]. Inflammation is intimately associated with renal fibrosis and indeed fibrosis is often the response to tissue damage caused by inflammation [3]. While we have gained insight into a number of pathways that regulate the fibrotic response in kidney disease (e.g. TGF-β/Smad3, Wnt/β-catenin, p38 MAPK), there is still much to learn regarding how the inflammation/fibrosis responses are regulated.

Spleen tyrosine kinase (Syk) is a cytoplasmic kinase which is required for the signal transduction triggered by the activation of various immune recognition receptors [4,5]. The best characterized function of Syk is in signalling via the B cell receptor and Fc receptors [4,5]. Recent studies have identified Syk activation in neutrophils and macrophages in crescentic glomerulonephritis, although Syk activation is also evident in interstitial cells across a range of glomerulonephritides [6–8]. Experimental studies using kinase inhibitors and Syk gene deletion have identified a pathogenic role for Syk in macrophages and neutrophils in experimental crescentic glomerulonephritis [8–13].

The contribution of Syk to progressive kidney disease may not be limited to antibody-based activation of myeloid cells. Indeed, Syk facilitates signalling via receptors not involved in antibody responses, such as leukocyte integrins [4,5]. Furthermore, Syk can be expressed by some non-haematopoietic cell types including vascular smooth muscle cells, fibroblasts and mesangial cells [6,14–16], suggesting a potential role for Syk in renal fibrosis. However, we have known little of Syk function in any type of fibrosis. Therefore, we investigated whether Syk plays a functional role in renal interstitial fibrosis. We studied the unilateral ureteric obstruction (UUO) model since this features inflammation and fibrosis driven by irreversible mechanical stretch and does not involve antibody deposition, thereby allowing the first investigation of Syk in antibody-independent renal injury.

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2. Materials and methods

2.1. Reagents

The selective Syk inhibitor, CC0482417 (abbreviated as CC0417), was manufactured by Celgene (San Diego, CA, USA). Data on the selectivity of CC0417 has been published previously [17]. We used antibodies recognizing: p-ERK1/2 (Thr202/Tyr204); p-Akt (Ser473); p-p38 (Thr180/Tyr182); Syk for immunostaining (D3Z1E) (all from Cell Signaling, CA, USA); Syk for immunoblotting (C20, Santa Cruz Biotechnology, Dallas, TX, USA); unconjugated and Cy3-conjugated smooth muscle actin (α-SMA; Sigma-Aldrich, Castle Hill, NSW, Australia), collagen IV (Southern Biotechnology, Birmingham, AL, USA), macrophages (F4/80, Serotec, Oxford, UK; APC-conjugated CD68 antibody from Biolegend, San Diego, CA, USA), proliferating cells (Ki67, BD Biosciences, San Jose, CA, USA), and; tubulin (Abcam, Cambridge, UK). Secondary antibodies included: FITC-conjugated sheep anti-rabbit IgG (Serotec, Oxford, UK); HRP-conjugated sheep anti mouse IgG and HRP-conjugated mouse anti-peroxidase complexes (Dako Australia, North Sydney, NSW, Australia); biotinylated rabbit anti-goat IgG and sheep anti-rabbit IgG (Zymed-Invitrogen, Carlsbad, CA, USA), and; goat anti-rabbit Alexa fluor 680 and donkey anti mouse IRDye 800 (Molecular Probes, Eugene, OR, USA).

2.2. Animals

Forty male C57BL6/J mice (10–12 weeks old) were randomized into four groups (n = 10 per group). Mice had the left ureter obstructed through a surgical procedure as previously described [18]. Mice were given twice daily gavage with SYK inhibitor CC0417 (30 mg/kg), vehicle alone (10% 2-hydroxypropyl-beta-cyclodextrin), or no treatment, starting at 60 min before surgery continued until mice were killed 7 days later. The normal control group consisted of 10 male mice without experimentation. Animal experimentation was done in line with National Health and Medical Research Council of Australia Guidelines for Animal Experimentation and was approved by the Animal Ethics Committee of Monash Medical Centre.

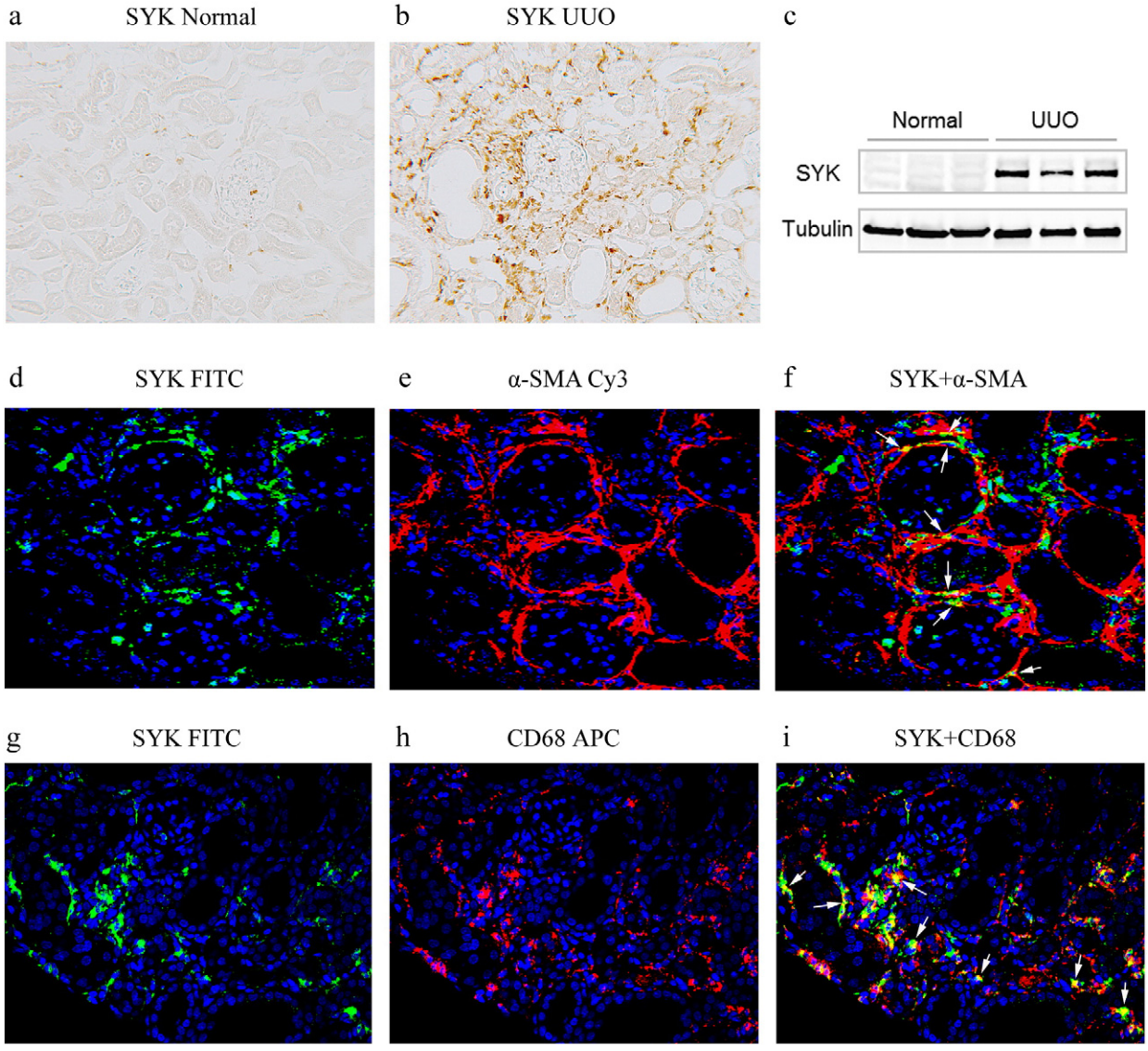

Fig. 1. Syk expression in the normal and UUO kidney. Immunoperoxidase staining for Syk in: (a) normal mouse kidney, and; (b) day 7 untreated UUO kidney. (c) Western blot of Syk (72 kDa) in normal mouse kidney and untreated day 7 UUO kidney which was reprobed for α-tubulin. (d–i) Two colour confocal microscopy of untreated day 7 UUO kidney with DAPI (blue) nuclear counterstain. (d) Syk (green), and (e) α-SMA+ myofibroblasts (red), with double stained cells indicated as yellow (arrows) in merged image (f). (g) Syk (green), and (h) CD68+ macrophages (red), with double stained cells indicated as yellow (arrows) in merged image (i).