Blocking class II histone deacetylase activity inhibits renal fibrosis

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INTRODUCTION and OBJECTIVES

■Renal fibrosis is the typical pathological outcome of many chronic kidney diseases. The process is characterized by excessive accumulation of extracellular matrix (ECM) components and the consequent destruction of normal tissue architecture.

■ Myofibroblasts are thought to be the prominant ECM-producing cells in renal fibrosis. The cells are derived through differentiation from local resident fibroblasts, bone marrow cells, epithelial-to mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndMT).

■Histone deacetylase (HDAC) remove acetyl groups from -N-acetyl lysine amino acids. They are classified into 4 main classes of enzymes: class I HDAC include HDAC1, 2, 3, and 8; class II HDAC include HDAC4, 5, 7, 9 (subclass IIa) and HDAC6, 10 (subclass IIb); class III HDAC are homologs of yeast Sir2 proteins; and the sole class IV HDAC is HDAC11.

■Inhibition of HDAC has strong anti-neoplastic effects through cytotoxic and proapoptotic mechanisms. There are also increasing data from nononcologic settings that HDAC inhibitors exhibit anti-inflammatory effects.

■Calss I HDAC enzymes are expressed in every cell, whereas class IIa HDAC enzymes have tissue-specific expression. Class I HDACs were mostly restricted to the nucleus, whereas class IIa HDAC enzymes shuttled between the nucleus and cytoplasm.

■Although HDAC has been reported to be involved in renal fibrosis, no previous studies have
implicated specific class of HDAC in the pathophysiology of renal fibrosis.

To investigate which class of HDAC is involved in pathogenic renal fibrosis and evaluate anti-fibrotic effect of the defined HDAC inhibitors.

METHODS

■EMT induction: Serum-starved HK2 cells were exposed to recombinant human TGF-β1 (10 ug/ml). Cell morphology was observed on light microscopy.

■Detection of collagen I and a-SMA expression: Total RNA was extracted from HK2 cells stimulated with TGF-β1 for 3 days and reverse transcribed into complementary DNA. Real-time PCR was performed using specific primer for collagen type I and α-SMA. The results are expressed as the mean ratio of collagen or α-SMA/GAPDH ± SD.

■ Measurement of HDAC activity: Cell extracts were prepared at 24 h after exposure and subjected to immune blotting with antibodies specific for the acetylated forms of histone H3 and α-tubulin.

■ Effect of SB939 on UUO model: The left ureter of C57BL/6 mice was exposed and permanently ligated twice with 4-0 nylon suture. The contralateral kidney was used as a control. Vehicle or SB939 were administrated i.p. at a dose of 50 mg/kg daily for 7 days (day 0-7). At day 7, both kidneys were obtained from the mice.

RESULTS

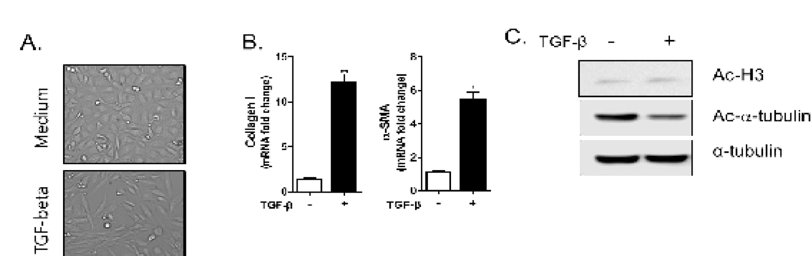

Figure 1. Enzyme activity of HDACs on TGF-β1-induced EMT. Serum-starved HK2 cells were exposed to recombinant human TGF-β1 (10 ug/ml). A) Morphologic examination. After 3 days, cells were observed using light microscopy. B) Expression of EMT markers. Total RNA was extracted from HK2 cells stimulated with TGF-β1 for 3 days and reverse transcribed into complementary DNA. Real-time PCR was performed using specific primer for collagen type I and α-SMA expression. The results are expressed as the mean ratio of collagen or α-SMA/GAPDH ± SD. C) Enzyme activity of HDACs. Cell extracts were prepared at 24 h after exposure and subjected to immune blotting with antibodies specific for the acetylated forms of histone H3 and α-tubulin.

[Fig.1A showed that TGF-β1 stimulated HK2 cells lost their typical epithelial cell morphology and became scattered and spindle-like, thus resembling mesenchymal cell morphology. TGF-β1 induced a significant increase in accumulation of collagen and α-SMA (Fig.1B). Class II HDACs are activated by TGF-β signal during EMT (Fig.1C).]

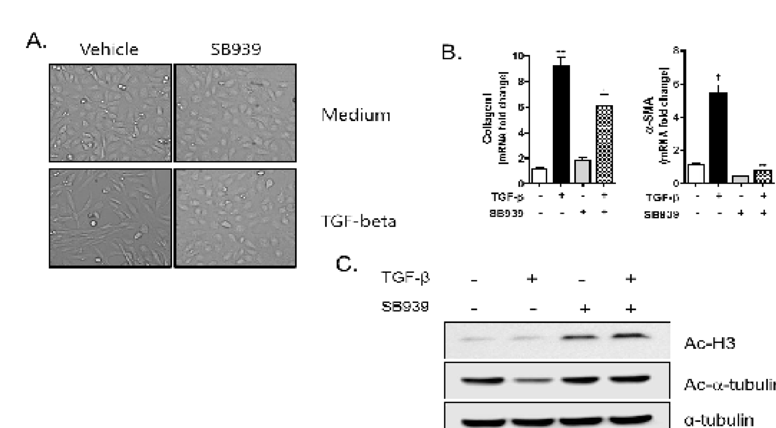

Figure 2. Effects of pan-inhibitor SB939 on TGF-β1-induced EMT. Serum-starved HK2 cells were exposed to recombinant human TGF-β1 and treated with SB939 (200 nM). Cell morphology (A), EMT markers (B) and HDAC activity (C) were analyzed as described in Fig. 1.

[These data indicate that pan HDAC inhibitor SB939 strongly suppressed TGF-β1-induced EMT through inhibiting Class II HDAC activation.]

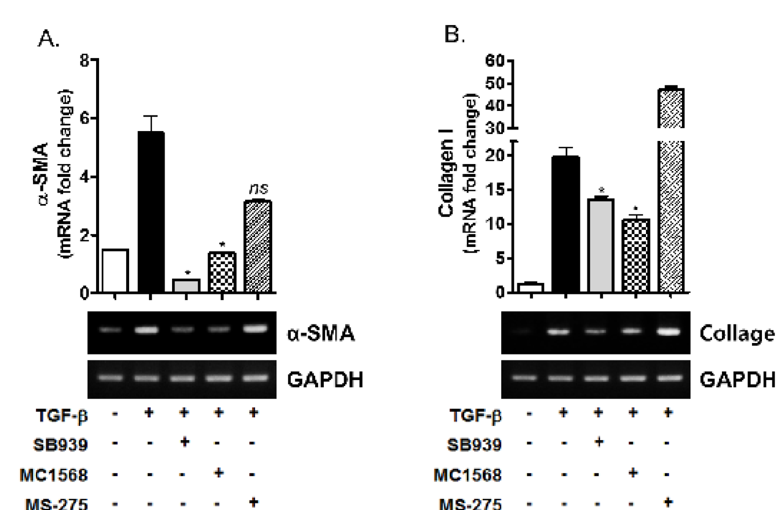

Figure 3. The role of class I and class II enzymes in TGF-β1-induced EMT. Serum-starved HK2 cells were exposed to recombinant human TGF-β1 and treated with pan hihibitor SB939 (200 nM), class II specific inhibitor MC1568 (300 nM), or class I specific inhibitor MS275 (1 μM). EMT markers were quantitated by real-time PCR analysis as described in Fig.1C.

[These data showed that class II-specific inhibitor MC1568 had the similar effects of SB939, but class I-specific inhibitor MS275 did not have the effects, suggesting that class II HDACs contribute TGF-β1-induced EMT.]

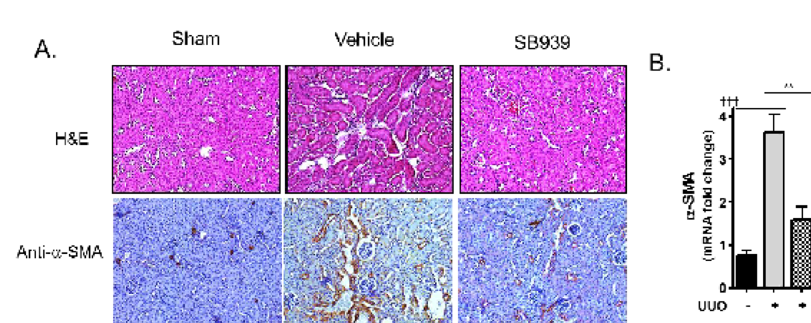

Figure 4. Effect of inhibiting HDACs on UUO model. The right ureter of C57BL/6 mice was exposed and permanently ligated twice with 4-0 nylon suture. Vehicle or SB939 were administrated i.p. at a dose of 50 mg/kg daily for 7 days (day 0-7). At day 7, both kidneys were obtained from the mice. A. Histological examination. Hematoxylin and eosin (H&E) staining (upper), and immunohistochemical staining for α-SMA expression (lower). B. Expression of fibrotic markers. Total RNA was extracted from kidneys of vehicle or SB939 treated mice. EMT markers were quantitated by real-time PCR analysis as described in Fig.1C.

[H&E results in vehicle kidney showed that morphologic changes manifested by tubular atrophy, tubular dilatation, and expanded interstitial space filled with numerous cells. Thus, α-SMA and collagen I were significantly accumulated. SB939 treatment was markedly inhibited deposit of α-SMA as well as histological fibrosis.]

SUMMARY & CONCLUSION

■Class II HDAC activity is induced in HK2 cells by TGF-beta during EMT.

■Inhibition of class II HDAC suppress TGF-beta induced EMT.

■Administration of SB939 suppress renal fibrosis on UUO model.

■Our results demonstrate that class II HDACs contribute to renal fibrosis and
suggest that class II-selective inhibitors have a therapeutic potential for
the treatment of renal fibrosis.

1. The poster number: MP003
2. The topic: Blocking class II histone deacetylase activity inhibits renal fibrosis
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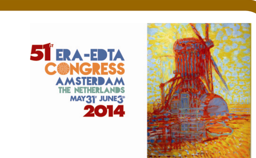
DOI: 10.3252/pso.eu.51era.2014



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