Diabetic Complications Consortium

Application Title: A potential role of CDA1 in regulating inflammatory pathway in diabetic complications

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1. Project Accomplishments:

The theme of this project is to explore a putative role of CDA1 in inhibiting SIRT6, a histone deacetylase, and their roles in diabetic kidney disease. The hypothesis is that CDA1 sequesters SIRT6 via direct protein-protein interaction, leading to reduced SIRT6 activity and enhanced expression of target genes related to renal fibrosis. Indeed, SIRT6 proteins levels were found to be higher in CDA1 KO mouse kidney and other tissues, suggesting that CDA1 is able to reduce SIRT6 protein levels. Co-transfection of CDA1 and SIRT6 in HEK293 cells demonstrated that CDA1 overexpression significantly reduced the transfected expression level of SIRT6 by WB. Co-transfection with increasing amount of CDA1 and constant amount of SIRT6 showed that CDA1 reduced co-transfected SIRT6 protein levels in a dose-dependent manner. This effect of CDA1 was demonstrated in an experiment using adenovirus delivered overexpression of CDA1, which reduced the protein levels of endogenous SIRT6 in human proximal tubule cells, HK-2. We are currently investigating whether this effect of CDA1 on SIRT6 requires direct protein-protein interaction and whether this effect is via increasing protein degradation of SIRT6. Preliminary data show that CDA1 deficiency in CDA1 KO mice appears to be associated with increased protein level of SIRT6, reduced level of H3 acetylation (H3K9Ac) and gene expression of target genes such as MCP1 and collagens I and III. Our data also showed a clear effect of diabetes on the overall H3K9Ac levels in kidney tissues from 20-week diabetic WT mice. These data support our hypothesis and CDA1 deficiency increases SIRT6 activity, leading to inhibition of expression of target genes via deacetylation of the histone H3.

2. Specific Aims:

Aim 1: To investigate TGF-β induced co-expression of SIRT6 and CDA1

In a preliminary experiment, TGF-β treatment (1ng/mL) in a time course showed that CDA and SIRT6 mRNA levels were increased and reached a peak level at 30 min. CDA1 mRNA levels remained at least until 6hrs, whereas SIRT6 levels quickly dropped to control level as seen at 1 hr after treatment (Fig 1). This result supports the notion that both CDA1 and SIRT6 are responding to TGF-β treatment and increasing CDA1 appears to be associated with reduction of SIRT6 expression.

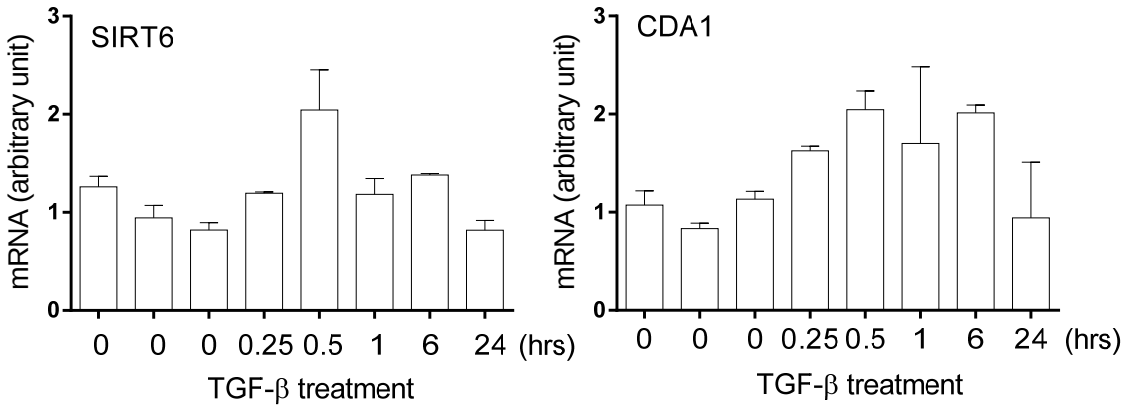

Figure 1: TGF-β treatment induces co-expression of SIRT6 and CDA1 in 3T3 cells. 3T3 cells were treated with TGF-β at 1ng/mL for specified time and mRNA levels of SIRT6 and CDA1 were measured by real-time RT-PCR.

Aim 2: To determine whether CDA1 inhibits the SIRT6 mediated histone H3 deacetylation in target genes of NFκB and/or TGF-β

We carried out experiment to investigate the relationship between CDA1 and SIRT6. Initially, we found that SIRT6 protein levels in mouse kidney was inversely related to CDA1. As shown in Fig 2A, in both kidney and testes tissues, protein levels of SIRT6 were higher in CDA1 KO mice than WT. Furthermore, in WT mice, SIRT6 protein was detected in kidney where CDA1 was moderately expressed, but SIRT6 was undetectable in testes where CDA1

was highly expressed (Fig 2A). This result suggests that CDA1 potentially reduces the protein levels of SIRT6.

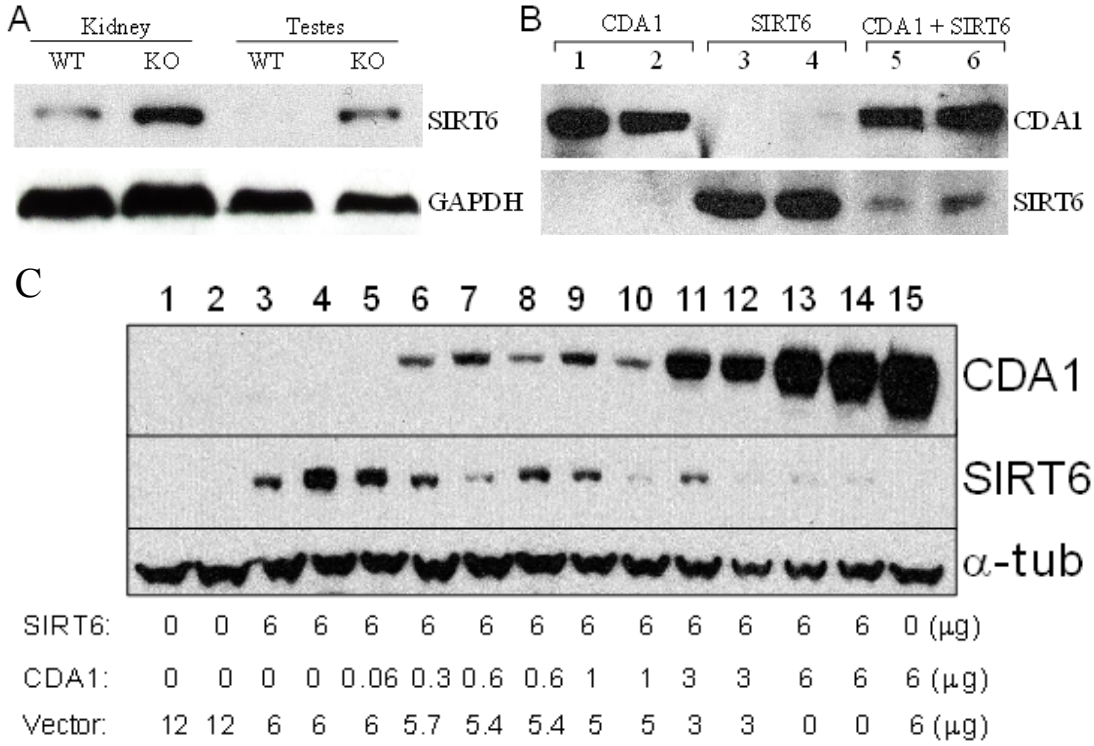

Figure 2: CDA1 reduces SIRT6 protein levels. **A:** WB shows SIRT6 in kidney and testes tissues from CDA1 WT and KO mice. **B:** WB shows CDA1 and SIRT6 in HEK293 cells transfected with CDA1 (lanes 1-2), SIRT6 (lanes 3-4) as well as co-transfected with CDA1and SIRT6 (lanes 5-6). **C:** WB shows CDA1, SIRT6 and α-tubulin in HEK293 cells transfected with 12 μg DNA containing SIRT6, CDA1 and Vector DNA in specified amount.

This putative effect of CDA1 in reducing SIRT6 protein level in vivo was further examined by an in vitro study where human embryonic kidney cells (HEK293) were transfected with CDA1, SIRT6 and co-transfected with both CDA1 and SIRT6. The WB (Fig 2B) showed that co-transfected CDA1 (lanes 5-6) was overexpressed to a similar level in single transfection of CDA1 (lanes 1-2). As a result of CDA1 overexpression, SIRT6 (Fig 2B, lower panel) in the co-transfected cells (lanes 5-6) were significantly reduced, when compared to the single transfection of SIRT6 (lanes 3-4). In a similar experiment, we co-transfected cells with constant amount of SIRT6 (6μg) and increasing amount of CDA1 (0, 0.06, 0.3, 0.6, 1, 3 and 6 μg). As shown in Fig 2C, CDA1 reduced the protein levels of co-transfected SIRT6 in a dose-dependent manner. These

results demonstrated the ability of CDA1 to reduce SIRT protein levels, likely by promoting SIRT6 degradation, being consistent with the inhibitory effect of CDA1 on SIRT6.

Effect of CDA1 on endogenous SIRT6 protein was examined in human proximal tubule cells, HK-2. HK-2 cells were infected with control (Ad-Go) and CDA1 expressing adenovirus (Ad-CDA1) at 25, 100, 250 and 1000 MOI to overexpress CDA1 at increasing levels. As shown in Fig 3, endogenous SIRT6 protein levels appeared to be lower in CDA1 overexpressing cells. As a result of reduced SIRT6 levels, histone H3 acetylation (H3K9Ac) levels were increased (Fig 3)

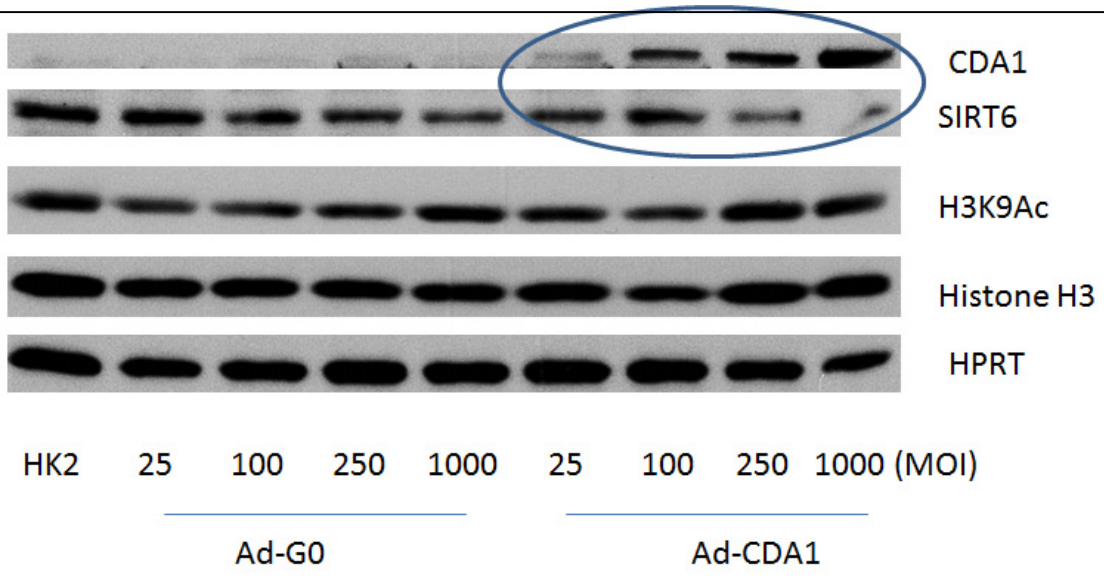

Figure 3: Adenovirus delivered CDA1 overexpression reduced SIRT6 expression level resulting in increased acetylation of histone H3 in HK-2 cells. HK-2 cells were infected with control Ad-Go and CDA1 expression Ad-CDA1 adenoviral constructs overnight. WB shows proteins of CDA1, SIRT6, acetylated histone H3 (H3K9Ac), histone H3 and a loading control protein, HPRT.

Aim 3: To determine histone H3 acetylation status in MCP-1, VCAM-1, CTGF and collagen I, III genes in kidney from control and diabetic WT and CDA1 KO mice

We have induced diabetes in WT and CDA1 KO mice and have collected kidney tissues after 10 and 20 weeks of diabetes from diabetic and age-matched non-diabetic mice as planned. We have assessed the mRNA levels of target genes in these samples, showing that diabetes increased expression of these

genes in WT mice. This diabetes associated effects on these target genes were attenuated in diabetic CDA1 KO mice. WB was carried out to assess the proteins such as histone H3 acetylation in these tissues. Our preliminary data showed that there is a significant reduction of H3K9Ac levels in 20-week diabetic WT mice when compared to the non-diabetic controls, showing an effect of diabetes on overall H3K9Ac (Fig 4). Whether diabetes has an effect on H3K9Ac on the specific target genes of TGF-β and NFκB is being investigated. Furthermore, H3K9Ac levels were significantly lower in CDA1 KO mice (Fig 4), which is consistent with the notion that CDA1 deficiency increased SIRT6 expression levels/activities, leading to deacetylation of H3 histone.

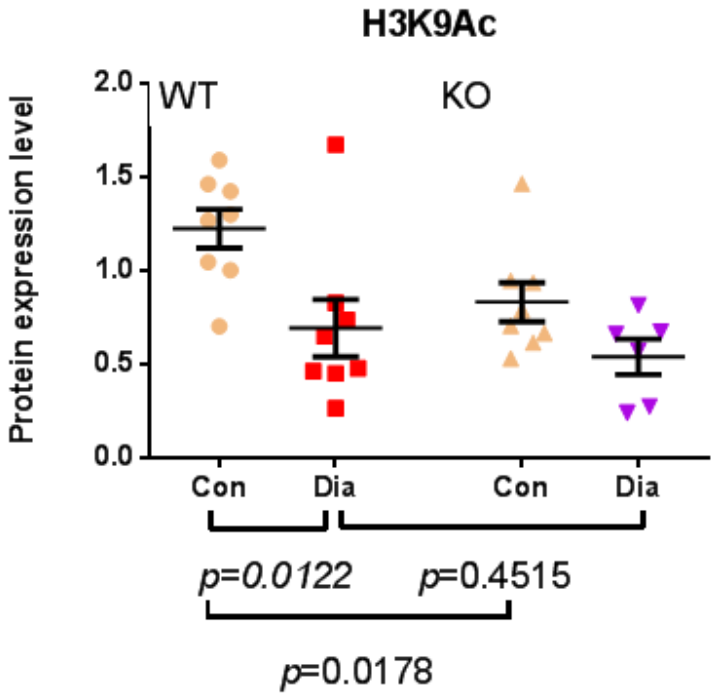

Figure 4: Mouse kidney homogenates from age-matched non-diabetic and 20-week diabetic WT and CDA1 KO mice were examined for acetylated histone H3 (H3K9Ac) by WB. The expression levels of H3K9Ac were normalized against internal GAPDH.

3. Publications:

No publication has been produced yet.