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Poly(ADP-ribose) polymerase-1 (PARP-1) regulates fibroblast activation in systemic sclerosis

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Yun Zhang, Sebastian Pötter, Chih-Wei Chen, Ruifang Liang, Kolja Gelse, Ingo Ludolph, Raymund E Horch, Oliver Distler, Georg Schett, Jörg H W Distler, Clara Dees. Ann Rheum Dis. 2018 May;77(5):744-751.

The enzyme poly(ADP-ribose) polymerase-1 also called PARP-1, is a member of the PARP family, a group of 18 enzymes that transfer ADP-ribose groups onto various substrate proteins. This mechanism called PARylation, exerts profound regulatory effects on many physiological and pathological processes. Among PARPs, PARP-1 is by far the most well characterized and it has been recently shown that PARP-1 could PARylate Smad proteins. As Smad proteins take part in TGFβ signaling, a key pathway involved in the pathogenesis of fibrosis is systemic sclerosis (SSc), modulation of PARP-1 and PARylation may also have regulatory effects on fibrosis. In this article, the authors have explored the links between PARP-1 and TGFβ signaling and the impact of a modulation of PARP-1-dependent PARylation on fibrosis both in vitro and in vivo.

The authors firstly demonstrated that the expression of PARP-1 was decreased in the skin fibroblasts and keratinocytes of SSc patients in comparison with healthy donors. PARP-1 was also decreased in the skin of TSk-1 mice when compared to controls. Then, they demonstrated that TGFβ could reduce the expression of PARP-1 in healthy fibroblasts in vitro, via a Smad-dependent canonical signaling. Using MeDIP assays, the authors demonstrated that this down-regulation of PARP-1 by TGFβ was induced by an hyper-methylation of PARP-1 promoter.

Using a specific PARP-1 inhibitor (3AB), the authors showed that TGFβ signaling was up-regulated after PARP-1 silencing, resulting in a higher stimulatory effect of TGFβ on myofibroblast differention, attested by an increased expression of ACTA2/aSMA and stress fibre formation in fibroblasts after co-treatment by 3AB and TGFβ when compared to fibroblasts treated by TGFβ alone. The authors secondly explored by which mechanisms PARP-1-dependent PARylation could modulate TGFβ signaling.

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They showed that PARP-1 was involved in a negative feedback of the TGFβ pathway. Indeed, although TGFβ down-regulated PARP-1 expression, TGFβ also promoted the direct binding of PARP-1 to Smad3, resulting in an increased PARylation of Smad3 with consecutive decreased expression of TGFβ/Smad target genes such as PAI-1, Smad7 and CTGF. The inhibition of PARP-1-dependent PARylation by 3AB limited this negative feedback, with restored expression of TGFβ/Smad target genes in TGFβ-stimulated fibroblasts.

In vivo, Parp-1-deficient mice developed more severe skin fibrosis after injection of bleomycin as compared with wild type littermates. The same pro-fibrotic effects were observed in wild type mice challenged by bleomycin after pharmacological inhibition of Parp-1 by two structurally non-related inhibitors, 3AB and PJ34. The inhibition of Parp-1 by 3AB also resulted in a more severe skin fibrosis in the inflammatory topoisomerase-induced SSc mouse model and in the Tsk-1 model, a model characterized by a fibrosis less dependent on inflammation.

Altogether, these results demonstrate that PARP-1 dependent PARylation of Smad3 is a key regulator of TGFβ signaling and fibrosis: the down-regulation of PARP-1 secondary to chronic TGFβ-dependent epigenetic mechanisms in SSc, may result in a decrease of its negative-feedback on TGFβ signaling with subsequent up-regulation of the canonical TGFβ pathway, increased fibroblast activation and more severe experimental skin fibrosis.

Molecular Basis for Dysregulated Activation of NKX2-5 in the Vascular Remodeling of Systemic Sclerosis

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NKX2-5 is a homeobox transcription factor involved in the embryonal development of the heart and vasculature and which expression disappears during the post-natal period.

The authors have previously shown this factor to be aberrantly expressed by pathological vascular smooth muscle cells (VSMCs) in atherosclerotic lesions. Thus, the authors made the hypothesis that NKX2-5 could be involved in the vascular remodeling of systemic sclerosis (SSc). Hence, they assessed the expression of NKX2-5 in pulmonary artery smooth muscle cells (PASMCs) of SSc patients. Next, they studied whether the single nucleotid polymorphisms (SNPs) of the NKX2-5 locus were associated with SSc. Finally, they studied the mechanisms by which these SNPs might influence the regulation of NKX2-5 gene in SSc in silico and in vitro.

The authors showed that NKX2-5 is expressed at both transcript and protein level in PASMCs of SSc patients with pulmonary hypertension but not in healthy controls. The meta-analysis of 2 SSc Caucasian cohorts from United Kingdom (n=1334) and Spain (n=1736) showed that rs3131917 was associated with SSc. The subgroup analysis revealed that this rs3131917 and rs3132139 SNPs were strongly associated with pulmonary hypertension and rs3095870 with pulmonary fibrosis in Spanish SSc patients.

Hence, the authors assessed whether these SNPs had a functional translation. The in silico analysis revealed that these SNPs were located in potential regulatory regions. The in vitro cloning of these SNPs and their study in luciferase reporter gene assays in PASMCs demonstrated that the three SNPs directly increased NKX2-5 transcription.

The location of rs3132139 and rs3131917 in a downstream activeenhancer region accounted for *NKX2-5* overexpression. In addition, the chromatin immunoprecipitation assays showed

that this downstream enhancer genomic locus displayed an increased binding of the transcription factors myocyte-specific enhancer factor 2C (MEF-2C), GATA-6 and c-Jun.

Rs3095870 was located in an upstream promoter region of NKX2-5 and was associated with an upregulation of NKX2-5 transcription. This regulation involved the transcription-enhancer factor domain 1 (TEAD1) binding as evidenced by DNA Protein- binding assays and chromatin immunoprecipitation assays.

Altogether the authors demonstrated the overexpression of NKX2-5 in SSc PASMCs. They identified SNPs located in the regulatory regions of NKX2-5 that are associated with SSc, pulmonary hypertension and fibrosis. They provided functional evidences on the role of these SNPs in NKX2-5 upregulation in human PASMcs. This mechanism could participate in the vascular remodeling observed in SSc.

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Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis

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The authors demonstrate that platelet-derived microparticles contribute to vasculopathy in SSc. In the past, they already demonstrated the presence of a high number of platelet-derived microparticles expressing HMGB1 in blood from patients with SSc, and that HMGB1 expressed on platelet microparticles is biologically active.

In this manuscript, they co-cultured the microparticles with neutrophils and showed that they activated fibrinolytic activity and autophagic flux. NET (neutrophil extracellular traps) formation was also greatest in neutrophils stimulated by the SSc platelet-derived microparticles.

In the same line, neutrophils from SSc patients were more autophagic than neutrophils from healthy donors.

In a murine part, platelet-derived microparticles were injected into immunodeficient mice recapitulating some of the SSc characteristics. Endothelial damages above all but also neutrophils flux to the lungs leading to pulmonary fibrosis. Their conclusion is that blocking the production of platelet-derived microparticles, or the effect of the HMGB1 they carry, could be a therapeutic approach to limit disease progression, or even reduce the clinical manifestations of SSc. They succeeded in neutralizing the effect of the microparticles by pre-treating them in vitro with a competitive antagonist of HMGB1 called BoxA. Altogether these results implicate neutrophils in SSc vasculopathy through the role of platelet-derived microparticle-associated HMGB1.

Methyl-CpG-binding protein 2 mediates antifibrotic effects in scleroderma fibroblasts

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The authors demonstrated a novel role for MeCP2 in skin fibrosis and identified MeCP2-regulated genes associated with fibroblast migration, myofibroblast differentiation and extracellular matrix degradation, which can be potentially targeted for therapy in SSc.

To test the hypothesis that MeCP2 is involved in fibrosis in SSc by regulating fibrotic genes and altering fibroblast functions, they examined the expression of MeCP2 in fibroblasts isolated from healthy controls or patients with dcSSc, and then probed the effects of MeCP2 on myofibroblast differentiation, fibroblast proliferation and migration. Unbiased RNA-seq and sequencing (ChIP-seq) were used to identify genome-wide transcriptional targets of MeCP2, followed by bioinformatics analyses and functional validations of identified targets.
They demonstrated that MeCP2 was significantly elevated in dsSSc fibroblast compared with normal fibroblasts.
MeCP2 attenuates pro-fibrotic responses, and that MeCP2 overexpression alters biological functions important in fibrosis, through antifibrotic MeCP2 target genes.
Therefore, the results suggest that increased MeCP2 in dcSSc fibroblasts might be a defense mechanism to counteract the pro-fibrotic nature of the disease in the early stages of dcSSc. Genes like COL1A1, α-SMA and PPAR-γ were responsive to MeCP2 regulation in normal fibroblasts, but they might also be co-regulated by other pro-fibrotic factors (eg, TGF-β), which exert prominent influence on dcSSc fibroblasts to maintain the 'SSc phenotype' compared with normal fibroblasts. For exemple COL1A1 expression was attenuated with MeCP2 overexpression in normal fibroblasts but not in dcSSc fibroblasts, indicating that the effects of MeCP2 on COL1A1 might be neutralized by other pro-fibrotic regulators amplified in dcSSc fibroblasts. By coupling RNA-seq with functional assays, not only were fibrotic genes like COL1A1, α-SMA and PPAR-γ confirmed as MeCP2-regulated genes, but also novel targets like plasminogen activator urokinase (PLAU), nidogen-2 (NID2) and adenosine deaminase (ADA) were identified as potential mediators in the antifibrotic effects of MeCP2. In summary, these results collectively imply that MeCP2 overexpression acts as protective mechanism against skin fibrosis in early dcSSc and that exploiting this mechanism might provide new avenues for therapeutic intervention in this disease.
Several canonical and novel fibrotic genes regulated by MeCP2 were identified and functionally characterised. Drugs or compounds modulating MeCP2 expression or targeting these MeCP2-regulated genes might provide attractive new strategies to prevent the progression of fibrosis in scleroderma.