NADPH oxidase (NOX) is a multi-protein complex producing reactive oxygen species (ROS) in response to growth factors. Among the seven members of the NOX family there are key differences in their activation, subunit composition, localization and expression. NOX overactivity or overexpression are often associated with chronic diseases, characterized by tissue damage and fibrosis. Such changes are consistent with the involvement of ROS in causing damage to biomolecules including protein, DNA, and lipid membranes. Scleroderma (SSc) is a chronic fibrotic disease which can affect skin and internal organs, resulting in significant morbidity. SSc fibroblasts isolated from lesional areas of patients overproduce ROS and overexpress type I collagen and α-smooth muscle actin (α-SMA), and show chromosomal aberrations. Although the evidence that oxidative stress contribute to the establishment of fibrosis, the role of the single NADPH oxidase members has not been previously investigated in scleroderma.

In this study we focused on the role of NOX enzymes in SSc fibroblast activation and on the possible link between ROS induced-DNA damage and fibrosis. Scleroderma skin fibroblasts show enhanced expression of NOX 2 and 4 mRNA and protein compared to normal fibroblasts. Incubation with DPI or transfection with siRNAs against specific NOX mRNA downregulate cell activation, DNA damage, and type I collagen expression in scleroderma cells. SSc fibroblasts show high levels of phosphorylated ATM (ataxia telangiectasia mutated, the major regulator kinase of the cellular response to DNA damage) compared to normal cells. Incubation with KU55933, a specific inhibitor of ATM, leads to downregulation of ROS in SSc fibroblasts as well as in normal cells stimulated by bleomycin, an inducing agent of fibrosis. Moreover inhibition of ATM causes a significant reduction of type I collagen expression, as confirmed in ATM-/- cells.

In the present study, we identified NOX 2 and 4 as critical components of NADPH oxidase complex in SSc fibroblasts and we provide evidence that ROS produced by NOX may play a important role in the pathological activation of dermal fibroblasts. These data strongly demonstrate the pathological link among ROS, DNA damage and collagen production, and suggest new targeting strategies in the treatment of fibrotic diseases, such as Scleroderma.

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Fig. 1: ROS production. SSc fibroblasts were transfected with a control siRNA (CTRL) or siRNA against NADPH oxidase subunits (NOX2 or NOX4). In the upper panel, cells were incubated with DHE and the fluorescence were analyzed using a confocal microscopy. In the lower panel, after treatment with DCF ROS production was measured using a microplate reader.

Fig. 2: Activation state. SSc fibroblasts were transfected with a control siRNA (CTRL) or siRNA against NADPH oxidase subunits (NOX2 or NOX4). In the upper panel, a-SMA expression was detected using a confocal microscopy. In the lower panel, collagen and fibronectin mRNA levels were measured in real-time PCR.

Fig. 3: DNA damage. SSc fibroblasts were transfected with a control siRNA (CTRL) or siRNA against NADPH oxidase subunits (NOX2 or NOX4) or treated with DPI. In the upper panel, phosphorylation of H2A.X was analyzed using a confocal microscopy. In the lower panel, the same marker was detected in western blot.