Discoidin Domain Receptor 1 phosphorylates the adaptor protein p66Shc promoting mitochondrial oxidative stress

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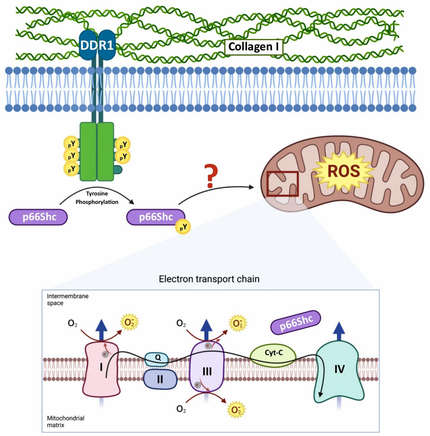

Highlights

- Describe a new molecular mechanism by which DDR1 regulates p66Shc-mediated mitochondrial ROS production.
- Advancing the understanding of how DDR1 contributes to tissue damage.

Background

Oxidative stress, characterized by an overproduction in reactive oxygen species (ROS), is a crucial factor in the pathogenesis of kidney fibrosis. The adaptor protein p66Shc is well-recognized for its role in promoting mitochondrial reactive oxygen species (mtROS) production[1]. Previous work has shown that p66Shc interacts with the Discoidin Domain Receptor 1 (DDR1)[2], a collagen-activated receptor tyrosine kinase that is upregulated in kidney injury and contributes to kidney fibrosis[3]. Upon collagen stimulation, DDR1 undergoes autophosphorylation on tyrosine residues and activates downstream, signaling pathways[4]. However, whether DDR1 phosphorylates p66Shc to promote p66Shc-induced mtROS production is yet to be investigated.

Here, we show that, upon collagen stimulation, DDR1-expressing human embryonic kidney cells that co-express p66Shc produce more mtROS than cells expressing a mutant form of p66Shc in which the tyrosine residues located on the collagen homology 1 domain (CH1) were mutated (Y239F, Y240F, and Y317F). Thus, the DDR1/p66Shc interaction represents a new molecular mechanism by which DDR1 regulates mtROS production, contributing to kidney damage and the progression of kidney fibrosis.

p66Shc structure

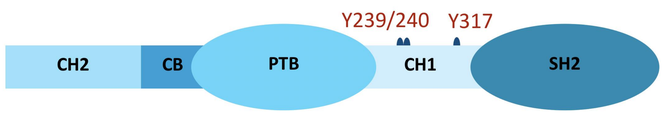

Figure 1. Diagrammatic representation of the adaptor protein p66Shc. The collagen homology domain 1 (CH1) contains three tyrosine residues (Tyr239, Tyr240, and Tyr317) important for cellular signaling.

DDR1 phosphorylates p66Shc

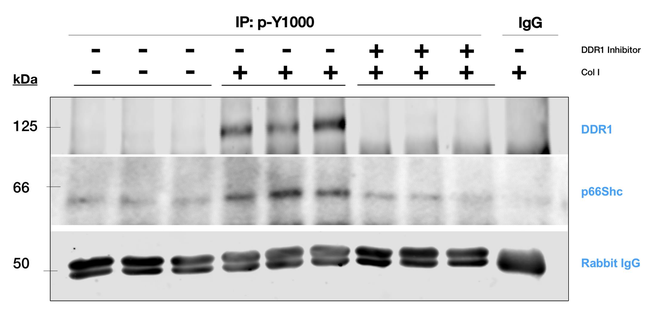

Figure 2. Western blot analysis of immunoprecipitated tyrosine phosphorylated proteins revealed that activated-DDR1 is able to phosphorylate tyrosine residue(s) on p66Shc.

Hypothesis

Does collagen activated DDR1 phosphorylate p66Shc to promote mitochondrial ROS production?

Experimental approach

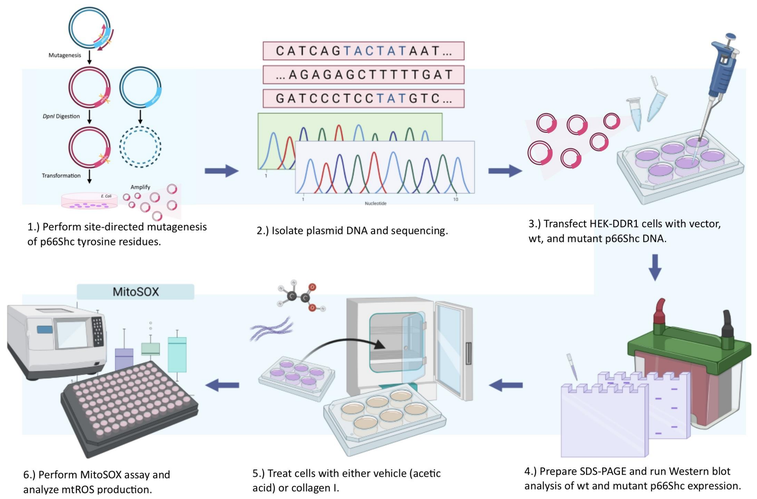

Figure 3. *Experimental Approach.* In this study, we mutated three tyrosine residues on p66Shc that are important for its signaling function (1) and sequenced for the targeted mutations (2). We then transfected human embryonic kidney cells that express DDR1 with our triple mutant p66Shc (3). The protein expression was analyzed by Western blot (4). The cells were treated with either vehicle or collagen I (5), and ROS production was measured for each condition (6).

Wild-type and mutant p66Shc expression

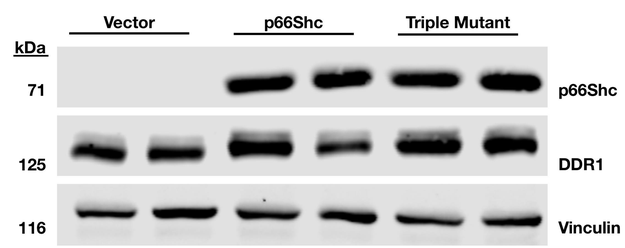

Figure 4. *Wild-type and mutant p66Shc expression.* A western blot analysis of p66Shc expression shows the presence of wt p66Shc and triple mutant p66Shc (top). DDR1 expressed in all samples (middle). Vinculin served as a loading control (bottom).

ROS production is reduced in p66Shc mutant

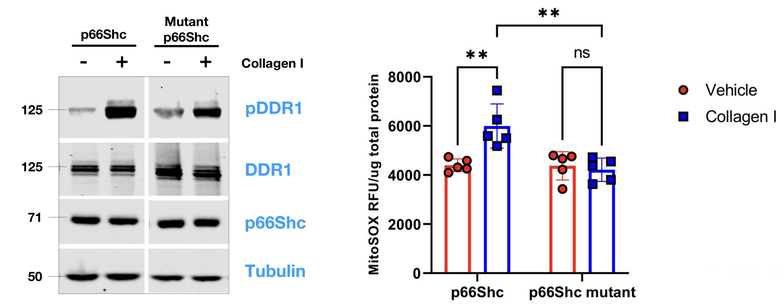

Figure 5. *ROS production is reduced in p66Shc mutant.* Transfected HEK-DDR1 cells were stimulated with vehicle or collagen I for one hour and treated with MitoSOX reagent to measure mitochondrial superoxide (mtROS) production. DDR1 activation and p66Shc expression were analyzed by Western blot. The results indicate that, after collagen stimulation, mtROS production is significantly decreased in the p66Shc mutant compared to the wild-type. The graph represents the mean ± SD of five independent experiments, of which values were normalized to the total protein amount, ns = not significant, **p-value < 0.005.

ROS production in HEK-DDR1 cells

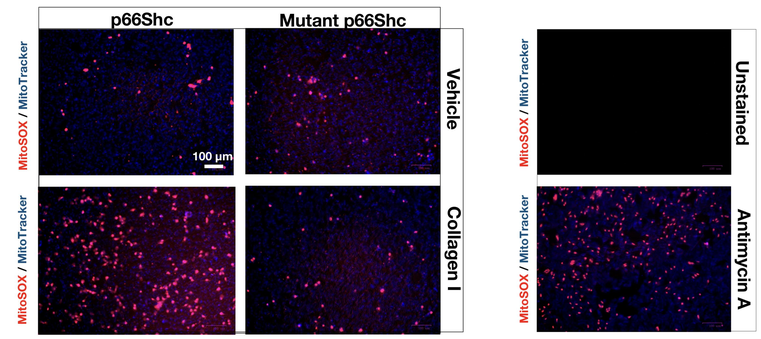

Figure 6. *ROS production in HEK-DDR1 cells.* Staining with MitoSOX Red Reagent and MitoTRACKER visually shows the presence of superoxide production (red) and mitochondria (blue) in HEK cells. Wt p66Shc cells subjected to collagen I treatment have visibly more mtROS than vehicle treatment. The triple mutant p66Shc shows that cells had similar levels of mtROS production upon vehicle and collagen I treatment.

Conclusion

- Collagen activated-DDR1 contributes to mitochondrial ROS production via phosphorylation of p66Shc tyrosine residues, indicating a novel molecular mechanism by which DDR1 promotes oxidative stress in the mitochondria.
- The significance of the DDR1/p66Shc interaction reveals a newly discovered role of DDR1 in mitochondrial oxidative stress, a pathogenic factor in the development of kidney damage.
- Future directions for research: the inhibition of DDR1 can be viewed as a promising therapeutic strategy to prevent mtROS overproduction and to ameliorate kidney injury.

References

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