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A MOUSE MODEL OF CONDITIONAL SMOOTH MUSCLE-SPECIFIC DELETION OF THE MANGANESE SUPEROXIDE DISMUTASE GENE ALLOWS EXAMINATION ROLE OF OXIDATIVE STRESS IN DIABETIC BLADDER DYSFUNCTION

Hypothesis / aims of study

Evidence strongly suggests a key role for exaggerated Oxidative Stress (OS) in decompensated phase of diabetic bladder dysfunction (DBD). We aimed to generate a smooth muscle-specific manganese superoxide dismutase (MnSOD) knockout mouse to examine the role of OS in DBD.

Study design, materials and methods

We crossed the floxed MnSOD ($MnSOD^{lox/lox}$) mouse with mouse containing a heterozygous knock-in of the $CreER^{T2}$ gene in the SM22α promoter locus ($SM\text{-}CreER^{T2}(ki)^{Cre/+}$), which is transgenic mouse lines expressing a tamoxifen-activated Cre recombinase. Both MnSOD alleles modified to contain loxP sites bounding exon 3, using a modified Cre recombinase estrogen receptor fusion protein, $CreER^{T2}$, to catalyze the knockout. SM22α is a calponin related protein that is expressed specifically in smooth muscle. $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ activated by a lower amount of 4-hydroxytamoxifen (OHT). Mature offsprings (8 weeks after birth) were injected with OHT at 40 mg/kg for 5 consecutive days. Three days after the final injection, 31 male mice were sacrificed, and tissues of detrusor of the bladder, urothelium, aorta, heart, liver, skeletal muscle and skin of the tail were examined for MnSOD exon 3 by polymerase chain reaction (PCR).

Results

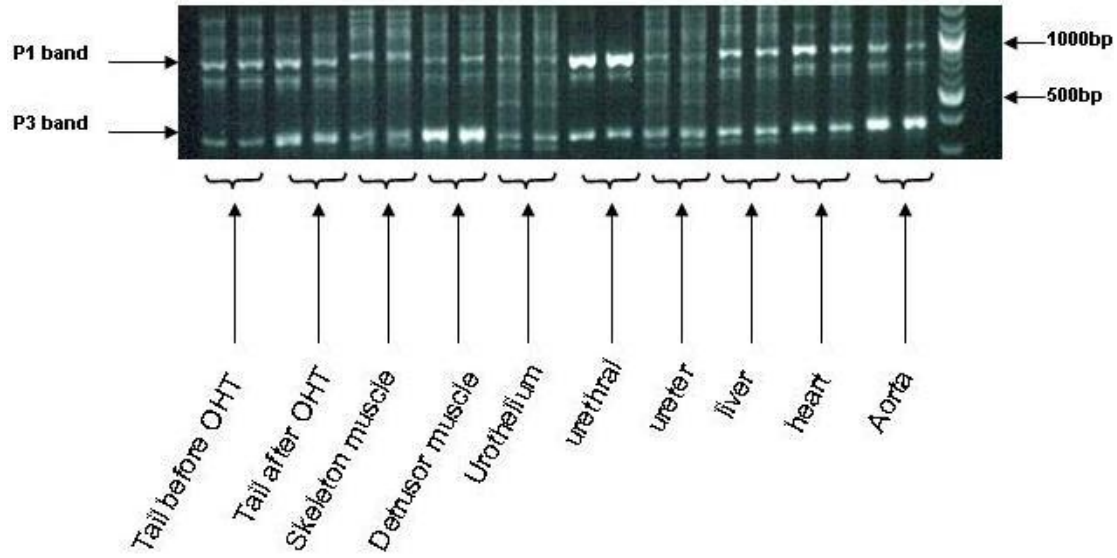

Fig 1. PCR result of MnSODlox/+,SM-CreERT2Cre/+ treated mice with OHT.
P1 band: without MnSOD exon 3 excision
P3 band: MnSOD exon 3 was excised

Interpretation of results

The phenotypical characterization of the created $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ mouse shows normal growth, and function with no gross abnormalities. Three days after OHT injection, the PCR of the harvested tissues shows deletion of MnSOD exon 3 in the bladder smooth muscle and aorta of the $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ mouse. The MnSOD exon 3 was present in heart, liver, skeleton muscle, urothelium, and tail of the mouse, suggesting a conditional and smooth muscle specific deletion of the MnSOD exon 3 in the created mice.

Concluding message

We have successfully deleted MnSOD exon 3 in the detrusor smooth muscle bladder of a $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ mouse in a time selective manner by activation of the Cre recombinase system. Upon induction of diabetes in these mice, we will be able to examine the mechanistic role of OS in remodeling of the bladder in a time specific manner according to the temporal alteration of the DBD previously described by us and other investigators.

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Is this a clinical trial?	**No**

What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**SUNY Upstate Medical University Committee for the Humane Use of Animals**