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Promoting Sustainable Kidney Health: The Asia-Pacific and Beyond

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The Function And Regulatory Mechanism of NOX4 in Rhabdomyolysis-induced Acute Kidney Injury

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Objectives : Emerging evidence has revealed that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) is implicated in the pathological processes of various kidney diseases, while its role in rhabdomyolysis-induced acute kidney injury (RIAKI) remains unknown.
Methods : In this study, we applied renal tubular epithelial cell (RTEC)-specific NOX4 knockout ($NOX4^{tecKO}$) and the NOX4 inhibitor GKT137831 to treat RIAKI in vivo and in vitro. In vivo, RIAKI mice was induced via intramuscular injection of 50% glycerol in hind thighs (8 ml/kg). In vitro, TCMK-1 (mouse kidney tubular epithelium cell line) cells were stimulated by 200 μM myoglobin. Serum and supernatant biochemical, inflammatory, and apoptotic parameters were measured and compared among groups. The activation of reactive oxygen species (ROS) and endoplasmic reticulum stress (ERS) signaling was also evaluated.
Results : RTEC-specific NOX4 deficiency and GKT137831 treatment both protected against glycerin-induced renal function and pathology in RIAKI mice. Moreover, NOX4 inhibition reduced inflammation, decreased apoptosis of RTECs, and downregulated cleaved-Caspase3 expression. Surprisingly, similar results were obtained with GKT137831 treatment in vitro. Mechanistically, inhibition of NOX4 suppressed the generation of ROS and the expression of ERS-associated proteins at both RNA and protein levels, such as CHOP, GRP78, p-JNK, and Caspase12 proteins.
Conclusions : Collectively, genetic and pharmacological inhibition of NOX4 protects against RIAKI by mitigating ROS generation and suppressing activation of ERS signaling pathway, which attenuates inflammation and apoptosis. Targeting NOX4 may be a novel therapy for RIAKI.

Figure1.png

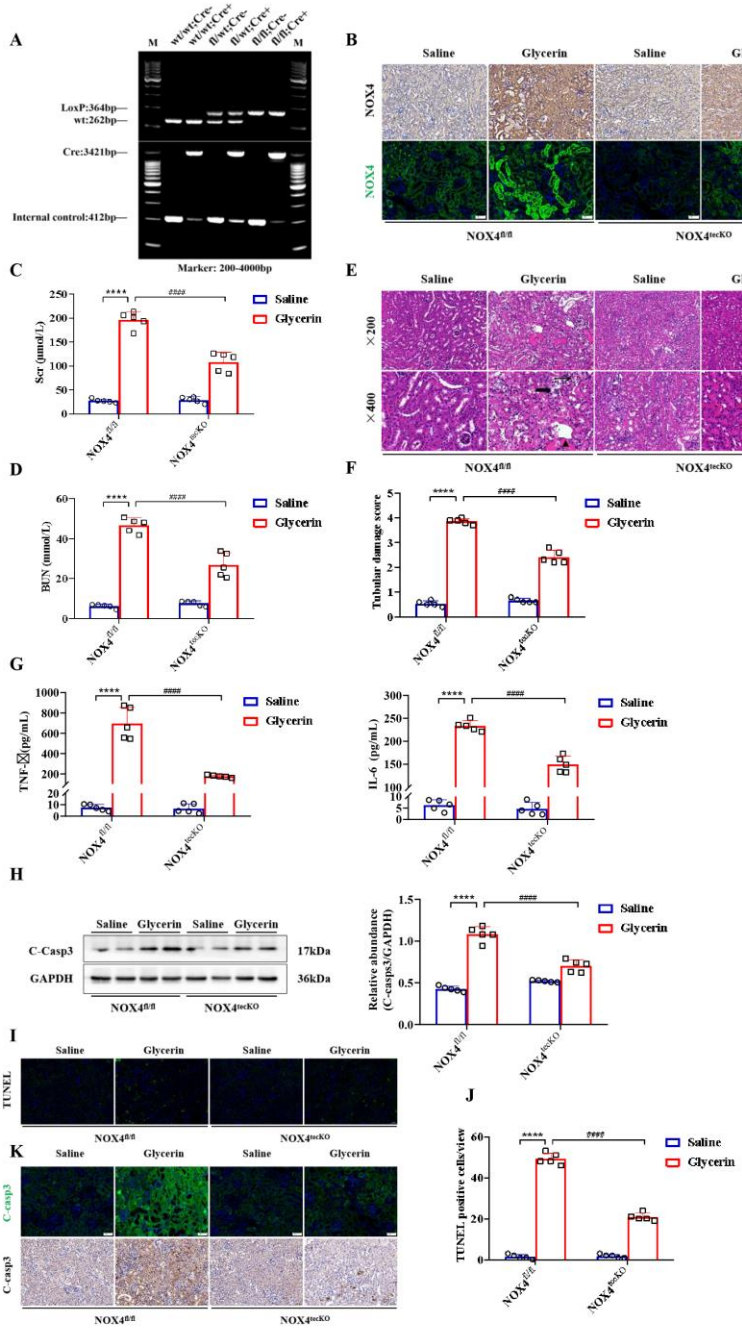
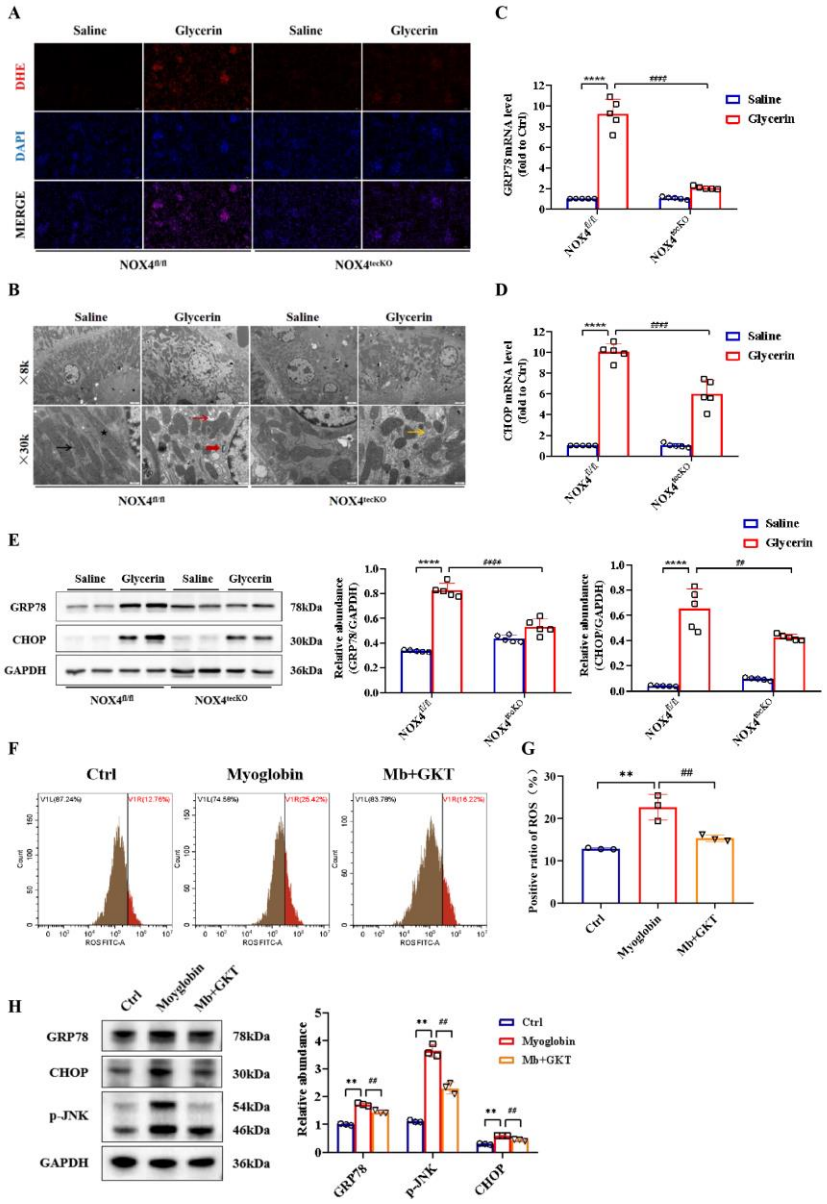

A
M
wt/wtCre-
wt/wtCre+
fl/wtCre-
fl/wtCre+
fl/flCre-
fl/flCre+
M
LoxP:364bp
wt:262bp
Cre:3421bp
Internal control:412bp
Marker: 200-4000bp
B
Saline
Glycerin
Saline
Glycerin
NOX4
NOX4
NOX4fl/fl
NOX4tecKO
C
Saline
Glycerin
Scr (μmol/L)
NOX4fl/fl
NOX4tecKO
D
Saline
Glycerin
BUN (mmol/L)
NOX4fl/fl
NOX4tecKO
E
Saline
Glycerin
Saline
Glycerin
×200
×400
NOX4fl/fl
NOX4tecKO
F
Saline
Glycerin
Tubular damage score
NOX4fl/fl
NOX4tecKO
G
Saline
Glycerin
TNF-α (pg/mL)
NOX4fl/fl
NOX4tecKO
Saline
Glycerin
IL-6 (pg/mL)
NOX4fl/fl
NOX4tecKO
H
Saline
Glycerin
Saline
Glycerin
C-Casp3
GAPDH
17kDa
36kDa
NOX4fl/fl
NOX4tecKO
Saline
Glycerin
Relative abundance (C-casp3/GAPDH)
NOX4fl/fl
NOX4tecKO
I
Saline
Glycerin
Saline
Glycerin
TUNEL
NOX4fl/fl
NOX4tecKO
J
Saline
Glycerin
TUNEL positive cells/view
NOX4fl/fl
NOX4tecKO
K
Saline
Glycerin
Saline
Glycerin
C-casp3
C-casp3
NOX4fl/fl
NOX4tecKO

Figure1.png


A
Saline
Glycerin
Saline
Glycerin
DHE
DAPI
MERGE
NOX4fl/fl
NOX4tecKO
B
×8k
×30k
C
GRP78 mRNA level (fold to Ctrl)
Saline
Glycerin
D
CHOP mRNA level (fold to Ctrl)
E
GRP78
CHOP
GAPDH
78kDa
30kDa
36kDa
Relative abundance (GRP78/GAPDH)
Relative abundance (CHOP/GAPDH)
F
Ctrl
Myoglobin
Mb+GKT
G
Positive ratio of ROS (%)
H
p-JNK
54kDa
46kDa
Relative abundance