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Identification of an adaptor protein that facilitates Nrf2-Keap1 complex formation and modulates antioxidant response

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ABSTRACT

Nrf2 plays a key role in the protection of the body against environmental stress *via* inducible expression of detoxification and antioxidant enzymes. Keap1 functions as a sensor for oxidative and electrophilic stresses and promotes Nrf2 degradation *via* its E3 ligase activity. Modulation of the Nrf2-Keap1 pathway has been extensively explored as a strategy to combat against drug toxicity and stress-induced diseases. Here we report a new player that modulates the Nrf2-Keap1 pathway. PAQR3, a membrane protein specifically localized in the Golgi apparatus, negatively regulates the expression of an array of Nrf2 target genes and alters cellular level of reactive oxygen species. PAQR3 tethers Nrf2 and Keap1, but not small MAF proteins to the Golgi apparatus. PAQR3 interacts with both Nrf2 and Keap1 and facilitates the interaction of Nrf2 with Keap1. PAQR3 promotes ubiquitination and degradation of Nrf2. Disruption of PAQR3 interaction with Nrf2 and Keap1 by a synthetic peptide reduces Nrf2 ubiquitination and elevates expression of Nrf2 target genes. At the animal level, deletion of PAQR3 increases Nrf2 protein level and the expression of Nrf2 target genes. In conclusion, our study pinpoints that PAQR3 functions as an adaptor protein to promote Nrf2-Keap1 complex formation, thereby modulating the Nrf2-Keap2 pathway and playing an important role in controlling antioxidant response of the cell.

1. Introduction

All living organisms are constantly challenged by internal and external oxidative stresses such as those from drugs, xenobiotics, heavy metals, and ionizing radiation. The reactive oxygen species (ROS) and electrophiles generated by these substances are detrimental to the cells *via* causing macromolecular damage, DNA mutations, apoptosis, and cell transformation. Therefore, the cells are equipped with protective systems to relieve these stresses. The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), a basic leucine zipper (bZIP) protein, plays a central role in coordinating the expression of detoxification and antioxidant genes in mammalian cells [1,2]. Nrf2 is activated by ROS and/or electrophiles and crucial for the transcriptional activation of an array of antioxidant and detoxification genes [1], such as xenobiotic metabolizing enzyme (NAD(P)H quinine oxidoreductase-1 (NqoI) [3], hemeoxygenase-1 (HO-1) that cleaves heme to form biliverdin [4], glutamate cysteine ligase (GCLC) and its modifier GCLM for detoxification of electrophilic compounds [5], and the family of glutathione S-transferase (GST) that catalyzes the conjugation of GSH with endogenous and xenobiotic electrophiles [6]. Nrf2, upon forming a complex with its coactivators small Maf proteins (sMAF), activates the expression of these genes *via* binding with the antioxidant response elements (AREs) located in the promoters of the downstream target genes [7].

The activity of Nrf2 is primarily controlled by kelch-like ECH-associated protein 1 (Keap1) [8]. Under quiescent conditions, Keap1 functions as a substrate adaptor for a Cul3-based E3 ubiquitin ligase, constantly targeting Nrf2 for ubiquitin-dependent degradation [9,10]. Upon oxidative and electrophilic stresses, Keap1 functions as a sensor for the stress signals and lose its activity to interact with Nrf2, leading to reduced degradation of Nrf2. As a consequence, Nrf2 is accumulated and translocated to the nucleus, resulting in upregulation of the detoxification and

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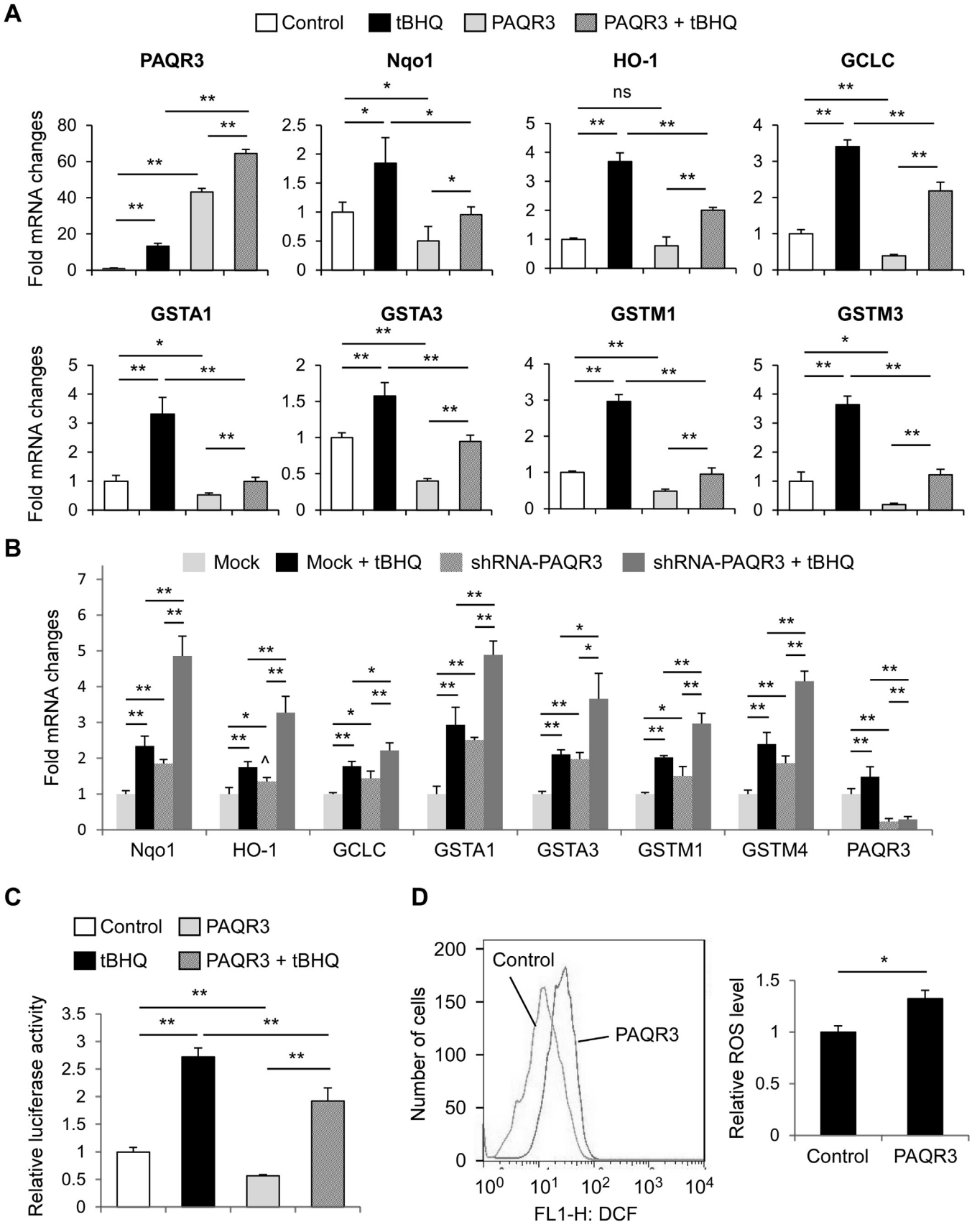
A
Control
tBHQ
PAQR3
PAQR3 + tBHQ
PAQR3
Nqo1
HO-1
GCLC
GSTA1
GSTA3
GSTM1
GSTM3
Fold mRNA changes
ns
B
Mock
Mock + tBHQ
shRNA-PAQR3
shRNA-PAQR3 + tBHQ
Fold mRNA changes
Nqo1
HO-1
GCLC
GSTA1
GSTA3
GSTM1
GSTM4
PAQR3
C
Control
PAQR3
tBHQ
PAQR3 + tBHQ
Relative luciferase activity
D
Control
PAQR3
Number of cells
FL1-H: DCF
Relative ROS level
Control
PAQR3