tert-Butylhydroquinone mobilizes intracellular-bound zinc to stabilize Nrf2 through inhibiting phosphatase activity

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The nuclear factor erythroid 2-related factor 2 (Nrf2) is required to combat increases in oxidative stress. The chemical compound tert-butylhydroquinone (tBHQ) can downregulate Kelch-like ECH-associated protein 1 (Keap1), a repressor of Nrf2, thus maintaining the stability of Nrf2. tBHQ can also increase intracellular "free" zinc in human bronchial epithelial (16HBE) cells. We aim to investigate whether the intracellular free zinc change plays a role in Nrf2 activation. tBHQ exposure dose-dependently increases intracellular free zinc concentrations within 30 min in 16HBE cells by mobilizing intracellular zinc pools. Active Nrf2 and the antioxidant enzyme heme oxygenase-1 (HO-1) increase at 3 h after tBHQ treatment. Chelating intracellular free zinc with tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) during tBHQexposure partially abrogates the tBHQ-induced activation of Nrf2 and HO-1 expression, while Keap1 is further decreased.

These results indicate that tBHQ-induced stability of Nrf2 is associated with the intracellular free zinc level. Because the activated Nrf2 is phosphorylated, the serine/threonine protein phosphatase activity, which is known to be inhibited by zinc, is assayed. The results showed that tBHQ treatment can suppress cellular protein phosphatase-2A (PP2A) and protein phosphatase-2C (PP2C) activity, which can be abrogated by adding TPEN. This finding is verified in a cell-free protein extract experiment by supplying zinc or by chelating zinc with TPEN. These results provide a novel mechanistic insight into Nrf2 activation in antioxidant enzyme induction involving zinc signaling. The increase of intracellular free zinc may be one mechanism for Nrf2 activation. The inhibition of PP2A and PP2C activity may be involved in Nrf2 phosphorylation modulation.

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