Nrf2 and p21 regulate the fine balance between life and death by controlling ROS levels

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Abbreviations: ROS, reactive oxygen species; CDK, cyclin dependent kinase; PCNA, proliferating cell nuclear antigen

Reactive oxygen species (ROS) are generated by normal metabolic processes or from environmental exposures, and are implicated in the development of cancer and cardiovascular disease, as well as the aging process. Under most conditions, ROS is tightly regulated by a number of enzymes and antioxidants to prevent damage. However, oxidative stress can occur when there is an imbalance in the production of ROS and the cells ability to detoxify the reactive intermediates. Consequently, oxidative stress can induce cellular damage which can accumulate and promote disease development.

Nrf2 is a transcription factor that plays a pivotal role in activating an antioxidant response that decreases ROS, detoxifies harmful chemicals, and ultimately protects against cellular damage. Nrf2 regulates a battery of downstream genes that play a role in a wide variety of functions, such as cellular redox homeostasis, cell growth and apoptosis, DNA repair, the inflammatory response, and the ubiquitin-mediated degradation pathway. Together, the induction of these genes is imperative for cells to counteract ROS and environmental or chemical toxicants. The diverse nature of Nrf2’s downstream target genes demonstrates its vital importance in cell survival and protection. Nrf2 is negatively regulated by Keap1, a substrate adaptor for the Cul3-dependent E3 ubiquitin ligase complex. Under basal conditions, Keap1 targets Nrf2 for ubiquitination and proteasomal degradation, maintaining low basal levels of Nrf2. Under oxidative stress conditions, the activity of the E3 ubiquitin ligase complex is suppressed, stabilizing the Nrf2 protein. In addition, we have shown that inhibiting Keap1-dependent Nrf2 ubiquitination, resulting in stabilization of the Nrf2 protein. In our recent study,1 we have revealed a novel mechanism by which p21 protects cells against oxidative stress through upregulation of the Nrf2 signaling pathway. Our data strongly suggests that upregulation of the Nrf2-dependent antioxidant response is another means for p21 to exert its tumor suppressor activity. We showed that p21 competes with Keap1 for Nrf2 binding, thus, inhibiting Keap1-dependent Nrf2 ubiquitination, resulting in stabilization of the Nrf2 protein. In addition, we have shown that Nrf2 is essential for p21’s antioxidant effects, by demonstrating that ectopic expression of p21 was able to enhance cell survival in response to H2O2 in MEF-Nrf2+/+, but not in MEF-Nrf2-/- cells. One may be concerned that overexpression of p21 may lead to cell cycle arrest, therefore, protecting cells from death independently of Nrf2. However, this is not the case in our current study, since most of the experiments were done in HCT116-p21-/- cells, HCT116-p21-/- cells transfected with small amounts of p21-cDNA, or in MEF-Nrf2+/+ or MEF-Nrf2-/- cells with endogenous p21 knocked down by p21-siRNA. Furthermore, we did not observe obvious changes in the growth rate of cells during the course of our experiments. Nevertheless, the requirement of Nrf2 for the p21-mediated antioxidant response is clearly demonstrated using many different approaches in this study. Moreover, we confirmed these results using p21-deficient mice, demonstrating the physiological significance of our findings. The essential role of p21 in protecting cells or mice from oxidative damage has been reported extensively. However, until our recent findings the mechanism of how p21 exerts its antioxidant effects was unknown.

Cyclin-CDK complexes mainly bind to the N-terminal of p21, containing a cyclin-binding motif, CY1 (amino acids 17–24) and a CDK2 binding motif (amino acids 53–59). The C-terminal of p21 also contains a cyclin-binding motif, CY2 (amino acids 155–157) as well as the PCNA-interacting domain (amino acids 143–160). Additionally, both termini have the ability to inhibit cell proliferation; however, the N-terminus has a greater capability.

The Nrf2-interacting motif is mapped to the C-terminal
The complexity and interplay between these two pathways demonstrates their vital role in the balance between cell survival and cell death and warrants further investigation.

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References