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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.[1] 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 and thus activating the antioxidant response.[2] The Nrf2-dependent antioxidant response has been shown to protect against oxidative-stress related diseases such as cancer, neurodegenerative diseases, aging, cardiovascular disease, inflammation, pulmonary fibrosis and acute pulmonary injury.[3-6]

p21 is a cyclin dependent kinase (CDK) inhibitor that regulates many cellular processes in a p53-dependent and -independent manner. p21 can promote cellular differentiation and senescence, and inhibit gene transcription and apoptosis. Furthermore, through inhibition of proliferating cell nuclear antigen (PCNA), p21 can modulate DNA replication and repair. p21 is the major target of p53-mediated cell cycle arrest. In response to DNA damage, p21 functions as a tumor suppressor and initiates cell cycle arrest between the G_1 and S interface allowing time for DNA repair.[7] On the other hand, p21 facilitates apoptotic processes when DNA damage is beyond repair.[8] Furthermore, cellular stress that does not damage DNA, such as hypoxia or exposure to ribonucleotide biosynthesis inhibitors, may also induce p53-dependent expression of p21.[7]

In our recent study,[9] 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 H_2O_2 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.[9] 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.[10,11] 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

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proliferation; however, the N-terminus has a greater capability.[12] The Nrf2-interacting motif is mapped to the C-terminal ^{154}KRR,[9] overlapping the CY2 motif as well as the PCNA interacting domain. Thus, it is most likely that p21-mediated upregulation of the Nrf2 signaling pathway and p21-dependent cell cycle arrest represent two separate cytoprotective mechanisms carried out by p21. However, the cytoprotective pathway that will be predominantly activated in response to a particular oxidative stress-inducing chemical is likely dependent on the nature of the chemical and the magnitude of oxidative stress. Conceivably, p21-mediated activation of the Nrf2 signaling pathway may be the first defense mechanism used to reduce ROS under low stress conditions (**Fig. 1**). At moderate levels of oxidative stress involving DNA damage, p21-mediated cell cycle arrest would be activated to allow time for DNA repair (**Fig. 1**). At high levels of oxidative stress, at the point of no return, apoptosis is induced. Since activation of apoptosis requires the accumulation of ROS, it is plausible that the Nrf2 antioxidant response pathway must be suppressed in order to induce apoptosis. In concordance with this presumption, at high levels of oxidative stress Nrf2 protein levels begin to decrease in a dose-dependent manner, although, this precise mechanism has not yet been elucidated (**Fig. 1**).[13]

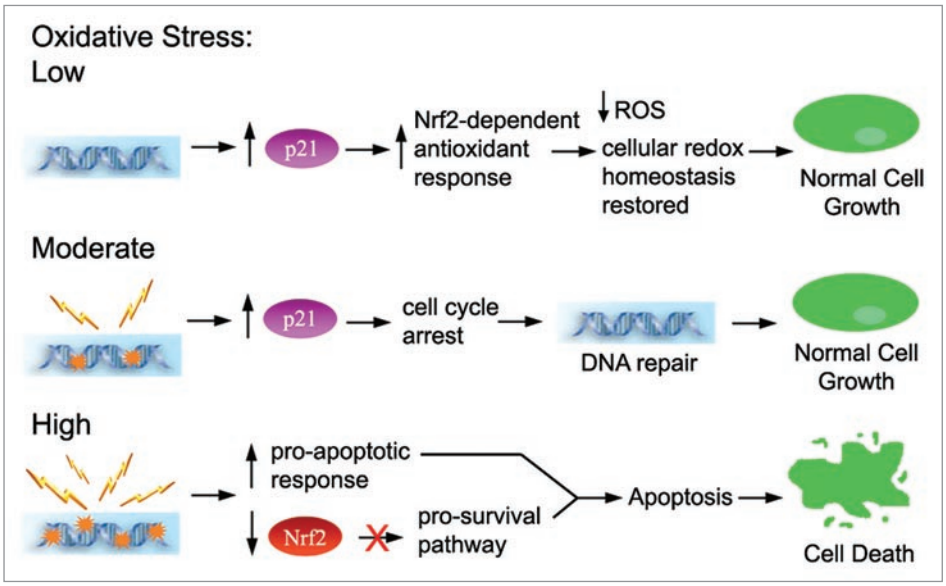

Figure 1. The effect of oxidative stress on cell survival. At low levels of oxidative stress, p21 activates the Nrf2-dependent antioxidant response which protects cells from ROS induced cellular damage. At moderate levels of oxidative stress, p21 induces cell cycle arrest to allow time for DNA repair. At high levels of oxidative stress, Nrf2 is destroyed and the Nrf2-dependent pro-survival response is inhibited and the pro-apoptotic response is initiated activating apoptosis.

Our recent finding, demonstrating that p21 is able to upregulate the Nrf2-dependent antioxidant response, revealed a novel mechanism by which p21 enhances cell survival.[9] In addition, 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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