The p53-Mdm2 Loop: A Critical Juncture of Stress Response

Yaara Levav-Cohen, Zehavit Goldberg, Osnat Alsheich-Bartok, Valentina Zuckerman, Sue Haupt and Ygal Haupt*

Abstract

The presence of a functional p53 protein is a key factor for the proper suppression of cancer development. A loss of p53 activity, by mutations or inhibition, is often associated with human malignancies. The p53 protein integrates various stress signals into a growth restrictive cellular response. In this way, p53 eliminates cells with a potential to become cancerous. Being a powerful decision maker, it is imperative that p53 be activated properly, efficiently and temporarily in response to stress. Equally important is that p53 activation will be extinguished upon recovery from stress, and that improper activation of p53 will be avoided. Failure to achieve these aims is likely to have catastrophic consequences for the organism. The machinery that governs this tight regulation is largely based on the major inhibitor of p53, Mdm2, which both blocks p53 activities and promotes its destabilization. The interplay between p53 and Mdm2 involves a complex network of positive and negative feedback loops. Relief from Mdm2 suppression is required for p53 to be stabilized and activated in response to stress. Protection from Mdm2 entails a concerted action of modifying enzymes and partner proteins. The association of p53 with the PML-nuclear bodies may provide an infrastructure in which this complex regulatory network can be orchestrated. In this chapter we use examples to illustrate the regulatory machinery that drives this network.

Introduction

The tumor suppressor p53 protein is pivotal in the prevention of cancer development. P53 determines cell fate through its activities as a transcription factor, and by engagement in critical protein interactions at the mitochondria (reviewed in ref. 1). P53 is normally labile, but in response to external and internal stress signals, it is triggered to become stable and active within the nucleus. As a transcription factor it controls the expression of genes that regulate cell growth and cell death (reviewed in refs. 2,3). Stabilized p53 induces either cell growth arrest (temporary, or permanent "senescence"), or programmed cell death (apoptosis). The growth restrictive activities of p53 prevent the proliferation of cells with damaged DNA or with a potential for neoplastic transformation; while p53-mediated permanent cell growth inhibition (apoptosis or senescence) drives tumor suppression. Given these functions of p53, it is not surprising that p53 serves as a serious obstacle to the step-by-step progression of cancer development. This barrier is very frequently removed at one of the steps, either by direct mutation of the p53 gene, or by indirect mechanisms, such as an elevation in the expression levels of p53 inhibitors, or by down-regulation of p53 co-activators, such as ARF.

*Corresponding Author: Ygal Haupt—Research Division, The Peter MacCallum Cancer Centre, St. Andrew’s Place, East Melbourne 3002, Victoria, Australia. Email: ygal.haupt@petermac.org

The lability of p53 under normal cellular conditions is largely attributable to its inhibitor Mdm2 (Hdm2 in humans), which ensures that p53 has a short half-life and consequent low basal activity. Stresses that dramatically elicit a change in p53 status include: DNA damage, deregulated oncogenes, hypoxia, and nucleotide depletion among others (reviewed in ref. 5). The activation of p53 involves stabilization of the protein, which is mediated by extensive post-translational modifications, and protein-protein interactions with cooperating factors. Once stable, p53 engages in enhanced DNA binding and transcriptional activity. The summation of the incoming signals and the cellular context, dictates whether activated p53 will direct cells to growth arrest, senescence or apoptosis (reviewed in ref. 6). This chapter focuses on the regulation of p53 by Mdm2. Particular emphasis will be given to current models explaining how the p53/Mdm2 auto-regulatory loop is modulated or interrupted in response to stress. The different mechanisms involved will be illustrated by specific examples. The intention of this chapter is to explain how such a busy network of regulation may be coordinated within a cell in a spatial and temporal manner in response to a given stress signal.

The p53-Mdm2 Feedback Loop

Almost two decades of research have passed since the identification of \textit{mdm2} as a p53 target gene\textsuperscript{7,8} (also reviewed in refs. 9,10). The revelation that p53 induces Mdm2 expression, which then inhibits the biochemical and biological activities of p53, defined the first and the most important auto-regulatory loop that governs p53 regulation. This loop proves to be even more powerful than initially thought, as additional multiple regulatory loops are being found to interweave with it\textsuperscript{4,11-14}. Several of these loops will be described in this chapter. Mdm2 binds p53 in the transactivation domain and blocks its ability to induce or suppress transcription (reviewed in refs. 9,10). The major and most efficient inhibitory effect of Mdm2 is to destroy the p53 protein via the ubiquitin-proteasome pathway\textsuperscript{15,16} (also reviewed in ref. 17). Thus, through this negative feedback loop, Mdm2 shuts off its own expression (Fig. 1). The physiological significance of this auto-regulatory feedback loop was demonstrated by the clinical observation that amplification of Hdm2 in human cancers often correlates with wild type p53 status, supporting the notion that high expression of Hdm2 is sufficient for relieving a cell from p53 regulation, in the absence of p53 mutation (reviewed in refs. 9,18). Further, a single nucleotide polymorphism (SNP) in the \textit{hdm2} gene that leads to increased Hdm2 expression,

Figure 1. The p53/Mdm2 autoregulatory loop. Activated p53 induces the expression of multiple target genes. One of the genes is Mdm2 which binds p53, inhibits its transcriptional activity and promotes it for proteasomal degradation.