Publication
CHIP Chaperones Wild Type p53 Tumor Suppressor Protein*

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Wild type p53 exists in a constant state of equilibrium between wild type and mutant conformation and undergoes conformational changes at elevated temperature. We have demonstrated that the co-chaperone CHIP (carboxyl terminus of Hsp70-interacting protein), which suppressed aggregation of several misfolded substrates and induced the proteasomal degradation of both wild type and mutant p53, physically interacts with the amino terminus of WT53 and prevented it from irreversible thermal inactivation. CHIP preferentially binds to the p53 mutant phenotype and restored the DNA binding activity of heat-denatured p53 in an ATP-independent manner. In cells under elevated temperatures that contained a higher level of p53 mutant phenotype, CHIP restored the native-like conformation of p53 in the presence of geldanamycin, whereas CHIP-small interfering RNA considerably increased the mutant form. Further, under elevated temperatures, the levels of p53 and CHIP were higher in nucleus, and chromatin immunoprecipitation shows the presence of p53 and CHIP together upon the DNA binding site in the p21 and p53 promoters. We propose that CHIP might be a direct chaperone of wild type p53 that helps p53 in maintaining wild type conformation under physiological condition as well as help resurrect p53 mutant phenotype into a folded native state under stress condition.

p53 is a transcription factor that is responsible to maintain the integrity of the genome and is mutated in over 50% of human cancers (1). Wild type (WT) p53 is a structurally unstable protein, which undergoes conformational changes at elevated temperatures (2, 3). p53 is normally expressed at low levels in a latent form that is unable to bind specifically to DNA, and several in vivo experiments suggest that WT p53 may exist in a constant state of equilibrium between the wild type and mutant conformation. In its mutant form, it could inactivate the protein from the wild type allele, and the formation of hetero-oligomers of wild type and mutant proteins could drive the wild type protein into mutant conformation (4). It was suggested that chaperone-mediated actions might decrease the probability for the formation of kinetically trapped, mutant-like intermediates that would allow a shift in the conformational equilibrium toward the active, wild type p53 conformation (5). Hsp90 was recently shown to bind to a folded, native-like conformation of p53 in vitro that was essential to stabilize p53 at physiological temperature (6). Hsp90 stabilized WT p53-DNA complexes at 25 °C as well as partially protected the WT p53-DNA binding conformation during long term exposures at 37 °C in an ATP-dependent manner (7) and might be involved in regulating the shift between wild type and mutant p53 conformation (8), whereas other human chaperones Hsp70 and Hsp40 failed to efficiently substitute Hsp90. The function of Hsp90 may be modulated by association with co-chaperones, such as Hsc70, Hsp40 (9), and Hop (10). A drug CP-31398 (11, 12) as well as a plant alkaloid ellipticine (13) have also been shown to maintain p53 in active conformation and can drive some mutant p53s into wild type conformation. Further, MDM2 binding to the p53 amino terminus could induce a conformational change in wild type p53, and this change was opposed by Hsp90 (14).

CHIP (carboxyl terminus of Hsp70-interacting protein) is a dimeric 35-kDa ubiquitin ligase (15) comprising three functional domains: a tetratricopeptide repeat (TPR) at the amino terminus, a U-box domain at the COOH terminus, and a highly charged region separating the two (16). The TPR domain mediates its interaction with Hsp90 and Hsp70 during the regulation of signaling pathways and during protein quality control (17) targeting Hsp70 (15) and its substrates, such as p53 (18) and GR (19) and for proteasomal degradation. Overexpression of CHIP in fibroblasts increased the refolding of proteins after thermal denaturation and inhibition of Hsp70 chaperone activity abolished the effects of CHIP on protein folding, indicating that the CHIP-mediated events were Hsp70-dependent (20). Although the full range of cellular substrates of CHIP remains to be explored, misfolded CFTR (17, 21), tau (22, 23), and polyglutamine aggregation (24) are suppressed by CHIP-assisted quality control.

In this study, we have analyzed in detail the interaction of p53 with CHIP and have discovered that the co-chaperone CHIP regulates p53 conformation and activity under physiological and elevated temperatures both in vitro and in vivo independent of Hsp90 function. CHIP prevents p53 from irreversible thermal inactivation and restores the DNA binding activity of heat-denatured p53 in an ATP-independent manner. In cells under elevated temperatures, CHIP helps resurrect the p53 mutant conformation into a folded native state. CHIP was shown to coassociate with WT p53 upon DBS in chromatin. We have proposed that CHIP might be a direct chaperone of WT p53 both under physiological and elevated temperatures.