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p53 reactivation
The link to zinc

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Lack of p53 expression or expression of mutant p53 is common in human cancers and is associated with increased tumor growth and resistance to therapies.[1] Significant efforts toward pharmaceutical reactivation of defective p53 by small molecules are therefore underway, targeting the different means that inactivate p53.[2,3] Indeed, reactivated p53 can lead to tumor destruction.[4]

A recent paper in *Cancer Cell* by Yu et al. describes reactivation of mutant p53 (mtp53) by thiosemicarbazone compounds.[5] These compounds induce wild-type (wt)p53 conformation, in particular for the p53H175 mutant, and this restores p53-dependent apoptosis and inhibition of xenograft tumor growth.[5] In this paper, the authors emphasize that the mechanism of p53H175 reactivation depends on the zinc ion chelating properties of the thiosemicarbazone compounds that allow the p53H175 mutant to change conformation into a wild-type folding.[5] p53 is a zinc-containing transcription factor that includes one zinc ion as an important cofactor, which is coordinated to the side chains of three Cys and one His residue in the DNA binding domain (DBD, residues 94–312).[6] Zinc stabilizes the second and third loops of the DBD and is needed for wtp53 function.[6] Many tumor-associated p53 mutations, classified as contact (e.g., R273H and R273C) or structural mutations (e.g., R175H, V143A, Y220C, G245S, R249S, F270L, R282W), may change the DBD conformation resulting in diminished DNA binding.[7] Interestingly, mutant p53 proteins are prone to the loss of the DBD-bound Zn^{2+} that promotes protein unfolding and aggregation.[8] Similarly, mutations of the coordinating residues (C176F, H179R, C238S, C242S and also R175H) result in loss of zinc and reduced affinity to DNA. Changes of p53 conformation are also achieved by the removal of zinc using chelating agents and reversed by adding zinc.[9,10] The important feature of these mtp53 structures is their flexibility and the reversibility of the conformational changes. The reversibility of conformational changes of p53 mutants is particularly noticed in many temperature-sensitive mutants of p53, where wild-type activity is lost at 37° and regained at 32°. Hence, the attempt to overcome the effect of mutations by changes of p53 conformation is becoming an important challenge and a hope in cancer therapy, even for mutants that are not temperature-sensitive.

In this regard, the thiosemicarbazone compounds switch the mutant conformation in the mtp53 (H175) protein toward p53 wild-type conformation, as evidenced by immunoprecipitation and immunofluorescence studies with conformation-specific antibodies. This conformational switch leads to restoration of p53 transactivation function in vitro and in vivo.[5] These findings strengthen the role of zinc in reactivating mutant p53 function. Moreover, they confirm and enhance our previous results based on the zinc supplementation approach to reactivate dysfunctional p53, both misfolded and mutant. We found that wtp53 acquires a misfolded "mutant-like" conformation in HIPK2-depleted cells due to deregulation of metallothionein and zinc.[11,12] In line with the original findings of the importance of zinc in p53 folding and stability,[9,10] we found that zinc supplementation reverts p53 misfolding, thereby restoring p53 wild-type conformation as well as DNA binding and transactivation of target genes.[11] These results were corroborated by in vivo studies in mice with the transgenic MMTV-*neu* spontaneous breast cancer model, where low HIPK2 expression correlates with misfolded p53.[13] Upon zinc supplementation, the misfolded p53 was reactivated, leading to wtp53 activity and tumor growth inhibition in response to drug.[13] More recently, we also explored the possibility of affecting mutant p53 by zinc. We demonstrated that zinc switches the conformation of two of the most frequent p53 mutants, such as R175H and R273H, toward wtp53 conformation, as evidenced by protein immunoprecipitation with conformation-specific antibodies.[14] Reactivation of both H175 and H273 leads to restoration of wtp53 binding to target promoters and apoptotic transcriptional activity in response to drug. The biological outcome resulted in increased cell death in vitro and inhibition of xenograft tumor growth in vivo. Moreover, we found that zinc supplementation to both H175 and H273 mutants inhibits one of the mtp53 pro-oncogenic activities, that is the interaction with p73 family member, with restoration of p73 binding to target gene promoters.[14] Altogether, our findings show that zinc supplementation can reactivate both H175 and H273 p53 mutants, restoring wtp53 functions in response to drugs and inhibiting some mtp53 pro-oncogenic functions. Therefore, zinc ions can be as beneficial to mtp53 reactivation as thiosemicarbazone compounds, as demonstrated by Yu et al.[5] These results strongly support translational studies in the clinic to modify mtp53 conformation by zinc in order to improve patient outcome. Yet the question remains whether

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zinc might also affect mtp53 proteins other than H175 and H273. Further experiments are necessary to answer this question.

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