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A novel facet of tumor suppression by p53
Induction of tumor immunogenicity

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Pharmacological reactivation of the p53 tumor suppressor is a promising strategy for anti-cancer therapy due to its high potential to elicit apoptosis or growth arrest in cancer cells. Recently we uncovered the mechanism of activation of the innate immune response by p53 upon its activation by small molecules.

Cell-intrinsic barrier mediated by tumor suppressors and cell-extrinsic barrier mediated by the immune system are the main defenses of our body against oncogenesis. Only those cells, which escaped from both barriers, have the opportunity to develop into tumor. Innate immunity among the cell-extrinsic barrier is a front-line defense against infectious diseases and malignancies, of which NK cells constitute an important component.[1,2]

p53 tumor suppressor is widely recognized as a master regulator of cell-intrinsic anti-tumor defenses via induction of growth arrest, apoptosis, senescence, autophagy, and inhibition of cancer cells metabolism.[3] Albeit functionally inactive, p53 is expressed in cancers, leading to the idea of p53 reinstatement as a novel therapeutic strategy against human cancers. Several p53-reactivating compounds are currently being tested in Phase I clinical trials, including mutant p53-reactivating PRIMA-1 analog APR-246, derivative of MDM2 inhibitor nutlin3a RG7112 and compounds from Johnson and Johnson.[3] For the successful application of p53-based therapies in the clinic, a more rigorous determination of p53 activities upon its pharmacological reinstatement in cancer cells is of utmost importance.

Strikingly, apart from well-characterized cell-intrinsic mechanisms, p53 might also play a role in modulating the cell-extrinsic anti-cancer defense. This is suggested by recent study in mice with "switchable" p53 which demonstrated p53-dependent tumor regression due to the elimination of senescent cancer cells by innate immune system.[4] This new facet of p53 tumor suppression function along with the regulation of cell-intrinsic defenses may greatly increase the probability to achieve a durable therapeutic success upon pharmacological reactivation of p53. However, the exact molecular mechanism underlying the stimulation of innate anti-cancer response by p53 is currently unknown.

In our recent study, we applied a set of p53-reactivating compounds, PRIMA-1^{MET}, Nutlin3a, RITA and a low dose of Actinomycin D, as a tool to address whether and how p53 can stimulate the immune response against cancer cells. We found that pharmacological reactivation of p53 enhanced the NK cell-mediated killing of samples derived from patients with metastatic tumors of different origin, including melanoma, pancreatic, breast, colon and lung carcinoma, as well as established lines, derived from different carcinomas, osteosarcoma and lymphoma. We further demonstrated that this effect is due to the induction of ULBP2, a ligand of NK cell receptor NKG2D, an important component of the front-line immune defense against infectious diseases and malignancies. Furthermore, we found that the binding of p53 to its response element (RE) within the first intron of *ULBP2* gene is required for the activation of its expression, thus establishing *ULBP2* as a bona fide p53 target gene and suggesting a direct effect of p53 on stimulation of anti-cancer immune response.[1] Notably, the induction of ULBP2 by p53 was also recently reported in an independent study by Textor et al.[2]

However, stabilization of p53 by different agents is necessary, but not sufficient for binding of p53 to *ULPB2* gene and ULPB2 induction. In spite of a similar extent of p53 stabilization, different p53 activating compounds have distinct effect on ULBP2 expression.[1]

Recently proposed model suggests that p53 activation in vivo includes three major steps: (1) p53 stabilization, (2) antirepression (i.e., release from MDM2), and (3) promoter-specific activation, involving the binding to different cofactors and distinct post-translational modifications, so called "barcode."[5,6] In addition, the binding of p53 to its specific RE in DNA can be determined by the epigenetic modifications of the promoter.

We found that the p53 RE in *ULBP2* gene is highly methylated in cancer cells, which prevents p53 binding. Demethylation of the p53 RE in *ULBP2*, achieved through repression of DNA methyltransferases (DNMTs), is required for the interaction of p53 with its binding site and the subsequent induction of ULBP2 by p53 (**Fig. 1A**).[1]

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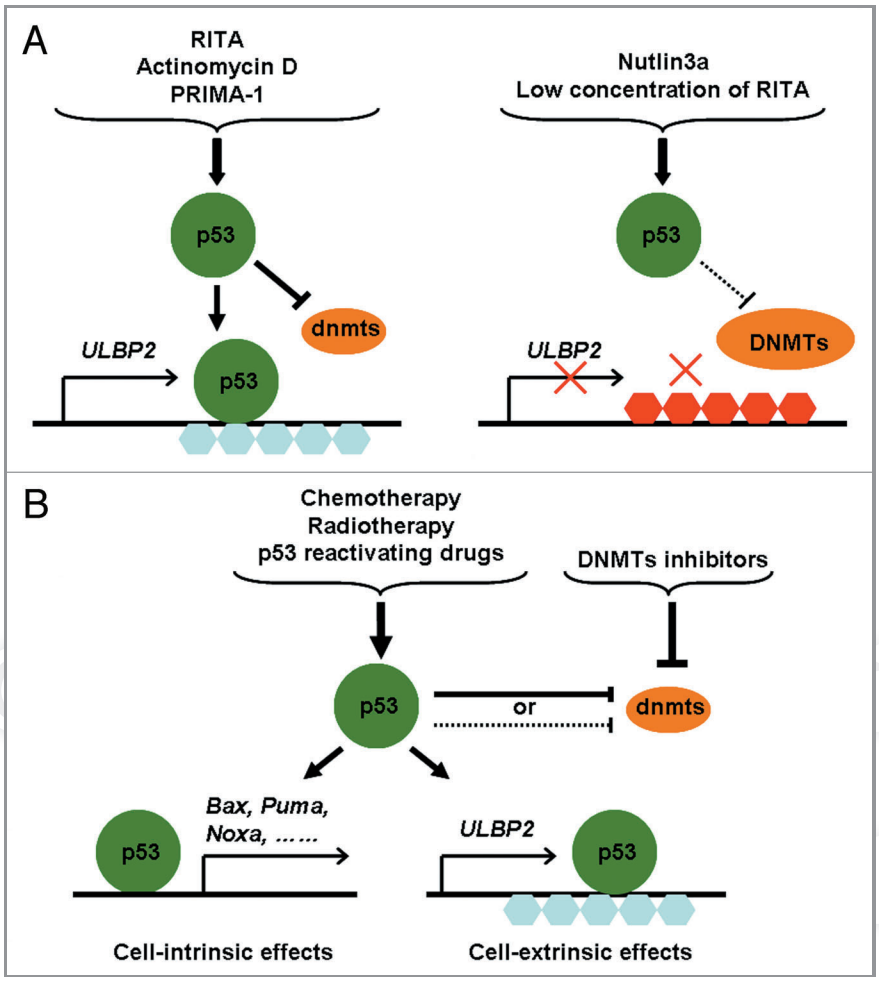

Figure 1. Model illustrating the mechanism underlying transcriptional control of ULBP2 by p53 and proposed therapeutic implications. (A) p53 activated by PRIMA-1^{MET}, Actinomycin D, and RITA, but not Nutlin3a and low concentration of RITA, can induce demethylation of its response element (RE) within the first intron of *ULBP2* gene via the repression of expression of DNMT's. This allows the interaction of p53 with its RE and the subsequent induction of ULBP2 transcription by p53, blue, non-methylated DNA; red, methylated DNA. (B) Proposed therapeutic benefit of combined administration of p53-activating agents and DNMT's inhibitors.

Although our study provides an evidence for the repression of DNMTs by p53 activation, the mechanism determining the distinct effect of p53 on DNMTs expression upon activation by different agents remains to be elucidated. It is tempting to speculate that the repression of DNMTs by p53 requires certain post-translational modifications and/or interaction with specific cellular cofactors induced by RITA, PRIMA-1^{MET} and Actinomycin D, but not by Nutlin3a or low concentration of RITA. Recently, wild-type p53 has been shown to negatively regulate DNMT1 expression by forming a complex with specificity protein 1 (SP1), which binds to the *DNMT1* promoter.[7] However, our data do not support the involvement of SP1 in DNMT repression by p53, although we found that SP1 is important for p53-mediated repression of genes involved in glycolysis.[8] It is plausible, that p53's post-translational modifications and cofactors that associate with p53 form the underlying basis of the heterogeneity of p53 response, depending on the type and dose of stimuli as well as cell type.[6] Since there are more than 50 known p53 partner proteins, which can modulate p53-mediated regulation of gene expression, high throughput approaches are required to identify the bars of the code, which confer the differential gene regulation in response to different p53-activating molecules.

Induction of ULBP2 by p53 we uncovered may contribute to the promotion of NKG2D ligand expression upon genotoxic stress reported previously.[9] Furthermore, cellular response to genotoxic stress could modulate inhibitory NK cell ligands as well.[10] Taken together, these findings are of particular interest as they link chemotherapy and immunotherapy and explain the synergistic effects of these therapeutic approaches observed in clinic. Further, our data indicate that the combined administration of p53 activating agents and DNMT inhibitors could be very beneficial, since it will not merely induce autonomous effects in tumor cells but also prevent their escape from immunosurveillance (**Fig. 1B**).

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