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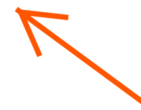
Structural basis for high-affinity peptide inhibition of p53 interactions with MDM2 and MDMX

Marzena Pazgier,[a,1] Min Liu,[a,b,1] Guozhang Zou,[a] Weirong Yuan,[a] Changqing Li,[a] Chong Li,[a] Jing Li,[a] Juahdi Monbo,[a] Davide Zella,[a] Sergey G. Tarasov,[c] and Wuyuan Lu[a,2]



ABSTRACT

The oncoproteins MDM2 and MDMX negatively regulate the activity and stability of the tumor suppressor protein p53—a cellular process initiated by MDM2 and/or MDMX binding to the N-terminal transactivation domain of p53. MDM2 and MDMX in many tumors confer p53 inactivation and tumor survival, and are important molecular targets for anticancer therapy. We screened a duodecimal peptide phage library against site-specifically biotinylated p53-binding domains of human MDM2 and MDMX chemically synthesized via native chemical ligation, and identified several peptide inhibitors of the p53-MDM2/MDMX interactions. The most potent inhibitor (TSFAEYWNLLSP), termed PMI, bound to MDM2 and MDMX at low nanomolar affinities—approximately 2 orders of magnitude stronger than the wild-type p53 peptide of the same length (ETFSDLWKLLPE). We solved the crystal structures of synthetic MDM2 and MDMX, both in complex with PMI, at 1.6 Å resolution. Comparative structural analysis identified an extensive, tightened intramolecular H-bonding network in bound PMI that contributed to its conformational stability, thus enhanced binding to the 2 oncogenic proteins. Importantly, the C-terminal residue Pro of PMI induced formation of a hydrophobic cleft in MDMX previously unseen in the structures of p53-bound MDM2 or MDMX. Our findings deciphered the structural basis for high-affinity peptide inhibition of p53 interactions with MDM2 and MDMX, shedding new light on structure-based rational design of different classes of p53 activators for potential therapeutic use.

p53 is best known as a tumor suppressor that transcriptionally regulates, in response to cellular stresses such as DNA damage or oncogene activation, the expression of various target genes that mediate cell-cycle arrest, DNA repair, senescence or apoptosis—all of these cellular responses are designed to prevent damaged cells from proliferating and passing mutations on to the next generation (1–3). In 50% of human cancers, p53 is defective due usually to somatic mutations or deletions primarily in its DNA-binding domain and, to a lesser extent, to posttranslational modifications such as phosphorylation, acetylation and methylation that affect p53 function and stability. Altered p53 fails to regulate growth arrest and cell death upon DNA damage, directly contributing to tumor development, malignant progression, poor prognosis and resistance to treatment (4). Conversely, restoring endogenous p53 activity can halt the growth of cancerous tumors in vivo by inducing apoptosis, senescence, and innate inflammatory responses (5–7).

As p53 mediates growth arrest and apoptosis, it is essential to keep its activity in check during normal development (2). Multiple mechanisms exist to negatively regulate p53 activity, among which the E3 ubiquitin ligase MDM2 and its homolog MDMX (also known as MDM4) play a central regulatory role in the developing embryo and in mature differentiated cells (8, 9). MDM2 consists of 491-aa residues, comprising an N-terminal p53-binding domain, a central domain preceded by nuclear export and localization signals essential for nuclear-cytoplasmic trafficking of MDM2, a zinc finger domain, and a C-terminal zinc-dependent RING finger domain that confers E3 ubiquitin ligase activity (10). Structurally related to MDM2, MDMX of 490-aa residues possesses domain structures arranged similarly to MDM2, except that MDMX lacks ubiquitin-ligase function (11, 12). Growing evidence supports that in unstressed cells MDM2 primarily controls p53 stability through ubiquitylation to target the tumor suppressor protein for constitutive degradation by the proteasome (13, 14), whereas MDMX mainly functions as a significant p53 transcriptional antagonist independently of MDM2 (15, 16). Under stress conditions, MDM2 and MDMX cooperate to activate p53 through mechanisms involving both MDM2 autodegradation (autoubiquitylation) and MDM2-depedent degradation of MDMX (17–20).