doi:10.1016/j.jmb.2010.03.024 J. Mol. Biol. (2010) 398, 489–496

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Structural Insight into p53 Recognition by the 53BP1 Tandem Tudor Domain

Siddhartha Roy[1], Catherine A. Musselman[1], Ioulia Kachirskaia[2], Ryo Hayashi[3], Karen C. Glass[1], Jay C. Nix[4], Or Gozani[2], Ettore Appella[3] and Tatiana G. Kutateladze[1]*

[1]*Department of Pharmacology, University of Colorado Denver School of Medicine, 12801 East 17th Avenue, Aurora, CO 80045-0511, USA*

[2]*Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA*

[3]*Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

[4]*Molecular Biology Consortium, Advanced Light Source Beamline 4.2.2, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*

Received 21 January 2010; received in revised form 11 March 2010; accepted 12 March 2010
Available online 20 March 2010

Edited by M. F. Summers

The tumor suppressor p53 and the DNA repair factor 53BP1 (p53 binding protein 1) regulate gene transcription and responses to genotoxic stresses. Upon DNA damage, p53 undergoes dimethylation at Lys382 (p53K382me2), and this posttranslational modification is recognized by 53BP1. The molecular mechanism of nonhistone methyl-lysine mark recognition remains unknown. Here we report a 1. 6-Å-resolution crystal structure of the tandem Tudor domain of human 53BP1 bound to a p53K382me2 peptide. In the complex, dimethylated Lys382 is restrained by a set of hydrophobic and cation–π interactions in a cage formed by four aromatic residues and an aspartate of 53BP1. The signature HKKme2 motif of p53, which defines specificity, is identified through a combination of NMR resonance perturbations, mutagenesis, measurements of binding affinities and docking simulations, and analysis of the crystal structures of 53BP1 bound to p53 peptides containing other dimethyl-lysine marks, p53K370me2 (p53 dimethylated at Lys370) and p53K372me2 (p53 dimethylated at Lys372). Binding of the 53BP1 Tudor domain to p53K382me2 may facilitate p53 accumulation at DNA damage sites and promote DNA repair as suggested by chromatin immunoprecipitation and DNA repair assays. Together, our data detail the molecular mechanism of p53–53BP1 association and provide the basis for deciphering the role of this interaction in the regulation of p53 and 53BP1 functions.

Keywords: Tudor domain; methylated p53; 53BP1; structure; cancer

Introduction

The tumor suppressor p53 is found to be mutated in about half of all human malignancies. It functions as a transcription factor that regulates the expression of genes essential for the initiation of cell cycle arrest, DNA damage repair, cellular senescence, and apoptosis.[1,2] The transcriptional activity of p53 is modulated by multiple posttranslational modifications.[3] Over 20 Ser and Thr residues throughout the p53 sequence can be phosphorylated, often leading to elevated activity of p53. Ubiquitination of Lys residues in the carboxy-terminal region directs p53 for proteasomal degradation, whereas acetylation of these residues protects p53 from ubiquitination, promoting p53 stability and accumulation, and alters DNA binding and interactions with cofactors.[4,5]

Three lysine residues—Lys370, Lys372, and Lys382—of the p53 carboxy-terminal regulatory domain undergo methylation.[6-9] Monomethylation of Lys372 by Set9 methyltransferase facilitates transcription of p53 target genes,[6] whereas monomethylation of either Lys370 or Lys382 by Smyd2 and Set8/PR-Set7, respectively, represses p53

*Corresponding author. E-mail address: Tatiana.Kutateladze@UCDenver.edu.

Abbreviations used: 53BP1, p53 binding protein 1; PDB, Protein Data Bank; DSB, double-strand break; ChIP, chromatin immunoprecipitation; Fmoc, 9-fluorenylmethoxycarbonyl.

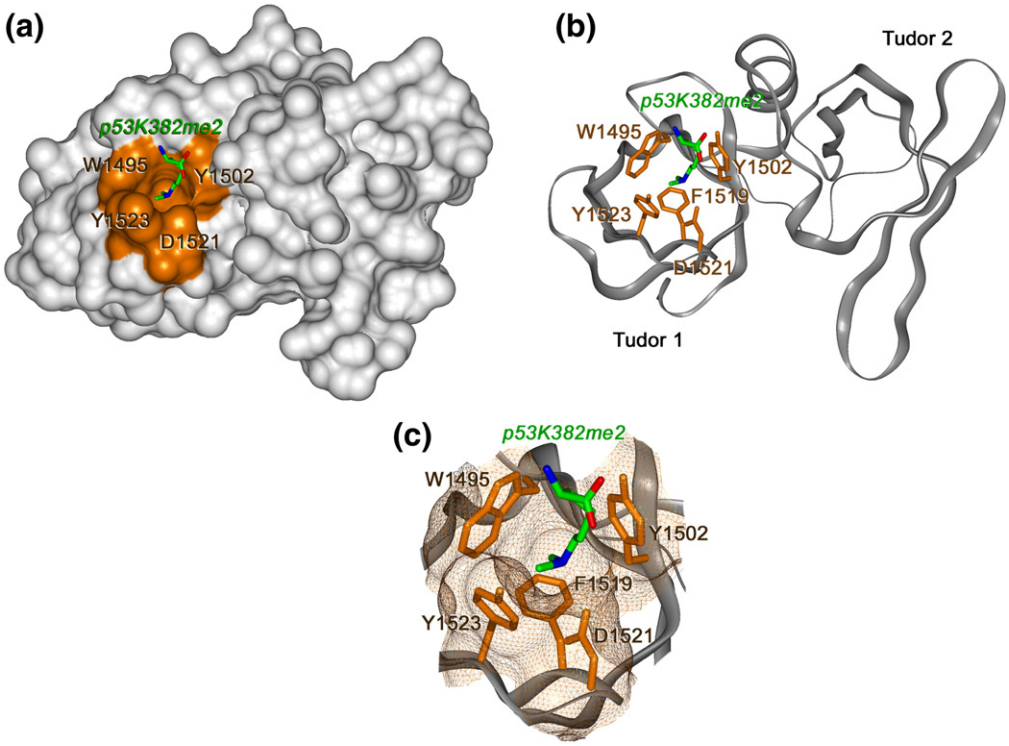

Fig. 1. Structure of the 53BP1 tandem Tudor domain in complex with a p53 peptide dimethylated at Lys382. (a) 53BP1 is shown as a solid surface, with the residues comprising the K382me2 binding cage shown in orange and labeled. Lys382me2 of the p53K382me2 peptide is shown as a stick model, with C, O, and N atoms shown in green, red, and blue, respectively. (b) Ribbon diagram of the structure. (c) Close-up view of the K382me2 binding cage.

function.[7,8,10] Similarly to dimethylation of p53 at Lys370 (p53K370me2),[7] dimethylation at Lys382 (p53K382me2)[8,11] enhances p53 stability and activation through association with the Tudor domain of p53 binding protein 1 (53BP1), a key DNA damage response mediator;[12] however, how the nonhistone methyl-lysine marks of p53 are recognized remains unknown.

Here we elucidate the molecular mechanism of p53 recognition by 53BP1 by determining a 1. 6-Å-resolution crystal structure of the human 53BP1 tandem Tudor domain in complex with a p53K382me2 peptide; establish the determinants of specificity for the p53 sequence by using a combination of NMR resonance perturbations, mutagenesis, measurements of binding affinities, and docking simulations, and by analyzing the crystal structures of 53BP1 bound to p53K370me2 and p53K372me2 (p53 dimethylated at Lys372) peptides; and propose the role of p53K382me2–53BP1 interaction in p53 accumulation and DNA repair.

Results and Discussion

Overall structure and recognition of dimethylated Lys

The p53K382me2-bound 53BP1 tandem Tudor domain folds into two almost identical modules, each consisting of four twisted anti-parallel β-strands and a short α-helix (Fig. 1). A longer carboxy-terminal helix α2 is tightly packed against both modules. The overall fold of 53BP1 in complex with p53K382me2 is similar to the fold of the ligand-free or histone H4K20me2-bound protein [Protein Data Bank (PDB) codes 1xni, 2g3r, and 2igo].[13,14] The structures of the p53K382me2-bound and unbound states superimpose with a root-mean-square deviation (rmsd) of 1.1 Å and 0.2 Å over C^{α} atoms, respectively, indicating that binding to the p53K382me2 peptide triggers only a minor conformational change in 53BP1.

The distinctive feature of p53 recognition by the 53BP1 tandem Tudor domain is an efficient coordination of dimethylated Lys382. The binding pocket for K382me2 is formed by four aromatic residues, W1495, Y1502, F1519, and Y1523, and an acidic residue, D1521, of the amino-terminal Tudor1 module (Fig. 1c). The aromatic side chains of W1495, Y1502, and Y1523, positioned perpendicular to each other and orthogonally to the protein surface, create three walls of the aromatic cage, whereas F1519 lies at the bottom of the cage. The fourth wall is formed by D1521. The aromatic residues make favorable hydrophobic and cation–π contacts with the dimethylammonium group of Lys382, whereas the carboxylate of D1521 forms a hydrogen bond with the only amino proton and a salt bridge with the ion. This mode of dimethyl-lysine recognition is reminiscent of the mechanism for discrimination of the low lysine methylation state, described previously for the interaction of 53BP1 tandem Tudor domain with H4K20me2 and