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Tetramerization-defects of p53 result in aberrant ubiquitylation and transcriptional activity

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ABSTRACT

The tumor suppressor p53 regulates the expression of genes involved in cell cycle progression, senescence and apoptosis. Here, we investigated the effect of single point mutations in the oligomerization domain (OD) on tetramerization, transcription, ubiquitylation and stability of p53. As predicted by docking and molecular dynamics simulations, p53 OD mutants show functional defects on transcription, Mdm2-dependent ubiquitylation and 26S

Abbreviations: OD, Oligomerization Domain; Bax, Bcl2-associated X protein; Bcl-XL, B-cell lymphoma-extralarge; PUMA, p5pregulated Modulator of Apoptosis; Noxa, Noxious stress induced protein; Bid, BH3 interacting domain death agonist; FAS/CD95, Fas (TNF receptor superfamily, member 6); p21, Chromosome 6p21.2 located Cyclin-dependent kinase inhibitor 1A; E2F1, E2F transcription factor 1; Mdm2, Mouse double minute 2 homolog ubiquitin ligase; PCNA, Proliferating Cell Nuclear Antigen; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; 14.3.3σ, 14-3-3 sigma protein eluted in the 14th fraction of bovine brain homogenate and migrating on position 3.3 after electrophoresis; MD, Molecular dynamics; FTDOCK, FFT-based docking program; ADR, Adriamycin; RMSD, Root mean square deviation.

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proteasome-mediated degradation. However, mutants unable to form tetramers are well degraded by the 20S proteasome. Unexpectedly, despite the lower structural stability compared to WT p53, p53 OD mutants form heterotetramers with WT p53 when expressed transiently or stably in cells wild type or null for p53. In consequence, p53 OD mutants interfere with the capacity of WT p53 tetramers to be properly ubiquitylated and result in changes of p53-dependent protein expression patterns, including the pro-apoptotic proteins Bax and PUMA under basal and adriamycin-induced conditions. Importantly, the patient derived p53 OD mutant L330R (OD1) showed the more severe changes in p53-dependent gene expression. Thus, in addition to the well-known effects on p53 stability, ubiquitylation defects promote changes in p53-dependent gene expression with implications on some of its functions.

1. Introduction

The tumor suppressor p53 is a major gatekeeper of the genome that tightly controls critical processes in the cell, acting as a central player within a large network of proteins involved in DNA-repair, apoptosis or cell cycle (Levine, 1997; Vogelstein et al., 2000; Zilfou and Lowe, 2009). P53 is a transcription factor that binds its DNA consensus sequence as a tetramer to activate transcription. The C-terminus of p53 contains the oligomerization domain (OD) that is required for the formation of the transcriptionally active tetramer. The C-terminus also contains sub-cellular localization signals and amino acids targeted by a wide variety of posttranslational modifications, including phosphorylation, acetylation, methylation, SUMOylation (Alarcon-Vargas and Ronai, 2002; Chuikov et al., 2004; Rodriguez et al., 1999) and ubiquitylation that control its degradation by the 26S proteasome (Maki, 1999; Rodriguez et al., 2000) and its function (Funk et al., 1992; Maki and Howley, 1997; Maltzman and Czyzyk, 1984). Data from *in vivo* experiments in mouse models have suggested that ubiquitylation of lysines located in C-terminal may play a role in the regulation of transcriptional activity in a manner of "fine-tuning" the expression of specific p53-dependent genes (Krummel et al., 2005) (Feng et al., 2005). Among the most important p53-dependent genes, the ubiquitin protein-ligase Mdm2, has been shown to be crucial for regulation of p53 function (Fang et al., 2000) and its proteasomal degradation (Chowdary et al., 1994; Maki, 1999; Maki et al., 1996). Other well-known p53-target-genes are Bax, PUMA, Noxa, Bid, Fas/CD95, which are involved in apoptosis, while p21 and E2F1 control cell cycle arrest and proliferation (Riley et al., 2008).

P53 is found mutated in about half of human cancers (Hollstein et al., 1991; Soussi and Beroud, 2001) with most of the mutations being missense and mainly located in the core DNA-Binding domain. To date, the database of *TP53* maintained at IARC, has registered more than 30,000 mutations in humans (www-p53.free.fr/www-p53.iarc.fr) (Olivier et al., 2002). Few mutations have been registered in its OD (Milner and Medcalf, 1991; Sturzbecher et al., 1992). After epidemiologic studies, 17% of germline mutations in patients with Li-Fraumeni syndrome occurred in the p53 OD (Petitjean et al., 2007). Transcriptionally silent p53 mutants generate transdominant negative effects over a WT allele through mechanisms that are not well understood. The aberrant accumulation of mutant p53 in tumor cells suggests an alteration in its degradation pathway. Intrigued by these aspects, we have introduced single point mutations in the β-strand of the p53 OD, mimicking the alterations in L330 observed in cancer, which is one of the crucial amino acids for correct tetramerization (Chene and Bechter, 1999). NMR and X-Ray crystallography analysis revealed that OD (residues 323–356) contains one β-strand (326–333) and one α-helix (335–354) with a V-shaped structure (Chene, 1997; Clore et al., 1995; Jeffrey et al., 1995; Lee et al., 1994; Miller et al., 1996; Mittl et al., 1998). The hydrophobic amino-acid L330 together with seven other residues (F328, I332, R337, F338, F341, N345 and D352) form a critical hydrophobic pocket, allowing association of two dimers, necessary for the formation of a transcriptionally active tetramer and for preservation of p53 stability (Clore et al., 1995; Friedman et al., 1993; Jeffrey et al., 1995; Kato et al., 2003; Kawaguchi et al., 2005; Mateu and Fersht, 1998).

2. Materials and methods

2.1. Modeling of the p53 dimer structure by docking simulation

We used the monomeric structure of the p53tet PDB 1AIE; 1.5 Å in its active tetrameric form as starting structure for the WT dimer model and as scaffold to build all the structures of the mutants described herein, using SCWRL3.0 program (Canutescu et al., 2003). The rigid-body docking simulations were performed using the FFT-based docking program FTDOCK with grid resolution of 0.7 Å and electrostatics (Gabb et al., 1997), which generated a set of 10,000 docking poses that were evaluated by the energy-based pyDock scoring scheme (Cheng et al., 2007). For each docking run, we selected the lowest-energy solution. RMSD was calculated for a monomer respect to its equivalent in the p53 dimer structure (as found in the p53 biological assembly) after superposition of the interacting partner monomer.

2.2. Molecular dynamics simulation of WT and mutant p53 dimer structures

The WT p53 dimer structure for the Molecular dynamics (MD) simulation was extracted from PDB code 1AIE. The mutants were built *in silico* from the WT structure, using the AMBER

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