Detailed Computational Study of Point Mutations of the TP53 Tumor-Suppressor Protein

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It is becoming progressively accepted that the progression of mammalian cells towards malignancy is an evolutionary process that involves an accumulation of mutations on both the molecular and chromosomal level. Inherent in models for malignant progression is the concept that an initial mutation in an important regulatory gene (protein) may be pivotal in this process. Once the initial mutation is introduced, loss of normal gene function or the acquisition of deleterious functions may lead to additional mutations furthering the malignant transformation of the cell.

A candidate for the involvement in this process is the tumor suppressor, p53. The p53 protein provides one of the key regulatory elements monitoring genomic integrity in mammalian cells and is involved in a multiplicity of cellular functions. This ubiquitous factor is kept in a repressed state in normal cells, but is activated by post-translational modifications in response to forms of stress, both genotoxic (such as irradiation, chemical carcinogens, or cytotoxic agents used in cancer therapy), or non-genotoxic (such as hypoxia, depletion of ribonucleotides, and oncogenic activation of growth signaling cascades).[1] When active, the p53 protein accumulates to high levels in the nucleus and acts as a multi-functional transcriptional factor to enhance or repress the expression of several genes involved in cell cycle progression, apoptosis, adaptive response to stress, differentiation and DNA repair[2].

Tumor-specific p53 mutations were first identified in 1989.[3] Loss of p53 function is the most common event in human cancer, with more than half of all invasive tumors involving the decrease or total loss of p53 functions. In contrast to many other tumor suppressors, which are often inactivated by deletion or frameshift mutations, most of mutations in TP53 are point mutations (missense mutations: 75%; nonsense mutations: 8%). These mutations are exceptionally diverse in their nature and position. Thus, it is possible to draw tumor-specific mutation spectra that show significant differences from one type of cancer to the other. This observation has two very important implications: first, the spectrum of mutations reveals information on the mutagenic process that cause human cancer, and second, the whole set of mutations observed in cancer can be analyzed as an immense, in vivo, random mutagenesis experiment aimed at identifying residues which are important in the maintenance of the tumor suppressive function of the protein.

The open reading frame (ORF) of human p53 codes for 393 amino acids, consisting of three major structural domains: an N-terminal domain, which contains a strong

transcription activation signal,[4] a DNA-binding core domain (CD), and a C-terminal domain, which mediates oligomerization (see Figure 1).

Figure 1. Core domain of the tumor-suppressor protein p53.

A detailed analysis of p53 mutations based on evolutionary conservation has been performed by Walker and coworkers.[5] The vast majority of the mutations of p53 cluster in conserved regions of the CD (residues 96-292), and some 20% of all mutations are concentrated at five "hotspot" codons in the core domain: 175, 245, 248, 249 and 273.

The notion that several categories of mutant may exist has received much attention since it was realized that not all mutants are functionally equivalent, and substitutions in p53 CD have been classified in a number of ways. In general, however, the substitutions found in cancer fall into two broad categories: 1) *contact mutations*, located at the p53/DNA interface and breaking crucial contacts between the protein and the nucleic acid, or between p53 and other proteins and 2) *conformational mutations*, located in the protein skeleton and thought to prevent its correct folding, thus thwarting high-affinity DNA binding. Indeed, accurate thermodynamic experimental analyses[6-8] have revealed that p53 is only marginally stable at body temperature, so any mutation which further reduces stability is likely to lead to unfolding/misfolding in vivo.

The work presented here is the first attempt to exploit the knowledge available on p53 protein structure and the power of sophisticated molecular modeling techniques to classify the various types of mutants into specific categories. To this purpose, we obtained a 3D molecular model of p53 starting from the relevant human p53 crystal structure (entry 1TSR in PDB), by applying a relaxation procedure[9] based on AMBER force field[10] and GB/SA continuum solvation model[11]. As expected, no relevant structural changes were observed between the p53 relaxed structure and the original 3D structure, as depicted in Figure 2. Accordingly, the following 20 cancer-associated mutants that are distributed throughout the CD and are representative of the p53 mutation database have been considered and modeled:

R175H	M237I	C238Y	C242S	R248Q	R273H
F134L	G245S	R249S	R282W	R282G	V143A
L145Q	P151S	V157F	I195T	Y220C	I232T
I255F	F270C				

where the mutations in red belong to the Zn^{2+} binding region, those listed blue are tumorigenic mutations at the two key DNA-contact residues, Arg 248 and Arg 273, those

written in green refer to the so-called DNA-region and the remaining mutations belong to the β-sandwich, the fundamental protein structural scaffold.
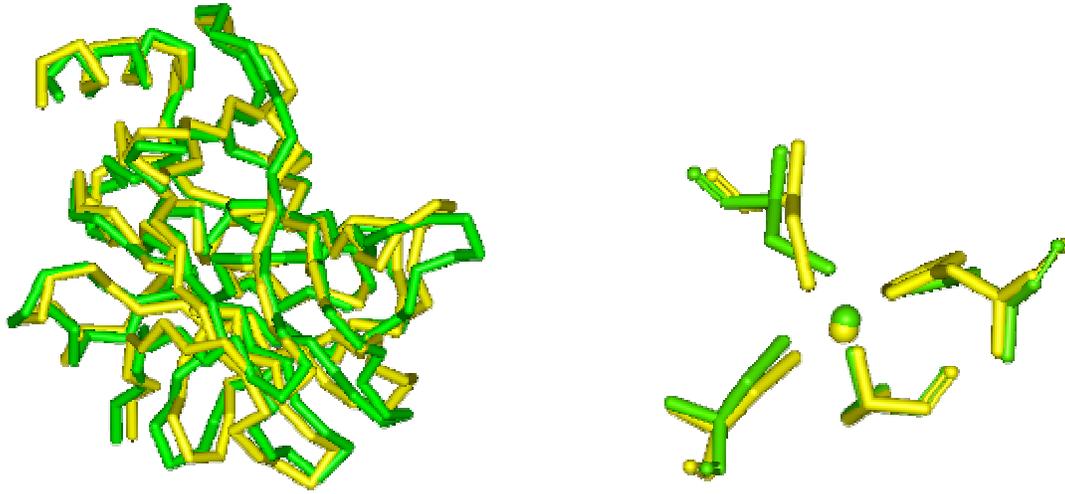

Figure 2. Overlapped images of the starting (green) and energy minimized (yellow) models of the entire CD (left, rmsd = 1.4 Å) and of the zinc-binding region (right, rmsd = 0.4 Å) of p53.

Several sets of molecular dynamics simulation experiments were carried out on the wild-type (WT) and its mutants. Molecular energies were estimated[9] from molecular mechanical energies (G_{MM}) and the solvation free energy ($G_{PB} + G_{NP}$). Solvent accessible surfaces of the considered residues were calculated using a method based on semi-empirical molecular orbital calculations[12]. Alanine scanning mutagenesis[13] was applied to evaluate the relative energy of each mutation with respect to the WT.

The results obtained so far revealed that, in agreement with the experimental findings, all mutants are destabilized with respect to the WT, and allowed us to rationalize the data set of mutations considered into 4 different classes, characterized by well-defined patterns of energy-structure relationship.

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