1556 Angels and demons of the guardian of the genome. A coupled modeling-biochemical study of p53 mutants in ovarian cancer

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Background
Ovarian carcinoma is the deadliest gynecologic malignancy, and the *TP53* tumor suppressor gene is found mutated in 60% of ovarian tumors. Some p53 protein mutations could be predicted to compromise p53 function. In this work, we reported a jointed modeling/biochemical approach to investigate several p53 core domain (CD) mutations, detected in ovarian cancer patients who were found to be either responding or non-responding to different therapeutic regimens.
Materials and methods
Using the so-called MM/PBSA computational technique, we calculated both the thermodynamic stability of each protein, and the relative affinity for DNA. Contemporarily, we introduced the same p53 mutations into a wild-type p53 expressing vector by site-specific mutagenesis and then transfected these vectors into a mammalian p53 null cell system (SaOs). By western blotting, we will detect the expression levels of different proteins involved in p53-dependent apoptosis.
Results
The calculated thermodynamic stabilities and DNA binding energies for some mutants were in excellent agreement with the available experimental data. Table 1 reports the comparison between calculated and experimental difference in free energy of denaturation between wild-type and mutated p53 for some mutations, as an example.
Globally, the calculations on both core domain and full length allowed classifying the different p53 mutants in two major classes:
- Class 1, for which the presence of the mutant residue induces an alteration of the structural/energetical features of p53, apparently affecting other active domains of the protein but not interfering with DNA binding;
- Class 2, for which the mutant residue also impairs DNA binding.
From the experimental standpoint, we are currently optimizing a biochemical approach to investigate the expression level of several proteins involved in p53-dependent apoptosis (Parp, caspase 3, Bax, p21) in SaOs cells transfected with wild type or mutated *TP53* gene. This with the ultimate goal of evaluating the possible impact of the mutations on the capacity of p53 to induce apoptosis, making the cell responding or non-responding to different therapeutic regimens.

Table 1. Comparison between experimental and calculated free energy difference

Mutation	$\Delta\Delta G_{exp}$ (kcal/mol)	$\Delta\Delta G_{calc}$ (kcal/mol)
R273H	0.45 0.04	0.68 0.15
Y220C	3.98 0.06	3.59 0.13
I195T	4.13 0.06	4.00 0.19

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