Tumour Suppressor p53: structure, aggregation and rescue

PL-10

A. Fersht[I]

[I]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The tumour suppressor p53 is an archetypical intrinsically disordered protein, having two stably folded domains, with the remaining 37% of its sequence disordered. If p53 and its apoptopic pathways are functional, cancer cells are doomed. Consequently, it has the most frequently mutated gene in cancer. Reactivation of inactivated mutant p53 is an attractive target for the development of anti-cancer drugs. To provide a rational basis for designing such drugs, we performed a rigorous biophysical analysis of the inactivation of p53 before designing candidate molecules and testing with cancer cell lines. Some 30% of inactivating mutations just lower the stability of p53. Further, p53 is kinetically unstable and those oncogenic mutants even more so, rapidly aggregating. We solved the structure of the most common oncogenic mutants. One highly destabilized mutant, Y220C, has a mutational surface cavity. We developed small compounds that raise its melting temperature and restore its activity. They rescue the activity of Y220C in cancer cell lines and induce apoptosis. To probe the feasibility of preventing aggregation by other routes, we analysed the aggregation mechanism. p53 aggregates to give an amorphous structure that gives the characteristic diffraction pattern and bind the dyes diagnostic of amyloid fibrils. A detailed phi-value analysis shows that the first step in aggregation is the extensive unfolding of the p53 core domain followed by the binding binding and extensive unfolding of a second core domain, with many beta strands participating. Several aggregation prone sequences in the protein self- and cross-aggregate. Mutation of any one of those sequences does not prevent formation of the amorphous aggregate. Our most successful molecule for the general rescue of p53 mutants reacts covalently with two surface-exposed thiol groups, stabilising p53 thermodynamically and also eliciting cytotoxic effects specifically in cancer cells.