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Review

Structural and sequential context of p53: A review of experimental and theoretical evidence

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ABSTRACT

Approximately 27 million people are suffering from cancer that contains either an inactivating missense mutation of *TP53* gene or partially abrogated p53 signaling pathway. Concerted action of folded and intrinsically disordered domains accounts for multi-faceted role of p53. The intricacy of dynamic p53 structure is believed to shed light on its cellular activity for developing new cancer therapies. In this review, insights into structural details of p53, diverse single point mutations affecting its core domain, thermodynamic understanding and therapeutic strategies for pharmacological rescue of p53 function has been illustrated. An effort has been made here to bridge the structural and sequential evidence of p53 from experimental to computational studies. First, we focused on the individual domains and the crucial protein–protein or DNA–protein contacts that determine conformation and dynamic behavior of p53. Next, the oncogenic mutations associated with cancer and its contribution to thermodynamic fluctuation has been discussed. Thus the emerging anti-cancer strategies include targeting of destabilized cancer mutants with selective inhibition of its negative regulators. Recent advances in development of small molecule inhibitors and peptides exploiting p53–MDM2 interaction has been included. In a nutshell, this review attempts to describe structural biology of p53 which provide new openings for structure-guided rescue.

Contents

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1. Introduction

Spatio-temporal information of diverse proteins correlating their function in the tumor milieu is emerging as an important aspect of studying protein function in cancer microenvironment. The major factor responsible for cancer is the cumulative dynamic changes associated at the genomic level (Hanahan and Weinberg, 2000). This information alone is not considered to be sufficient to explain the equally intricate complexity in function of protein over time and space. Understanding how diverse proteins come together spatially as well as temporally and the translation of their unambiguous interactions into specific cellular response is therefore crucial. Linking structural changes of protein in the course of mutation with its altered cellular localization is therefore far more important to illustrate its function with respect to uncontrolled cellular proliferation. Thus the recent approach for studying molecular events in cancer is focusing more on the regulation of spatio-temporal distribution of proteins and their activity which can coordinate sequence–structure–function relationship with the progression of the disease. Tumorigenesis can be explained by a process of acquiring successive genetic mutations that transforms a normal cell into a malignant one. These genetic errors give rise to sustained proliferative signaling, enabling replicative immortality, evasion of growth suppression signals, resistance to cell death and tumor angiogenesis. This accelerates event like tumor cell invasion and metastasis. It is the deregulated expression of transcription factors, during tumorigenesis which promote proliferation and differentiation of the neoplastic population (Libermann and Zerbini, 2006). The relative expression of these proteins is found to be altered in cancer compared to that of the normal cells. The tumor suppressor protein p53 acts as a cell cycle regulator, involved in maintaining the genetic integrity and is popularly known as guardian of the genome. Control over the cell cycle machinery, apoptosis and DNA repair (Levine, 1997), are the critical activities of the tumor suppressor that elicits an anti-cancer response. Activation of p53 is triggered by events like DNA damage, hypoxia, heat shock and various other stress signals. Depending on the nature of the stress it decides the specific cellular outcome in an order to restrict any anomaly at the genetic level. Once activated, the confirmation of p53 undergoes modifications at both N- and C-terminal regions (Jenkins et al., 2012). Mutations affecting the three-dimensional structure of p53 have been reported to cause aberrant nucleo-cytoplasmic shuttling, cytoplasmic retention or mis-localization, thereby resulting in loss of its tumor suppressor functions.

The p53 family of transcription factors comprises the gene products of TP53, TP63, and TP73 genes. These proteins share a high-degree of homology in structure as they have evolved from the common ancestor in course of evolution (Belyi et al., 2010). p53 mutation develop variety of cancers including breast carcinomas, sarcomas, brain tumors, adrenal cortical carcinomas, defining the Li-Fraumeni and Li-Fraumeni-like syndromes (Olivier et al., 2003).

The family members of p53 are not only found in vertebrates but also in many invertebrates including mollusks, insects, and worms (Yang et al., 2000). Within the higher vertebrates, p63 and p73 have taken on new functions in the development of tissues and organs, whereas p53 has become the guardian of the somatic genome and a tumor suppressor as well. Mutation in p63 gene show developmental disorder like cleft palate, skeletal abnormalities, skin pathologies, but they do not develop cancers at high rates (Yang et al., 1999). p73 is involved in the development of the central nervous system and immune system (Belyi and Levine, 2009). It is to note that p73 can act as a back-up for p53 in response to various stress signals and can initiate apoptosis (Yang et al., 2000).

In the present review, an attempt has been taken for understanding the sequence and structural architecture of p53 protein and its importance with respect to cancer biology. Along with the information detailing structure–function affairs of proteins elucidated with experimental techniques, it is of high value to consider the computational aspects for exploring important physiological functions of biologically relevant molecules like p53 (Lane et al., 2011). Adoption of ideology with appropriate techniques, such as homology modeling, molecular docking and molecular dynamics to account the dynamicity and conformational attributes, could be useful in addressing important biological questions. Similar concepts such as free energy evaluation, energy landscape and water dynamicity have been discussed here with reference to the reported literature in context to p53 family. Thus evidences of sequential and structural information of p53 using relevant experimental and computational methods have been reviewed in this article, which explains functional signature of p53 in the biology of cancer (Brown et al., 2009).

2. Structure and function

2.1. Individual p53 domains

The general understanding of the biological concept necessitates an integration of experimental as well as computational research. An overview of the reported experimental and computational studies has been discussed in context to structure and function of p53. The theoretical studies have either correlated or supplemented additional information in conjunction to the experimental approach. Such an attempt will not only present a detailed overview of p53, but will also motivate to develop robust way-out to explore the missing link from structure to drug-design.

TP53 gene that resides on chromosome 17p13.1 encodes the 393 amino acid long protein-p53, which is the most frequent target for mutation in human cancer (Olivier et al., 2010). The functional state of p53 is homo-tetramer, where each monomer consists of an intrinsically disordered N-terminal transactivation domain (Met1-Asp42), a proline-rich domain with multiple copies of PXXP sequence (Asp61-Ser94), a central DNA binding core domain (Thr102-Lys292) and a C-terminal domain (Pro301-Asp393) containing a tetramerization domain (Asp324-Ala355) (Fig. 1).

The N-terminal domain of p53 is required for making contacts with the transcriptional co-activators or co-repressors (Fig. 2A). Biochemical and biophysical studies have revealed that this region of helix with double β-turn, occupy many structural motifs. It is noteworthy to mention that the secondary structure of this region is transiently stable. This region is crucial for binding with MDM2; where upon binding, there is a change in secondary structure form random coil to helix (Thr18 to Leu26) (Dawson et al., 2003). Kussie et al. have deciphered a part of p53 N-terminal domain structure in complex state with MDM2 (Fig. 2A) (Kussie et al., 1996). Espinoza-Fonseca et al. have further explored the helical property of the N-terminal domain using molecular dynamics (MD) simulation (Espinoza-Fonseca and Trujillo-Ferrara, 2006). It is interesting to find that the region of p53 (Phe17 to Leu22), depicts a stable picture of helical conformation in the simulation studies. The aromatic stacking (π–π interaction) between Phe19 and Trp23 is also believed to be crucial in perturbing the secondary structure. Mutational studies in conjunction to MD simulation also reveal these two amino acids to be critical for functional role and maintaining structural stability of p53 (Lee et al., 2000). It can be concluded from these studies that the short region (Phe19 to Leu22), have anchor role for interaction between p53 and MDM2. Considering the residue-wise energetics contribution, it is Phe19 (−6.6 kcal/mol), Leu22 (−2.5 kcal/mol), Trp23 (−5.5 kcal/mol) and Leu26 (−3.9 kcal/mol) of p53 which are the key amino-acids for its interaction with MDM2 (Joseph et al., 2010). Binding of MDM2

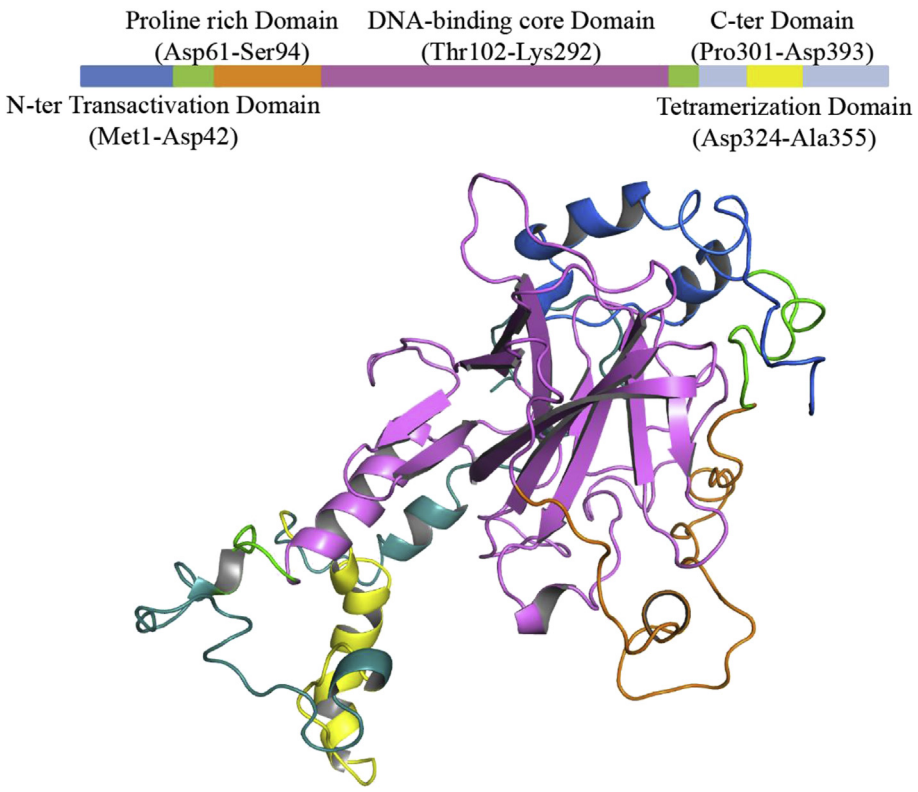

Fig. 1. Figure depicting sequence and structure of full length p53. Individual domains of the protein have been highlighted using various colors. The full length structure of p53 has been prepared with homology modeling and molecular dynamics (MD) simulation.

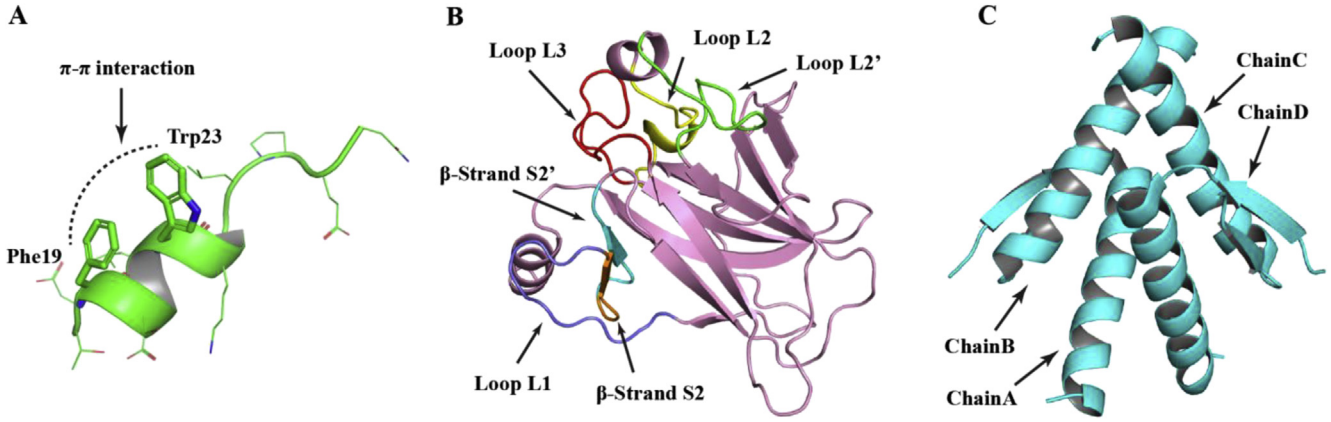

Fig. 2. Details of individual domains of p53. (A) N-terminal transactivation domain showing π−π interaction between Phe19 and Trp23 (PDB code 1YCR). (B) Loop-Sheet-Helix arrangement of DNA binding core domain (PDB code 1TUP). (C) C-terminal region showing close packing of tetramerization domain of p53 tetramer (PDB code 2J0Z).

deploys p53 from functioning with transcriptional machinery and also exposes it for ubiquitination. Using this information, much emphasis is being focused on this region so as to develop small molecules and peptides to disrupt the MDM2—p53 complex formation.

DNA binding region of p53 can be narrated to contain antiparallel β-sheet sandwiched framework (Joerger and Fersht, 2007b). The secondary structural arrangement is composed of traditional loop-sheet-helix motifs, containing loop L1 (Phe113 to Thr123), two beta strands S2 and S2′ and C-terminal domain (Fig. 2B), which bind to the major groove of DNA. Another structural part of the DNA binding domain which binds to minor groove are two large loops L2 (Lys164-Cys176 and Cys182-Lys194) and L3 (Met237 to Pro250) (Cho et al., 1994). L2 also communicates with L1 for establishing a stable DNA-p53 complex. A remarkable feature of loop L2 and L3 is accommodation of Zn^{2+} ion which aids in structural stability. This Zn^{2+} ion use to be tetrahedrally co-ordinated by Cys176, His179, Cys238, and Cys242 (Joerger et al., 2004). An increase in aggregation propensities that are further accompanied by structural fluctuations affecting DNA binding specificity is the outcome of Zn^{2+} ion loss. MD simulation studies also predict a high degree of structural fluctuation in the adjacent loops due to the loss of Zn^{2+} ion (Duan and Nilsson, 2006). It is significant to note that many of the authors did not attempt to fully investigate the Zn^{2+} ion and associated residues (His179, Cys176, Cys238 and Cys242) by computational means. The reason associated being the

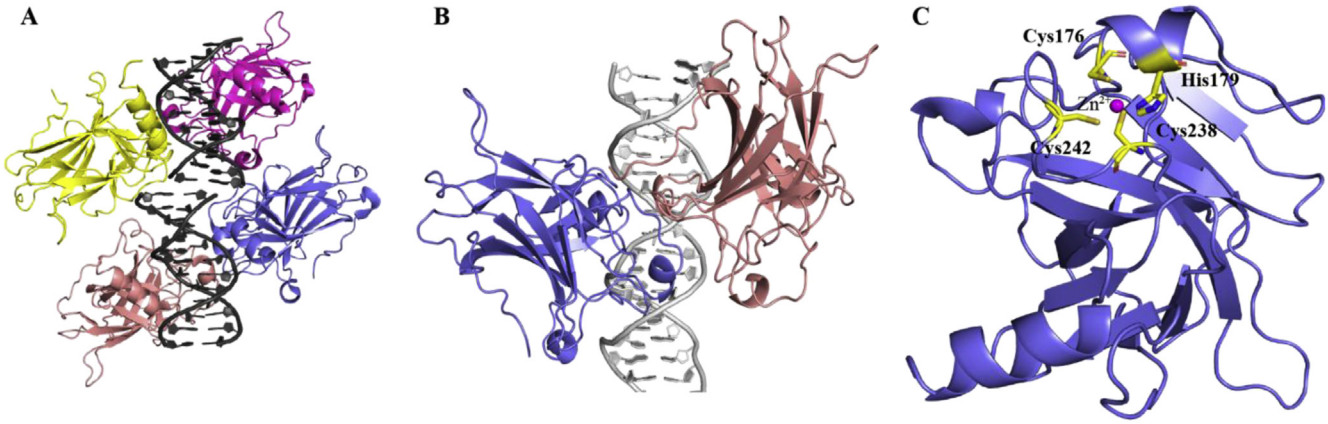

Fig. 3. Structural arrangement of p53 complex with DNA. (A) Close packing of p53 tetramer binding to major and minor grooves of DNA (PDB code 2ATA). (B) Symmetrical arrangement of p53 dimer in complex with DNA (PDB code 1TUP). (C) Zn^{+2} binding region in the DNA binding domain of p53. The amino acids involved in coordination with Zn^{2+} ion have been highlighted in yellow color (PDB code 2OCJ).

requirement of quantum calculations and sufficient parameterization. Lu et al. have attempted the work with the help of RESP charges and antechamber mediated GAFF utilization for similar investigation. It is hence essential to focus on exploring crucial information in future for finding novel therapeutic means by using more complex computational calculations (Lu et al., 2007).

Loop L1 is known to be highly flexible containing disordered secondary structure, which is important for p53 functioning (Lukman et al., 2013). L1 contains few amino acids with low incidence of mutational rate, designated as 'cold-spot' mutation (Lukman et al., 2013). The DNA binding core domain of p53 also shows low intrinsic thermodynamic and kinetic stability (Cañadillas et al., 2006; Khoo et al., 2009). According to International Agency for Research of Cancer (IARC) TP53 Mutation Database, more than 95% of the cancer mutations turned out to be in this domain (Olivier et al., 2002). It was observed that there is a high frequency of mutation in few residues, which are popularly known as 'hot-spot' mutations (Harris and Hollstein, 1993; Ma and Levine, 2007; Petitjean et al., 2007).

Lu et al. have explored the conformational analysis of DNA binding domain with the help of MD simulation in presence and absence of DNA (Lu et al., 2007). The major conformational difference in p53 structure was found near to the DNA-binding interface. Both the loops L1 and L3 were found to be perturbed in absence of DNA. The hydrophobic core in the β-sandwich region (Ilu195 and Val143) was restrained in the simulation studies emphasizing on the fact that these residues are responsible for protein stability (Lu et al., 2007). The interactions within the protein such as Arg175-Ser183, Ser183-Arg196 and Gln198-Asn235 were found to be decisive for DNA binding (Lu et al., 2007). Furthermore it was also revealed that it is the symmetries of p53 structure which helps in DNA recognition for binding (Kitayner et al., 2006). Reports by Ma et al. and Pan et al. independently suggest that p53 tetramer can bind with DNA using two monomers and thereby induce DNA bending (Ma and Levine, 2007; Pan and Nussinov, 2007). Using MD simulation it was later revealed that the interaction between p53 and DNA complex is mediated not only in sequence specific but also in geometry specific manner (Fig. 3). The individual DNA binding domain of monomeric and multimeric p53 was found to be similar structurally as well as thermodynamically (Lubin et al., 2010). The sequence dependent concept was earlier explored by computation means where it was found that different DNA sequences account for DNA bending in a range of 20°–35° (Nagaich et al., 1999). This domain is connected to the tetramerization domain via a short linker. This tetramerization domain regulates the oligomerization process of p53.

2.2. Other family members of p53

For over two decades, p53 was considered to be the only gene of its kind in the vertebrate genome, which was proved to be wrong in successive years (Levrero et al., 1999). There is a high-degree of functional specificity between p63 and p73 with p53, owing to their duplication and divergence in the course of evolution. This trace of p53 gene evolution has been accounted using bioinformatics tool by Pintus et al. (2007). The oligomerization domain of paralogs p63 and p73, have a conserved C-terminal sterile alpha motif (SAM) which confers protein stability (Dötsch et al., 2010). Alternative splicing of both p63 and p73 give rise to various isoforms, known as ΔNp63/p73α, ΔNp63/p73β, and ΔNp63/p73γ. These N-terminal deleted p63/p73 isoforms fail to activate transcription of target genes and impede the transactivation functions of full length p53, p63 and p73 (Levrero et al., 2000; Yang et al., 1998).

The use of computational methodology has also been found in the relevant context where a comparison of structural motifs for p53, p63 and p73 has been attempted in lieu of its functional aspects. Falconi et al. explored the interaction and association of two helices in the structure of p73 with respect to C-terminal SAM (Falconi et al., 2004). It was revealed that changes in the conformation of one helix perturb the dynamicity of another helix and thereby the dimerization was not possible. In conjunction to p63 C-terminal SAM, Cicero et al. have prepared the molecular models of mutant Gly534Val, Thr537Pro to compare the dynamic profile with wild type using MD simulation (Cicero et al., 2006). The mutations in SAM domain use to cause Hay–Wells syndrome, and was found to impair the protein–protein interaction (McGrath et al., 2001). It was revealed that the wild type p63 C-terminal SAM lacks any flexible region, whereas the mutant imposes various degrees of freedom in flexibility of the protein which explains the biological question of structural collapse. Similar studies should be explored in the future to investigate the dimer and tetramer formation of p53 with respect to the globular protein structure (Dawson et al., 2003; Lee et al., 2000). In another study, Mavinahalli et al. have explored the nature of trans-activation domain residues from sequence number 17 to 29 of p53, p63 and p73 using MD simulation. Various mutants of these three proteins have also been taken in the study to elucidate why MDM2 interacts preferentially with p53 and p73. It was revealed that the order of helix stabilization for the

transactivation domain was high for p53 and p73, and least for p63, which is responsible for respective interaction with MDM2 (Mavinahalli et al., 2010).

'Gain of function' effect of mutant p53 arises due to its ability to bind and inactivate p63 and p73 when they are ectopically or endogenously expressed. Both "contact" (e.g. Arg248Trp, Arg273His, Arg273Cys) and "structural" (e.g. Arg175His, Arg249Ser, Arg282Trp, Gly245Ser) p53 mutants can engage in direct protein–protein interaction with p63/p73 isoforms through their respective conserved core domains and render them inactive. A comparative analysis of p53, p63 and p73 with DNA and their dimer complex have been evaluated in terms of energy by Madhumalar et al., where the importance of DNA binding domain has been elucidated (Madhumalar et al., 2009).

3. Post-translational modification: route to stabilization

Post-translational modification (PTM) of proteins is considered to be an essential mechanism, where different functional groups are added to the amino acids. Active p53 is subjected to a complex and diverse array of PTMs, which involve the covalent addition of functional groups, such as phosphorylation of Ser/Thr, acetylation, ubiquitination and sumoylation of Lys residues. The consequence of PTM has been conferred by various experimental methods and the effect over the conformational stability and protein functioning has also been explored with the help of computational methodologies. Computation with fixed-charged molecular mechanics energy function using force-field like OPLS, CHARMM, GROMACS and AMBER can be typically used in simulation of proteins for studying PTM (Narayanan and Jacobson, 2009). The common methods include, conventional MD, accelerated MD, torsional angle based sampling and replica-exchange MD simulation. As for example, McCammon and coworker have explored with their method of MD simulations that the rate of Cis/Trans conformational exchange of Pro residue use to slow down upon phosphorylation of preceding Ser (Hamelberg et al., 2005). Phosphorylation of p53 increases its stability and sequence specific DNA-binding (Ashcroft et al., 1999). A wide array of protein kinases that are involved in the process includes ATM, ATR, CHK1/CHK2, CDK, JNK, MAPKAPK2, p38 kinase, GSK3β, HIPK2, PKC and ERK. It was revealed that in response to DNA damage, p53 is subjected to phosphorylation/dephosphorylation at 17 probable target sites. Human p53 includes phosphorylation of (i) Ser 6, 9, 15, 20, 33, 37 and 46, Thr18 and 81 in the N-terminal region; (ii) Ser149, Thr150 and 155 in the central DNA binding region; (iii) Ser315 and 392 in the C-terminal region (Chehab et al., 1999). *In-silico* analysis revealed that phosphorylation of Thr18 does not disrupt the helical conformation of trans-activation domain; but phosphorylation of Thr18 and Ser20 leads to disruption of a crucial H-bond between Thr18 and Asp21, which abrogates p53–MDM2 complex formation (Craig et al., 1999; Schon et al., 2002). Electrostatic analysis using APBS (Adaptive Poisson-Boltzmann Solver) shows that a local anionic patch in the complex formed with MDM2 at position where Thr18-p docks is responsible for weak affinity with MDM2 (Lee et al., 2007). On the contrary, in a report by Mavinahalli et al. it was revealed that Thr18-p results in loss of helical propensity of N-terminal domain where a long range communication between phosphate group and Lsy24 was said to be responsible (Mavinahalli et al., 2010). Phosphorylation at Ser269 and Ser215 of the DNA binding domain of p53 reduces the affinity and specificity for DNA (Fraser et al., 2010a). Fraser et al. showed that the negatively charged phosphate group of Ser269-p was involved in interaction with Gln100 and Thr102, whereas phosphorylation at Ser215-p is interacting with two positively charged residues Arg215 and Arg213 (Fraser et al., 2010b). Owing to the phosphorylation of Ser269-p and Ser215-p, there is a conformational change in p53 which disrupts the structural integrity and results in weaker interaction with DNA. In addition, certain residues like Thr55, Ser376 and Ser378 residues are constitutively phosphorylated in normal cells (Gatti et al., 2000; Waterman et al., 1998).

Tibbetts et al. showed that phosphorylation of Ser15 is stimulated by DNA-damaging agents like ionizing radiation (Tibbetts et al., 1999). This phosphorylation on Ser15 and Ser37 is mediated by DNA-activated protein kinase. Similarly, ATM phosphorylates Ser15 and ATR phosphorylates both Ser15 and Ser37, which induces apoptosis. The phosphorylation at Ser15 is important as it stabilizes p53 through allosteric modification, which results in dissociation of MDM2 from p53. In this context, few interesting evidence were found in the report of Kalid et al. evaluating the energetics of p53 upon mutation and phosphorylation in complexation with MDM2 (Kalid and Ben-Tal, 2009). The energetics of Ser15p, Thr18-p and Ser20-p were found to be −293, −217.9 and −279 kcal/mol respectively, which was found to be higher, compared to non-phosphorylated p53. In case of double phosphorylations, Thr18-p/Ser20-p, Ser15-p/Thr18-p and Ser15-p/Ser20-p, the energetics were found to be −265.1, −292.5 and −361.8 kcal/mol respectively, which confers that the stability of the molecule is favored by the phosphorylation event. In response to ionizing radiation, CHK1/CHK2 phosphorylates Ser20, which also leads to disruption of p53–MDM2 complex. *In-vivo* experiments by Matsumoto et al. showed that mice model bearing both Ser15Ala and Ser20Ala mutations display severe type of tumor development including defect in pro-apoptotic response (Matsumoto et al., 2006). MD simulation of p53 (fragment)-MDM2 complex containing Ser20Ala mutation suggests that there is a complete loss of anchoring phenomenon by Trp23 and Leu26, which causes a rapid drifting in the docked site of MDM2 and hence the complex is not stable (Kalid and Ben-Tal, 2009; Schon et al., 2002).

Phosphorylation at Ser46 use to happen only after stimuli of very high level of DNA damage (Oda et al., 2000). Sakaguchi et al. reported phosphorylation at Ser392 which stabilizes p53 tetramer formation (Sakaguchi et al., 1997). Another study by Jiang et al. using knock-in mice with Ser389Ala (human Ser392Ala) mutation shows normal stability of p53 with high predisposition to UV induced skin cancer (Jiang et al., 2011). It is noteworthy to mention that a significant redundancy in phosphorylation sites of p53 exist, which are found to be shared by multiple protein kinases. A definite set of phosphorylation at defined residues is the minimal requirement for further phosphorylation of p53. In parallel context, Dumaz et al. showed phosphorylation of Ser15 is a pre-requisite factor for phosphorylation at Thr18 (Dumaz et al., 1999). This sequential event invokes maximum activation of the tumor suppressor p53. Considering the atomic insights, side chain orientation of The18 and Ser20 are in close proximity, compared to Thr18 with Ser15, which have different orientation in wild type p53. Phosphorylation can be found to have anionic charge repulsion more in case of Thr18p/Ser20p, and hence Ser15p/Thr18p have little way in interrupting p53–MDM2 interaction (Kar et al., 2002). Dephosphorylation, on the contrary, also contributes to activation of p53. Ser376 and Thr55 are dephosphorylated in cells exposed to ionizing radiation (Li et al., 2004).

Site-specific acetylation also contributes to p53 stabilization, increasing its half-life and transcriptional activation (Barlev et al., 2001). p53 acetylation results in activation of p53 independent of its phosphorylation status. Luo et al. and Ceskova et al. independently found that the acetylation motif of p53 gets exposed upon

Table 1
Site-specific PTM on p53 (Phosphorylation, Acetylation, Ubiquitination and Sumoylation) and their respective cellular consequences.

Sites & type of PTM	Cellular outcome	References
Ser15, 37 phosphorylation	Apoptosis Disruption of p53–MDM2 complex	(Tibbetts et al., 1999; Shieh et al., 1997)
Ser20 phosphorylation	Disruption of p53–MDM2 complex, apoptosis	(Unger et al., 1999; Chehab et al., 1999)
Ser315 phosphorylation	Ubiquitination and degradation of p53	(Katayama et al., 2004)
Ser6,9, Thr18 (requires prior phosphorylation of Ser15)	p53 stabilization through MDM2 inhibition	(Dumaz et al., 1999; Sakaguchi et al., 1997)
Ser392 phosphorylation	Increased p53 activity	(Keller et al., 2001)
Ser315, Ser376 phosphorylation	Inhibits p53 mediated apoptosis	(Qu et al., 2004)
Thr81 phosphorylation	p53 stabilization	(Buschmann et al., 2001)
Ser33, Ser46 phosphorylation	Stabilization of p53, apoptosis	(Bulavin et al., 1999)
Thr150, Thr155, Ser149 phosphorylation	Degradation of p53	(Bech-Otschir et al., 2001)
Ser376, Thr55 dephosphorylation	Activation of p53	(Waterman et al., 1998; Li et al., 2004)
Lys120 acetylation	p53 stabilization activation of target genes involved in apoptosis	(Sykes et al., 2009)
Lys320 acetylation	Promotes p53-mediated activation of cell-cycle arrest genes	(Sakaguchi et al., 1997);
Lys382 acetylation	Reactive oxygen species production, BAX activation and apoptosis	(Yamaguchi et al., 2009)
Lys382 deacetylation	Expression of target genes involved in apoptosis is negatively regulated	(Vaziri et al., 2001)
Lys370, Lys372, Lys 373,Lys381, Lys 382, Lys386 ubiquitination	Promotes p53 degradation	(Xu, 2003)
Lys386 sumoylation	Activate transcriptional response of p53	(Rodriguez et al., 1999)

binding of specific DNA (Cesková et al., 2006; Luo et al., 2004). Acetylation at Lys120 in p53 is known to affect its functioning of transcription independent apoptosis (Sykes et al., 2009). Two closely related HATs, P300/CREB Binding Protein (CBP) and P300/CBP Associated Factor (PCAF) acetylate the C-terminus of p53 at Lys373 and Lys382 (p300/CBP) and at Lys320 (PCAF). In another report, Eichenbaum et al. took an attempt to elucidate the role of acetylation of Lys382 by computational techniques (Eichenbaum et al., 2010). The acetylation was found to be an important event to for providing stability of p53-CBP complex, which act as a molecular switch in p53 mediated transcriptional activation.

In response to DNA damage by γ-irradiation, both p53 and MDM2 show repeated pulses characterized by damped oscillations (Lev Bar-Or et al., 2000). Fan et al. shows the dynamical insights of PTMs with the help of simulation studies (Fan et al., 2014) and revealed that p53 phosphorylated-state (p53p) goes through several oscillations for a period of 6 h and then p53 acetylated (p53ac) form accumulates. p53 thus elicit distinct cellular response depending on the dynamical pattern of its accumulation in cell. Sumoylation is similar to ubiquitination where an iso-peptide bond is formed between the C-terminal carboxyl group of the small ubiquitin like protein (SUMO1) and the ε-amino group of Lys residue in target protein. The target for sumoylation in p53 is Lys386 (Rodriguez et al., 1999). Sumoylation of p53 is regulated by MDM2- and ARF-mediated nuclear targeting (Chen and Chen, 2003). Covalent conjugation of one or more ubiquitin molecules to the target protein leads to degradation of p53. MDM2 in this context functions as E3 ligase and directs the addition of ubiquitin molecules. Lys residues within the C-terminal regulatory domain of p53 (Lys370, 372, 373, 381, 382 and 386) are the targets for ubiquitination by MDM2 (Xu, 2003). A precise summary of the crucial amino acids involved in PTM, and their respective cellular consequences has been given in Table 1.

4. Structural basis of sequence-specific target gene recognition

p53 tetramer endures sequence-specific co-operative binding with its target DNA (Weinberg et al., 2004). It regulates gene transcription by binding to a specific double stranded DNA sequence of two decameric motifs (half-sites) of the general form RRRCWWGYYY (R = A, G; W = A, T; Y = C, T), separated by a spacer sequence of 0–13 base pairs (el-Deiry et al., 1992; Funk et al., 1992). This geometry favors four p53 molecules to self-assemble on two DNA half-sites, forming dimer of dimers (Fig. 3). It should be noted that the DNA–protein interfaces within the tetramer are identical (McLure and Lee, 1998). The variation in the structure observed among different crystals and NMR structures suggest that p53 dimer–dimer interfaces and the DNA conformations are polymorphic (Nagaich et al., 1999; Pan and Nussinov, 2007). With the help of MD simulation studies performed by Ma et al., it was elucidated that formation of salt-bridges by four pairs of Glu180-Arg181 (electrostatic interaction) is crucial between p53 monomers, in p53 tetramer complex with DNA (Ma and Levine, 2007). DNA–p53 tetramer complex shows association with core domain-p53REs (Response Elements), which are 20 base-pairs (bps) long stretch of sequence on the target gene (Pan and Nussinov, 2009). In a computational study of full length p53 sliding over DNA by Khazanov et al., it was found that if the p53REs length were reduced, it will reduce the sliding speed (Khazanov and Levy, 2011). p53 with more exposed interface to DNA allows it to bind with higher affinity and is attributed to higher electrostatic interaction.

The self-complementary core domain is formed by the residues from loop L3 and the short helical segment within L2 (Figs. 2B and 3). Key residues at interface that make a direct contact with a DNA half site are Lys120, Ser241, Arg248, Arg249, Arg273, Ala276, Cys277, and Arg280. Impact of crucial interactions (Lys120, Arg181,

Arg248 and Arg249) have been studied in detail with the help of MD simulation by Pan and Nussinov (2010b). Lys120 is the main key residue involved in recognition of various p53REs (Pan and Nussinov, 2010a). It was found that, p53 recognize the DNA in non-specific manner where the unstructured C-terminal domain of p53 is involved. The sliding takes place for finding suitable major groove binding site in a cooperative molecular recognition pattern. Lys120 (loop L1) and Arg280 possess anchor role for finding suitable hydrogen bonding partner, and subsequently DNA bending occurs (Pan and Nussinov, 2010a). The loop L3 binds to the DNA minor groove via Arg248 and involves either direct or water mediated contacts with the DNA backbone (Joerger and Fersht, 2008). Zn^{2+} ion plays a crucial role in maintaining the structural integrity of loop L2 (Duan and Nilsson, 2006). In complex state with DNA, Zn^{2+} has a direct contact with backbone phosphate group which attracts Arg248 (loop L3) into the minor groove. Similarly, guanidinium group of Arg249 stabilizes the conformation of loop L3 via a network of hydrogen bonds involving the carbonyl group of Gly245 and Met246 and a salt bridge with Glu171 (Wang et al., 2007). The side chains of Ser241 and Arg273 make direct contacts with the phosphate backbone (Kitayner et al., 2006). The sequence specific major groove contacts are formed by residues from the C-terminal helix (Ala276, Cys277, and Arg280) and Lys120 from the loop L1 (Joerger and Fersht, 2007a). It was also reported that, in particular, one structural water molecule forms four hydrogen bonds with the DNA backbone, the main chain of Cys277, the side chain of Arg280 and the side chain of Asp281 in p53–DNA complex. The same fact has been proved by Ferrone et al. and Barakat et al. using MD simulation (Barakat et al., 2011; Ferrone et al., 2006).

5. Mutants

Mutations in p53 mostly accounts for single amino-acid substitution which provokes its tendency to accumulate in tumor cell (Rotter, 1983). Cho et al. solved the crystal structure of p53 in complex with DNA that provided the basic framework for studying the deleterious effect of point mutations commonly involved in cancer (Cho et al., 1994). The “hot spot” mutants fall into two broad categories, viz. contact mutants and structural mutants (Joerger et al., 2006). The most popular hot-spot mutations are Arg175His, Gly245Ser, Arg248Trp, Arg249Ser, Arg273His and Arg282Trp (Hainaut and Hollstein, 2000).

Crystallographic data revealed that the contact mutations Arg273His and Arg273Cys remove crucial DNA contacts without perturbing the surrounding residues (Bullock et al., 2000; Joerger et al., 2006). Ang et al. reported that although Arg273His exhibits the same thermodynamic and kinetic stability similar to wild type protein, the selectivity of DNA binding is reduced to a greater extent (Ang et al., 2006). In a recent study, Li et al. have found using MD simulation that mutations offer a higher degree of flexibility in the core domain of p53 (Li et al., 2014). Report by Ma et al. suggests that the mutation Arg273His in a single monomer is sufficient enough to perturb the binding of p53 with DNA (Ma and Levine, 2007). On the contrary, at least three monomers need to have Arg273His mutation, to destabilize the tetrameric structure of p53. In a recent study by Eldar et al., the pivotal role of Arg273 mutations (His/Cys) has been elucidated which infers the machinery for specificity of DNA binding (Eldar et al., 2013). Likewise in other cases, mutation in contact residues Arg248 and Arg280 attributes to small local changes in the p53 structure which affects the DNA binding affinity (Joerger and Fersht, 2007b). Joerger and Fersht have discussed in their report that due to the loss of crucial DNA–protein contacts, large hydrophobic chains are also introduced (Ser241Phe, Arg248Trp and Cys277Phe) leading to overall loss of DNA binding specificity (Joerger and Fersht, 2007b). Mutation at Arg248Trp results in loss of DNA–protein interaction. Arg248Trp attains a long range communication from loop L3 (Arg248Trp/Gln), and induces conformational flexibility in loop L1 (His115Asn) (Chen et al., 1994). There are some counter mutations also, which can help the mutant p53 in gaining function as like the wild type. In case of Arg248Trp, the loss of tumor suppressive function cannot be compensated, whereas if the mutation is Arg248Gln, the functional loss can be restored with His115Asn counter mutation (Merabet et al., 2010).

Structural mutations like Arg175His, Arg249Ser, Arg282Trp and Arg110Pro affect the thermodynamic stability of the protein by various degrees as inferred from both experimental and theoretical studies (Koulgi et al., 2013; Rauf et al., 2010). Molecular modeling and artificial neural networking studies have indicated that most of the hot-spot residues having statistical significance show a relatively high evolutionary conservation (Cho et al., 2009; Greenblatt et al., 2003). In the wild type protein, Arg175 is embedded within the side-chains of loop L2 and L3, which is located in the Zn^{+2} ion binding domain. Along with a salt-bridge with Asp184, Arg175, it also forms hydrogen bonds with Pro191 and Met237 (Joerger and Fersht, 2007b). Arg175 lies very close to Zn^{2+} ion binding residues; introduction of the bulky imidazole ring (Arg175His) results in structural distortions which in turn affect the Zn^{2+} ion binding (Joerger and Fersht, 2007b). It is interesting to note that the substitution at codon 175 can either be C to T (Arg to His) or G to A (Arg to Cys), whereas in nature Arg175His gets selective advantage over Arg175Cys and shows a high incidence of mutation at this point compared to Arg175Cys (Rodin et al., 1998). *In-silico* studies suggests that His at codon 175 interferes the local structure of p53 sterically, making the mutant more deleterious; whereas substitution with Cys does not impart such steric clashes (Walker et al., 1999). Arg249 is responsible for stabilizing the DNA minor groove-p53 interface at L3 region, is also a target site for structural mutation. Crystal structure shows the loss of critical interactions induced by Arg249Ser mutation, which imparts a high degree of flexibility (Joerger et al., 2005).

Involvement of a peculiar methionine switch (Met243 and Met246) plays a crucial role in determining the outcome of this Arg249Ser mutation (Joerger et al., 2005). p53Met243 is solvent exposed in free-state, whereas it is buried in the interface of p53 monomers in complex-state with DNA. On the same hand, another methionine residue Met246 is buried in hydrophobic pocket of p53 in both states. In case of Arg249Ser mutation; upon binding to DNA, Met243 displaces Met246 from the hydrophobic pocket of Zn^{2+} ion binding domain. With the displacement of Met246, the loop conformation adopts a weak α-helical structure, leading to strong perturbation in the conformation of p53. This in turn displaces Arg248 from making contact with the phosphate backbone. In another structural mutation Arg282Trp/Gln, the substitution of Trp results in its steric interference with other residues like Thr118, Ser127 and Phe134, distorting the native structure globally (Walker et al., 1999). Cys242Ser is also a structural mutation which perturbs the Zn^{2+} ion binding site (Arg175His), causing alteration in the thermodynamic stability of the core domain (Bullock et al., 1997). In this context, β-sandwich mutants (Val143Ala, Phe270Cys and Tyr220Cys) are also greatly destabilized (Friedler et al., 2003). Friedler et al. have reported that in case of highly destabilized mutants like Val143Ala, Cys242Ser and Arg249Ser, the process of unfolding is 10 times faster than the wild type core domain (Friedler et al., 2003). In contrast to this, contact mutant Arg273His shows roughly the same half-life as that of the wild type p53 (Friedler et al., 2003). The degree of structural changes in the p53 mutant Gly245Ser is comparatively much smaller as the overall conformation of loop L3 in this mutant is conserved. According to Joerger et al., Gly245Ser mutation leads to a small structural shift which in turn affects the protein–protein interaction between p53

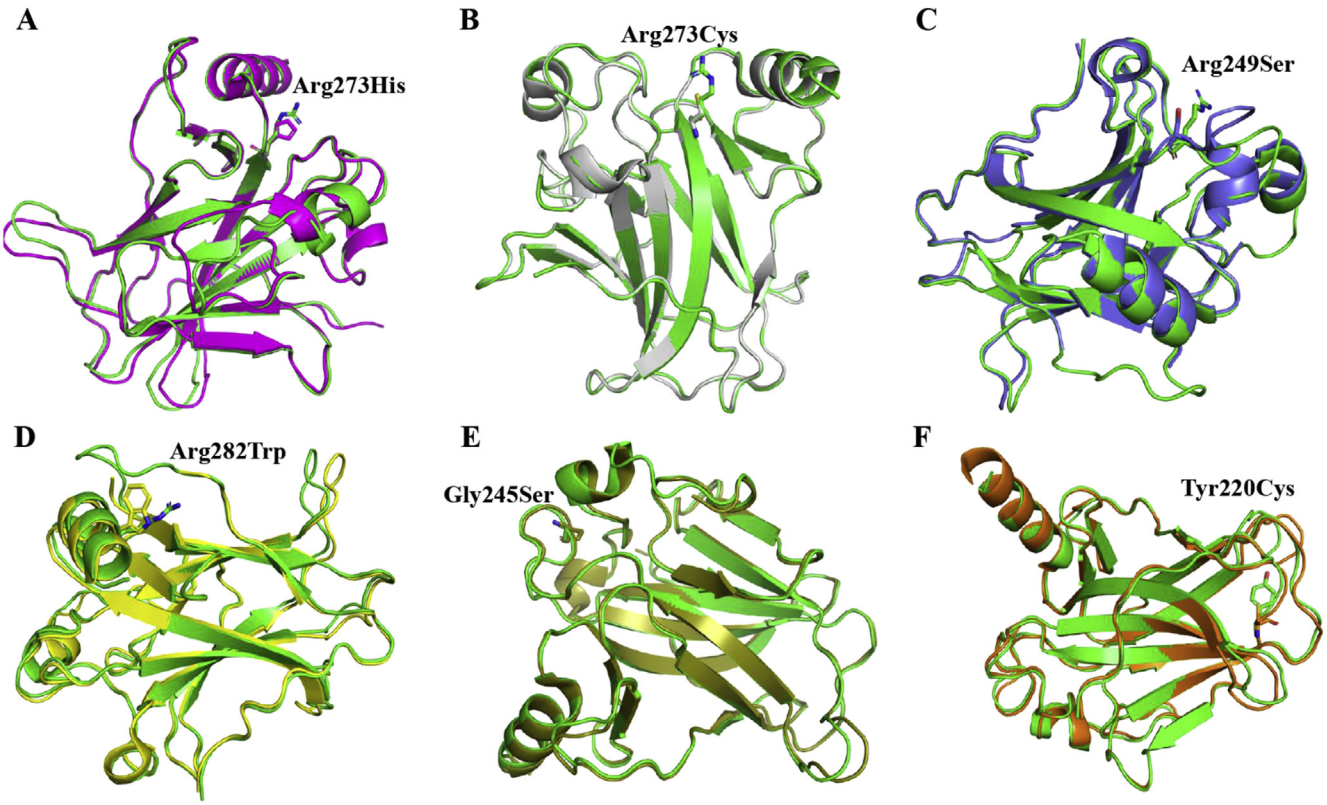

Fig. 4. Superimposed structure of wild-type p53 with contact and structural mutants, showing varying degree of structural perturbation. PDB code 2OCJ is being used to represent wild type p53 (green color). (A) Arg273His (PDB code 4IJT); (B) Arg273Cys (PDB code 4IBQ); (C) Arg249Ser (PDB code 3D05); (D) Arg282Trp (PDB code 2J21); (E) Gly245Ser (PDB code 2J1Y); (F) Tyr220Cys (PDB code 4AGN).

monomers (Joerger et al., 2006). Mutations in p53 not only affect the interaction phenomenon, but also affect the globular packing of the molecule, causing denaturation or unfolding. In other words, mutant p53 often find difficulty to get transported into the nucleus due to loss of its native structure.

MD simulation in relation to p53 mutants such as Arg175His, Arg249Ser and Arg282Trp explores that structural mutations tends to increase the overall plasticity of the p53, retaining up to 5% activity of wild type protein (O'Farrell et al., 2004). Demir et al. suggested some mutations, using ensemble based conformational sampling approach, which can act as rescue mutations (Demir et al., 2011). As for example, Asn263Val, Asn200Gln and Asp208Thr can act as rescue mutations for Arg175His. Similarly, Asn239Tyr and Thr123Pro for Gly245Ser; Ala138Gly and Leu137Arg for Tyr220Cys can act as rescue mutation helping them to restore the transcriptional activities. Strong selection for maintained expression of this selective group p53 mutants results in the GOF. In a recent report, Huang et al. have compared the mutation and GOF for p53 using computational evolutionary analysis in conjunction to MD simulation (Huang et al., 2014). Mutant p53 GOF not only signifies its loss of tumor suppressor functions but also the gain of new oncogenic property (Walerych et al., 2012; Xu et al., 2011). In conjunction to the mutants, Fig. 4 represents the structural comparison of p53 mutants available in protein data bank with the wild type p53. A tabulated summary of the illustrated p53 mutants, their crucial biophysical information and additional information regarding disease consequences has been presented in Table 2.

6. Fluctuation in thermo-dynamic stability

It would be wise to mention that the contribution from the computational techniques in conjunction to experimental methodology is crucial for understanding the molecular basis of thermodynamic instability. A review by Dell'Orco in similar context discusses many of the valuable time-effective information with respect to predicting of thermodynamics and kinetics profile for proteins in accordance to point mutations (Dell'Orco, 2009). Biophysical studies like circular dichroism, urea denaturation, protein folding-unfolding dynamics and MD simulation have been useful in determining relevant thermodynamic properties of the wild type versus mutant proteins. It is significant to mention that due to single point mutations, free energy (ΔG) or relative free energy (ΔΔG) of protein gets increased (Guerois et al., 2002). A detailed illustration of this fact has been presented in reports by Ang et al. (2006), Nikolova et al. (1998) and Wright et al. (2002). Increase in ΔG on the other hand perturbs the globular packing, which results in exposure of the hydrophobic core toward the solvent phase. The proteins therefore, either remain denatured or unfolded, which might accelerate their aggregation in cytosol. The core DNA binding domain dictates the stability of full length p53. The ΔG of unfolding of p53 core domain at 25 °C is 7.5 kcal/mol (Bullock et al., 2000). However, the stability of p53 core domain sharply falls with the increase in temperature that ultimately results in irreversible denaturation. The structure of p53 is more flexible than it is predicted from the crystal structure as revealed with the help of MD simulation studies (Wells et al., 2008). p53 core domain has a low thermal and chemical stability, therefore shows a low melting temperature (T_m, 44 °C) (Bullock et al., 1997). It is to be noted that point mutations in the core domain make the protein prone to aggregation which is attributed with highly fluorescent spectra (Klein-Seetharaman et al., 2002). According to Bell et al., the full length p53 is composed of large unstructured region in its N-and C-terminal regions that accounts for limited stability of the protein (Bell et al., 2002). This effect of the N-and C-terminal disordered

Table 2
Different biophysical parameters and disease consequences of p53 core domain hot-spot mutants.

Mutation residue	Structural regions affected	% folded (37 °C)	Structure (37 °C)	ΔΔG (kcal/mol)	Disease consequences	References
Arg273His/Cys	DNA contact	98%	native	0.45 ± 0.04	Disordered proliferation in 3D cultures, induction of migration-related mutant p53 activated genes, inhibition of apoptosis, altered growth and cell polarity in 3D cultures, EMT induction, anchorage independent growth	(Bullock et al., 2000; Lim et al., 2009; Zhang et al., 2011)
Arg248Trp	DNA binding region	>85%	Distorted	<2.0	Altered growth and cell polarity in 3D environment, EMT induction, drug resistance, genomic instability	(Zhang et al., 2011; Agapova et al., 1996)
Arg249Ser	DNA binding region	85%	Locally Distorted	1.92 ± 0.04	Increased migratory potential, cell cycle progression, uncontrolled proliferation, polyploidy	(Vaughan et al., 2012; Noll et al., 2012)
Arg175His	Zinc-binding region	30%	Globally denatured	3.52 ± 0.06	Increased tumorigenic potential, induction of pro-angiogenic genes, altered growth and cell polarity in 3D environment, chemo-resistance, inhibition of apoptosis, cytosolic sequestration of p63 and p73 (GOF), induction of EMT, invasion, anchorage-independent growth, stem cell dedifferentiation. polyploidy	(Kogan-Sakin et al., 2011; Xu et al., 2011; Yi et al., 2012; Adorno et al., 2009; Muller and Vousden, 2013; Muller et al., 2009)
Gly245Ser	DNA binding region	95%	Weakly destabilized	1.21 ± 0.03	Altered growth and cell polarity in 3D cultures, weak gain of function effect, EMT	(Zhang et al., 2011)
Arg282Trp	DNA binding region	38%	Globally denatured	3.30 ± 0.10	Cytosolic sequestration of p63 and p73 (GOF), dominant negative activity on wild type p53	(Xu et al., 2011)
Tyr220Cys	β-sandwich region	17%	Globally denatured	3.98 ± 0.06	Interacts with p73 and inhibits its ability of transactivation	(Bullock et al., 2000; Gaiddon et al., 2001)

domains on the globular packing of p53 has been well correlated with NMR studies in conjunction to MD simulation in a recent report by Pagel et al. (2013). The tumor-associated mutants thermally destabilize the protein further at body temperature. Barakat et al. have elucidated such effect considering the example of Arg248Gln and related mutations using essential collective dynamics by varying the theoretical temperature of the MD simulation system (Barakat et al., 2011). DNA contact mutations usually impose minimal effect on the overall stability of the protein whereas mutations inducing local distortion in the structure usually destabilize the protein by ~2 kcal/mol. Structural mutations on the other end causes global unfolding of the protein that are highly destabilizing (>3 kcal/mol).

Friedler et al. have studied the kinetics of p53 core domain mutants unfolding, to get an insight of the molecular mechanism of their inactivation. A strong correlation exists between the thermodynamic and kinetic instability of p53 core domain mutants where it is suggested that the instability is directly proportional to the shorter half-life of mutant (Friedler et al., 2003). The stability of wild-type protein is 6 kcal/mol at 25 °C and 9.8 kcal/mol at 10 °C (Bullock et al., 1997). The DNA binding mutant Arg273His is similar compared to the wild type in terms of ΔG, whereas Cys242Ser and Arg175His are destabilized by 2.9 Kcal and 3.0 kcal/mol respectively. In similar context, the energetics evaluation of these residues surrounding the Zn^{+2} ion is reported by Wang et al. comparing the gas phase and solvent phase simulation system (Wang et al., 2011). The corresponding alanine mutant, Arg175Ala is minimally destabilized (0.7 kcal/mol). On the contrary, a significant variance between the thermo-dynamic stability is observed in these mutants, which shows difference in folding of Arg175His (30% folded at 37 °C) compared to Arg175Ala (98% folded at 37 °C). The energetic contribution from mutant Arg175His, as characterized by cluster based study of MD-generated trajectories of various point mutants, is remarkably higher compared to other mutants (Wright et al., 2002). In the NMR and fluorescence studies, Arg175His and Arg249Ser show precise difference in the native spectra compared to the wild type protein, which indicates local changes in structure and dynamics. In contrast to this Gly245Ser and Arg273His shows similar spectra as compared to wild type. Arg273His having the least effect, destabilized by only 0.4 kcal, thus preserving the wild type functional specificity (Bullock et al., 1997). In Arg282Trp mutation, substitution of a tryptophan moiety invites a large thermodynamic compensation of 3.3 kcal/mol. Fluorescence spectra of this mutant indicate the increase of solvent-accessible surface which give inference of being in unfolded state. A report by Canadillas et al. states that there exist several buried polar groups, which accounts for thermodynamic instability of core domain (Cañadillas et al., 2006). Two polar residues Tyr236 and Thr253 buried in the hydrophobic core contribute to such instability of p53. A change from a highly directional polar interaction (Tyr236, Thr253) to non-directional non-polar interaction (Phe238, Ile255) makes the structure more stable as suggested by *in-silico* free energy in a report of Madhumalar et al. (2008). Reports by McCoy et al. and Mateu et al. illustrate the role of p53 tetramerization domain (Glu326-Ala351) with respect to its thermodynamic stability (Mateu and Fersht, 1998; McCoy et al., 1997). Truncation of each amino acid side chains into alanine/glycine within this p53 tetramerization domain affects the stability as well as the oligomerization status. Nine hydrophobic residues in each of the monomer have been reported to be responsible for determining the stability of the protein (Fig. 2C).

7. Structural plasticity facilitating binding to proteins or small molecules: route to reactivation

Structural plasticity of p53 core domain is directly linked to its interaction with different protein partners or peptides, which adds more degrees in its complex regulation. Small molecules and

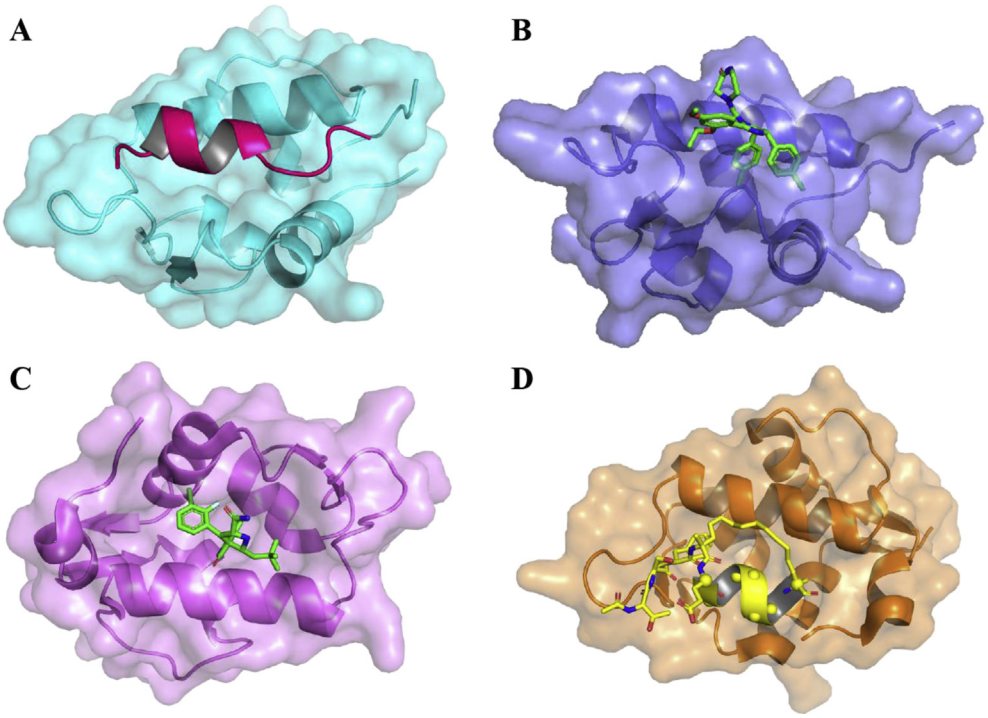

Fig. 5. Figure representing small molecule rescue of tumor suppressor functioning of p53 using MDM2 inhibition approach. (A) Binding of p53 N-terminal transactivation domain within the hydrophobic cleft of MDM2 (PDB code 1YCR). (B) Nutlin-3A interaction within the extended N-terminal domain of MDM2 (PDB code 4J3E). (C) Small molecule MI-63 bound to MDM2 in the p53 docking site (PDB code 3LBL). (D) Docked pose of designed staple peptide within the hydrophobic core of MDM2 (PDB code 4UMN).

peptides, hence play a pioneering role in reactivating p53 which have been an integral part of designing potential drug candidates (Brown et al., 2011). Using arrays of small molecule ligands, computer guided identification of binding pockets have been screened to predict possible peptide interaction site (Wassman et al., 2013). Reactivation of mutant p53 in parallel to the strategy of expressing wild type p53 has become a matter of great concern in the p53 biology, because of its increased resistance to chemotherapy (Levine, 1997). In this section focus will be on the approaches that are currently being undertaken to target p53 and its regulators.

7.1. Small molecules that disrupt MDM2–p53 interaction

MDM2 is a major negative regulator of p53 that is over-expressed in cancer (Joerger and Fersht, 2008). N-terminal transactivation domain of p53 (residue 15–29) interacts with a large cleft on the N-terminal surface of MDM2 (residue 25–109). The interaction between MDM2–p53 relies on the steric complementarity between the MDM2 cleft and the hydrophobic face of p53 α-helix (Fig. 5A). The interface between both the proteins is relatively small which is indicated by the calculated accessible surface area in the interface on MDM2 and p53 to be about 600 Å and 809 Å respectively (Chène, 2003). Inhibitors of p53–MDM2 interaction will have to fit in the binding cleft of MDM2 in order to rescue p53.

MDMX is another negative regulator of p53, control the stability and activity of p53 in an independent mechanism (Moll and Petrenko, 2003). MDMX is highly homologous to MDM2, with the difference that it does not possess any ubiquitin ligase activity (Stad et al., 2001). Inhibition of p53–MDM2/MDMX interaction with small molecules and peptides has been exploited as a novel therapeutic strategy (Shangary and Wang, 2009). Recently, class of cis-imidazoline analogs, Nutlins have been used to displace recombinant p53 protein from its complex with MDM2 (Fig. 5B) (Joerger and Fersht, 2010). It competes with p53 for binding to the extended hydrophobic-cleft on the N-terminus of MDM2. Mutations on Met62Ala and Gln24Arg of MDM2 impart resistance to nutlins by selectively decreasing the affinity (Wei et al., 2013). Nutlin-1, Nutlin-2 and Nutlin-3 have been discovered by chemical library screening, of which Nutlin-3 is most potent (Secchiero et al., 2011). Various computational studies involving the usage of Brownian dynamics simulation have proved to be effective in this regard which helps in explaining the interaction of Nutlin with p53–MDM2 and p53–MDMX (ElSawy et al., 2013a, 2013b; Hernychova et al., 2013).

Another fungal (*Fusarium*) metabolite, chlorofusin, has been reported as an inhibitor of p53–MDM2 interaction (Duncan et al., 2001). Chlorofusin binds at the N-terminal of MDM2, though the exact binding mode of this compound is not yet elucidated. Another bioactive small molecule, Tenovin-1 has been reported which elevates the amount of p53 protein as revealed from *in-vitro* studies (Lain et al., 2008). The MI series of spiro-oxindole compounds (MI-219, MI-63, and MI-43) structurally mimic the MDM2 binding residues on p53 (Phe19, Trp23, leu22 and Leu26) (Fig. 5C) (Popowicz et al., 2010). From chemical phenotypic screen another compound RITA has been discovered (Issaeva et al., 2004) that binds with the N-terminal of p53 with high affinity and inhibits p53–MDM2 interaction. It suppresses the tumor growth both *in-vivo* and *in-vitro* by inducing programmed cell death in various cancer cell models (Vassilev, 2007). Stapling of peptides of wild-type p53 sequence, in this context, is also coming up as an additional tool for reactivation. A new approach called “stapled peptide” has been introduced that selectively targets HDMX (MDM4) and prevents p53-HDMX binding (Crunkhorn, 2011). Stapled peptides consist of a covalent linkage bridging adjacent turns of α-helical peptide (Walensky et al., 2004). Stapling peptides increase their affinity toward the protein target by reducing the entropic cost of binding by improving their proteolytic stability, which in turn significantly increase their cellular uptake (Brown et al., 2013; Kim

et al., 2011; Verdine and Walensky, 2007). One such stabilized α-helix (SAH) of p53, SAH-p53-8 prevents HDMX mediated p53 sequestration (Fig. 5D).

7.2. Small molecules re-activating p53 mutations

Mutant p53 reactivation has been the recent strategy in the treatment of cancer. Rational designing with molecular docking and high throughput virtual screening approaches has led to the generation of diversified set of small molecules and peptides that can restore mutant p53 to wild type structure. Kaar et al. have suggested a series of compounds having an α, β unsaturated double bond which is a characteristic of Michael acceptor molecules (Kaar et al., 2010). These compounds can covalently bind to p53Cys residues, resulting in increase of melting temperature of hot-spot mutants. The best studied and promising of all those molecules are PRIMA-1 and PRIMA-1^{Met}, currently undergoing phase I/II clinical trials (Bykov et al., 2002; Zache et al., 2008b). Recently, Wassman et al. have reported the existence of a transiently open reactivation pocket in the region of loop L1 and sheet S3 of DNA binding core domain. This L1/S3 pocket is found to be around Cys124, Cys135, and Cys141 (Wassman et al., 2013). The pocket thus can be considered as possible target for reactivation of tumor suppressor by small molecules. Mutation of Cys124, located at the center of the pocket, abolishes p53 reactivation of mutant Arg175His by PRIMA-1. Cys124Ala mutation in Arg175His background impairs the activity of PRIMA-1 in osteosarcoma cell line (Bykov and Wiman, 2014). Reiber et al. also suggest the role of PRIMA-1 in hypoxic background (Rieber and Strasberg-Rieber, 2012).

STIMA-1 (2-vinylquinazolin-4-3H-one), having structural similarity with CP-31398, is another Michael acceptor being used with an effort to reactivate mutant p53 (Zache et al., 2008a). It enhances mutant p53 DNA binding *in-vivo*, induces expression of p53 target genes and induces apoptosis. It targets human tumor cells expressing two hot-spot mutants Arg175His and Arg273His. MIRA-1, another maleimide derivative compound, can reactivate DNA binding (Bykov et al., 2005), consisting of a reactive carbon–carbon double bond which is capable of reactivating mutant p53 (Bykov et al., 2005). MQ, an electrophilic small molecule, on the other hand binds to cysteine residues of wild-type p53 with a higher affinity (Lambert et al., 2009). Docking simulations have revealed that diverse p53-targeting compounds MQ, MIRA-1/2/3, STIMA-1 selectively bind to the L1/S3 pocket of p53 and invoke p53-downstream response. Yu et al. reported that compound NSC319726 restores the wild-type p53 structure and function in case of Arg175His mutation and is capable of inducing apoptosis (Yu et al., 2012). Zn^{2+} ion chelating property of the compound is suggested to be the reason of its functional efficacy.

7.3. Reactivation through exploiting other protein–protein interactions

p53 interactome is also a crucial part of the study involving mutant p53 reactivation. Small molecules that destabilize the complex between mutant p53 with other family members also have potential to act as new generation anticancer therapeutics. RETRA a small molecule has also been reported to impede mutant p53 carrying tumors *in-vitro* and in xenograft model (Kravchenko et al., 2008). In the same context, short-interfering mutant p53 (SIMP) peptides are also being designed with the aim to destabilize mutant p53/p73 interaction in tumor cells. These peptides are capable of rescuing p73 from being sequestered with mutant p53, and thus can restore p73 tumor suppressive function.

8. Conclusion

In conclusion, this report illustrates various experimental and computational evidence, reflecting tumor suppressor p53 as an attractive therapeutic target in cancer. The central packing of p53 consisting of beta-sandwiches, flexible loops and unstructured regions in DNA-binding core domain makes it structurally challenging to rescue. Computational studies such as MD simulation, molecular docking, free energy evaluation, key residues involved in protein–protein or protein–ligand interaction has provided relevant biological information in context to p53. A general understanding of the structural organization involving disease-causing mutations is helpful in correlating the loss of tumor suppressor function and novel oncogenic gain of function. Therefore, the reactivation of mutant proteins with small molecules and stapled peptides needs intense computational study to explore the docking site on the surface of p53 and interacting biomolecules such as MDM2/MDMX and DNA. In future, the extensive application of algorithm-based computational analysis supported with experimental studies is believed to produce appropriate findings in order to overcome the limitations of p53 reactivation in the way to develop novel cancer therapeutics.

Conflict of interest

There is no conflict of interest.

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