Aims and Objectives
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 the 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 the first section, insights into structural details of p53, diverse single point mutations affecting its core domain, thermodynamic understanding and therapeutic strategies for the 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 the development of small molecule inhibitors and peptides exploiting p53-MDM2 interaction have been included. In a nutshell, this section attempts to describe the structural biology of p53 which provide new openings for structure-guided rescue.

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 believed to be the cumulative dynamic changes associated at the genomic level. This information alone is not considered to be sufficient to explain the equally intricate complexity in function of protein over time and space. Linking structural changes of protein in the course of mutation with its altered cellular localization is therefore far more important to illustrate its function on uncontrolled cellular proliferation. Thus the recent approach for studying molecular events in cancer is focusing more on the regulation of the spatiotemporal distribution of proteins and their activity which can coordinate sequence–structure–function relationship with the progression of the disease.

Alteration in the protein structure in the course of mutation has been recognized as an important factor that affects p53 localization. Mutation in its DNA-binding domain (DBD) is a frequent event in cancer which contributes to the proliferation and differentiation of the
neoplastic population. According to IARC TP53 mutation database, single point mutations in p53DBD accounts for more than 95% of the reported malignant mutations (Olivier et al., 2002). Six amino acid residues in p53DBD have been mapped, having the highest frequency of mutation, popularly known as sites of “hot-spot" mutations. The DNA-binding core domain p53 mutants can be distinguished into contact and structural mutants. In response to genotoxic stress, weakly destabilized p53 contact-mutants (R273H and R248W), predominantly show nuclear distribution. On the contrary, structural-mutants, R175H, R248Q, R249S, R250L, and R110P typically show perinuclear localization of protein aggregates (Xu et al., 2011), indicative of impaired nuclear import.

Microtubule track and its associated minus ‘−’ end-directed motor dynein have been reported to regulate nuclear translocation of p53 (Giannakakou et al., 2000). Recently, actin has also been found to play a critical role in vesicular transport in animal cells (Schuh, 2011). Notably, wild-type p53 has been found to be very closely associated with the cytoplasmic actin filaments at the time of DNA synthesis (Metcalfe et al., 1999). Polymerization of actin has been reported to negatively influence p53 nuclear import (Wang et al., 2013). This evidence suggests that regulation of this crucial cellular event involving p53 nuclear trafficking is probably based on its interaction with cellular actin. It is noteworthy to mention that the precise role of actin-forms involved in interaction with p53, and their contribution to nuclear transport is not clear till date. Here it is to mention that both F- and G-forms of actin are sequentially same, each monomer consisting of 375 amino acids long protein, yet structurally they are different.

In the present study, an attempt has been taken for understanding the sequence and structural architecture of p53 protein and its importance in cancer biology. In the first part, we have studied the varying degree of structural perturbation in cancer-associated p53 mutants, and also suggested small-molecule based rescue of p53 activity. Adoption of ideology with appropriate techniques, such as homology modeling, molecular docking and molecular dynamics simulation, free energy evaluation to account the dynamicity and conformational attributes, has been useful in addressing important biological questions. In the next part, we have searched for the involvement of cellular actin in determining p53 nuclear transport. Our study explores the contribution of monomeric G-actin in mediating p53 nuclear transport. To address atomistic details of the complex, constraint-based docked
model of p53:G-actin complex was generated based on crystal structures. MD simulation further reveals that p53 DNA-binding domain arrests very well the G-actin protein. Docking benchmark studies have been carried out for a known crystal structure, 1YCS (complex between p53DBD and BP2), which validates the docking protocol we adopted. Furthermore, to confirm our assumption, functional p53 mutants that either alter the 3-D structure of the protein or compromise with its DNA-binding activity, have been included in our study. The inclusion of these variants is expected provide a robust way out for shedding light on our hypothesis.

We, therefore, have undertaken the following aims and objectives in our present study:

**Objectives:**

**Chapter-1: Elucidating the structural and sequential context of p53: evidence from experimental and theoretical studies.**

I. Homology modeling of full-length (393aa) tumor suppressor p53.

II. Delineating individual domains of p53.

III. Studying the post-translational modification of p53, and structural arrangement of p53 in complex with DNA.

IV. Studying the varying degree of structural perturbation in super-imposed structure of wild-type p53 with contact and structural mutants.

V. Studying the fluctuation in thermo-dynamic stability in p53 contact and structural mutants.

VI. Studying small-molecule rescue of p53 functioning, using MDM2 inhibition approach based on the steric complementarity between MDM2-cleft and hydrophobic faces of p53.

**Chapter-2: Nuclear trafficking machinery of tumor suppressor p53 guided by monomeric G-actin.**

I. Tracing the p53 nuclear translocation in the presence of actin stabilizing/depolymerizing drugs.

II. Studying the correlation between fluctuation of F/G-actin ratio and p53 nuclear transport in actin disrupted conditions.
III. Determination of interaction between Wild-type p53 with cellular actin.

IV. Elucidation of physical interaction between Wild-type p53 and polymerization-deficient G-actin, input from 3-dimensional confocal staining.

V. Purification of His6-p53 in Ni-NTA column and further identification of an in-vitro interaction between His6-p53 and purified monomeric G-actin.

VI. Molecular docking between crystal structures of p53 DNA-binding domain and G-actin to define the atomistic details of the protein-protein interaction.

VII. Elucidation of the interaction of p53 at the ‘Target-binding cleft’ of G-actin.

VIII. Comparative study of the molecular interface in cross-linked actin dimer with p53:G-actin interface to find out the basis of p53:G-actin interaction.

Chapter-3: Defining the basis of altered localization of p53 “hot-spot” mutants.

I. Study of subcellular localization of p53 wild-type/mutants.


III. Identification of a physical interaction between p53-contact/structural mutants and G-actin and correlating it with the degree of p53 nuclear import.

IV. Elucidation of cytosolic sequestration of structural p53 mutants.