Chapter 8

Summary and future prospects

BRCA1 interacts with different molecules through its functional domains and is responsible for multiple functions like cell-cycle regulation, DNA damage repair and transcription [89]. BRCA1 BRCT interacts with different proteins via their (pS)-X-X-F motifs and forms three distinct complexes-the BRCA1 A, BRCA1 B and BRCA1 C complexes [90]. BRCA1-BRCA2-containing complex (BRCC36) is a member of the four-subunit of BRISC (BRCC36 -isopeptidase complex) that contain ABRAXAS paralog, KIAA0157/Abro1 [92]. The BRISC complex contains three additional protein components RAP80, BRCC45 and MERIT40. In addition, the BRCA1-complex contains a five-member stoichiometric complex consisting of RAP80, ABRAXAS, BRCC36, BRCC45 and MERIT40 [13]. However, the structural organization of the complex is not known.

8.1 RAP80

The Receptor Associated Protein 80 (RAP80) identified as a retinoid-related protein comprises different functionally important domains that interact with BRCA1. RAP80 is responsible for recruitment of BRCA1 to the site of DNA repair. Alteration in BRCA1 and its associated partner may affect the recruitment of BRCA1 complex to DNA damage site, and hence disturb the DNA repair process also. RAP80 acts upstream of CCDC98 and BRCA1 in DDR [6, 16, 112]. RAP80 knockdown cells have shown hypersensitivity to IR and ultraviolet (UV) light, cell- cycle dysfunction and defective homologous recombination (HR) repair [6, 7, 16, 87]. RAP80 and p53 auto-regulate each other and influence apoptosis [154]. Loss of RAP80 alleles (RAP80 $^{-/-}$) increase the susceptibility to lymphoma, and promote tumor development in both p53$^{-/-}$ and p53$^{-/+}$ mice [155].

RAP80 (1-130 amino acids) wild-type and ΔE81 proteins are soluble in bacterial system. Thermal and proteolytic stability of wild -type was found to be significantly higher as compared to ΔE81, albeit both unfold likely with two-state transition. RAP80 UIMs are found in equilibrium between random-coil and helical states. This fact is supported by low Tm values of both wild- type and ΔE81. The reason behind dynamic nature of UIMs is to provide immense flexibility of dissociation and association of ubiquitin molecules during the protein trafficking process. Perhaps UIMs also use this mechanism for multiple mode of binding (monovalent and multivalent) so as to achieve cooperativity in binding interactions. This dynamic nature is essential for a flexible and transient initiation mechanism of the DNA damage repair process. Deletion of 81E residue alters the helical state conformation, thus shifting equilibrium towards a random structure. Helical to random structure transition results in loss of several weak intermolecular hydrogen bonds and hydrophobic interactions between the UIMs and Di-Ub (K-63 linked), thereby making the binding interactions unfavorable for ubiquitin. Since binding affinity of individual UIM for mono-ubiquitin is low [161], an avidity-based mechanism probably makes the interaction between RAP80 and Lys 63-linked polyubiquitin highly robust. Co-operative binding between multiple UIMs and ubiquitin chains likely occurs, which favors the interaction of second UIM with ubiquitin after positioning of the first [159]. It has been reported [5] that expression of RAP80 ΔE81 allele abates recruitment of BRCA1 complex at DSB site, which further augment chromosomal aberration (chromatic breaks). The results presented in this study also suggest that deletion of 81 Glutamic acid residue significantly obliterates RAP80 structure and impairs it's binding with polyubiquitin chain. Unstable nature of mutant and di-ubiquitin complex may be

responsible for defective recruitment of RAP80-BRCA1 complex to the DNA damage sites. Defective DNA damage repair perhaps leads to chromosomal aberration. Prolific comparison of RAP80 ΔE81 with wild- type will help in understanding its role in various diseases and repair defects. It will further explore the possibility of structure based inhibitor design for therapeutic application that can compensate the effect of such mutation.

8.2 MERIT40

MERIT40 is a key molecule in the BRCA1 complex and plays a decisive role in complex stabilization. It facilitates recruitment of BRCA1 to the site of DNA damage and favors homologous recombination repair. MERIT40 can be classified as an intrinsically disordered protein due to the presence of N-and C-terminal disorder region. However, its middle region showed well defined compact structure which indicates a possibility of crystallization of middle region for X-ray diffraction study. Structural homologous of MERIT40 suggests its plausible role in complement activation pathway. It perhaps sets up an interactions network in BRCA1 complex which is being utilized for stabilization of ABRAXAS since knockdown of MERIT40 significantly reduces the ABRAXAS and RAP80 levels [13, 19]. Stabilization of ABRAXAS probably further helps in maintaining the integrity of BRCA1-complex. This study will provide insights into the diverse interactions involved among various members which are essential for DNA repair function of BRCA1 complex. MERIT40 could be a multifunction molecule having role in DNA damage repair and complement activation.

8.3 ABRAXAS

ABRAXAS is the key member of BRCA1 complex and acts as a bridging molecule among various members. ABRAXAS expression was significantly correlated with lower chance of tumor response in patients with advanced non small-cell lung cancer receiving first-line platinum–gemcitabine chemotherapy[246]. Knockout studies of ABRAXAS showed defective recruitment of BRCA1 complex and hence the defective DNA repair[6, 7, 247]. Thus, it is a multifaceted molecule which plays a dispersive role in cancer progression, and BRCA1 mediated homologous recombination repair.

Multiple sequence alignment of ABRAXAS considering various species in phylogenic order has shown highly conserved nature of Arg361 residue. The modeled structure of ABRAXAS wild- type and R361Q mutant showed structural alteration. However, the observed structural changes were not contributing in oligomeric properties. Wild- type and mutant have shown similar secondary structural composition while the relative orientation of Trp and Tyr was slightly disturbed. This indicates that R361Q mutation bringing several localized changes in structure pattern of ABRAXAS which altogether furnish a different conformational stability of structure in a cumulative manner. These conformational changes are very minor and hence could not be detected at secondary structural level while their relative positions were traced during three dimension unfolding pathway. The relative redundancy of intermediate species in case of wild -type suggest the existence of different unfolding pathway which is partially followed by mutant. Altogether, the localized changes in the mutant structure brings down its thermal and chemical stability which further perturb interaction with RAP80. The cumulative global changes in mutant structure was sufficient to disturb critical interaction

necessary for BRCA1 complex integrity and localization. Therefore, in the presence of R361Q mutation, ABRAXAS could not extend its bridging interaction through RAP80 which perhaps reduce the recruitment of BRCA1 complex to the DNA damage site. Consequently, the nuclear retention of BRCA1 is adversely affected which further agitates G2/M checkpoint and homology-directed DNA repair [11]. These finding would substantially list ABRAXAS as a new susceptibility gene to cancer predisposition. It also opens the vast perspective of considering R361Q mutation role in disease progression such as cancer.It will further explore the opportunity of inhibitor design for therapeutic application that can recompense the effect of such adverse mutation.

8.4 BRCA1-complex

Protein-Protein Interactions in BRCA1 complex plays very significant role and alterations in interaction profile leads to stabilization/destabilization of whole complex. MERIT40 is an essential component of BRCA1 complex. MERIT40 showed direct interaction with ABRAXAS and BRCA1-BRCT as indicated with significant heat change during reaction. The binding association between MERIT40 and ABRAXAS was found K_d=2.56 ± 0.24 μM. Interactions between BRCA1-BRCT domain and MERIT40 with a affinity constant K=521±7μM was established. MERIT40 showed a direct interaction with BRCA1-BRCT. In order to validate this finding, pull-down assay was performed, which confirm the interaction between MERIT40 and ABRAXAS. It is reported that ABRAXAS form phosphodependent interaction with BRCA1 BRCT and phospho-independent interaction with RAP80, thus acting as a bridging molecule in BRCA1 complex. Binding interaction between MERIT40 and ABRAXAS probably help

in extension of bridging interaction among various members of BRCA1 complex and thereby maintaining its integrity.

8.5 Future perspective

The structural and functional analysis of BRCA1 complex was studied to understand its role in DNA damage repair. Two important mutations and their disease correlation have been analyzed in addition to dissecting the stabilization mechanism of BRCA1 complex through the MERIT40. The next approach could be the expression of MERIT40 stable domain (72-300 amino acids) in higher system such as yeast or insect cells lines in order to get a well folded protein with all desired post translation modification. The main issue in bacterial system was oligomerization which highly reduce the propensity of MERIT40 crystallization. However, a rational approach of cloning into higher expression system can result a well folded homogenous protein which would most likely form the crystal and good diffraction pattern. The next approach could be co-crystallization of BRCA1 members such as RAP80-ABRAXAS and MERIT40-BRCA1 BRCT. In this regard, the major problem was the purification of functional and homogenous ABRAXAS protein. The issue can be significantly resolve by its co-expression with other members either in bacterial or other expression system. In addition, role of MERIT40 can be explored in complement activation pathway due to its structural resemblance with complement factor B.