CHAPTER 10

SUMMARY AND FUTURE PROSPECTS

Summary and Future prospects

10.1. Overview of results

The main theme of the current thesis involves characterization of structure and dynamics of the human LMTK3 domain which is implicated in progression, invasion and metastasis of breast cancer. Here we investigated the molecular interaction (interface area, interacting residues) between LMTK3 with its binding partner proteins (ERα, GRB2, CDK5) that are involved in signaling and promoting endocrine therapy resistance, invasion and metastasis in breast cancer. Therefore, we made an attempt to design LMTK3 inhibitors in order to have control over breast cancer.

In the first part of the thesis we characterized the salient structural features of LMTK3 domain. We found LMTK3 structure to be stable and flexible in the coils and loop region, there are significant number of intermolecular hydrogen bonds and hydrophobic contacts which are essential for the proper folding of the protein structure. We also predicted the probable binding cavities in LMTK3 domain. In addition, we determined hydrophobic clusters and patches in LMTK3 which may be crucial for folding and stabilization. Possible interaction sites in LMTK3 domain were then studied from electrostatic potential analysis based on positive and negative surfaces. In LMTK3 domain, we identified the ATP binding pocket and analyzed the key residues involved in interaction with ATP.

In the second part of the thesis, we examined the protein-protein interactions between LMTK3 and its binding partner proteins as it plays an important role in signaling, At first we investigated the intermolecular interactions between LMTK3 and ERα, as ERα get phosphorylated by LMTK3. This study revealed the significant number of hydrogen bonds, salt bridges and non-bonded contacts between the residues of LMTK3 and ERα.

We also studied the interactions between LMTK3 and GRB2 which promote invasion and metastasis in triple negative breast cancer. We identified the significant number of bonded and non-bonded interaction holding the two proteins and stabilizing

the complex structure. Further from MM-PBSA calculation, the total binding free energy (BFE) was found to be favorable towards the formation of the complex and substantiates the stability of the complex**.** In addition to interface area and interacting residues, we also identified the probable hotspot residues at GRB2 -LMTK3 interface.

Then we studied the interactions between LMTK3 and CDK5, as LMTK3 get phosphorylated by CDK5 and leads to tumorigenesis in breast cancer. Here, we identified the probable interaction that takes place between CDK5 and LMTK3, and this interaction study helps us to identify the probable phosphorylation sites in LMTK3 at activation loop. Then we studied the effect of activation loop phosphorylation on the conformational dynamics of LMTK3 domain. The phosphorylation of LMTK3 domain at activation loop imposes a conformational change and may have significant effect on LMTK3 activity. As a result it may lead to breast cancer progression.

In the protein-protein interaction study, the knowledge of interacting interface area, interacting residues may accordingly be useful in designing potential inhibitors, therefore, protein-protein interaction could be obstructed and signaling can be controlled. And in the last part of the thesis, we demonstrated two different methods to design potential inhibitors against LMTK3.

In the first method, we made an attempt to identify ATP competitive inhibitors against LMTK3 using virtual screening and molecular docking. Our findings provide the information about the key residues of LMTK3 involved in the formation of hydrogen bonds and hydrophobic interaction with potential inhibitors. MD simulation study and MM-PBSA/GBSA gave assurance to the high binding affinity of the inhibitors as compared to ATP. Further, potential of mean force (PMF) calculation results revealed that the ATP has the lowest dissociation energy barriers. And inhibitors have high dissociation energy barriers. Thus, the residence time of ATP is less and can be pulled out easily. Thereby, we suggested these inhibitors to be the probable ATP competitive inhibitors.

In the second approach, using Schrodinger Drug Designing suite, we identified top three lead compounds (BAS12945106, BDF24025570, BAS01313675) based on Glide XP score. These three compounds bound to the LMTK3 domain at a region distinct from the ATP pocket. MD simulation and MM-PBSA calculation showed the complex stability and high binding affinity of these three compounds. Thus, these three lead compounds can be considered as a potential non-ATP competitive inhibitors. And based on the binding mode of all the three compounds, E-pharmacophore model has been determined with four pharmacophoric features including two aromatic rings, one hydrogen bond donor and acceptor. These pharmacophoric features may be helpful in the development of new potential inhibitors to target LMTK3 for effective breast cancer therapeutics.

10.2. Future prospects

This thesis gave an atomistic insight about structural features of LMTK3, different protein-protein interactions involved in LMTK3 with other proteins and finally designing of inhibitor against LMTK3 to reduce breast cancer load. There are further possibilities that include:

(i) One can develop experimentally (X-ray or NMR) determined 3-D structure of LMTK3 domain. This will shed light on the development of effective LMTK3 inhibitors for effective therapeutic intervention.

(ii) Experimental validation of *in silico* designed potential inhibitor will be more helpful for the effective therapeutics to control breast cancer.