CHAPTER 1

MOTIVATION & OUTLINE OF THE THESIS

1. Motivation & outline of the thesis

1.1. Motivation of the present work

Breast cancer is known to be the most commonly diagnosed cancer, making up to 25% of all cancer cases and leading cause of mortality in female Worldwide, [1, 2]. Breast cancers originate from the ducts that are lined with luminal epithelial cells of the normal mammary gland [3]. Even with the great improvement in survival rates in the last two decades, disease with metastasis still remains the most poorly understood aspect of cancer pathogenesis [4]. Therefore, there is a growing need to identify targets implicated in this process. Over the past decade, many studies have reported, the dys-regulations, hyper-activations or mutations in protein kinases, cause human cancers including breast cancer, making the protein kinases into a valid candidates for targeted therapies. In the recent past, Giamas and group in 2011 identified a new protein that is Lemur tyrosine kinase-3 (LMTK3), implicated in ERα positive breast cancer growth and endocrine resistance [5, 6]. Moreover increased LMTK3 expression associates with poor overall and disease-free survival, revealing a potential involvement of LMTK3 in breast cancer progression [7, 8]. In ERα positive breast cancer LMTK3 regulates ERα activity through phosphorylation and protects ERα from proteosomal degradation which leads to cancer growth and endocrine therapy resistance [5]. Thus LMTK3 is considered as a master oncogenic modulator and novel therapeutic target in ERα-positive breast cancer. Therefore, inhibition of LMTK3 functioning by potential inhibitors may down regulate ERα protein level and may result in controlling breast cancer progression. Since, there is no effective inhibitor against LMTK3 till now, therefore these studies motivated us to study the salient structural features of LMTK3 domain in more details. Structural study will be useful in designing potential inhibitor against LMTK3. And identification of probable molecular interactions between ERα and LMTK3 domain is also critical.

In addition to ERα regulation, LMTK3 also involved in invasion and metastasis in triple negative breast cancer. Similar to other receptor tyrosine kinase (RTKs), LMTK3 can directly interact with the adaptor protein Growth factor receptor bound protein (GRB2) which activates RAS-GTPase family members subsequently leading to

the up-regulation of serum response factor (SRF) activity and then increases the binding of SRF to integrin promoter which leads to the transcriptional activation of integrin β_1 and finally results in breast cancer invasion and metastasis [9]. Thereby molecular interaction between GRB2 and LMTK3 is critical. With the knowledge of probable interface area and interacting interface residues at GRB2 and LMTK3, protein-protein interaction can be controlled by designing drugs accordingly.

Recently, in vitro studies have identified the ability of CDK5 to phosphorylate LMTK3 and activates it which results in breast cancer tumorigenesis. [10]. Thereby molecular interaction between CDK5 and LMTK3 is critical. Generally before a protein kinase catalyse the phosphorylation reaction, they themselves get activated and regulated through phosphorylation by other kinases and the phosphorylation event occurs at activation loop of kinases, [11, 12]. The activation loop phosphorylation induces and catalyses the phosphoryl transfer reaction of γ-phosphate of an ATP molecule to its substrate [13-15]. In addition we also studied the effect of activation loop phosphorylation at probable phosphorylation site of LMTK3 domain in the dynamic system. Overall, from these studies we can see that protein–protein interaction between LMTK3 and its binding partners is critical in signaling pathways which causes breast cancer progression, invasion and metastasis.

Therefore, it will be worthwhile to investigate on (i) Structural and dynamic characteristic features of LMTK3 domain and the ATP binding pocket in it (ii) Molecular interaction profile between LMTK3 domain and its substrates/binding partners (iii) Identification of potential inhibitors against LMTK3 functional domain.

1.2 Outline of the thesis

Chapter 2 briefs about the Breast cancer and its types, the therapies to treat breast cancer and the reason behind the development of resistance to therapies. Also the implications of Lemur tyrosine kinase 3 (LMTK3) in breast cancer and other cancers.

Chapter 3 describes the computational techniques and the key principle of Molecular Dynamics (MD) simulation and other computational tools and software used in this thesis.

Chapter 4 presents the salient structural features of LMTK3 domain and ATP binding mechanism.

Chapter 5, 6 and **7a** present the interactions of LMTK3 with its binding partners (ERα, GRB2 and CDK5) which are critical in signaling and breast cancer progression, invasion and metastasis. These studies may be helpful to hinder the protein-protein interactions between them.

Chapter 7b presents the effect of activation loop phosphorylation of LMTK3 domain in dynamic system, wherein *in silico* phosphorylation has been performed by adding phosphate group to the probable phosphorylation site in the activation loop of LMTK3 domain.

Chapter 8 presents the designing of potential inhibitors against LMTK3 using ATP competitive approach. Using virtual screening and molecular docking we narrowed down to six potential inhibitors that bind to the ATP binding pocket with high binding free energy.

Chapter 9 presents energy optimized (E-) pharmacophoric features for the LMTK3 inhibitor.

Chapter 10 summaries the important findings and future prospects of this work.