PUBLICATIONS

(first page)

Current Biotechnology

Title: Salient Structural Features of Human Lemur Tyrosine Kinase 3 (LMTK3) Domain from Molecular Dynamics Simulation Study

VOLUME: 7 **ISSUE:** 4

Author(s): Himakshi Sarma and Venkata Satish Kumar Mattaparthi*

Affiliation: Molecular Modelling & Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam, Molecular Modelling & Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam

Keywords: Breast cancer, estrogen receptor-α, tamoxifen resistance, computational tools, endocrine resistance, hormone.

Abstract: Background: Estrogen receptor-α (ERα) positive breast cancer is considered to be one of the most common metastatic diseases. Estrogenic signalling in breast cancer is one of the most critical oncogenic pathways. Recently Lemur Tyrosine Kinase 3 (LMTK3) was identified as a potential oncogenic ERα regulator with a significant role in endocrine resistance. Therefore, targeting LMTK3 in breast cancer would control ERα modulation and may provide a better diagnostic development and a new therapeutic target to fight these resistant and aggressive tumours.

Objective: The study aimed to understand the salient structural features of LMTK3 using molecular dynamics simulation.

Methods: In this computational study, we modelled 3D structure of LMTK3 domain using Iterative Threading ASSembly Refinement (I-TASSER) and studied conformational dynamics using molecular dynamics simulation. We used online computational tools and software to perform comprehensive investigation on the cavities, hydrophobicity, electrostatic potential, secondary structure topology and intra- molecular interactions in LMTK3.

Results: The LMTK3 structure was observed to be stable during Molecular Dynamics (MD) simulation. We also predicted the probable binding cavities in LMTK3. In addition, we determined hydrophobic clusters and patches in LMTK3 which may be crucial for folding and stabilisation. Possible interaction sites in LMTK3 were then studied by electrostatic potential analysis based on positive and negative surfaces. From the secondary structure topology analysis, we noticed nine antiparallel β-sheets forming β- sheets topology and five hairpins were involved in forming the secondary structure.

Conclusion: Our inferences from this study would be helpful in understanding the structure–function relationships of LMTK3 and also help in designing suitable inhibitors for LMTK3.

Current Proteomics

Title: Unveiling the Transient Protein-Protein Interactions that Modulate the Activity of Estrogen Receptor(ER)-α by Human Lemur Tyrosine Kinase-3 (LMTK3) Domain: An In Silico Study

VOLUME: 14 **ISSUE:** 2

Author(s): Venkata Satish Kumar Mattaparthi* and Himakshi Sarma

Affiliation: Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam, Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam

Keywords: Breast cancer, endocrine resistance, molecular dynamics simulation, PatchDock, PDBsum.

Abstract: Background: Estrogen receptor-α positive breast cancer is the most common dreadful disease and leading cause of death among women. In majority of human breast cancer, the interactions between kinases and ERα are considered to be critical in signaling pathway. Many kinases are known to regulate ERα activity. Recently Lemur tyrosine kinase-3 was identified as predictive oncogenic ERα regulator with a vital role in endocrine resistance. The role of LMTK3 in ERα regulation can be known by studying the interactions between them.

Objectives: To understand the transient interactions between ER-α and LMTK3 using computational technique.

Method: ERα -LMTK3 complex structure was obtained using PatchDock server. The interacting residues and interface area between ERα and LMTK3 were identified using PDBsum. Molecular dynamics simulation was used to study the conformational dynamics of ERα -LMTK3 complex.

Result: The approximate interface area of ERα-LMTK3 was found to be 3175 Å2 with atomic contact energy of 191.77 kcal/mol. PDBsum results revealed that some of the residues in C-terminal region of LMTK3 displayed non-bonding interactions with the residues in the phosphorylation sites (Ser104 and Ser106) of ERα. We noticed the total number of interface residues in ERα-LMTK3 complex to be 50 and the interface area for ERα as well as LMTK3 chain involved in interaction to be more than 2380 Å2. From conformational dynamics study, ERα-LMTK3 complex structure was found to be stable.

Conclusion: The outcomes of the current study enhance the understanding of interactions between ERα and LMTK3 which are thought to be critical in signaling pathway in majority of human breast cancers.

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RESEARCH ARTICLE

Unveiling the Transient Protein-Protein Interactions that Regulate the Activity of Human Lemur Tyrosine Kinase-3 (LMTK3) Domain by Cyclin-Dependent Kinase 5 (CDK5) in Breast Cancer: An *in silico* Study

Himakshi Sarma and Venkata Satish Kumar Mattaparthi*

Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam, India

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Abstract: ***Background***: In many human diseases protein kinase are known to play a central role. Protein kinases phosphorylate its substrates and they themselves regulated through phosphorylation of their activation loop and become catalytically active. LMTK3 is an oncogenic protein kinase, implicated in breast cancer progression and endocrine resistance. Recent report says phosphorylation of LMTK3 by CDK5 results in breast cancer tumor progression. Thereby information about interface residues and probable phosphorylation site on LMTK3 is critical.

Objective: To understand the transient protein – protein interactions between CDK5 and LMTK3 using computational techniques.

Methods: LMTK3 structure was superimposed with known kinases to determine the probable activation segment and phosphorylation sites in LMTK3. PatchDock was used to obtain CDK5-LMTK3 complex structure. PDBsum server was used to identify the interface residues between CDK5 and LMTK3. The stability of CDK5-LMTK3 complex was studied using Molecular dynamics (MD) simulation.

Results: From PatchDock, interface area between CDK5-LMTK3 complex was found to be 2081 $Å^2$ with atomic contact energy of -228.80 kcal/mol. PDBsum result reveal that, CDK5 interact and displayed non-bonding interactions with the probable phosphorylation sites of LMTK3. Total number of interface residues across CDK5-LMTK3 was found to be around 50 and the interface area found to be 1274 $Å^2$ (in CDK5) and 1224 $Å^2$ (in LMTK3). From MD simulation, CDK5-LMTK3 complex was found to be stable.

Conclusion: This study enhances the understanding of interactions between CDK5 and LMTK3 that may be helpful in understanding the LMTK3 phosphorylation by CDK5 which is considered to be a new cellular pathway in breast cancer tumor progression.

Keywords: Breast cancer progression, activation segment, phosphorylation sites, PatchDock, PDBsum, molecular dynamics simulation.

1. INTRODUCTION

Lemur tyrosine kinase-3 (LMTK3) was identified as an oncogenic Serine Threonine Tyrosine kinase which is found to be involved in various types of cancer including breast [1], lungs [2] and colorectal cancer [3]. In estrogen receptor α (ERα) positive breast cancer, LMTK3 has been reported to regulate ERα through phosphorylation with a significant role in endocrine resistance [1, 4, 5]. In addition cytoplasmic elevation of LMTK3 in triple-negative breast cancer, promotes breast cancer cell motility, migration and invasion through transcriptional activation of intigrins [6].

Generally, protein kinases consist of two lobes, the N-terminal (small N-lobe) and C-terminal (large C-lobe). These two lobes form a deep pocket that accommodates an ATP molecule [7]. The C-lobe contains an activation segment which is 20-35 residues stretch located between a conserved DFG and APE motif which is conformationaly very flexible, its conformation effect on both substrate binding and catalytic efficiency [8, 9].

Phosphorylation is a common posttranslational modification of proteins in eukaryotic cells [10] that regulate several important cellular processes, such as cell growth and differen-

*Address correspondence to this author at the Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam, India; Tel: 08811806866; Fax: 03712-275443; E-mails: mvenkatasatishkumar@gmail.com; venkata@tezu.ernet.in

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Effect of Activation Loop Phosphorylation on Lemur Tyrosine Kinase 3 (LMTK3) activity: A Molecular Dynamics Simulation Study

Himakshi Sarma and Venkata Satish Kumar Mattaparthi
Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam, India

(Corresponding author: Venkata Satish Kumar Mattaparthi)
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ABSTRACT: Protein kinases catalyze the phosphorylation reaction, and they themselves become catalytically activated through phosphorylation of their activation loop. LMTK3 is an oncogenic kinase, reported in various types of cancer. Recent study highlights LMTK3 phosphorylation by CDK5 results in breast cancer tumourogenesis. We determined the probable activation loop in LMTK3 and carried out *in silico* phosphorylation at probable phosphorylation site (Thr189) in activation loop and studied the effects of phosphorylation on conformational dynamics. We substituted Glu for phosphorylated Thr189 and noticed Glu does not mimic the effect of phosphorylation. From Molecular dynamics analysis, phosphorylated, unphosphorylated and mutated LMTK3 found to be stable. But phosphorylated loop region shows much fluctuation. Thereby ATP binding mode was observed to be different in phosphorylated as compared to unphosphorylated LMTK3. Phosphorylation mediated conformational change in the ATP binding site of LMTK3 may facilitate phosphoryl transfer reaction to its substrates, and may leads to breast cancer progression.

Keywords: Breast cancer tumourogenesis; Phosphorylation sites; Molecular docking; Activation segment; Conformational dynamics.

INTRODUCTION

Eukaryotic protein kinases are the largest gene family that regulates several important cellular processes, such as cell growth and differentiation (Ban *et. al.,* 2011; Waldrop, 2014). Protein kinases share a conserved core consisting of two lobes, the N-terminal (small N-lobe) and C-terminal (large C-lobe). These two lobes form a deep pocket that accommodates an ATP molecule (Kornev *et. al.,* 2010). The N-lobe consists of five β-strands and an α-helix (called αC-helix). The C-lobe contains α-helices and includes the activation segment which is 20-35 residues stretch located between a conserved DFG motif and APE motif that is conformationaly very flexible and its conformation can influence both substrate binding and catalytic efficiency (Huse *et. al.,* 2002; Nolen *et. al.,* 2004).

Phosphorylation is the commonest posttranslational modification of proteins in eukaryotic cells (Olsen *et. al.,* 2013). Eukaryotic protein kinases (EPKs) are the family of enzymes that catalyse the phosphorylation reaction, and themselves regulated by phosphorylation (Beltrao *et. al.,* 2012; Nolen *et. al.,* 2004). The regulatory phosphorylation event occurs in most EPKs at activation loop of kinases, which is part of the activation segment (Beltrao *et. al.,* 2012; Nolen *et. al.,* 2004). Phosphorylation of the activation loop is a key mechanism that induces a dynamic changes in activation process (Kornev *et. al.,* 2006; Kornev *et. al.,* 2015; Meharena *et. al.,* 2013; Taylor *et. al.,* 2012), and leads to structural changes which stabilize the active conformation. This mechanism catalyses the transfer of γ-phosphate of an ATP molecule to the phosphoacceptor site of substrate (Beltrao *et. al.,* 2012; Kornev *et. al.,* 2015; Johnson *et. al.,* 1996). Therefore activation-loop phosphorylation is crucial because it is required for the interconversion from an inactive to an active conformation of kinase. Generally the protein kinase domain is a structurally conserved protein domain containing the catalytic function of all protein kinases (Hanks *et. al.,* 1991; Hanks *et. al.,* 1995; Scheeff *et. al.,* 2005).

Lemur tyrosine kinase-3 (LMTK3) is an oncogenic Serine Threonine Tyrosine kinase implicated in various types of cancer including breast (Giamas *et. al.,* 2011) lungs (Xu *et. al.,* 2014) and colorectal (Shi *et. al.,* 2014).

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ORIGINAL RESEARCH ARTICLE

Structure-Based Virtual Screening of High-Affinity ATP-Competitive Inhibitors Against Human Lemur Tyrosine Kinase-3 (LMTK3) Domain: A Novel Therapeutic Target for Breast Cancer

Himakshi Sarma[1] · **Venkata Satish Kumar Mattaparthi**[1]

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Abstract
Human lemur tyrosine kinase-3 (LMTK3) is an oncogenic kinase known to regulate ER-α through phosphorylation and is considered to be a novel therapeutic target for breast cancer. In this work, we have studied the ATP-binding mechanism with LMTK3 domain and also carried out virtual screening on LMTK3 to identify lead compounds using Dock blaster server. The top scored compounds obtained from Dock blaster were then narrowed down further to six lead compounds (ZINC37996511, ZINC83363046, ZINC3745998, ZINC50456700, ZINC83351792 and ZINC83364581) based on high-binding affinity and non-bonding interactions with LMTK3 using Autodock 4.2 program. We found in comparison to ATP, the lead compounds bind relatively stronger to LMTK3. The relative binding free energy results from MM-PBSA/GBSA method further indicate the strong binding affinity of lead compounds over ATP to LMTK3 in the dynamic system. Further, potential of mean force (PMF) study for ATP and lead compounds with LMTK3 have been performed to explore the unbinding processes and the free energy barrier. From the PMF results, we observed that the lead compounds have higher dissociation energy barriers than the ATP. Our findings suggest that these lead compounds may compete with ATP, and could act as probable potential inhibitors for LMTK3.

Keywords Virtual screening · Molecular docking · Potential inhibitors · MM-PBSA/GBSA · Potential of mean force

1 Introduction

Breast cancer is the most common invasive cancer among women which leads to severe health complications and ultimately, death [1]. Among the different types of breast cancers, 70% of cases express estrogen receptor-α (ERα) [2] and are thus termed as ERα-positive breast cancer. ERα is a transcription factor whose activity is regulated by estrogen binding [3, 4]. In ERα-positive breast cancer, the key target has been reported to be the estrogen-signaling pathway [5]. Various endocrine therapies have been used to treat ERα-positive breast cancer, which includes antiestrogenic drug, tamoxifen which is an estrogen receptor modulator [6], aromatase inhibitors to inhibit the peripheral estrogen synthesis [7], and fulvestrant which induce receptor degradation. These therapies have been found to be effective and also improve disease-free survival [8]. But then again, resistance commonly occurs against these therapies in breast cancer due to the phosphorylation of ERα [9] by various protein kinases (MAPK, CDK2-CyclinA, and protein kinase A) [10–12] which modules ERα transcriptional activity and alter its stability [13, 14]. Therefore, protein kinases have been reported to be the key target in ERα-positive breast cancer [15].

Human lemur tyrosine kinase 3 (LMTK3) is a serine–threonine–tyrosine kinase [16, 17] recently identified to regulate ERα activity through phosphorylation that leads breast cancer progression and endocrine resistance. It has been reported that in most of the aggressive types of breast cancers, the LMTK3 expression level is very high, thus its level correlates with the disease-free survival [18–20]. In addition, the high cytoplasmic

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✉ Venkata Satish Kumar Mattaparthi
mvenkatasatishkumar@gmail.com; venkata@tezu.ernet.in

[1] Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam 784 028, India

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