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Research paper

Mutations and interactions in human ERα and bZIP proteins: An in silico approach for cell signaling in breast oncology

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ABSTRACT

I. Background: Metastasis of breast cancer serves the most aggravating cause for transience in breast cancer patients. Accumulating evidences suggest that signal transduction in human breast cancers commences in estrogen-reliant pattern via signaling of the estrogen-receptor α-subunit (ERα) and XBP-1 (bZIP-domain) proteins. Furthermore, earlier investigations from SAGE and GST pull-down assay, also state that a point mutation in ERα leads to a risky factor by resulting into hyper-responsiveness towards estrogen and increased proliferation of breast cancer cells. So, a molecular-level exploration into the signaling mechanism is a prime requisite for future clinical and therapeutic progress.
II. Methods and results: Present study explores primarily the residual participation of the two essential proteins from humans to boost the signaling mechanism in malignant breast tumors. So, 3D structures of the respective monomer proteins were demonstrated and mutated protein was homology modeled after the satisfaction of the stereo-chemical features. The functionality was observed to be conserved after mutation. Abrupt increment in protein-protein interactions was studied for the individual optimized and Molecular Dynamics simulated protein complexes. Revelation from supportive statistical significances for several energy calculations, solvent accessibility areas, electrostatic surface potentials and interaction studies led to confer that after mutation, the complex and the individual protein were the most stable and the best interactive one. For metastasis in breast cancer cells, polar charged residues hold a significant contribution.
III. Conclusion: Therefore, this investigation provides a cogent framework for the interactive studies associated with breast cancer and an exposure towards the lethal impact on mutation.

1. Introduction

Metastatic breast cancer has deadly impact for the patients struggling against it. In the progress of breast cancers, estrogen cell signaling mechanism is aided by the estrogen receptor (ER) (Sudipa and Ratna, 2012). The major subunit of ER is ERα (Warner et al., 1999; Hewitt et al., 2000). In the mammary epithelium, ERα plays a vital role in the advancement of breast cancer (Warner et al., 1999; Hewitt et al., 2000). After the binding of estrogen to ERα, the ligand-activated ERα gets translocated to the nucleus (Sudipa and Ratna, 2012). Now, after interacting with the promoter of the target gene, there occurs initiation of gene transcription via the genomic or nuclear signaling which is executed by ERα (McKenna et al., 1999; McDonnell and Norris, 2002). This ERα is further assisted by the X box-binding protein 1 (XBP-1). X box-binding protein 1 (XBP-1) is one more essential protein in humans. It accompanies and facilitates the performance of ERα and estrogen receptor not only in vivo but also in vitro for breast tumors (Ding et al., 2003; Sengupta et al., 2010). Studying the research studies from GST pull-down assay, it was deduced that XBP-1 binds to the ERα protein for the progression of metastasis (Ding et al., 2003; Ding et al., 2004). Documentation documents it to promote and increase the activity of transcription in ERα, without being reliant on any ligand (Ding et al., 2003).

A special and sole domain of human XBP-1 is known as basic region leucine zipper (bZIP) domain. This bZIP domain is competently responsible for vital interactions with ERα (Liou et al., 1990). It has been well documented with the performance of SAGE (serial analysis of gene expression) that bZIP-domain from human XBP-1 expresses at highly amplified stages in ERα-positive malignant breast tumors (Ding et al.,

Abbreviations: WT, wild type; MT, mutant type; MD, Molecular Dynamics; DS, Discovery Studio; P.I.C, Protein Interaction Calculator; SD, standard deviation.

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2003). In the progression of breast cancer after its initial establishment, cell signal transduction aided by the ERα protein plays a determining role. At the basic residual and computational level, the critical role of XBP-1 (bZIP-domain) protein to enhance and facilitate the transcriptional activity of ERα remains yet unexplored.

From evidences available from previous research studies, it is indicated that there is high risk factor in breast cancers when estrogen receptor alpha gene, *esr1* undergoes mutation in the lysine residues (Conway et al., 2007; Conway et al., 2005). It makes the estrogen receptor alpha protein to be highly hyper-sensitive to estrogen (Conway et al., 2007; Conway et al., 2005). In addition to that, all the lysine residues in ER alpha protein are known to be essential (Conway et al., 2007; Conway et al., 2005). This is because mutation in the lysine residues hampers the significant lysine methylation necessary for ER alpha signaling mechanisms (Conway et al., 2007; Conway et al., 2005). Therefore, upon mutation of lysine residue, the cell signaling mechanism gets enhanced highly by estrogen receptor alpha protein, resulting into increased progression of breast cancer cells (Conway et al., 2007). It was primarily examined in hyperplasias of breast (Conway et al., 2007; Conway et al., 2005).

Therefore, in this study, the structures of the two essential human proteins (ER-alpha and bZIP protein domain from PDB ID: 1GU4) were analyzed and demonstrated. As the crystal structure of XBP-1 protein was unavailable from PDB, so the crystallographic structure of the common domain (bZIP protein domain) from PDB ID: 1GU4 was utilized after several comparative studies. Predominantly, the crystallographic structure of human bZIP protein from PDB ID: 1GU4 was found to possess the same pfam accession number as that of bZIP protein domain from XBP-1 protein (from humans). The essential domain of ER alpha, responsible for the interaction with bZIP protein was identified from the X-ray crystal structure. The necessary lysine residue underwent point mutation in the sequence of the parent protein of ERα. The mutated protein (MT) was further remodeled by homology or comparative modeling after the satisfaction of its varied stereo-chemical properties. The functionality and fold of the mutated protein was observed and compared with the wild type (WT) protein. Finally, energy minimization of the 3D structure was executed for obtaining a stable protein conformation. To find out the residual binding mode, the bZIP protein was individually docked with the 3D functional structures of WT and MT ER-alpha protein using protein-protein docking procedure. Next, energy optimization followed by Molecular Dynamics (MD) simulation was executed for either of the docked complexes. The evaluation of several stability estimating energy parameters, net solvent accessibility area values and electrostatic surface potential calculations were utilized to analyze the firmness and steady impact upon their binding. Firm residual involvement of the relevant proteins to interact with bZIP domain was discerned and examined. Earlier investigations through computational studies (Bhattacharya et al., 2014; Bagchi, 2015) were carried out for several other diseases. Hitherto no such *in silico* based detailed molecular level research work was carried out to explore the root level basis of life-threatening disease metastasis in breast cancer.

This present computational exploration is focused on the base source regarding participation of the two important proteins in the cell signaling event for the proliferation and progression of malignant estrogen controlled tissues. The study is further extended to particularize the effect for point mutation in the estrogen receptor alpha protein consequent to its altered interaction with bZIP protein and change in the stability in MT. The revelation and analysis of residual dependencies of the relevant proteins in the respective WT and MT cases, from this probe would be additionally favorable for upcoming therapeutic research in future.

2. Experimental

2.1. Structure analysis of wild type (WT) ERα and bZIP protein

The search results lead to the identification of the X-ray crystal structure for ERα and bZIP proteins. The PDB ID of ERα and bZIP proteins are 2OCF, chain A (Koide et al., 2002) and 1GU4, chain A (Tahirov et al., 2001) from *Homo sapiens*, respectively. The former protein was bound to a ligand and the latter protein was bound to a DNA fragment. So, the essential protein structures were extracted with the help of Discovery Studio (BIOVIA Discovery Studio 4.1 software (USA)). Furthermore, the protein structures were individually analyzed and demonstrated in their independent forms, respectively.

2.1.1. Comparative study for the selection of human bZIP protein domain from X-ray crystallographic structure

The X-ray crystallographic structure of XBP-1 protein was not available in PDB. So, after necessary comparisons, the common domain (bZIP protein domain) from the X-ray crystallographic structure of C/EBP-β (PDB ID: 1GU4) was selected for our present study. The comparison between the two protein domains (bZIP from X-ray crystallographic structure and from XBP-1 protein) was performed in three consecutive steps: (i) Conserved domain identification (through pfam), (ii) sequence alignments (pair-wise) and (iii) preservation of folds and functionality from the two tertiary structures (through TM-score).

The pfam (Punta et al., 2011) accession number: PF07716.10 was similar in both the proteins (X-ray crystal bZIP protein domain and bZIP domain from the amino acid sequence of XBP-1 protein (GI: 47678753, Accession no: CAG30497.1) from NCBI). This thereby affirms the domain to similar to perform its function independently. The protein domain- bZIP, in either of the cases holds same functionality and similar fold.

The two protein sequences underwent pairwise alignment. Earlier studies have analyzed that proteins can possess same fold but might not exhibit proper sequence identity (Friedberg and Margalit, 2002; Sander and Schneider, 1991). It was also documented that proteins might have low alignment scores through pairwise alignment but that did not signify that they were unrelated to each other (Friedberg and Margalit, 2002). More specifically, in >90% cases, two proteins having a rough cut-off of 30% sequence identity were observed to be homologous in nature (Rost, 1999). They were observed to be structurally similar too (Rost, 1999). In fact, most analogous protein structure pair shows to exhibit <12% sequence identity upon pairwise alignment (Rost, 1999). The sequence identity in this present study was observed to be >30%, that is 33.3% and sequence similarity was found to be nearly 60% (Suppl. Fig. 1).

The bZIP domain of XBP-1 protein from *Homo sapiens* (GI: 47678753, Accession no: CAG30497.1) was also modeled through homology modeling approach. Now to analyze and compare the fold and functionality of the protein structures, this modeled bZIP protein and the bZIP protein having X-ray crystallographic structure (PDB ID: 1GU4) was subjected to TM-Align (Zhang and Skolnick, 2005). TM-Align helps to evaluate the TM-score between the two provide d protein 3D structures. The TM-score > 0.5 indicates that the two proteins share a common SCOP/CATH fold and preserve their functionality (Zhang and Skolnick, 2005). It helped to analyze the proper alignment of the 3D structures of the two proteins and further generated protein residue-to-residue alignment based on structural similarity (Zhang and Skolnick, 2005). In this study, the TM-score between the two bZIP proteins (one modeled structure and the other having PDB ID: 1GU4) was found to be 0.6204. This depicts the protein to share same functionality having same SCOP/CATH folds. The alignment after Cα superimposition of the two bZIP protein structures has been depicted in Suppl. Fig. 2. So, the X-ray crystallographic structure was selected for the further investigations in the present study as this structure (PDB ID: 1GU4) was experimentally validated being deposited in the PDB database.

2.2. Analysis of the residue to be mutated, template search and comparative modeling for the mutated (MT) ERα protein

2.2.1. Analysis of the residue to be mutated

Firstly, in the X-ray crystal validated structure of ER-alpha protein, the most essential residue from the interactive part (Ligand-Binding