Molecular Computing and Residual Binding Mode in ERα and bZIP Proteins from *Homo Sapiens*: An Insight into the Signal Transduction in Breast Cancer Metastasis

Arundhati Banerjee and Sujay Ray

Abstract The most provoking reason for death in breast cancer patients is the metastasis of breast cancer. Accumulating documentation states that signal transduction in human breast cancers initiate in estrogen-dependent manner with the signaling of estrogen receptor α-subunit (ERα) and XBP-1 (bZIP-domain) proteins. So, molecular level insight into the signaling mechanism is indispensable for future pathological and therapeutic developments. Thus, this current study discloses the stable residual participation of the two crucial human proteins for enhancing the signaling mechanism in breast tumor malignancies. For this purpose, 3D homology models of the respective proteins were prepared after the satisfaction of their stereo-chemical features. The protein–protein interaction was studied and protein complex was energy optimized. Revelation from the stability calculating parameters, solvent accessibility areas and interaction probes led to the inference of the most stable optimized complex and its residual participation (exceptional contribution of polar charged residues) for metastasis progression in breast cancer cells.

Keywords Homology modeling · Cell signal transduction · Docked protein–protein interaction · Energy optimization and breast cancer metastasis

A. Banerjee
Department of Biotechnology, National Institute of Technology, Durgapur, West Bengal, India
e-mail: arundhati.92star@gmail.com

S. Ray
Department of Biochemistry and Biophysics, University of Kalyani, Kalyani, West Bengal, India

S. Ray (✉)
Department of Biotechnology, Bengal College of Engineering and Technology, Durgapur, West Bengal, India
e-mail: raysujay@gmail.com

1 Introduction

One of the life-threatening phases of cancer is occupied by the metastatic breast cancer and has a lethal impact for the patients fighting against breast cancer. In the development and advancement of breast cancer, estrogen cell signal transduction accompanied by the estrogen receptor (ER) is highly associated [1]. In mammary epithelium, a major ER subunit, ERα participates in a pivotal role in breast cancer advancement [2, 3]. Estrogen binds to ERα, which is followed by the translocation of the ligand-activated ERα to the nucleus [1]. Now, post binding to the promoter of the target gene, transcription of the gene gets stimulated by genomic or nuclear signaling is performed by ERα [4, 5]. Another important human protein, human X box-binding protein 1 (XBP-1) is coupled with ERα activity in vitro and in vivo for breast tumors [6, 7]. It is known to elevate the transcriptional activity of ERα, in a ligand-free mode [6].

Human XBP-1, comprises a unique domain known as basic region leucine zipper (bZIP) domain which is efficiently responsible for important interactions including the one with ERα [8]. With the aid of SAGE (serial analysis of the expression of gene), bZIP-domain from XBP-1 has been properly documented to be expressed at elevated levels in ERα-positive malignant tumors in breast [6]. Therefore, in the progression of breast cancer advancement and succession, cell signal transduction by ERα protein holds a prior significance. This essential investigation in the efficient role of XBP-1 (bZIP-domain) in the transcriptional activity of ERα, at its molecular and computational level remains yet unexplored.

Therefore, in this present probe, the two vital proteins were modeled by homology or comparative modeling techniques after their sequence analysis. Each of the models was prepared finally after satisfying their varied stereo-chemical properties. Overall energy minimization of the 3D-modeled tertiary structures was executed for achieving a stable protein conformation. The protein monomers underwent protein–protein docking phenomena to interact with each other. Further energy optimization was performed for the docked complex. The stability and the strengthening impact on their binding were observed by the calculation of several stability determining parameters. Electrostatic surface potential calculations also aided to investigate the stable interactive complex. Residual participation from the respective proteins to cooperate among one another firmly was discerned and investigated. Earlier investigations [9, 10] documents the molecular level studies for many such diseases but breast cancer metastasis, a deadly life-threatening disease, was dealt nowhere.

In a digest, this extant computational exploration aimed into the root-source for the important responsibilities of the two indispensably important proteins in the signaling phenomena for the proliferation of malignant estrogen-controlled tissues. The examination of residual dependencies of the respective proteins from this study would be further beneficial for future therapeutic investigation. It might endow with

an upcoming scope for the clinical research for the investigation of any small modulators for ERα or/and any specific discovery of drug to motivate the protein to reduce its tendency for causing the proliferation of the malignant breast cancer cells.

2 Materials and Methods

2.1 Analysis of Sequences and Template Search for Comparative Modeling

The initial step for homology modeling lies in analyzing the amino acid sequences for the proteins. So, the amino acid sequences of XBP-1 protein and ERα proteins from *Homo sapiens* were obtained, individually from NCBI (GI: 47678753, Accession No.: CAG30497.1 for XBP-1 and GI: 11907837, Accession No.: AAG41359.1 for ERα). The sequence analysis was verified by using Uniprot KB. For the purpose of the study, the only functionally most interactive and conserved region (domain) [11, 12], bZIP-domain from XBP-1 protein was extracted from EMBOSS from EMBL-EBI software packages [13], after identifying and analyzing the occupying region of the domain in the parent protein from pfam [14]. Results from BLASTP [15] against PDB [16] helped to deduce the templates for building homology models. HH-Pred, a very sensitive algorithm that uses homologous relationships between distantly related proteins [17], was also utilized for template search. Templates for bZIP-domain and ERα were obtained from the X-ray crystal structure of *Schizosaccharomyces pombe* and *Homo sapiens,* respectively, (PDB code: 1GD2_E with 99.46 % probability sharing 40 % sequence identity for bZIP-domain from HH-Pred search and 2OCF_A with 55 % sequence coverage and sharing 99 % sequence identity for ERα).

2.2 Comparative Modeling of bZIP-Domain and ERα Monomers

Homology modeling technique, also often familiar as comparative modeling technique was utilized with the aid of MODELLER9.14 software tool [18], for building up the individual 3D tertiary structures of the proteins. Back bone superimposition on each of the modeled protein's respective crystal templates (i.e., 1GD2_E for bZIP-domain and 2OCF_A for ERα), by using PyMOL [19], yielded the appropriateness for the modelled structures. For instance, the root mean squared deviation (RMSD) of ERα protein was perceived to be 0.291Å. So, Fig. 1 shows the ribbon-like representation for the superimposition of the modeled ERα protein (pink) on its crystal template (blue).
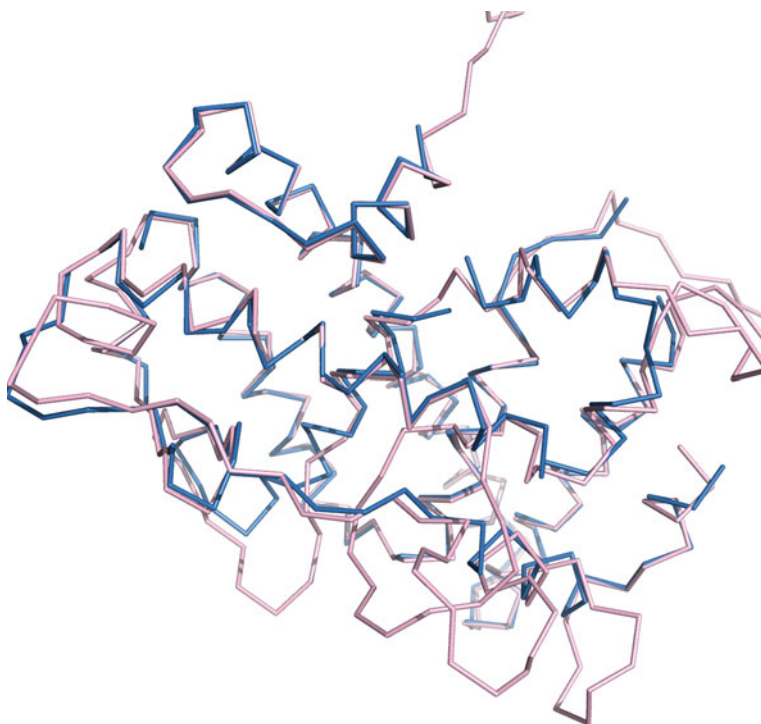
Fig. 1 Superimposition of the ERα protein on its X-ray crystal template (PDB ID: 2OCF_A)

2.3 Optimization and Conformational Stability of Loop Regions Using ModLoop

The homology modeled proteins were subjected further to ModLoop [20] for loop optimization. ModLoop [20] reduces and corrects the inaccuracies due to conformational discrepancy in the loop regions of the proteins. So, each of the structures were remodeled and optimized using ModLoop for proper conformation of ψ–φ angles.

2.4 Energy Optimization of the Loop-Optimized Modeled Protein Structures

Clearance the hostile geometries in the 3D models followed by protein refinement of the individual monomers was performed by ModRefiner tool [21]. The entire conformational search is done by physics based and knowledge based force field together. Thus native states of proteins are achieved from initial models

(where, maximum stability among residues exists) with respect to positioning of backbone and spatial restraints [21]. It also thereby helps to minimize the overall energy of the protein structures, leading to a firmer and steady conformation [21].

2.5 Stereo-Chemical Validation of the Modeled Proteins

Verification of the stereo-chemical summary and overall quality of the modeled structures was performed with the assistance of PROCHECK [22] and ERRAT [23]. As per the predictions, quantitatively satisfying protein models were observed. There were no such amino acid residues that were perceived in the unfavored regions of the Ramachandran Plots for each of the modeled protein monomers [24].

2.6 Protein–Protein Docking Simulations

For the protein–protein interaction study, docking of the monomers were performed employing Cluspro2.0 [25] docking server. The ERα protein, having more amino acid residues, was uploaded as receptor and on the other hand, bZIP protein having comparatively lesser amino acid residues, was uploaded as ligand. Operating the advanced choice of ClusPro2.0 for modification of protein structures, the unstructured residues were reduced. A total of 10 docked bZIP-ERα complexes were presented by Cluspro2.0. The best cluster size among all the complexes was opted for further analysis. GRAMM-X [26] and ZDOCK [27] was also used to perform the protein–protein interactions. They offered with an inclusive outcome for the purpose.

2.7 Molecular Dynamics Simulation of the Modeled Complex

Improvement of high-resolution protein structures is increasingly essential to achieve a stable conformation of the protein with a diminished overall energy. For the purpose, FG-MD (Fragment-Guided Molecular Dynamics) was operated [28]. Besides implementing high resolution, it carries out atomic-level refinements for the protein structure using knowledge-based template information and physics-based MD simulations simultaneously [28]. To re-prepare the energy path of the MD simulation, special criteria was applied from the divided templates. Improvement of the local geometry of the refined protein structure was performed by eliminating steric clashes and upgrading torsion angle. Thus, it draws the structure near to its native state with higher level of accuracy [28].

2.8 Stability Examination of the Energy Minimized and Simulated 'bZIP-ERα' Complex

Overall optimization of energy leads to a better pattern of interaction and increased structural stability of the protein complex. So, the investigation of the stability of the modelled complex was scrutinized using FastContact server [29, 30]. Additionally, to infer a comprehensive result regarding a stable complex (that is, pre- and post-energy minimization complex protein structure), the net solvent accessibility area was estimated.

2.9 Calculation of Interaction Patterns and Binding Modes in the Complex

To explore the residues dependable for the protein–protein complexes from their respective positions on the protein structure, protein interaction calculator (P.I.C) web server [31] was utilized. The results were supported from the findings of the same by Discovery Studio software packages from Accelrys and PyMOL [19]. It helped to analyze the net hydrogen bonding interactions, ionic interactions, aromatic interactions, and so on.

2.10 Surface Electrostatic Potential Assessment

Before and after energy optimization, the electrostatic potential on the surface for the 'bZIP-ERα' complex was computed and compared. The surface electrostatic potential was generated in vacuum electrostatics with the aid of PyMOL [19]. It was performed by mapping onto the protein surface using the units; kT/e.

3 Results

3.1 Model's Structural Description for bZIP Monomer

The functionally active human bZIP protein domain of XBP-1 protein had a Pfam accession number of PF07716.10. It occupied residue range of 69–120 amino acid residues in the parent XBP-1 protein. After homology modeling, the prepared model was observed to be analogous to its crystal template belonging from *Schizosaccharomyces pombe* (PDB Code: 1GD2; E chain). The 52 amino acid long protein begins with five residues in coil region, followed by α-helices (amino acid

Fig. 2 The bZIP protein showing α-helices (*blue*) with tiny coil (*red*) regions at the N- and C-terminals of the protein
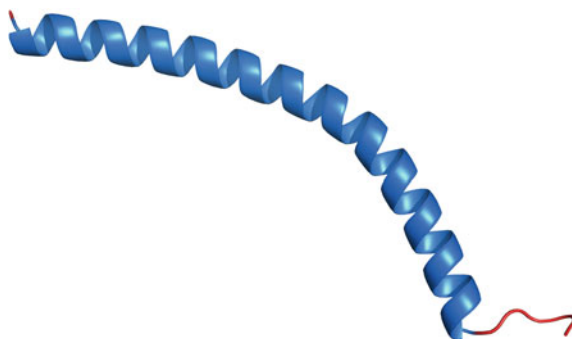
residues: 6–51) and ends with a single residue in coil region again. The structure is well depicted in Fig. 2 with α-helices in blue shades interspersed with red shaded coils.

3.2 Model's Structural Description for ERα Monomer

The functionally active human ERα protein from *Homo sapiens* after homology modeling was observed to be analogous to its X-ray crystal template (PDB Code: 2OCF; A chain). The 288 amino acid residue long protein monomer begins with methionine forming coil region and then followed by a helical region (amino acid residues: 2–5). Rest of the protein mainly forms several α-helical regions (amino acids: 18–23, 56–76, 91–93, 111–118, 120–126, 151–156, 160–177, 189–194, 212–232, 236–270 and 276–286) interspersed with coil regions and a set of antiparallel β-sheets. Six residues form antiparallel β-sheets (residues: 140–142 and 148–150). The structure again winds up with two residues in coil regions. The structure is presented in Fig. 3 with cyan and red shades illustrating α-helices and β-sheets, respectively, with interspersing magenta shaded coils.

3.3 Deduction of the Stability of bZIP-ERα Protein Complex

The variation in net interaction energy values of the bZIP-ERα protein complex (before energy optimization) and the energy minimized complex with MD simulations are well depicted in Table 1. It is lucid from the table that at the protein interfaces, the net interaction energy became stronger after the overall energy optimization of the modeled bZIP-ERα complex. Furthermore, from Table 1, the
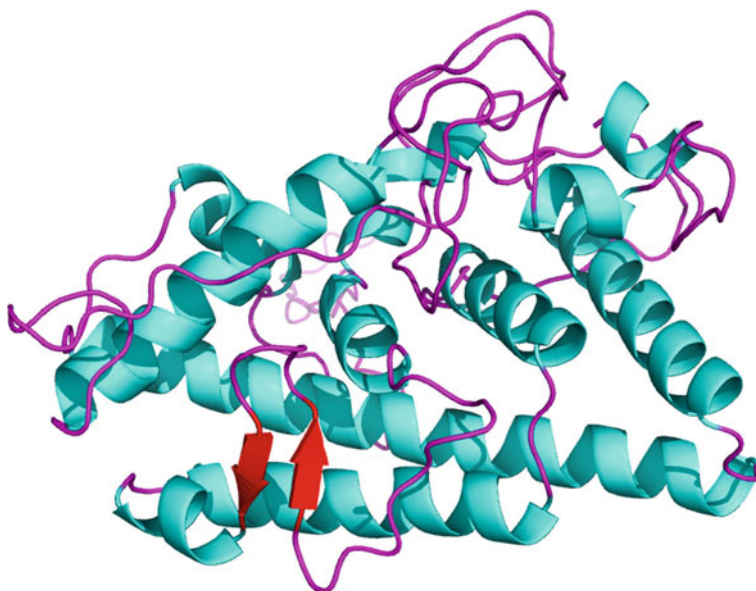
Fig. 3 ERα protein comprising tiny antiparallel β-sheets (*red*) and α-helices (*cyan*) with interspersing magenta coils

Table 1 Stability calculation of the optimized and simulated bZIP-ERα complex

Parameters/bZIP-ERα complex	Total interaction energy (kcal/mol)	Net solvent accessibility ($\mathring{A}^2$)	Stabilizing/destabilizing
Before MD simulation	(−) 1615.04	18237.08 $\mathring{A}^2$	Stable
MD simulated complex	(−) 1734.06	17572.69 $\mathring{A}^2$	More stabilizing

reduction in the net solvent accessibility value for the complex implies that the energy optimized complex structure appeared to be a more interactive one.

3.4 Protein–Protein Interactions in bZIP-ERα Complex

The homology modeled complex structure of bZIP-ERα protein is well illustrated in Fig. 4. The examination using P. I. C web server [31] shows bZIP and ERα cooperate strongly with one another, not only through H-bonding but preponderantly by ionic–ionic interactions. Ionic–Ionic interactions that lead to a stronger and most interactive complex [32] were found to be increased in number after optimization and simulation. Tables 2 and 3 represent the ionic–ionic interactions
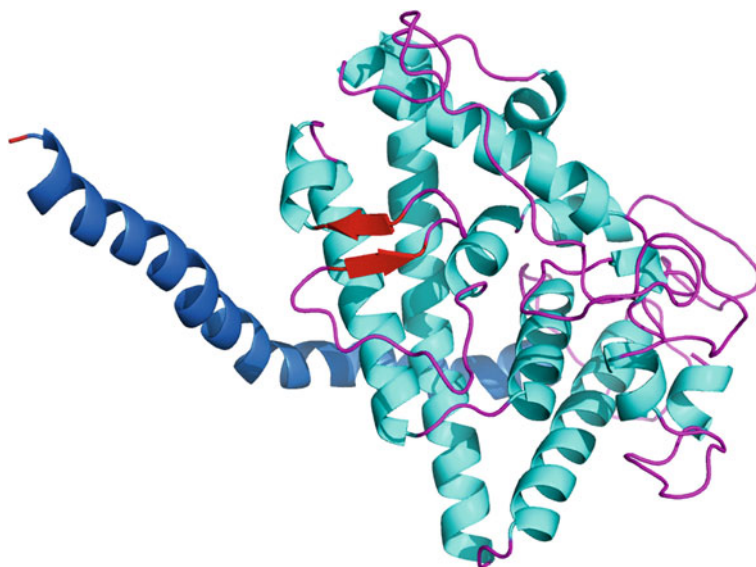
Fig. 4 The bZIP-ERα docked complex firmly interacting with bZIP showing α-helices (*blue*) and beginning with a tiny coil region (*red*) and ERα comprising tiny antiparallel β-sheets (*red*) and α-helices (*cyan*) with interspersing magenta coils

Table 2 Ionic–Ionic interactions in the bZIP-ERα complex before simulation

Residue number	Residue	Chain	Residue number	Residue	Chain
151	ARG	A	36	ASP	X
155	LYS	A	36	ASP	X
155	LYS	A	39	GLU	X
155	LYS	A	40	GLU	X
173	ARG	A	21	ASP	X
219	ASP	A	9	LYS	X
252	HIS	A	21	ASP	X

Chain A and Chain X represents ERα and bZIP, respectively

accomplished by the bZIP-ERα complexes before and after optimization of overall energy (followed by the MD Simulation).

3.5 Surface Electrostatic Potential Estimation

Fascinatingly, the vacuum electrostatic potential calculation also infers the energy minimized complex bZIP-ERα structure to be a more stable and highly interactive one. Figure 5 portrays the pictorial view for the comparable study of the

Table 3 Ionic–Ionic interactions in the bZIP-ERα complex after simulation

Residue number	Residue	Chain	Residue number	Residue	Chain
173	ARG	X	36	ASP	A
210	GLU	X	13	ARG	A
210	GLU	X	9	LYS	A
252	HIS	X	36	ASP	A
254	ARG	X	21	ASP	A
255	HIS	X	29	GLU	A
259	LYS	X	29	GLU	A
262	GLU	X	22	ARG	A
281	GLU	X	7	ARG	A

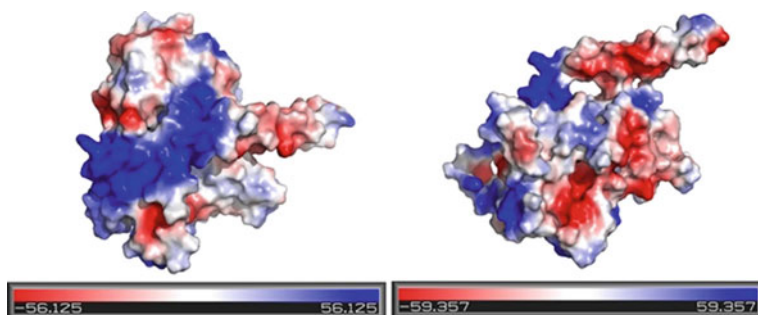Chain A and Chain X represents ERα and bZIP, respectively

Fig. 5 Comparable view of the surface electrostatic potential change on the surfaces before (*left*) and after (*right*) energy minimization and simulation of bZIP-ERα protein complex

electrostatic potentials for the complex structures before and after energy minimization and corresponding MD simulation. The electrostatically positive zones are depicted by blue areas whereas electrostatically negative ones are depicted in red shades, in either of the two cases.

4   Discussion

In the contemporaneous work, the functional tertiary modeled protein structures of the bZIP-domain and ERα were built and analyzed. From the human XBP-1 protein, bZIP-domain is the only domain and the most important interactive zone for the detection of the proliferation of breast cancer cells [6, 8]. The varied interaction pattern in bZIP-ERα complex were analyzed, calculated, and illustrated. The net interaction energies were observed to get a turn-down from −1615.04 kcal/mol (prior-to optimization and simulation) to −1734.06 kcal/mol, after optimization of energy and simulation. Fascinatingly, in addition to that a descent was also observed

in the value of net accessibility area for solvent. The reduction was rapidly from 18237.08 Å^2 (before optimization and simulation) to 17572.69 Å^2 after minimizing and simulating the complex structure. The electrostatic potential values also further, ensure that the optimized and simulated complex structure possessed an exceedingly compact, steady, and a firm interaction between one another. From the strengthening ionic–ionic interactions amongst the complexes, the final simulated complex was perceived to be more firmly interacting with greater number of ionic bonds, which is an increment to nine bonds from seven bonds. Solely, among total nine bonds, five positively charged residues from bZIP protein interacted with five negatively charged residues from ERα protein. Mainly, the positively charged arginine and negatively charged glutamine residues dominated the strengthening of the ionic bonds. From, bZIP protein, Glu210 alone, forms two ionic bonds with Arg13 and Lys9 from ERα. Again, Glu262 and Glu281 of bZIP protein were observed to interact with Arg22 and Arg7 from ERα. Negatively charged aspartic acid was found to interact only from the ERα protein. Asp36, therefore, formed two bonds with Arg173 and His252 of bZIP protein. Asp21 accompanied the interactions by binding Arg254. From ERα, Glu29 forms only two bonds with His255 and Lys259 from bZIP protein. So, in a digest, for the most stable complex, all the five types of polar charged residues (three positively charged-Lys, Arg, His and two negatively charged Asp and Glu) were observed to be satisfactorily indulged to fortify the interaction by the formation of the cavity to accommodate the bZIP protein.

Consequently, this contemporary study presents an acquaintance in the interaction between ERα and bZIP proteins from *Homo sapiens*. This residual level computational study to scrutinize the basis of the interaction is one of the most essential zones to be explored into. Previously, several molecular level studies [9, 10] were documented for other diseases but none dealt with the cell signal transduction in the enhancement of metastasis of breast tumors. This in silico discern therefore, unveils the residual participation, binding demonstration and analysis of the most stable complex (i.e., the energy optimized simulated complex) of ERα-bZIP protein for the signaling mechanism in the breast cancer malignancies. It endows with an avenue for the future therapeutic research in a lucid mode.

5 Conclusion and Future Prospect

The cooperative participation of the residues from the two essential human proteins (ERα and bZIP) for metastasis of breast tumors was the prime focus of this present investigation. Signals are triggered by the respective breast tumor cells at the time of progression toward invasion from tumorigenesis. This further triggers the extranuclear pathways for the cell signal transduction of ERα. As a result, it further provides an increment in the migratory functions of the responsible cells, followed by metastasis. This ERα protein is also associated with the bZIP protein for the increased signal transduction and metastasis. This duo protein interaction enhances during the advanced breast cancers. Thus, this study poses a cogent framework for

the extranuclear cell signal transduction involving the metastatic control of ERα-positive tumors in collaboration with bZIP protein.

So, the present structural and computational molecular contribution of ERα–bZIP interactions was essential to be elucidated not only for the clinical progress in novel therapeutics for breast cancers but also for the improvement of the future production of refined modulators for ERα. Future scope lies in the in silico investigation of any mutation in either ERα or bZIP protein which might shed an impact on the cancer progression. It will further pave an outlook in the clinical and pharmaceutical research for the investigation of any small modulators for ERα or any certain drug discovery to mold the protein to lower its tendency for causing the efficient progression of metastasis in the breast cancer cells.

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