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Original Article

MOLECULAR MODELLING AND DOCKING STUDIES OF HUMAN ACROSIN BINDING PROTEIN (ACRBP/OY-TES-1)

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ABSTRACT

Objective: We have made an attempt to identify inhibitors that are bound with Acrosin binding protein (ACRBP/OY-TES-1) through *In silico* molecular docking studies.

Methods: Modeling of ACRBP/OY-TES-1 was performed using Iterative Threading Assembly Refinement (I-TASSER) software. Docking calculations were carried out using Glide. Glide Score (GS core) was used to rank the ligands on the basis of their relative binding affinities.

Results: *Food and Drug Administration (FDA)*-approved drugs were docked with ACRBP/OY-TES-1 to identify potent inhibitors. Leuprolide a decapeptide interacts with the protein at residues Tyr116, Gly421, Leu433, Asp480 and Gln483 with Glide score-14.188. Other compounds that showed high affinity to the protein are triptorelin, nafatarelin, goserelin and sincalide.

Conclusion: The investigation concluded that these drugs could be used as potential inhibitors against ACRBP/OY-TES-1 in cancer treatment.

Keywords: ACRBP/OY-TES-1, Docking, Glide, Immunotherapy, Modeling.

INTRODUCTION

Cancer remains a fundamental burden to public health despite substantial efforts aimed at developing effective chemotherapeutics and significant advances in chemotherapeutic regimen. The major challenge in anti-cancer drug design is to selectively target cancer cells with high specificity [1]. Target-based therapies are widely considered to be the future of cancer treatment and much attention has been focused on developing inhibitors of the target proteins [2]. Hence the identification of target proteins and the understanding of their interaction with ligands are key elements of modern biomedical research [3]. The interaction with target proteins is the molecular origin of most drugs, their particular importance for molecular biology, molecular pharmacy and pharmaceutical sciences is obvious [4, 5]. Many small-molecule identification or drug discovery efforts start by selecting a target that is expected to modulate a pathway or disease of interest [6]. Different types of proteins are involved in different types of cancers which are sensitive to small molecules. The anticancer drugs that are currently available for the treatment of cancer are not specific. The major cause of limited success of chemotherapy is an acquisition of drug resistance by tumors, inadequate target drug delivery due to abnormal tumor vasculature, and its toxicity [7, 8]. Hence there is a need to identify a novel protein molecule as a target for development of therapeutic agents.

One such protein Acrosin binding protein (ACRBP) or OY-TES-1 is expressed in normal testis and over expressed in many cancers such as melanoma, breast cancer, bladder cancer, prostate cancer, esophageal squamous cell carcinoma and hepatocellular carcinoma [9]. Acrosin binding protein or cancer/testis antigen/OY-TES-1 is a 61359 Da protein consists of two domains-Proacrosin binding sp32 (1–240) and the kazal domain (469–509). ACRBP/OY-TES-1 maps to chromosome 12p12-p13 and contains 10 exons [9]. This protein is located in the sperm acrosome and is thought to function as a binding protein to proacrosin for packaging and condensation of the acrosin zymogen in the acrosomal matrix. This protein is a member of the cancer/testis (CT) family of antigens and it is found to be immunogenic [10]. Luo *et al.*'s finding demonstrates that ACRBP/OY-TES-1 is frequently expressed in colorectal cancer (CRC) and can induce humoral immune response spontaneously in CRC patients, suggesting that it might be a promising immunotherapy target for CRC [11]. In ovarian cancer high ACRBP/OY-TES-1 expression is significantly correlated with poor prognosis, both with respect to overall survival and time to disease recurrence [12]. Studies showed that ACRBP/OY-TES-1 supports mitotic spindle fidelity in tumor cells by regulating NUMA1 protein levels and thereby reinforcing bipolar spindle assembly in the presence of paclitaxel [12]. NuMA over expression was suggested to cause mitotic perturbations required for the plasticity of the preneoplastic genome, with co-evolving over expression of ACRBP as tumors progress [13]. Fig. 1 provides an overview of ACRBP's role in promoting cancer cell proliferation.

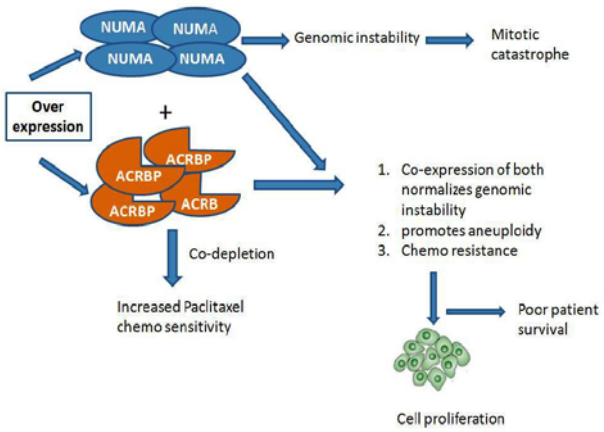

Fig. 1: Role of ACRBP/OY-TES-1 in tumors

Given the extensive expression correlation with cancer and restricted expression pattern in normal tissues, we thought to model the protein ACRBP/OY-TES-1 by computational methods and perform docking studies to identify a novel compound.

Drug discovery is a lengthy and most expensive step with high failure rates. Whereas approved drugs have acceptable pharmacokinetics, safety profiles and are accepted by regulatory agencies for human use, and it is important to address whether it is

preferable to use them as a whole or as a template for further design of new molecules [14]. So, we took FDA approved drugs for docking studies to avoid false-positive predictions and to design new drugs with high accuracy and selectivity.

In a protein targeting drug discovery, 3D structure of the protein should be known. Crystal structure of ACRBP/OY-TES-1 is not available in the protein data bank. So, this study aims to model the protein and identify the binding sites computationally. Docking studies were carried out to design, optimize and identify potential inhibitors for ACRBP/OY-TES-1.

MATERIALS AND METHODS

Protein modeling

The Amino acid sequence of ACRBP/OY-TES-1 was obtained from Uniprot Acc NO: Q8NEB7. Modeling of ACRBP/OY-TES-1 was performed using I-TASSER software. I-TASSER is a hierarchical protein structure modeling approach based on the secondary-structure enhanced Profile-Profile threading Alignment (PPA) [15, 16] and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program [17]. The I-TASSER method has been described in detail previously [18, 19]. Maestro 9.8 protein preparation wizard (Schrodinger) was used to correct errors in the modeled structure.

Active site identification

Site Map, Schrödinger's program was used for identifying, evaluating, and visualizing ligand binding sites. A receptor grid was generated around the active site and was given a dimension sufficient to accommodate compounds with a length ≤15A°. The ligand-midpoint box was given a side of 10A°. No scaling factors were applied to the van der Waals (vdW) radii of the receptor atoms [20].

Ligand preparation

The FDA approved drugs were downloaded from e-LEA3D: Chem Informatic Tools and Databases (http://chemoinfo.ipmc.cnrs.fr/download.html). Ligands were subjected to automatic preparation process, performed with Lig Prep (LigPrep, version 3.2, Schrödinger, LLC, New York, NY, 2014.) tool of the Schrodinger package. It generates all possible protonation and tautomeric states available within a pH range of 7.0±2.0.

Molecular docking

Docking calculations were carried out using Glide (Glide, version 6.5, Schrödinger, LLC, New York, NY, 2014). The prepared and optimized ligands were flexibly docked in the grid box of the protein using MCSA (Monte Carlo based simulated algorithm) based minimization [21, 22]. Glide Score (GScore) was used to rank the ligands on the basis of their relative binding affinities.

RESULTS

A phylogenetic analysis was done to determine ACRBP/OY-TES-1 evolutionary history between different species [23]. Evolutionary relationships can determine how many species are closely related or deviated from Humans. Neighbor joining tree for ACRBP/OY-TES-1 is shown in fig. 2. The phylogenetic analysis reveals that there are 51 speciation nodes of ACRBP/OY-TES-1 with one duplication event. The human gene was orthologous to Chimpanzees and also showed a close relationship with gorillas, Orangutans and Gibbons. The sequence similarities between these species are around 60%. Other species such as rodents, birds and amphibians are paralogs of humans. The tree shows the evolutionary descent of the ancestral gene to paralogs and orthologs following gene duplication in the species Xenopus.

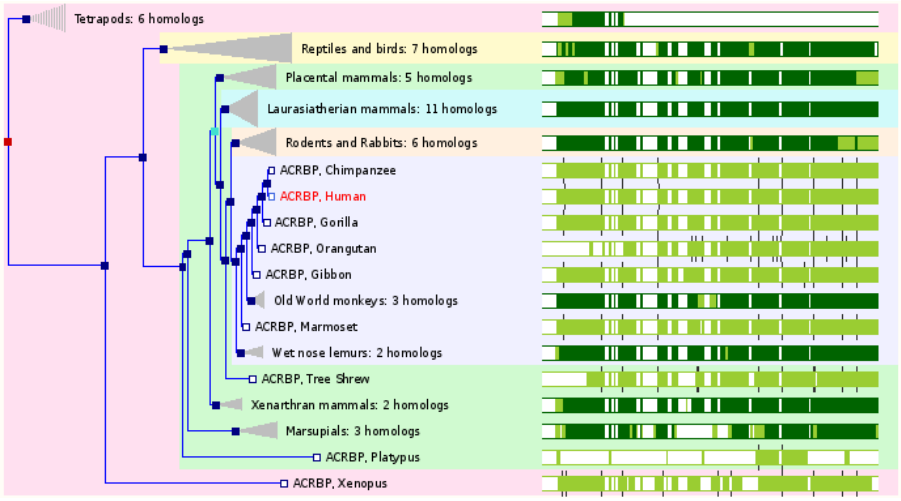

Fig. 2: Gene tree for the ACRBP/OY-TES-1 Gene

The protein sequence of ACRBP/OY-TES-1 consists of 543 amino acids. Protparam analysis revealed the presence of 72 negatively charged and 48 positively charged residues. The molecular weight was found to be 49161.6 Daltons and theoretical isoelectric point was 5.09 [24]. The secondary structure of ACRBP/OY-TES-1 was predicted by the Chou and Fasman Secondary Structure Prediction Server. It was noticed that ACRBP/OY-TES-1 consists of Helix 61.9%, extended strand 68.3% and turn 13.1% [25]. The functional domain predictions with SMART tool predicted 3 low complexity regions from 9–25, 177-191, 209–243. It shows two domains (PBP_sp32 domain & Kazal Domain). The PBP_sp32 domain shared similarity with Pfam database. This family consists of several mammalian specific proacrosin binding protein sp32 sequences. sp 32 is a sperm specific protein, which is known to bind with 53 kDa proacrosins and the 49 kDa acrosin intermediate. This domain starts at position 1 and ends at position 240 with e value of 3.1 e-138. The kazal domain is starting from 469 and ends at position 509. It is kazal type proteinase inhibitors and is distributed in a wide range of organisms from all kingdoms of life and plays a crucial role in various physiological mechanisms [26] (fig. 3).

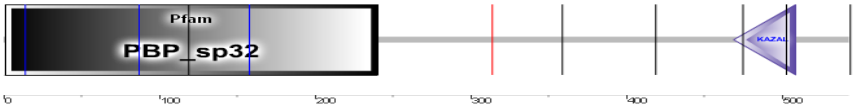

Fig. 3: Domain architecture of ACRBP/OY-TES-1

Modeling

The protein data bank (PDB) was checked for the 3D structure of the selected protein, and it was confirmed that no 3D structure had been predicted to date. Therefore, we decided to predict the three-dimensional (3D) model of the ACRBP/OY-TES-1 protein by using homology modeling, threading and ab-initio methods. As low of percentage of sequence identity, not more than 25%, we modeled the protein with ab-initio method by using I-TASSER simulations, where 5 predicted models were obtained. The modeled structure was imported into protein preparation wizard to correct its bond orders and atomic charges. Hydrogen atoms were added, bond orders were assigned to certain amino acid residues and tautomeric states at pH 7.0 were generated. Protonation states of hydroxyl, histidine groups and side chain atoms of the protein were optimized (fig. 4).

Fig. 4: Ribbon view of ACRBP/OY-TES-1

Impref minimization was carried out using the OPLS 2005 constant dielectric force field. The final minimized model was used to identify active binding pockets and for further docking studies (fig. 5).

The active binding pockets were identified by SiteMap. It calculates the binding site by an energetic grid based method. SiteMap identified five potential binding sites with site scores>0.9. The predicted active site, Site I had a site score of 1.081 and amino acids in this region were 115-119, 331, 335, 338, 339, 342, 343, 408, 409, 412, 413, 422–42, 430-435, 477, 480, 481, 483, 484, 486. This site was used for further screening and docking studies.

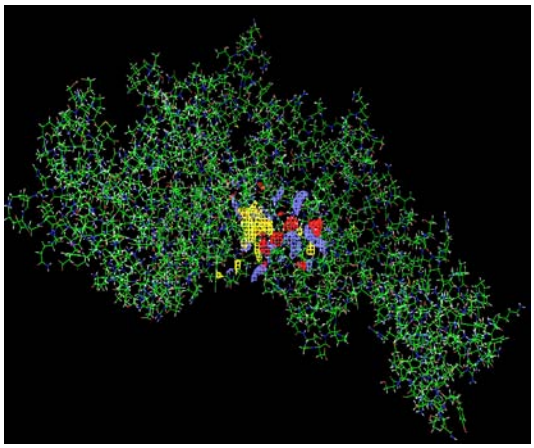

Fig. 5: Binding pocket

Docking

The modeled and refined protein was docked against FDA-approved drugs. Table 1 shows the compounds which had a high docking score. For the docking score, mainly four parameters were considered: (i) G-Score (ii) Glide energy (iii) H bonds and (iv) Good van-der-walls interactions.

Table 1: Docking score of FDA approved drugs

Compound	Glide score
Leuprolide	-14.188
Triptorelin	-13.512
Nafarelin	-12.71
Goserelin	-11.363
Sincalide	-11.105
Colistin	-10.673
Abarelix	-10.446
Cetrorelix	-10.275
Saralasin	-9.86
Ritonavir	-9.838

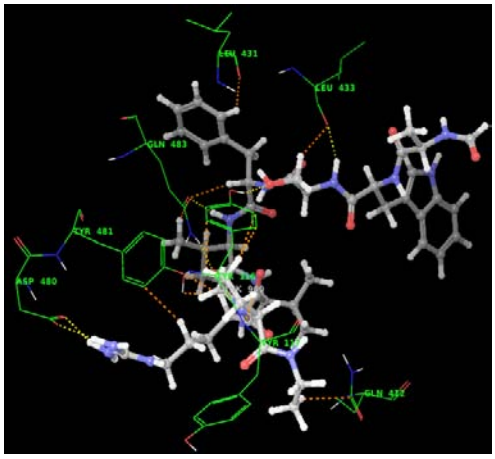

Fig. 6: Leuprolide

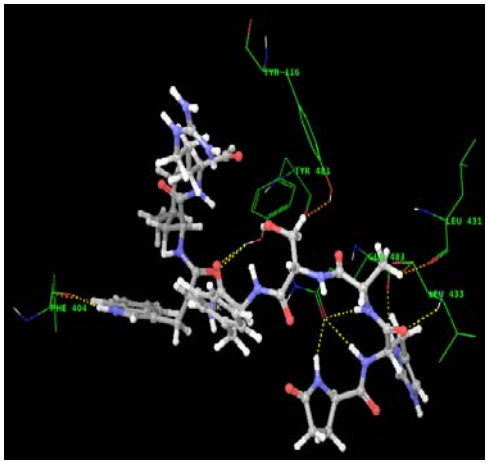

Fig. 7: Triptorelin

Higher the negative value of the docking score, better the binding affinity of the ligand and receptor. The minimum energy for binding and higher number of hydrogen bonds also indicates good binding affinity of ligands towards the receptor. The docking score of Leuprolide is-14.188. The complex (fig. 6) indicates that amino acids Tyr116, Tyr117, Leu431, Leu433, Asp480, Tyr481 and Gln 483 are involved in hydrogen bonding. Leuprolide forms seven hydrogen bonds with ACRBP/OY-TES-1. Triptorelin (fig. 7) forms hydrogen bonds (Tyr116, Phe404, Leu431, Leu433, Tyr481, Gln483) with a docking score of-13.512. The residues Tyr 116,Tyr 117,Ser 411, Ser 415, Tyr 434, Asp 478, Tyr 481, Gly483 are involved in hydrogen bonding with Naferelin (fig. 8).

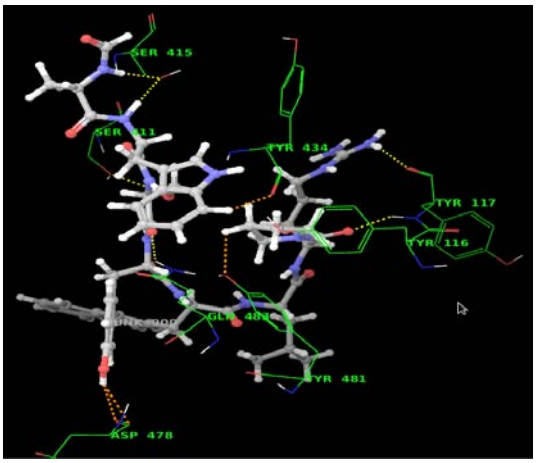

Fig. 8: Naferelin

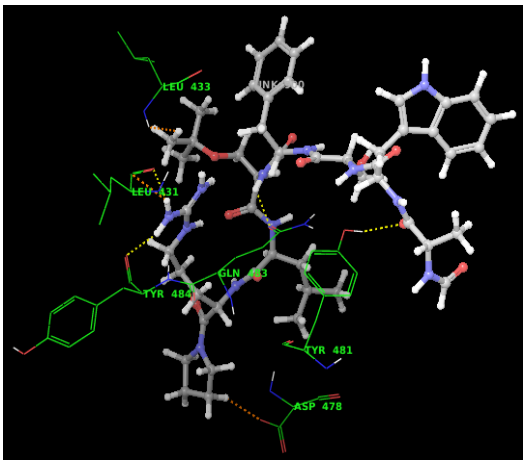

Fig. 9: Goserelin

DISCUSSION

Acrosin binding protein (OY-TES-1) is normally expressed in developing and adult testis and anomalously expressed in a variety of tumor types, including lung, breast, and liver [9]. It is a member of a class of tumor-specific proteins that emerge as a consequence of tumor evolution. They do not drive the proliferation or survival, rather, they normalize the perturbed mitotic infrastructure responsible for disease-promoting genetic variation. In such context, if it is targeted, it is likely to represent therapeutic intervention targets with large therapeutic windows. It is also an important target due to its high expression in a variety of cancers and restricted expression in normal adult tissues, except for testis. The down-regulation of ACRBP/OY-TES-1 expression in mesenchymal stem cells caused cell growth inhibition, cell cycle arrest, and apoptosis induction and attenuation of migratory ability [27].

Luo *et al.* [11] had previously reported an over expression of ACRBP/OY-TES-1 in breast, epithelial ovarian, lung, liver colon and bladder cancer. Its mRNA levels have been detected in various cancers, including bladder, breast, lung, liver, and colon cancers while protein expression was reported in a majority of epithelial ovarian cancer. Thus, chemotherapeutic agents that bind to the protein can help to reduce levels of ACRBP/OY-TES-1 and induce cell death.

The crystal structure of ACRBP/OY-TES-1 is not available in the Protein Data Bank so far. Hence we modeled the protein by using *in silico* methods and evaluated potential small molecules by docking them with the modeled protein structure. Analysis of the docked ligands showed that the glide score range was between – 14.188 kcal/mol and-1 kcal/mol. Docked molecules showed intermolecular hydrogen bonding interactions between receptor-ligand molecules. The amino acids involved were hydrophobic (Leucine & Phenylalanine), polar (tyrosine) and charged amino acids (aspartic acid and glutamic acid). The binding modes and geometrical orientation of all compounds were almost identical, suggesting that all them occupied a common cavity in the receptor. The top four compounds were peptides and formed hydrogen bonds with Tyr. Hence we speculate that these compounds may successfully target and bind the ACRBP/OY-TES-1 molecule, We also hypothesize that these lead drug-like small molecules may serve as inhibitors of the ACRBP/OY-TES-1 protein in-vivo.

Apart from being a promising target for chemotherapeutic intervention, ACRBP/OY-TES-1 expression in several cancers presents an opportunity for immunotherapeutic targeting. A cell-mediated immunotherapy based approach not only ensures tumor elimination but also offers immune cell memory against any tumors that may develop in the future. Eliciting active immune surveillance may be difficult since the protein is intracellular and not membrane bound. Hence studying the mechanism of ACRBP/OY-TES-1 turnover and its association with MHC Class I for presentation need to be studied. Identifying specific HLA Class I alleles that could present these turned over peptides may help engineer an immune intervention against tumors. One such an approach has been used for designing a fusion protein consisting of an IL-2 molecule linked to a soluble single chain TCR that recognizes an octapeptide (from p53 turnover) linked to HLA A*0201. This method may present a possible strategy to overcome the challenge of targeting intracellular proteins. Cell-mediated immunity may also be elicited with the help of dendritic cells, which are the most potent antigen presenting cells and can process and present antigens to both CD4 and CD8 T cells [28]. This approach may, as stated earlier, evince a memory response as well, virtually guaranteeing long term immune surveillance. But a dendritic cell vaccine route may not automatically translate to good clinical outcomes. The response to such vaccines as shown by clinical trials is far from encouraging and hence other approaches may need to be evaluated [29]. Apart from this, mimetic TCR like antibodies against peptide-MHC complexes which have been evaluated against several targets like MART-1, NY-ESO-1, HER2 [30] etc. may also be used against ACRBP/OY-TES-1 expressing tumors. With the availability of numerous such approaches, it is possible to exploit the expression of the tumor associated protein for tailoring the best possible immunotherapeutic approach.

CONCLUSION

In this study, we computationally modeled the tertiary structure of the protein ACRB/OY-TES-1. Molecular docking was applied to explore the binding mechanism of ACRBP/OY-TES-1 with FDA-approved drugs. Results indicate that peptides have a better binding affinity towards ACRBP/OY-TES-1. These potential drug candidates can further be validated in wet lab studies for their role as inhibitors of ACRBP/OY-TES-1. This protein that belongs to the CT family of antigens is frequently expressed in most cancers and can induce humoral immune response spontaneously in cancer patients, suggesting that it might be a promising immunotherapy target for different cancers as well.

CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

1. Mak L, Liggi S, Tan L, Kusonmano K, Rollinger JM, Koutsoukas A, *et al.* Anti-cancer drug development: computational strategies to identify and target proteins involved in cancer metabolism. Curr Pharm Des 2013;19:532-77.
2. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007;26:3291-310.
3. Hanzlik RP, Fang J, Koen YM. Filling and mining the reactive metabolite target protein database. Chem Biol Interact 2009;179:38-44.
4. Chan SY, Loscalzo J. The emerging paradigm of network medicine in the study of human disease. Circ Res 2012;111:359-74.

5. Yıldırım MA, Goh KI, Cusick ME, Barabási AL, Vidal M. Drug-target network. Nat Biotechnol 2007;25:1119-26.
6. Hoelder S, Clarke PA, Workman P. Discovery of small molecule cancer drugs: Successes, challenges and opportunities. Mol Oncol 2012;6:155-76.
7. Makley LN, Gestwicki JE. Expanding the number of "Druggable" Targets: non-enzymes and protein-protein interactions. Chem Biol Drug Des 2013;81:22-32.
8. Tejasvi K, Mayuresh A, Gauri T, Venkatesan GK, Krishnan K. *In silico* molecular docking of marine drugs against cancer proteins. Adv Chem Sci 2013;2:24-8.
9. Ono T, Kurashige T, Harada N, Noguchi Y, Saika T, Niikawa N, *et al.* Identification of proacrosin binding protein sp32 precursor as a human cancer/testis antigen. Proc Natl Acad Sci 2001;98:3282-7.
10. Tammela J, Uenaka A, Ono T, Noguchi Y, Jungbluth AA, Mhawech-Fauceglia P, *et al.* OY-TES-1 expression and serum immunoreactivity in epithelial ovarian cancer. Int J Oncol 2006;29:903-10.
11. Luo B, Yun X, Fan R, Lin YD, He SJ, Zhang QM, *et al.* Cancer testis antigen OY-TES-1 expression and serum immunogenicity in colorectal cancer: its relationship to clinicopathological parameters. Int J Clin Exp Pathol 2013;6:2835-45.
12. Whitehurst AW, Xie Y, Purinton SC, Cappell KM, Swanik JT, Larson B, *et al.* Tumor antigen acrosin binding protein normalizes mitotic spindle function to promote cancer cell proliferation. Cancer Res 2010;70:7652-61.
13. Brüning-Richardson A, Bond J, Alsiary R, Richardson J, Cairns DA, McCormac L *et al.* NuMA overexpression in epithelial ovarian cancer. PLoS One 2012;7:e38945. doi: 10.1371/journal.pone.0038945. [Article in Press]
14. Abagyan R, Totrov M. High-throughput docking for lead generation. Curr Opin Chem Biol 2001;5:375-82.
15. Wu ST, Zhang Y. LOMETS: A local meta-threading-server for protein structure prediction. Nucleic Acids Res 2007;35:3375-82.
16. Zhang Y, Skolnick J. Automated structure prediction of weakly homologous proteins on a genomic scale. Proc Natl Acad Sci 2004;101:7594-9.
17. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. Nat Methods 2015;12:7-8.
18. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc 2010;5:725-38.
19. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 2008;9:40. doi: 10.1186/1471-2105-9-40. [Article in Press]
20. Halgren T. New method for fast and accurate binding-site identification and analysis. Chem Biol Drug Des 2007;*69:*146-8.
21. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, *et al.* Glide: a new approach for rapid, Accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 2004;47:1750-9.
22. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, *et al.* Glide: a new approach for rapid, accurate docking and scoring. 1. method and assessment of docking accuracy. J Med Chem 2004;47:1739-49.
23. Vilella AJ, Severin J, Ureta-Vidal A, Heng L, Durbin R, Birney E. Ensembl Compara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. Genome Res 2009;9:327-35.
24. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 2003;31:3784-8.
25. Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. Adv Enzymol Relat Areas Mol Biol 1978;47:45-148.
26. Schultz J, Copley RR, Doerks T, Ponting CP, Bork P. SMART: a web-based tool for the study of genetically mobile domains. Nucleic Acids Res 2000;28:231-4.
27. Cen YH, Guo WW, Luo B, Lin YD, Zhang QM, Zhou SF, *et al.* Knockdown of OY-TES-1 by RNAi causes cell cycle arrest and migration decrease in bone marrow-derived mesenchymal stem cells. Cell Biol Int 2012;36:917-22.
28. Schuler G, Steinman RM. Dendritic cells as adjuvants for immune-mediated resistance to tumors. J Exp Med 1997;186:1183-7.
29. Qiu J, Lil GW, Sui YF, Sun YJ, Huang YY, Si SY. Truncated TERT mRNA transfected dendritic cells evoke TERT specific anti tumor response *in vivo*. Hepatogastroenterology 2007;54:681-7.
30. Fishman MN, Thompson JA, Pennock GK, Gonzalez R, Diez LM, Daud AI, *et al.* Phase I trial of ALT-801,an interleukin-2/T-cell receptor fusion protein targeting p53 (aa264–272)/HLA-A* 0201 complex, in patients with advanced malignancies. Clin Cancer Res 2011;17:7765-75.