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ORIGINAL ARTICLES

In-silico evaluation of the potential of a viral protein (Apoptin) as an anticancer agent

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Abstract: *As a significant immunosuppressive virus, chicken anemia virus (CAV) is common among natural poultry hosts and newborn chickens. It contains a tiny single-stranded negative circular DNA genome, encoding three proteins vp1, vp2, and vp3. Apoptin, encoded by the vp3 gene of the CAV virus, is a small protein whose subcellular localization appears to be crucial for tumor-selective activity. Residing in the cytoplasm of normal cells, it translocates into the nucleus in cancerous cells. Apoptin has attracted much attention because of causes specific death in transformed and cancer cells and has toxic effects on cancer cells. In-silico analysis of apoptin revealed the unknown structural and functional features. Physicochemical features and sequence analysis and secondary structure prediction were also conducted using Expasy server tools. Furthermore, the global structure of apoptin was modeled using the I-TASSER server and antigenic features were obtained via the application of the IEBD web tools. The results of in-silico analysis indicated that this protein had a stable structure, was a suitable choice for therapeutic goals, capable of acting as a good anti-cancer agent.*

Keywords: *apoptin; in-silico analysis, chicken anemia virus; cancer*

INTRODUCTION

Cancer is defined as the Irregularity, growth, and proliferation of the cell cycle and sometimes the abnormal proliferation of cells in the body (metastasis). Since the onset of cancer is associated with mutations in some of the natural cell genes, it is considered a genetic disease. Tumor cell mass which is different in appearance and function from normal cells can be either benign (progressive and invasion free with slow growth) or malignant (with rapid growth, metastatic potential), ultimately leading to patient death [1]. Due to the high cost of the medications and cancer treatment facilities, numerous studies have concentrated on finding methods to prevent the disease or effectively treat it [2]. In previous studies, different viruses and their proteins have been used to find a treatment for cancer [3].

Viruses' ability to attach to the cancer cells has attracted the researchers' attention as a novel strategy comparable with conventional therapies, including surgery, radiation therapy, and chemotherapy [4]. The application of recombinant proteins has recently given rise to an interest in understanding and recognizing the physiology of tumor cells. In particular, the identification of these proteins as a new drug and therapeutic cancer vaccine which can use different mechanisms to defeat cancerous cells is crucial for cancer researchers. Unlike prophylactic vaccines, which are generally prescribed to healthy people, cancer vaccines are mostly prescribed to cancer patients. These vaccines are designed to kill cancer cells by boosting the patient's immune response. They kill different immune effects or

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mechanisms created by therapeutic vaccination, especially cancer cells and normal cells. The apoptin protein is one of these proteins encoded by the chicken anemia virus (CAV) genome [5, 6].

Infectious anemia virus is one of the most significant immunosuppressive viruses in newborn chicks which are regarded as the natural host of this virus. The virus has three protein sequences as follows: The 50kDa viral protein (VP1) which is the only protein isolated from virus components the 30kDa non-structural VP2 protein functioning as a scaffold protein during the vision accumulations, and the third viral protein VP3 weighing 14kDa, which is located the nucleus of infected cells, but not found in the purified virus components [7]. VP3 is called apoptin, a potent stimulant of apoptosis in and lymphoblastoid cell lines and chicken thymocytes [8]. VP3 gene product due to its apoptotic effect was renamed apoptin [9]. This protein causes apoptosis to be independent of the p53Bcl-2-dependent tumor inhibitor. Apoptosis-induced apoptosis included the induction of caspase, leading to mitochondrial changes and dispersion of cytochrome C [10]. The C-terminal region of apoptin has a bilateral nuclear localization sequence (NLS). NLS1 amino acids from 82 to 88 and NLS2 amino acids remain in the range 111 and 121 [11]. In addition, in the range of 75-107 amino acids, apoptin has a putative nuclear export sequence that transfers the apoptin through the nucleus. Apoptin proteins cause apoptosis in the altered cells (transformed cells), while they leave no effect on normal cells [12, 13].

Bioinformatics refers to the information technology used in molecular biology. Bioinformatics tools can easily identify and predict the primary and secondary structures in a new sequence. This diagnostic tool is applied to find patterns, detect several protein families and show the oligonucleotide primers for a polymerase chain reaction. It also serves as an essential prelude to molecular evolutionary analysis [14]. To the best of the author's knowledge, not much is known about the structural characteristics of Apoptin as an anticancer agent. The main objective in the current study is to evaluate the physicochemical features and determine the secondary and tertiary structure and antigenic features of the apoptin protein of CAV, using bioinformatics tools and databases.

MATERIALS AND METHODS

Sequence analysis

The primary sequence of a given protein (Apoptin) was retrieved from GenBank (ACC. No. AEB91668.1). The first structure of the protein in terms of aliphatic index, extinction coefficient, molecular weight, instability index, isoelectric point, amino acid composition, and half-life in vitro and in vivo are considered as physicochemical features which can be evaluated using various bioinformatics tools.

Physicochemical Features of Apoptin were evaluated using ProtParam, Predict protein, Compute PI/MW tools, and ExPASy server (http://expasy.org). ProtParam database calculated the parameters, such as extinction coefficient, estimated half-life, amino acid composition, theoretical PI, instability index, molecular weight, atomic composition, aliphatic index, and hydropathic mean (GRAVY).

Predict Protein tool can integrate feature prediction for globular regions, B-values, disorder regions, subcellular localization, transmembrane helices secondary structure, intra-residue contacts, structural switch regions, coiled-coil regions, solvent accessibility protein-protein, and protein-DNA binding sites, disulfide bridges, protein-protein, and protein-DNA binding sites, metal-binding sites, domain boundaries, beta-barrels, cysteine bonds.

Structural analysis

The secondary structure components include Alpha Helix, B-Sheet, Turn, Loop, and Coil. The tertiary structure is also formed by the twist of the protein chain caused by the bonding between amino acids which gives rise to functional domains.

Secondary structure was performed to identify Alpha Helix, B- strands, etc, using the Gor, Jpred tools from ExPASy server (http://expasy.org/cgi-bin). According to its amino acid composition and similarity to the sequences of the known secondary structure, the possible secondary structure of a protein can be predicted. RAMPAGE software (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used to determine the validity, stability, and quality of the spatial structure of the protein. Then, the stability of protein structure was evaluated based on the PDB model.

A pattern of comparative modeling was used by the I-TASSER server predicting the third structure based on Homology modeling. I-TASSER provides the biological function of protein molecules from amino acid sequences and the prediction of high-quality models of 3D structure. Energy minimization of the tree-dimensional structure obtained from I-TASSER was performed by the Swiss-Pdb Viewer 4.1.0 software.

Antigenicity

The http://www.iedb.org server was employed to identify antigenic fragments of the protein. IEDB contains experimental data on antibodies and T cell epitopes studied in the fields of infectious diseases, allergies, autoimmunity,

and organ transplants in humans and other animal species. Predictions of B cell space epitopes were determined with ElliPro and Discotope servers.

We employed vaxijen server to estimate the antigenicity of the chimeric protein.

RESULTS

Primary sequence analysis

Table 1 shows the results obtained from the Prot param database, the number, and type of amino acids, and atomic components of Apoptin. This protein has 121 amino acids, most of which are Arg and the least are Met and Tyr. The molecular formula of the protein is C566H945N179O180S5. The protein's extinction coefficient is assumed to be 1740 if the number of cys is reduced to 1490. The protein's extinction coefficient is calculated using the amino acid composition (Table 1) of the protein. The estimated half-life span in mammals is 30 hours, in yeast 20 hours, and E. coli 10 hours. The protein instability index was calculated at 68.45.

The aliphatic index of protein was 65/37. The hydropathic mean (GRAVY) was also estimated to be -0.748

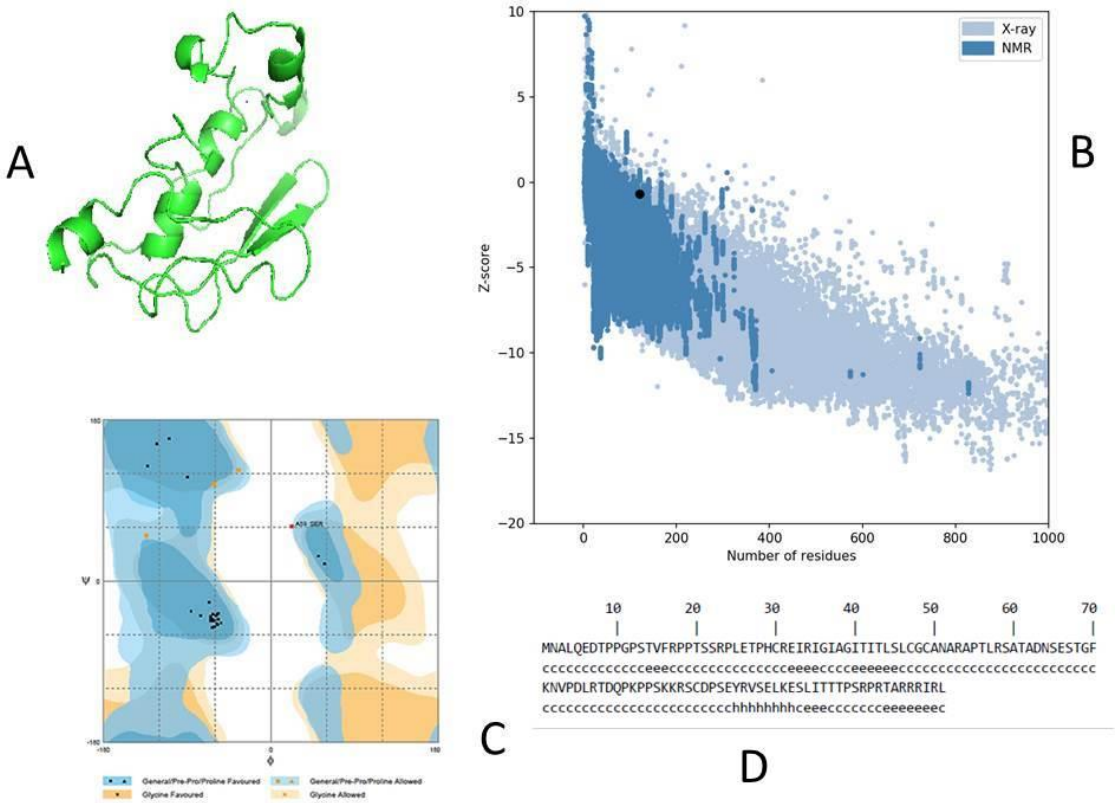
Table 1: Proteins parameters database

Number of amino acids	121
Molecular weight	13298/12
Theoretical pI	10/05
Formula	$C_{566}H_{945}N_{179}O_{180}S_5$
Total number of atoms	1875
Estimated half-life	30 hours (mammalian reticulocytes, in vitro)
	>20 hours (yeast, in vivo)
	>10 hours (Escherichia coli, in vivo)
Instability index	The instability index (II) is computed to be 68/45
Aliphatic index	65/37
Grand average of hydropathicity (GRAVY)	-0/748

The mean pI/Mw was calculated based on the Compute PI/MW database (PI=10/05, MW=13298/12)

According to the Ramachandran map obtained from RAMPAGE software, the protein is the apoptotic protein (Figure 1).

Figure 1: A (Image of 3D forms of the apoptin protein obtained from the i-Tasser database). B (prosaweb result). C (Image depicts the Ramachandran map of apoptin protein). D (Indicates the composition of the secondary structure of the protein)



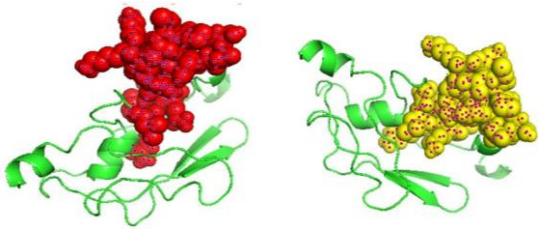
Prediction of tertiary structure

As shown in Figure 1, i-Tasser protein simulated models were calculated quantitatively by a C score based on the importance of pattern rules and convergence parameters of the structure assembly simulation. The energy minimizer of apoptin protein was obtained by using simulated models by the i-Tasser database in spdbv software indicating that apoptin is a stable protein.

Prediction of linear and discontinuous epitopes

According to Figure 2, the spatial structure of apoptin was obtained from the IEDB database, predicting an antigenically active peptide in the apoptin protein.

Figure 2. A (The Linear Epitopes spatial structure obtained from Ellipro). B (Discontinuous Epitope)



A B

DISCUSSION

CAV is a small non-enveloping virus that has a single-stranded circular DNA genome measuring 2.3 KB. The major structural protein (VP1) of viruses is encoded by the genomic strand, while at least two other proteins (VP2 and VP3) are encoded by the antigenomic strand. VP3, called apoptin, is a non-structural protein made up of 121 amino acids. Apoptin contains some residues of proline, serine, and threonine and has a positive charge at the C- terminus [15].

Apoptin protein induces apoptosis just in altered cells (cancer cells) but does not affect normal cells. The specificity of the effect of Apoptin depends on its location in the cell. Its transfer from the outside to the nucleus of the cell in the attachment process to the cancer cells increases its specific effect compared to other normal cells. This protein, in cancer cells, is located in the nucleus and regulated by phosphorylation. Apoptin can induce specific apoptosis of tumor cells in a p53-independent pathway. Apoptosis is moderated by intracellular cysteine proteases (caspases) as the initiator and executor of the apoptosis process [16].

Zhuang et al. indicated that apoptin is capable of inducing apoptosis in human osteosarcoma cells. Subsequent studies found evidence that apoptin induces apoptosis in various human hematologic tumor cells. Also, another study on the effect of apoptin protein by bacteriophage carriers on human breast tumor cell line (BT-474) showed that it influences the cancer cells, while it has no inhibitory effect on normal cells [8].

Traditional laboratory-based cancer research consists of costly experimental trial and error strategies applied to humans, animals, and their harvested tissues. “In-silico experimentation,” combines computational technologies with the mathematical or theoretical properties of cancer bio cells and presents a new approach to advancing cancer treatment [17].

This study showed a simulated 3D model of apoptin generated by using homology modeling as a backup alternative to the conventional, crystallography- or NMR-based methods. In addition, bioinformatics tools for different purposes such as primary and secondary structures and computational modeling of protein structures can help predict new choices for cancer therapy [18, 19].

The results of physicochemical features of apoptin protein including instability index, GRAVY index, solubility index, and aliphatic index evaluated by Prot param database help predict desirable apoptin protein. Since no 3D structure has been identified to date, the present study aimed to evaluate the structure of apoptin using the I-TASSER server. The results stored in pdb were assessed for stable and appropriate energy conditions by Swiss-Pdb Viewer 4.1.0 software, and then energy minimization was analyzed. The image obtained from the server along with the prediction results compared to the Ramachandran graph prediction generally showed structural stability as a fusion protein. Lee et al. (2012) demonstrated that direct engineering of apoptin protein was a suitable and cost-effective strategy to enhance the expression of TAT-Apoptin protein in E. coli [20].

Despite having drawbacks targeting only one epitope or several epitopes of tumor-associated antigen (TAA), protein/peptide-based vaccines are more cost-effective than autologous or personal vaccines. It is believed that the induction of antigen-specific CTLs and antigen-specific helper CD4 + T cells is essential for the optimal efficacy of the cancer vaccine. Some polypeptide vaccines (eg, Stimovax) potentially contain the CD4 and CD8 epitopes. Altering the peptide sequence of TAAs to introduce agonist-boosting epitopes is another method to increase the immunity of its antigen, which enhances the binding of the peptide to the MHC molecule or T cell receptor, resulting in a higher T cell response and/or T cells with higher or higher power [21]. In the present study, epitope mapping apoptin showed that this protein can be an appropriate candidate for therapeutic cancer vaccines.

To the best of the authors' knowledge, not much is known about the structural characteristics of apoptin. The main objective of the current study was to assess the potency and specificity of small apoptin protein in cancer therapy. However, more studies are needed for designing new small apoptin-like peptides to improve the anti-tumor activity of apoptin and avoid their undesirable side effects.

CONCLUSION

As the in-silico results indicate, apoptin has a stable structure and can be regarded as a suitable candidate for cancer treatment. Furthermore, the appropriate structure of apoptin may lead to its selection as a therapeutic cancer vaccine capable of stimulating both the humoral and cellular immune systems.

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Ethical Statement

Ethical approval was granted by the Medical Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1397.360).

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