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In Silico Analysis of an Epitope-Based Bladder Cancer Vaccine

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Abstract

Bladder cancer is a malignancy that arises from the tissues of the urinary bladder and is one of the most prevalent cancers worldwide for which there is no cure. Previous investigations have determined that amyloid-beta precursor protein (APP) is overexpressed in bladder cancer and has a significant role in its malignancy. In an effort to speed up cancer vaccine development and offer a probable immunotherapy option, we decided to work on the in silico development of a vaccine that targets APP and thus bladder cancer. The secondary structure of APP was analyzed, and immunogenic B cell and T cell epitopes were predicted. These epitopes were further evaluated for MHC 1 and 2 binding, allergenicity, and toxicity. The C-ImmSim server was used in two different immune response simulations to target the complete APP protein and each predicted epitope. Our results demonstrate that APP is a good candidate target protein that, if administered along with an adjuvant, can elicit a strong immune response against bladder cancer.

Keywords: In silico, bladder, cancer, vaccine

Introduction

Cancer

Bladder cancer is a malignancy that arises from the urinary bladder tissues and is one of the most prevalent cancers worldwide (Chen et.al., 2017). According to Bray et.al. (2018), there were 549,393 new cases reported in 2018, and about 12,000 men and 4,700 women died from the disease. The main types of bladder cancer are: 1) urothelial carcinoma and 2) squamous cell carcinoma. Urothelial carcinoma occurs in the urothelial cells that line the inside of the bladder, while squamous cell carcinoma commonly occurs in the lateral wall and trigone. The trigone is a triangular area formed by three openings in the floor of the urinary bladder located within the fundus (Jones, 2020).

There are 5 main stages in bladder cancer that range from stage 0 to stage 4. In Stage 0, abnormal cells are localized within the inter-tissue lining of the bladder. Stage 1 is characterized by the spread of the malignant cells into the inner lining of the bladder. Stage 2 involves the metastasis into the muscular layer of the bladder (Kaseb, 2021). In stage 3, the neoplasm has usually invaded the adipose tissue that surrounds the bladder, reproductive organs, and/or pelvic lymph nodes. Stage 4 is characterized by the metastasis to distant tissues or organs by way of the lymph nodes surrounding the common iliac arteries (Kaseb, 2021).

Treatment

Currently, bladder cancer is treated using one of three traditional methods: surgery, radiation therapy, and/or chemotherapy (DeGeorge et.al., 2017). The most common types of surgery are transurethral resection and radical cystectomy. Transurethral resection is where a cystoscope is inserted through the urethra and into the bladder to remove any tumors. Radical cystectomy is

surgery to remove the bladder. For men, the prostate and seminal vesicles are also removed along with the bladder, while in women, the uterus, ovaries, and part of the vagina is often removed along with the bladder (DeGeorge et.al., 2017). Radiation therapy utilizes high doses of radiation to destroy or shrink cancer cells; however, this process is also detrimental to normal cells in treatment. Chemotherapy uses powerful chemicals to kill hyper proliferating cells by damaging the cell's control center that helps it divide, therefore interrupting the chemical processes involved in cell division, but it also has deleterious effects on normal cells (DeGeorge et.al., 2017).

Protein

Amyloid-beta precursor protein (APP) is a 120 kDa polypeptide whose current speculated function within the bladder is to bind to other proteins on the surface of cells and aid in cell-to-cell adhesion. This protein is highly conserved among mammals, as seen in this phylogeny tree where four species were chosen, and it was determined that the squirrel monkey had the closest similarity to humans (Figure 1). Below is a structural analysis of APP that shows its strands, helices, and coils (Figure 2). According to the Entrez database, APP is expressed within a wide range of tissues, but has a high expression rate in the human brain (Figure 3).

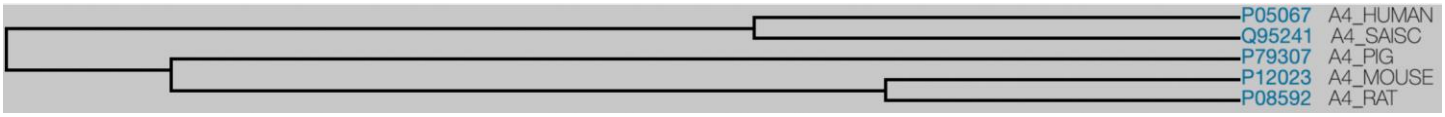

FIGURE 1: Phylogeny tree of Amyloid-beta precursor protein. The phylogeny tree showed the squirrel monkey to be the closest to humans followed by the pig, mouse, and rat.

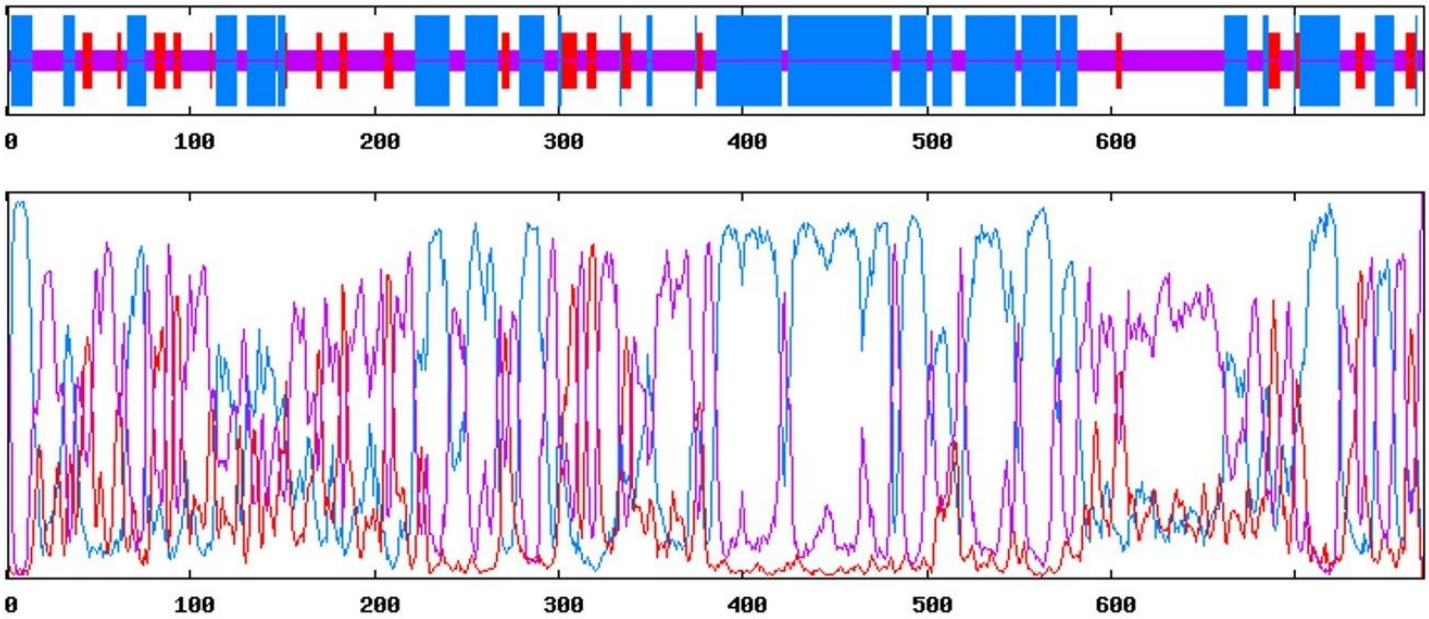

FIGURE 2: APP structure analysis. The secondary structure of the APP protein was analyzed using the Prabi server. The red are extended strands; blue are alpha helix configurations; and purple are random coils.

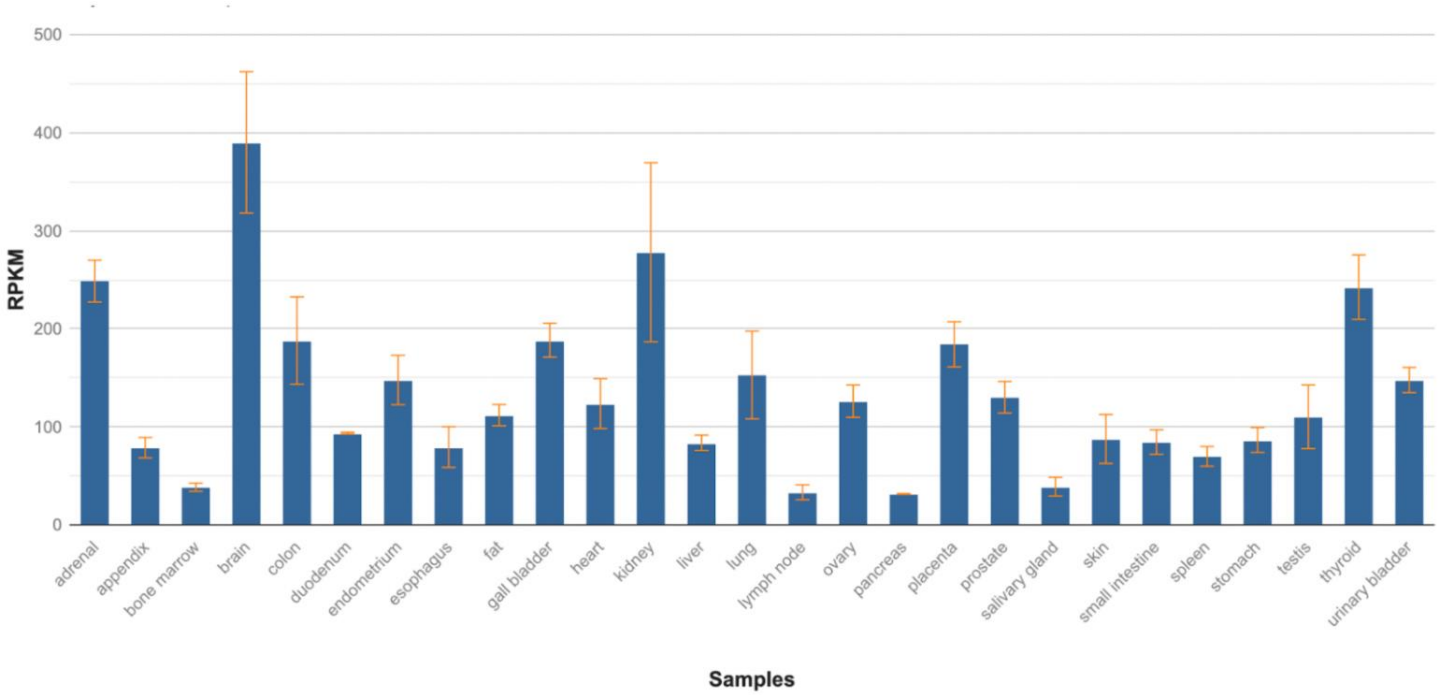

FIGURE 3: APP expression levels in the human body. Graph showing the varying APP expression levels in different tissues (NCBI, Entrez).

In Silico

In silico methods allow for the computational development of new vaccines that may ultimately save on time and money. For example, in the recent COVID-19 pandemic, to quickly create a vaccine to target a novel virus, an in silico approach for its development was employed by numerous pharmaceutical companies. In silico trials helped predict therapeutic failures while minimizing undesired effects and these tactics allowed the COVID-19 vaccines to become the fastest vaccines ever developed (Russo et.al., 2020). These methods can also be employed in the development of a vaccine against bladder cancer.

Vaccine

Previous studies have determined that immunization against a tumor-specific protein can elicit a targeted autoimmune attack that may provide protection and therapy against tumors (Tuohy et.al., 2016). Other investigations have determined that APP is overexpressed in bladder cancer and has a significant role in its malignancy (Zhang et.al., 2018). To speed up vaccine development and offer an immunotherapy option, we decided to work on the development of a vaccine that targets APP and thus bladder cancer. This vaccine might help strengthen the body's natural oncologic defenses against the cancer and it could also be administered as adjuvant therapy in conjunction with or after standard surgical and chemotherapeutic approaches.

Methods

Protein Sequence

Once a target protein was identified through an initial search, NCBI's Entrez database was accessed to obtain APP's accession number (NP_000475.1), FASTA sequence (Figure 7), and locus (21q21.3). The given APP transcripts were obtained along with the positions using NCBI's Splign (Table 1). The 3D structure of APP was generated with the use of UCSF Chimera software. To conduct a background search for any previously designed bladder cancer vaccines, Vaxquery (VIOLIN, 2019) was accessed, and the amino acid and transcript accession numbers

inserted (NP_000475.1 and NM_000484.4 respectively). The output indicated that at that time, there were no records of any other bladder cancer vaccines in development.

	Exo 1	Exo2	Exo 3	Exo 4	Exo 5	Exo 6	Exo 7	Exo 8	Exo 9	Exo 10	Exo 11	Exo 12	Exo 13	Exo 14	Exo 15	Exo 16	Exo 17	Exo 18	Exo 19
NM_000484.4		26170559-26170770	26111964-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187	25997355-25997421	25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911735-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
NM_001136131.3	26170980-26171128		26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047			25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
NM_001136129.3		26170559-26170770		26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047			25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880536-25881776
NM_001136130.3		26170559-26170770		26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187	25997355-25997421	25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
NM_001204303.2		26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047			25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967		25897568-25897678	25891717-25891873	25880550-25881776
NM_201414.3		26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047			25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
NM_001385253.1		26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047		25997355-25997421	25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
NM_001204302.2		26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187		25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967		25897568-25897678	25891717-25891873	25880550-25881776
NM_201413.3		26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187		25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776
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NM_001136016.3	26140148-26140390	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187	25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694	25911736-25911967	25905019-25905082	25897568-25897678	25891717-25891873	25880550-25881776		
XM_024452075.1	26170559-26170770	26111974-26112151	26089938-26090077	26053231-26053353	26050995-26051198	26021835-26022047	26000010-26000187	25982339-25982482	25975949-25976033	25975065-25975233	25955622-25955760	25954585-25954694							

TABLE 1: Exon sequence alignments. NCBI's Splign program was utilized to obtain all the exon positions for each of the APP transcripts.

>NP_000475.1 amyloid-beta precursor protein isoform a precursor [Homo sapiens]
MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTKTCIDTKEGILQYCQEVYP
ELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVGEFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKE
TCSEKSTNLHDYGMLLPCGIDKFRGVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEV
AEVEEEEADDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPCRAMISRWYFDVT
EGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSAMSQSLLKTTQEPLARDPVKLPTTAASTPDAVDKYLETPGDENEHAH
FQKAKERLEAKHRERMSQVMREWEEAERQAKNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDR
RRLALENYITALQAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYERMNQSLS
LLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTETKTTVELLPVNGEFSLDDLQPWHSF
GADSVPANTENEVEPVDARPAADRGLTTRPGSGLTNIKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAI
IGLMVGGVVIATVIVITLVMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

FIGURE 7: Amyloid-beta precursor protein amino acid FASTA sequence. The research database Entrez was accessed and used to obtain and confirm the amino acid sequence of APP.

B cell and T cell Epitopes

B cell epitopes were predicted with the use of the database ABCpred (Suipto, 2018). The amino acid sequence NP_000475.1 was used with a threshold setting of 0.51, window length of 16, and with the overlapping filter on. The top 10 B cell epitopes were chosen and recorded. T cell epitopes were analyzed and predicted using Vaxign which is a database that utilizes precomputed Vaxign results of over 350 genomes to calculate the efficacy of peptides in vaccine development (He et.al., 2010). This program was able to generate several epitopes of which 8 qualified as accurate and efficient.

Epitopes	MHC-1	MHC-2	Antigenicity	Allergenicity	Toxicity
LKTTQEPLARDPVKLP	+	+	Non-antigen	Non-allergen	Non-toxin
VEPVDARPAADRGLTT	+	+	Non-antigen	Non-allergen	Non-toxin
MREWEEAERQAKNLPK	+	+	Non-antigen	Allergen	Non-toxin
TTAASTPDAVDKYLET	+	+	Antigen	Allergen	Non-toxin
YEEATERTTSIATTTT	+	+	Antigen	Non-allergen	Non-toxin
DEVEEEAEEPYEEATE	+	+	Antigen	Non-allergen	Non-toxin
VVEVDAAVTPEERHLS	+	+	Antigen	Non-allergen	Non-toxin
AADRGLTTRPGSGLTN	+	+	Antigen	Non-allergen	Non-toxin
GGCGGNRNNFDTEEYC	+	+	Antigen	Non-allergen	Non-toxin
EEEEADDDEDDEDGDE	+	+	Non-antigen	Non-allergen	Non-toxin

TABLE 2: ABCpred B-cell epitopes. The top ten b-cell epitopes for APP were found using ABCpred. The binding epitopes to MHC-1 and MHC-2 molecules were predicted by the IEDB database. Then the b-cell epitopes were tested for their antigenicity, allergenicity, and toxicity on VaxiJen 2.0, AllerTOP v. 2.0, and ToxinPred respectively.

MHC Class I and II Predictions

The IEDB Analysis Resource (NIH, 2021) was used for the MHC class I binding predictions. IEDB uses several binding prediction methods including the Artificial Neural Network (ANN), Stabilized Matrix Method (SMM), SMM with a Peptide:MHC Binding Energy Covariance matrix (SMMPMBEC), Scoring Matrices derived from Combinatorial Peptide Libraries (Comblib_Sidney2008), Consensus, NetMHCpan, NetMHCcons, PickPocket and NetMHCstabpan. The top 10 B cell and T cell epitopes were entered in FASTA format with the default prediction method. Human was selected as the source species along with the HLA allele reference set. Peptides were sorted by predicted score. IEDB uses several binding prediction methods for MHC class II including the Consensus method, Combinatorial library, NN-align-2.3 (netMHCII-2.3), NN-align-2.2 (netMHCII-2.2), SMM-align (netMHCII-1.1), Sturniolo, NetMHCIIpan-3.1, and NetMHCIIpan-3.2. The top epitope sequences were entered along with the following settings: 1) default prediction method, 2) Human, HLA-DR as the species, 3) default length, and 4) adjusted rank for sorting.

Epitopes	MHC-1	MHC-2	Antigenicity	Allergenicity	Toxicity
ALLLLAAWTARALEV	+	+	Non-antigen	Non-allergen	Non-Toxin
FNMLKKYVRAEQKD R	+	+	Probable Antigen	Non-allergen	Non-Toxin
MTHLRVIYERMNQSL	+	+	Non-antigen	Probable Allergen	Non-Toxin
VIYERMNQSLSLLYN	+	+	Non-antigen	Probable Allergen	Non-Toxin
MNQSLSLLYNVPAVA	+	+	Probable Antigen	Non-allergen	Non-Toxin
MVGGVVIATVIVITL	+	+	Non-antigen	Non-allergen	Non-Toxin
VIATVIVITLVMLKK	+	+	Non-antigen	Non-allergen	Non-Toxin
IVITLVMLKKKQYTS	+	+	Non-antigen	Non-allergen	Non-Toxin

TABLE 3: IEDB database T-cell epitopes. The top eight t-cell epitopes for APP were found using IEDB. The binding epitopes to MHC-1 and MHC-2 molecules were predicted by the IEDB database. Then the t-cell epitopes were tested for their antigenicity, allergenicity, and toxicity on VaxiJen 2.0, AllerTOP v. 2.0, and ToxinPred respectively.

Immunogenicity, Allergenicity, and Toxicity

The VaxiJen database was used for the in silico screening of genomic information for immunogenicity (Doytchinova & Flower, 2007). All epitopes were entered, and "Tumour" selected as the "select target organism." VaxiJen uses tumor datasets to help predict whole protein immunogenicity with a prediction accuracy of 70 to 89% (Doytchinova & Flower, 2007). AllerTOP is a bioinformatics-based allergen prediction database that uses two primary approaches. The first approach scans peptides for sequence similarity while the second approach searches for motifs that may be probable allergens (Dimitrov & Doytchinova, 2013). In this database, the top epitopes were entered and then "Get the result" was clicked. ToxinPred is another bioinformatics tool that can be used to predict and design toxic and non-toxic peptides (Gupta et.al., 2013). Here, each of the previously generated peptides were entered with a fragment length of 10 and with an SVM (Swiss-Prot) base. An E-value cut-off of 10 was selected with a 0.0 SVM threshold. The physicochemical properties chosen to be displayed were Hydrophobicity, Hydropathicity, Hydrophilicity, Charge, and Molecular weight.

Physicochemical Properties and Solubility Prediction

The SCRATCH Protein Predictor is a tool used for the prediction of protein tertiary structures and other structural features. According to Cheng et.al. (2005), "The SCRATCH software suite includes predictors for secondary structure, relative solvent accessibility, disordered regions, domains, disulfide bridges, single mutation stability, residue contacts versus average, individual residue contacts and tertiary structure."

All epitopes were entered with the following selected predictions: Solubility upon Overexpression, Domains, Continuous B-cell Epitopes, and Protein Antigenicity. Expasy allows users to perform protein computational analysis to obtain several physical and chemical parameters (Wilkins et.al., 1999). Epitopes were copied and pasted into the search box and parameters computed.

IUPred was used to analyze protein disorder and binding regions. This server offers text and graphical results of these analyses including the localization of redox-sensitive regions (Mészáros et.al., 2018). All parameters were kept on the default settings (IUPred2 long disorder and ANCHOR2). PepCalc (https://pepcalc.com/) was used to calculate molecular weight, extinction coefficient, net charge, iso-electric point, and water solubility of the epitopes. All properties were kept on their default value.

Structure Prediction

The residues and arrangements of APP's secondary structure were analyzed using the PSIPRED 4.0 server on Prabi. Pepfold was used to predict the structure of the immunogenic epitopes and analyze them for critical energy and population-related conformations. The server uses over 50 simulations for its analysis (Alland et.al. 2005). The Prabi/PHD secondary structure prediction server allows users to analyze and integrate secondary structure predictions (Deléage, 2017). Epitopes were analyzed with the output width of 70. The Sequence Manipulation Suite randomly shuffles a protein sequence to evaluate the significance of the sequence analysis results (Stothard, 2000). The complete FASTA sequence (NP_000475.1) was submitted followed by the epitopes and a control group was generated.

Immune Response Simulation

C-ImmSim is a web-based immune response simulator that incorporates Miyazawa and Jernigan protein to protein potential measurements in its assessment (Rapin et.al., 2010). The complete APP protein sequence (NP_000475.1) was entered and all parameters were kept on their default values.

Results

B cell and T cell Epitopes

The database ABCpred was used to scan the full length of APP for the analysis of B and T cell epitopes followed by a subsequent analysis with the use of IEDB for HLA binding affinities. The best B cell and T cell epitopes were then chosen (Tables 2 and 3). The resultant epitopes were then mapped to APP to ensure they were not found in any areas of the polypeptide chain that may be cleaved during processing (Figure 4)

FIGURE 4: B cell and T cell epitopes. B cell and T cell epitope positions were mapped to the reference APP amino acid sequence. None were found within cleaved areas post APP processing.

Immunogenicity, Allergenicity, and Toxicity

All B cell and T cell epitopes were rigorously tested with VaxiJen 2.0 (threshold of 0.4), AllerTOP 2.0, and ToxinPred (peptide fragment length of 10) for immunogenicity, allergenicity, and toxicity respectively. Our data show that most chosen epitopes are predicted to be good primers of the immune system (Tables 2 and 3).

Epitope Docking Analysis

All epitopes were processed along with an HLA molecule and fitted with a docking grid to maximize affinity and accuracy. The resultant 3-dimensional structure demonstrates a good quality ligand-receptor fit predicted by in silico means (Figure 5).

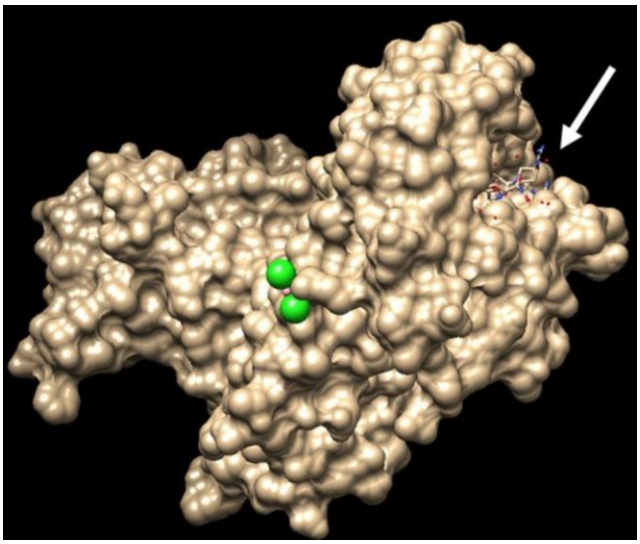
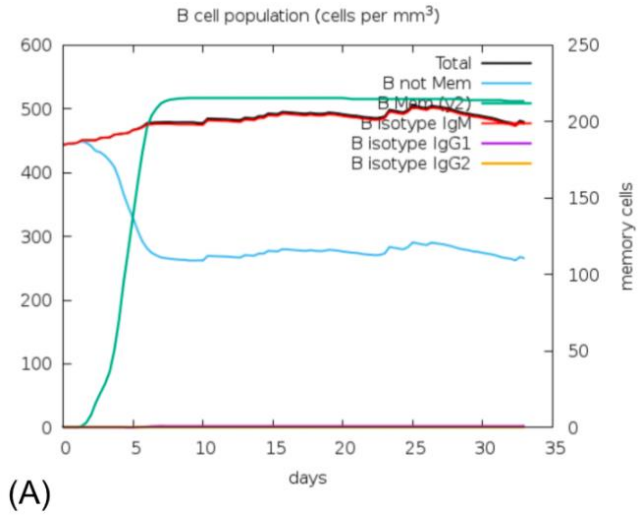
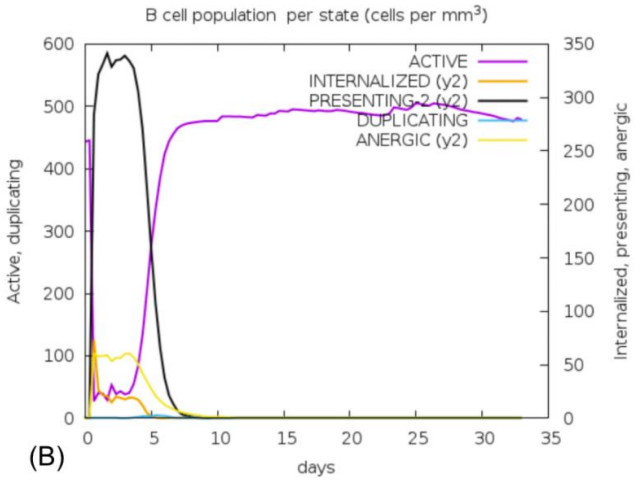


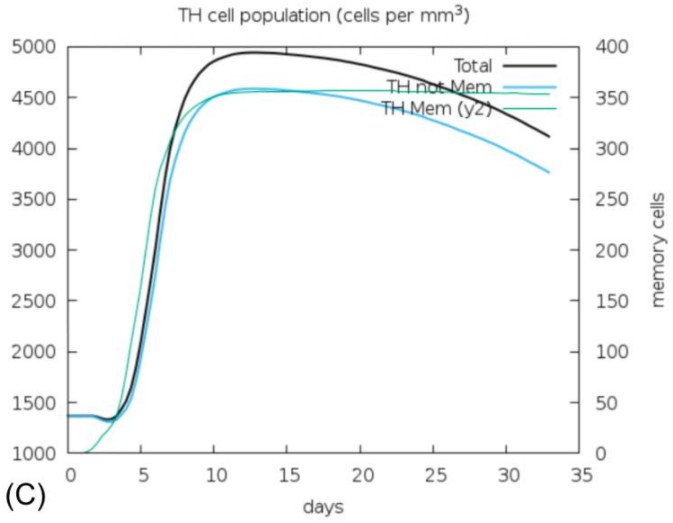
FIGURE 5: Ligand docking. Simulation of HLA class I and epitope docking.

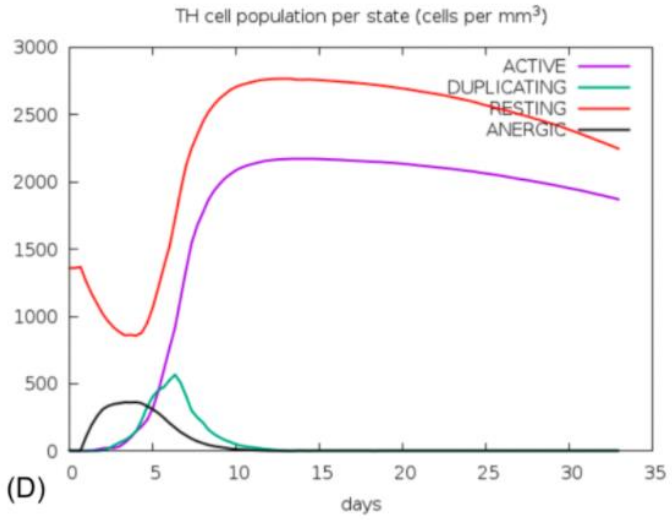
Immune Response

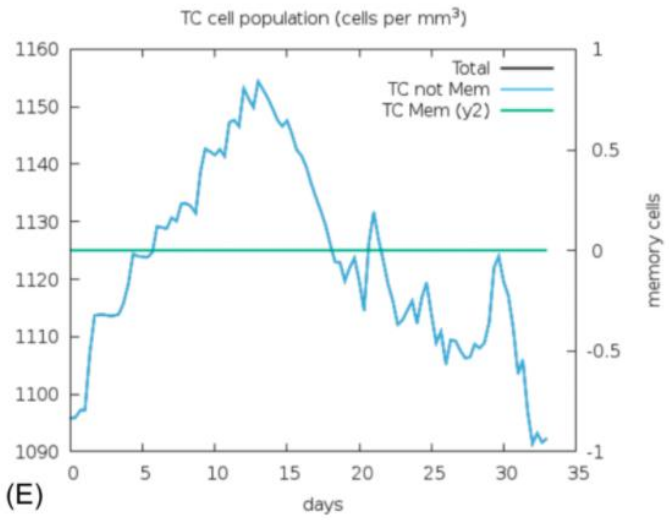
The server C-ImmSim was used to model the immune response of all B cell and T cell epitopes. This simulation was conducted without the use of an adjuvant and was processed to render an immunological study that included cell population state, lymphocyte subset, and immunoglobulins (Figure 6).

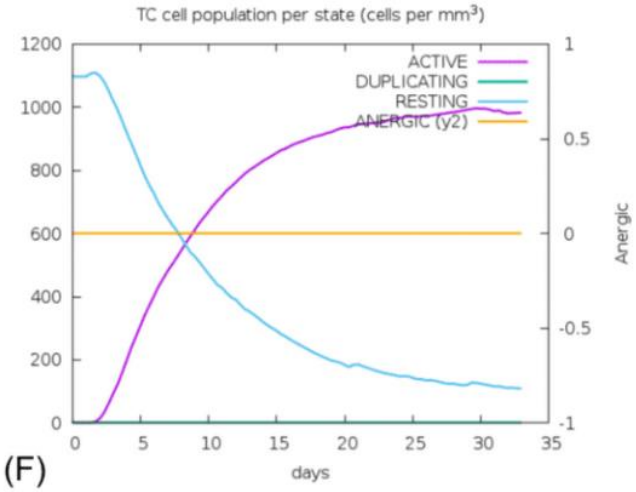


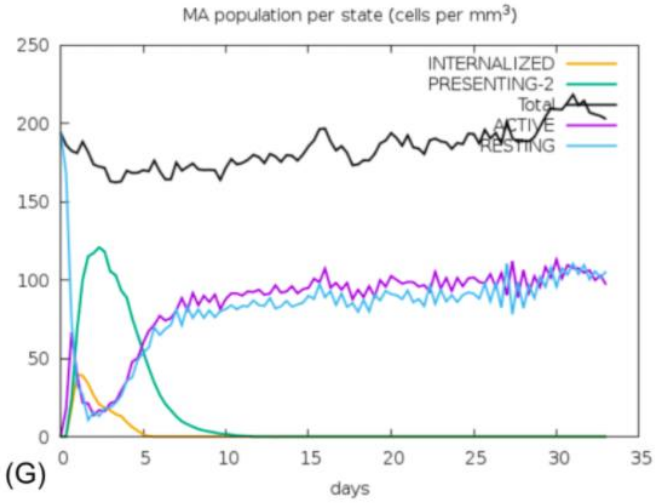


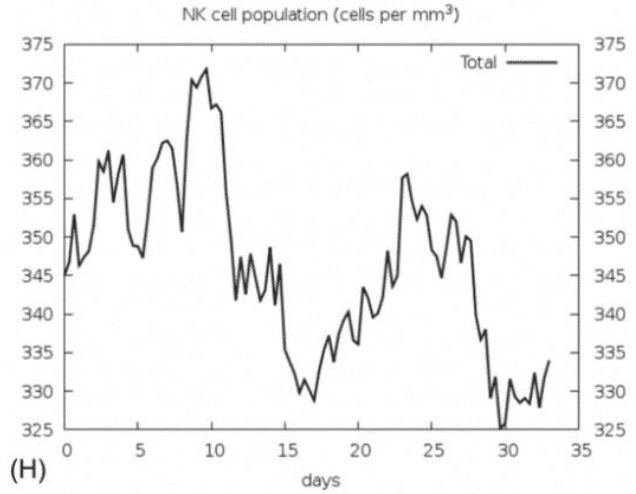



TH cell population per state (cells per mm3)
ACTIVE
DUPLICATING
RESTING
ANERGIC
days
(D)


TC cell population (cells per mm3)
Total
TC not Mem
TC Mem (y2)
memory cells
days
(E)


TC cell population per state (cells per mm3)
ACTIVE
DUPLICATING
RESTING
ANERGIC (y2)
Anergic
days
(F)


MA population per state (cells per mm3)
INTERNALIZED
PRESENTING-2
Total
ACTIVE
RESTING
days
(G)


NK cell population (cells per mm3)
Total
days
(H)

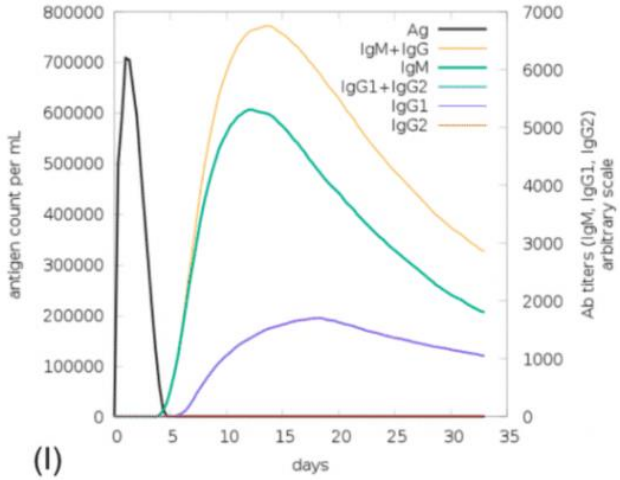

Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2)
arbitrary scale
days
(I)

FIGURE 6: Immune response simulation. The C-ImmSim server was used to run a simulation of an in silico immune response of the APP vaccine. Graph description in order from top to bottom and left to right. (A) B-cell population. (B) B-cell population per state. (C) T helper (TH) cell population. (D) TH cell population per state. (E) T cytotoxic (TC) cell population. (F) TC cell population per state. (G) Macrophage (MA) cell population. (H) Natural killer (NK) cell population. (I) Immunoglobulins. This simulation revealed the production of memory B and T cells that can present the antigen and maintain a stable population throughout the challenge. These data also predicted a 15-day peak in primed cytotoxic T cells, natural killer cells, and immunoglobulins.

Discussion

We show that bioinformatics analysis tools can be employed in the evaluation of a candidate protein as a potential antigen to be included in a vaccine. This is based on an in silica evaluation of its immunogenicity, allergenicity, and toxicity. A total of 18 B cell and T cell epitopes were rendered and demonstrated to have high immunogenicity and low allergenicity and toxicity. An immune response simulation revealed that this vaccine is likely to result in the production of memory B and T cells that can present the antigen and maintain a stable population throughout the challenge. These data also predicted a 15 day peak in primed cytotoxic T cells, natural killer cells, and immunoglobulins (Figure 6).

Previous research conducted by Jaini et.al. (2010) has determined that endogenous proteins that are overexpressed in cancer cells can be targeted with a vaccine and this can result in the prevention or treatment of breast cancer. The same methods have been employed successfully by Altuntas et.al. (2012) and Aguilar et.al. (2016). The only component that is missing here is the adjuvant that must be emulsified along with APP. In these mentioned studies, Complete Freund's Adjuvant was used which contains a subset of over 300 proteins of Mycobacterium tuberculosis (Dube et.al., 2020). Since this kind of adjuvant is known to cause severe reactions when injected into humans, we propose a safer alternative, a GMP-grade adjuvant from Saccharomyces cerevisiae. This adjuvant has been developed and used to target various human pathogens and tumors safely and with great efficacy (Grover et.al., 2016).

One major challenge of our vaccine is that although, according to the NCBI database (2021), the human urinary bladder has a high APP expression level with a mean of 147.782 Reads Per Kilobase Million (RPKM), APP has a higher expression level (almost 400 RPKM) in the brain (Figure 3). Administering a vaccine that targets high expression levels of APP at this point might seem unsafe. However, according to Sweeney et.al. (2019), the Blood Brain Barrier (BBB) is a highly selective semipermeable border that protects the brain from toxins or pathogens that could potentially harm it. The BBB is known to filter endogenous proteins such as albumin, which consists of 609 amino acid residues and has a molecular weight of 69 kDa, while APP consists of 770 residues and has a molecular weight of 87 kDa. Although APP will be lysed into several peptides during antigen presentation, this is an intracellular event that will sensitize antigen-presenting cells to APP, but these cells will not be able to cross the BBB.

The systemic autoimmune consequences of APP vaccination must also be considered. NCBI further lists what are considered high expression levels of APP in normal adrenal glands, kidneys, and the thyroid gland of 250, 275, and 230 RPKM, respectively (Figure 3). Guo et.al. (2013) reported that gene expression levels in tumor samples are 58 times greater than that in normal samples (measured in RPKM). Taking this information into account, a targeted autoimmune response against normal cells seems less likely. According to research conducted by Aguilar et.al. (2016), the autoimmune consequences of their developed testicular cancer vaccine were

relatively benign and quite tolerable, save for the targeted attack on the tumors. This was further bolstered by Mazumder et.al. (2017) with their ovarian cancer vaccine which targeted the ubiquitously expressed receptor protein of anti-Mullerian hormone with little effect on normal cells. Although this vaccine may be useful in inhibiting the growth and metastasis of human bladder tumors expressing high levels of APP, it needs to be further investigated for possible side effects.

Conclusion

Computational approaches may help detect epitopes with a higher degree of efficacy that are more difficult to acquire through in-vitro studies. Our results demonstrate that APP is a good candidate target protein that, if administered along with an adjuvant, can elicit a strong immune response against bladder cancer.

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