MINI-REVIEW

Current Trends in Cancer Vaccines - a Bioinformatics Perspective

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Abstract

Cancer vaccine development is in the process of becoming reality in future, due to successful phase II/III clinical trials. However, there are still problems due to the specificity of tumor antigens and weakness of tumor associated antigens in eliciting an effective immune response. Computational models to assess the vaccine efficacy have helped to improve and understand what is necessary for personalized treatment. Further research is needed to elucidate the mechanisms of activation of antigen specific cytotoxic T lymphocytes, decreased TREG number functionality and antigen cascade, so that overall improvement in vaccine efficacy and disease free survival can be attained. T cell epitomic based *in sillico* approaches might be very effective for the design and development of novel cancer vaccines.

Keywords: Cancer vaccine - tumor specific antigens - T cell epitomics - docking

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Introduction

Vaccinination begin with development of vaccine against small pox by Edward Jenner in 1798 (Jenner, 1798). Vaccination is derived from Latin word *Vaccinia*, which is meant for cow pox. Louis Pasteur further developed the concept through his innovative work on microbiology. Vaccination is considered to be very effective method used to prevent infectious diseases (Hellstorm, 2003). The need of the vaccine is to present a particular antigen or set of antigens against person's immune system to evoke an immune response. Vaccines are generally raised against ones immune system against invading pathogen. The main components of the vaccine may be inactive but attenuated (bacteria or viruses) which may be still be in active form and its efficiency is evaluated and tested (Chang et al., 2009). The administration of some vaccine is conducted after the exposure of pathogen. The small pox vaccine administered after three days of exposure is said to attenuate the disease, whereas same administered after one week, reduces the severity of the disease (Mortimer, 2003). There is lot of experimental vaccines for diseases like AIDS, Alzheimer Disease and for Cancer.

Types of Vaccines

There are different types of vaccines such as: *i*) Inactivated Vaccine in which virus particles grown in cell culture are inactivated using the formaldehyde or high temperature and the capsid proteins, which remain intact are able to produce an immune response against it; *ii*) Live Attenuated vaccine, in which live virus particles remain less virulent and slowly replicate for long periods of time making the antigen available for longer periods and less need of booster doses to stimulate the immune response against it (Payeete and Davis, 2001); *iii*) Subunit vaccines are those dealing only with antigenic subunits which would cause an better immune response against it. Hepatitis B vaccine containing Hepatitis B surface antigen is the the example of subunit vaccine (Szmuness et al., 1981); *iv*) Virus like particle vaccine uses viral proteins and have greater immunogenicity when compared to subunit vaccines. Examples of Virus like particle vaccine are vaccines against human pappilloma virus, Hepatitis B virus and Chikungunya virus being recently developed. (Akahata et al., 2010); *v*) Toxoid vaccines are those which are raised against the toxin, secreted by the bacteria, making them ineffective by means of treating toxin with formalin. The examples of Toxoid vaccine are diphtheria and tetanus; *vi*) DNA vaccines are still in experimental model of developing with focus on induction of humoral and cellular immunity with integration of plasmid DNA into host genome or induction Anti-DNA antibodies. (Manam et al., 2000); *vii*) Peptide vaccines based on epitope was first developed by Jakob et al. (1985). Epitope based vaccines are designed on the basis of B and T lymphocytes (Meloen et al., 2001). The T cell epitopes consists peptide fragment and B cell epitopes usually consists of proteins, lipids, nucleic acids or carbohydrates (Lehner et al., 1990) Peptide vaccines against various types of cancer had been developed owing to their easy

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production, stability and no infectious materials present in them (Naz and Dabir, 2007).

Cancer Vaccines

Cancer is the leading cause of death after heart disease and 1 in 4 deaths in USA are due to cancer (Jemel et al., 2009). Cancer is still a major health problem worldwide requiring new treatment modalities and new strategies to combat the disease. Cervical cancer is said to affect about 500,000 women in developing countries due to genital infection of human papilloma virus. Human Papilloma virus is an DNA virus that infect basal epithelial cells and HPV vaccines are prepared from virus like particles using the recombinant DNA technology (Zhou et al.,1991). Current HPV vaccine protect against the risk types HPV 16 and 18 and the Quadrivalent HPV vaccine also protect against the risk genotypes 6,11,16 and 18 (Harper et al., 2004). Compared to all other treatment modalities like chemotherapy, radiotherapy and adaptive immunotherapy, an vaccine based immune response against the tumor may be the only way to prevent the cancer for lifetime (Dougan and Dranoff et al., 2009). Tumor immunotherapy generally consists of administration of cells/antibodies ex vivo (Passive) and (Active) with administration of vaccine, eliciting the specific immune response against the Tumour Specific Antigen (TSA) and Tumor Associated Antigens (TAA) (Dougan and Dranoff et al., 2009). Cancer vaccines can be broadly classified into two types such therapeutic and prophylactic vaccines, with former focused on cancer immunotherapy and later on cancer immunoprevention according to Pappalardo et al., 2013).Vaccines had been used in the research more recently in the prevention and treatment of cancer(Guinn etl., 2007). Therapeutic vaccines had been more recently used in research on transgenic mice prone to cancer development to prove that it can lead to complete tumor prevention and normal life restoration. (Lollini et al., 2006). Prophylactic vaccines are only in research mode in animal models and far away from the application in human subjects. The development of prophylactic vaccine would definitely play a greater role in prevention of cancer. One of the major problems in the development of cancer vaccine is the lack of specific Tumor specific antigen and weakness of the Tumor Associated Antigen, which could be overcome by the development of Cell based vaccines, DNA, RNA based vaccines, Peptide based vaccines and vector based vaccines. (Palena et al., 2006). Recently carbohydrate based vaccine (Globo H Hexasaccharaide) with glycolipid as adjuvant had been found to effective against the breast cancer (Huang et al., 2013). One of the most commonly used strategies is to enhance the ability of Antigen presenting cells like dendritic cells in Cytotoxic T lymphocyte mediated immune response. Recently, several mechanisms had been proposed to instruct Antigen presenting cells like dendritic cells to enhance immune response such as a) by infecting dendritic cells with yeast, viral, bacterial vectors, b) Pulsing dendritic cell with peptides, protein and loading dendritic cells with tumor cells or tumor lysates, c) Transfecting dendritic cells with DNA, RNA. (Palena et al., 2006). Therapeutic cancer vaccines uses patients own immune system in targeting and eliminating cancer cells. Targeting of Tumor Specific Antigen using vaccine is difficult, since Tumor Specific Antigen(found in tumor cells), in tumors varies from individual to individual due to the somatic mutations occurring on the protein sequence, so personalized immune response to individuals system is needed(Bendle et al., 2005). Tumor Associated Antigens (TAA) found in tumors with similar histology and of different origin are weakly immunogenic due to tolerance to self antigens acquired by immune system in developmental stages, so targeting them for vaccine production is difficult. (Schietinger et al., 2008). Nanoparticles had been recently used as the delivery system for the Tumour Associated Antigen and adjuvants to dendridic cells so as to elict an effective immune response against the tumour cells. (Silva et al., 2013)

Cancer Immunoediting and Immuno -therapeutics

Cancer immunoediting is the one in which cancer cells are eliminated using the own immune system (Dunn et al., 2004) and those which escape the immune surveillance, develops into tumours. The development of cancer vaccine is problematic in the sense that it should make use of use of patients own immune system in destroying existing cancer cells. Cytotoxic T cells, Dendridic cells and antibodies represent cancer immunotherapeutics. (Begley and Ribas, 2008). Anti-idiotype antibodies are used as vaccine to prevent against development of growth of tumors in mice, and in curing mice with established tumors (Bhattacharya-Chatterjee et al., 2002). Dendritic cells pulsed with tumor antigens confer therapeutic and protective Anti-tumor immunity (Nestle et al., 2005). Tumor vaccines are raised against poorly immunogenic tumors to have more antigenic potential (Dunn et al., 2004). TLR ligands have shown to be effective against the target specific antigen stimulation through Dendridic cell activation and reversal of tumour driven immunosuppression (Silva et al., 2013). Nanocarriers such as Aliphatic polyesters like polyglycolic acid and Polylactic acid had been used for biomedical applications because of their biocompatibility and biodegradability to deliver Antigen to Dendridic cells (Silva et al., 2012).

Computational Modeling of Cancer Vaccines

Computation biology can play an effective role in prediction and optimization of therapeutic effects at the organism level. (Kumar et al., 2006). Computational immunologist use simulation of immune system using computer and mathematical modeling to study different pathogens and its responses to immune system.(He et al., 2010). The Designing of *in sillico* models would be helpful in understanding the molecular interactions taking place at cellular and molecular level and simulation of laboratory based experiments. The first step in designing cancer vaccine is design an *in sillico* model consisting complex biological system comprising of molecules, cells, organs and different organisms. Different computational model

have been proposed for the cancer vaccine design such as SimTriplex Model and Metastasim Model (Pappalardo et., 2013). In Sim Triplex Model, "Triplex" had been stimulated, Tumor preventive cell vaccines had been used in HER 2/Neu Transgenic mice prone to mammary carcinogenesis (Pappalardo et al., 2005) resulting in very long tumour free survival and blocking of mammary carcinogenesis in six week old mice (Palladini et al., 2010). The major limitation of Triplex vaccine is that it is only effective only in long term vaccination of more than 60 week and not effective in short term vaccination in preventing mammary carcinogenesis (Palladini et al., 2010). The "Sim Triplex model" simulation is designed in such a way that new tumor cells appear and existing tumor cells replicate and stops at the threshold levels signifying the formation of tumour mass (Pappalardo et al., 2013). The" Sim Triplex model" simulation helped to evaluate the the optimal vaccination schedule like that chronic protocol with reduced vaccine administrations and had been verified by in vivo experiment (Pappalardo et al., 2010). Another model Metastasim model was discovered by Pennisi et al., 2010, which has high similarity to that of Sim Triplex model and some differences, completely different in the case of cancer growth kinetics. The Metastasim model suggests that in prevention of lung metastases, initially massive vaccine dosage should be followed by few vaccine recalls (Pennisi et al., 2008). The Metastasim model is able to simulate multiple different metastatic nodules with different growth rates. The chemotherapy and radiotherapy both carry major side effects for the patients. Computational modeling combined with mathematical modeling had been major focus on immune system related approach to cancer. (Alemani et al., 2012). Joshi et al. (2008), had proposed a mathematical model describing the growth kinetics of immunogenic tumors in the presence of immune response with cytotoxic T cell interacting with that of the cancer cells. Personalized mathematical model, having the real-time inputs of patients clinical parameters, and immune response to ongoing therapy had been formulated, which suggests that increase in vaccine dose and frequency helps in the stabilization of the disease (Kogan et al., 2012). Recently Wilson and Levy (2012) had devised a mathematical model with Transforming growth factor beta, Activated cytotoxic cells, to be used for future designing of the experiments based on immunotherapy.

Vaccine with Bacteria, Yeast, Viral Vectors against Cancer

Vectors such as bacteria, yeast and virus had been used in cancer immunotherapy to induce immune response against Tumor Associated Antigens (TAA) (Kass et al., 1999). Recombinant vector based vaccine had been used to direct strong immunogenic response against the weakly immunogenic Tumor Associated Antigens (Kantor et al., 1993). Inflammatory responses are increased against genes of interest that are inserted inside the vector, and recombinant proteins that are produced, are more immunogenic than protein injected with adjuvants. (Kass et al., 1999). Dendritic cells provide the T cells with antigenic signal, and activates in the presence of co-stimulatory molecules, for the generation of potent T cell responses towards weak Tumor Associated Antigens (TAA) (Vergati et al., 2010).

PSA-TRICOM Vaccine (Prostrate Specific Antigen plus Triad of CO stimulatory molecules) consists of Transgenes for PSA against the epitope, and three CO stimulatory molecules such as(B 7.1, ICAM-1 and LFA-3) and also consists of recombinant vaccinia(rV-)PSA-TRICOM and booster vaccinations with recombinant Fowl pox (rF-) (Terasawa et al., 2002). In the Phase 2, clinical trial involving PSA-TRICOM VACCINE, in patients with metastatic hormone refractory prostrate cancer, PBMC from the pre and post vaccination were analyzed by the ELISPOT assay to evaluate the specific immune responses against the HLA-A2 PSA peptide and PSA-3 (Gulley et al., 2002). Out of 29 patients, 13 patients had enhanced PSA-3 specific T cell immune response. On post vaccination, patients who had >6 fold response against the epitope were found to live longer than those who had less <6 fold response (Gulley et al., 2002). Prostrate cancer vaccine are viable candidate for the development,since current treatment methods are not adequate for the treatment of Castration Resistant Prostrate Cancer (Geary and salem, 2013). Prostrate specific cancer vaccines responds against the patients immune system by production of tumour specific cytotoxic T lymphocytes. (Geary et al., 2013).

PANVAC-VF

It consists of Recombinant vaccinia expressing the transgene encoding for the Carcinoembryonic Antigen (CEA) and MUC 1 (Mucin glycoprotein) with single amino acid substitution to enhance the immunogenicity (Zaremba et al., 2002). Clinical trials using PANVAC-VF vaccine for the treatment in patients having advanced pancreatic cancer showed no improvement in survival. (Goldmann and Defrancesco, 2009).

Vaccines with Proteins and Peptides against Cancer

Protein or peptide had been used to stimulate immune response against the cancer employing broad range of proteins such as heat shock proteins (HSP), Agonist Peptides, Anti-idiotype antibodies (Palena et al., 2007; Bolhassini and Rafati, 2008; Ai et al., 2009). Vaccines based on proteins have stronger response on the generation of CD4+ lymphocytes, and less effective on production of Cytotoxic T lymphocytes (Ostrand Rosenberg, 2008). Various factors such as Weak immunogenicity of single protein, Tumors evading antigen recognition through mutation loss and HLA restriction of proteins, for vaccine development can be overcome by use of long peptides and using dendritic cells loaded with protein as adjuvant to stimulate the immune system (Cerundolo et al., 2004). Tumor Associated Antigen (TAA) can be identified using the genetic approach, in which Cytotoxic T lymphocyte clone is screened using, the patient derived target cells, transinfected using the Tumor derived cDNA libraries

which leads the increase in cytokine levels, due to the recognition by Tumor specific cytotoxic T lymphocyte clone, which further leads to selection of cells containing antigen encoding cDNA. These cells are further recloned and rescreened to further identify antigen encoding cDNA (Van der Bruggen et al., 1991). Recently, reverse Immunology is used for epiopte prediction screening using the algorithms, and Epitope validation is further tested using the invitro assays (Campoli and Ferrone et al., 2008).

SIPULEUCEL (Provenge) is an immunostimulatory product designed to stimulate T cell immunity against the the Prostatic Acid phosphatase for the treatment of patients with mHRPC. Recent clinical trials of Provenge showed that the patients treated with it had encouraging effects in terms of disease progression (Higano et al., 2009). ONCOPHAGE consists of HSP gp 96 preparation from the tumor complex and is used effectively to treat patients with melanoma and renal carcinoma. STIMUVAX is designed to stiumalte immune response against the MUC 1 (glycoprotein widely expressed in tumors of lung cancer, breast cancer, prostate cancer and colorectal cancer). The clinical trials conducted against the Non-small cell lung cancer patients (NSCLC) using the STIMUVAX signals the efficacy of the vaccine (Butts et al., 2007).

Vaccines with Tumor Cells or Tumor Cell Lysates against Cancer

Tumor vaccines can target both unknown and different antigens and the resulting Immune response is not restricted to HLA. Tumor antigens are phagocytosed by dendritic cells and presented to CD 8+ cells by the MHC molecules (Hamilton et al., 2006). ONCOVAX consists of irradiated tumor cells, directed against human colon cancer, and showed no improvement in stage 3 of the disease, whereas it showed significant improvement of recurrent free survival in stage 2 of disease (Vermorken et al., 1999). RENIALE (Liponova) vaccine is used for the treatment of Renal Cell Carcinoma and clinical trials using, Reniale showed that patients with higher risk showed greater improvement with adjuvant (Doehn et al., 2006). GVAX vaccine consists of two irradiated human prostrate cancer cell lines with LNCaP, and PC-3 with viral vector encoding the Human GM-CSF gene and combination therapies of GVAX vaccine along with the CTLA-4 blocking antibodies show activity in melanoma and ovarian carcinoma (Hodi et al., 2008).

DNA and RNA Vaccines against Cancer

DNA Vaccination had been widely used as alternative approach to develop efficient vaccines for cancer therapy. DNA vaccines are made using the Tumor specific antigens which direct them and amplify. Improved delivery systems such as gene gun, Cationic liposome's, simultaneous administration of cytokines (GM-CSF or IL-2) are used to enhance the potency of DNA vaccines (Pasquini et al., 1999; Ren et al., 2006; Best et al., 2009). DNA based vaccines are used to enhance the immunogenicity of plasmid encoded antigens (Binder and Srivastava, 2005). Different Tumor Associated Antigens (TAA) like the Prostate specific antigen, PAP, HSP 65, gp 100 are used as as target for DNA based vaccines for the treatment in patients with prostate cancer (Pavlenko et al., 2004), Melanoma (Yuan et al., 2009), Colorectal Cancer (Conry et al., 2002), Head and Neck carcinomas (Michaluart et al., 2008). Recently mRNA based gene transfer vaccines involving the mRNA expression in the target cells generated by the invitro transcription of bacteriophage promoter equipped plasmid DNA, composed of a cap structure at the 5'end, coding RNA for target antigen (PSA, CEA) and Tail of polyadenosine (Poly A-Tail) are used as immunotherapeutic approach towards cancer (Kreig and Melton, 1984). The mRNA based vaccine encoding the mRNA for Tumor Associated Antigen is transfected into dendritic cells and translated into proteins thus entailing the the Antigen specific cytotoxic T lymphocyte Immune response (Pascolo, 2004). Injection of mRNA directly into patients with breast cancer (Su et al., 2005), lung cancer (Morse et al., 2003), renal cell carcinoma (Su et al., 2005), brain cancer (Caruso et al., 2005), ovarian cancer (Danull et al., 2005), Neuroblastoma (Caruso et al., 2004) and melanoma (Kyte et al., 2006) was able to increase the Tumor Associated Antigen specific cytotoxic T lymphocyte response. In recent clinical trials in patients with renal cell carcinoma, involving the mRNA transfected with the Dendritic cells with total RNA extracted from clear cell carcinoma, there is an increase in tumor specific CD 4+ and CD 8+ cells. Further research is needed to elucidate the mechanism of activation of Antigen specific cytotoxic T lymphocytes, decreased TREG number functionality and antigen cascade to understand the immunological mechanisms, so the overall, improvement in the vaccine efficacy, disease free survival can be improved.

Futuristic T Cell Epitope Based Cancer Vaccine Design by Immunoinformatics

Epitopes are part of antigen that is recognizable by the immune system particularly by antibodies, B-cells and T cells. T cell epitopes are found on the surface of antigen presenting cells bound attached to Major Histocompatability molecules (MHC) (Madden, 1995). Class I MHC molecules are present on the nucleated cell of the body and Class II MHC molecules are present on the specialized cell types such as Dendritic cells, Macrophages and B cells (Janeway, 2001). T cells recognize the epitope to induce the immune system of an individual and in the case of diseases like human immunodeficiency virus, Hepatitis C virus, the viruses avoid the recognition of T cells by relying more on mutations that effectively alter the amino acid sequences of the proteins encoded by viral

genes.(Letvin and Walker, 2001). Predictions of T cell epitope play an important role in design of vaccines and pose a significant problem in immunoinformatic approach towards vaccine designing (Mishra and Sinha, 2009). Recently selection of T cell epitope towards the development of epitope based vaccine design had been carried out with help epitope enhancement by sequence modification (Lurescia et al., 2012). T cell epitope binding prediction is done on the basis of prediction of binding of the MHC using Bioinformatic prediction methodologies. T cell epitope prediction usually involves the peptide binding specificity with class I or II MHC molecules and then predicting the epitopes *in sillico*. T cell epitope based alogarithms have been constructed using, the peptide sequence binding data with experimental affinity binding data using any of the methods such as Motif based systems, support vector machines, Hidden Markova models, Quantitative-structure based relationships and structure based approach had been used for T cell epitope prediction. Immunoinformatics can be named as branch of bioinformatics dealing with *in sillico* analysis of Immunology related problems and data (Brusic and Petrovsky, 2005). Using the tools of Immunoinformatics, sequence areas of potential binding sites of pathogen genome with potential antigenic proteins (reverse vaccinology) can be analyzed for new vaccine design (Tomar and De, 2010). The conventional method of growing pathogen in lab, extraction of proteins and analyzing the proteins is time consuming and complicated process, so several *in sillico* methodologies have been created to identify epitopes, Normally the binding affinity of antigenic peptides to MHC molecules is been analyzed to predict epitope (Tomar and De, 2010). Sequence based methods such as: *i*) Motif search method is used for the prediction of Epitope (Joyce and Nathenson, 1994). One of the widely used epitope prediction tool is SYFPEITHI which is also based on motif based search method (Rammensee et al., 1999); *ii*) Artificial neural networks is used for the prediction of epitopes associated with MHC haplotypes (Bellard et al., 1998); *iii*) Support vector machine is used for the analysis of support vector machine data to predict the T cell epitope (Nanni, 2006); *iv*) Hidden Markov model is used for the prediction of peptide binding affinity to HLA proteins (Mamitsuka, 1998). Structure based methods such as Docking of peptides and screening of peptide libraries use structural information and computational methods to potentially find good binders. Combinatorial peptide library screening and ligand docking had been used mainly in the bioinformatics. Docking studies are mainly used in the investigation of peptides that are used in the analysis of MHC molecules (Sezerman et al., 1993). EPIDOCK is a widely used, structure based server for the MHC binding prediction of peptides using docking score based Quantitative matrices (Atanasova. et al., 2011).

Conclusion

Cancer vaccine development is problematic due to lack of specific Tumor Specific antigen and weakness of Tumor Associated antigen to stimulate strong immunogenic response. Targeting of Tumor specific antigen is difficult, since Tumor Specific antigen found in tumors varies from individual to individual necessating the need for personalized treatment. Tumour vaccines raised against poorly immunogenic tumors have more antigenic potential. Recently, Recombinant Vector based vaccine had been used recently to direct strong immunogenic response against weakly immunogenic Tumor Associated Antigens. The efficiency of cancer vaccine can be improved by understanding the mechanism of activation of Antigen Specific cytotoxic T lymphocytes, Decreased TREG numbers functionality and antigen cascade mechanism during immunological reactions. Computational and mathematical modeling to analyze the efficacy of cancer vaccines is new field of research for anti cancer approach. T cell epitope prediction and binding using Docking and peptide library screening is very much helpful to find potential antigenic determinant for binding of MHC molecules using the *in sillico* approach towards highly successful development of effective cancer vaccine design and development.

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