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Therapeutic Targets Prediction of Tumor Specific Antigen for Vaccine Design

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Abstract—*The difficulty of treating cancer lies in their similarity with normal cells in terms of antigen expression. Cancer cells are transformed cells that gains immortality by mutagenic transformation of ormal cells. The cross reactivity of surface antigens on tumor cells and normal cells makes them difficult to be identified by immune system. However , T Cytotoxic cells, NK cells and dendritic cells plays important role in immune response to tumor cells. T cell epitopes of tumor specific antigens were predicted as vaccine candidates so as to increase immunity against tumor cell. Tumor cells express tumor specific antigen peptide on their surfaces which are recognised by T-cytotoxic cells and hence give immune response against tumor. Dendritic cell phagocytose the tumor cell and present their peptides to the T-helper cell and hence result in tumor rejection. MHC class I binding prediction tool and $CD8^+$ immunogenicity prediction tools were used to predict class I epitopes. MHC II binding prediction tool and $CD4^+$ immunogenicity prediction tool were used to predict class II epitopes. Population coverage of the epitopes of both MHC I and MHC II binding peptide was calculated in Indian population. A set of most population covered epitope was selected for each antigen. Epitopes were predicted for ACTN4, BRAF, CAMEL, CASP5, CASP8, CDC27, CDK4, CDKN2A, CTNNB1, EEF2, FLT3, GPNMB, HSPA1B, KRAS, MUM1, PAPOLG,TPI1, UBXD5. A combinatorial epitope prediction would be effective in designing multifunctional immune stimulation response against tumor specific antigen.*

Introduction

Tumor is the abnormal mass of tissue when uncontrolled cell division takes place. Tumor antigen is the protein product of the tumor cell, which is identified by immune cell. By identification of tumor antigen immune cell respond to the tumor cells and try to eliminate these cells. Tumorantgen is classified into three different type, these are tumor specific antigens, shared antigen, unclassified[1]. Tumor specific antigen are further classified into substitutional mutation, alternative ORF, intron encoding, chromosomal translocation, internal tandem repeat[2]. There are no. of antigen in these like in substitution mutation ACTN4, BRAF, CASP8, CDC27 etc., in alternative ORF CASP5, CDKN2A,OGT etc., in intron encoding MUM1, in chromosomal translocation ABL-BCR, BCR-ABL, DEK-CAN etc., internal tandem repeat FLT3[3][4].

The tumor specific antigens are the main target of the immunogenic response against the tumor cell. These are the tumor specific antigens (not present on the normal cell) so if immunotherapy using these as targets for immune cells, then there is no harmful effect of it on the normal cell[5].

MHC class l presents the short peptide on the surface of tumor cell. T cytotoxic cell recognise this peptide and MHC l complex and give immune response against it[6]. MHC class II present the short peptide on the surface of tumor cell. T helper cell recognise this peptide and MHC II complex[7]. Helper T cell never give direct immune response instead of that they help in the activation of cytotoxic T cell and macrophages to give immune response to target cell. Or the T helper cell stimulate B cells to secrete antibodies. APCs ingest microbes, degrade them, and export small part of it(antigen) to the cell surface with the help of mhc ll. This MHCll and peptide complex then recognise by the receptors present on the helper cell surface known as $CD4^+$ receptors[7]. But this is not the only signal which is required for the activation of T cell. It required one more signal for its activation. It must be given by one of these two signal either by stimulation by a cytokine or by a stimulatory signal that is between B7 and CD28. B7 found on the surface of APCs. Receptor protein CD28 found on the surface of helper T cell. So, when the first signal and one of second signal are received then the helper T cell becomes activated[8][9].

So, Here we have to predict the epitopes, which can bind to receptors of both T helper cell as well as T cytotoxic cell. There are lots of tool which can use to predict MHC l and MHC ll binding peptides[10][11][12].

They give an accuracy of about 90-95%. Then we have to select those alleles which cover most of the population frequency. So the vaccine we want to form can be bind to the most HLA alleles. We can collect these data from different papers in which population frequency of different HLA alleles given[13].

Once the epitope selection takes place, then we can select a set of those epitope which cover atleast 80% of the population. So the vaccine we formed from these epitopes can be effective on the large population size.

METHODS

DATA COLLECTION

Accession id.oftumor specific antigen and their isomers were retrieve from TANTIGEN database. There were 45 tumor specific antigens and Consensus sequences were find by multiple sequence alignment of all the isomers of that tumor specific antigens. Seventeen antigens consensus sequence were selected which could be selected for the further prediction. For all other protein we either not able to get full length sequence or they have too short sequence so we can't predict targets from them.

In some cases the complete sequence of one isomer was selected that is known as reference sequence. The peptide part of the sequences were selected which is common in most of the isomers. From these sequences we have to predict the mhcl binding peptide andmhcII binding peptide.

Epitope prediction of T-cytotoxic cell

The antigen sequences are firstdevided into small peptide which binds to MHC1 then these MHC1-peptide complexs bind to the $CD8^+$ receptor of T-cytotoxic cell. So we first predicted MHC1 binding peptide then $CD8^+$ receptors binding.

MHC l binding peptide prediction
IEDB prediction tool Tepitool was used for the prediction of MHC l binding peptide. As this is a user friendly tool. And Different attributes were manully selected.

Alleles selection
A set of alleles selected on the bases of their population frequencies. Thirtyeight most abundant alleles set of HLA class l were used which covers almost 99% population of the world.

IC^{50} value selection
The ic^{50} value of all these alleles are more than 100 nm , so we select a threshold of less than or equal to 100 nm for best prediction results.

Prediction method selection
NetMHC1pan method was used for the prediction of mhc1 binding peptides. This is the only method which predict peptide on the bases of IC^{50} value.

$CD8^+$ immunogenicity prediction
IEDB CD8+ immunogenicity prediction tool was used with the default setting. In this tool automatically masking of 1,2 and C-terminal of amino acid take place. Prediction score was calculated for all mhc l binding peptide and only those peptide were selected whose prediction score is more than zero.

Population coverage of T-cytotoxic epitope
POPULATION ANALYSIS tool was used for the calculation of population coverage of selected peptide and those peptide were selected which cover most of the population.

T-helper cell epitope prediction

Tumor antigen are the internal peptides so presented by mhc1 but these tumor cell phegocytosis by dendritic cells which are antigen presenting cells . They present these antigens peptides to the T-helper cell.

MHC ll binding peptides would predict followed by their $CD4^+$ immunogenicity prediction.

MHC ll binding peptide prediction
IEDB prediction tool Tepitool was used for the prediction of MHC ll binding ppeptide and different attributes would select here.

Alleles selection
Twentysix most aboundant alleles of MHC II were used. These alleles IC^{50} value threshold cutoff selected here is 1000.

Prediction method selection
A set of methods was used for prediction of most promisiouse binding of HLA ll alleles. Only those peptide were selected which bind to atleast 50% of alleles from these Twentysix alleles.

$CD4^+$ immunogenicity prediction
IEDB prediction tool were used for the prediction of immunogenicity of the mhcll binding peptides. Immunogenicity score of all the peptide were predicted and only those peptide were selected which have immunogenicity score more than zero.

Population coverage
IEDB population coverage prediction tool was used for the analysis of population coverage in India. Those peptidewere selected which cover most population.

Combined population coverage of MHC1 and MHC11

Combined population coverage was calculated of the selected peptide of both mhc1 and mhc11 by population analysis tool.

RESULTS

A set of epitopes was predicted for different antigen which have maximum population coverage in Indian subcontinent.

Antigen Name	Predicted epitopes of T-Helper and T-Cytotoxic cell	Total population coverage in Indian subcontinent
ACTN4	KALDFIASK, MTYVSSFYH, VQNFHISWK, MTLGMIWTI, LAFNALIHR, SSFYHAFSGAQKAET, ALDFIASKGVKLVSI, LEINFNTLQTKLRLS, LQTKLRLSNRPAFMP, SNHIKLSGSNPYTTV	93.51

CAMEL1	FLMAQGAML, RMAVPLLRR, RPWKRSWSA, LMAQEALAF, CTSRCLSRR, AVPLLRRMEGAPAGP, FLMAQGAMLAAQERR, MLMAQEALAFLMAQG	87.32
CASP5	SVLRAFAAR, KSFEVPQAK, LLYDTIFQI, SSTPHNVSW, NVSWRDRTR, GAHYDIVGMKRLLQG, STFLVLMSHGILEGI, RDMESVLRAFAARPE, DFIAFCSSTPHNVSW, LWVRDSPASLAVISS	88.64
CASP8	IINNHNFAK, TVNNCVSYR, ILTEVNYEV, LSFLKELLF, MLEESNLSF, SFLKELLFRINRLDL, DALMLFQRLQEKRML, RAQISAYRVMLYQIS, RGYCLIINNHNFAKA, DLASLKFLSLDYIPQ	87.36
CDC27	HSGHFVALK, CIFAEMFRR, RIYSYQMAL, NPHKRISAF, LTPVVVTLW, KEAINILSHLPSHHY, TTLWHLQKDVALSVL, QTFKFTSLQNFSNCL, AAIWQALNHYAYRDA, YAYAYTLLGHEFVLT	91.5
CDK4	HSGHFVALK, CIFAEMFRR,RIYSYQMAL, NPHKRISAF, LTPVVVTLW, PHSGHFVALKSVRVP, RISAFRALQHSYLHK, SYQMALTPVVVTLWY, DFGLARIYSYQMALT, RQFLRGLDFLHANCI	94.41
CTNNB1	IMRTYTYEK, TTAPSLSGK, RLHYGLPVV, YAAAVLFRM, ATYAAAVLF, GQYAMTRAQRVRAAM, EKLLWTTSRVLKVLS, QALVNIMRTYTYEKL, WPLIKATVGLIRNLA, GLIRNLALCPANHAP	91.62
EEF2	IMRTYTYEK, TTAPSLSGK, RLHYGLPVV, YAAAVLFRM, ATYAAAVLF, PLMMYISKMVPTSDK, GRVFSGLVSTGLKVR, GGIYGVLNRKRGHVF, CITIKSTAISLFYEL, NRLYMKARPFPDGLA	85.41
FLT3	RMPEAAPPV, KTYQGSYGF, CTYSPALNK, RVRAMAIYK, TAKSVTCTY, APPQHLIRVEGNLRV, RAMAIYKQSQHMTEV, TYQGSYGFRLGFLHS, VTCTYSPALNKMFCQ	86.13
GPNMB	TIVEGILEV,ITFAVNNLIF, YVLNGTGSL, RTFNGSGTY, YVFHTLGQY, TVISLLVYKKHKEYN, DPASPLRMANSALIS, LSVFLNRAKAVFFPG, DVDEMCLLTVRRTFN, GNVVRSKGLSVFLNR	89.68
HSPA1B	LLLLDVAPL, AEAYLGYPV, RTTPSYVAF, VIAGLNVLR,GVMTALIKA, GLNVLRIINEPTAAA, QNKRAVRRLRTACER, TKDAGVIAGLNVLRI, GGVMTALIKRNSTIP, VGVFQHGKVEIIAND	86.34
KRAS	KSFEDIHHY, CVFAINNTK, LVREIRQYR, CLLDILDTA, QYMRTGEGF, TLVREIRQYRLKKIS, ALTIQLIQNHFVDEY, GFLCVFAINNTKSFE	87.68
MUM1	YAADISYPV, KMKGFTVSL,RSFEVGMLV, GMLVWHKHK, LSSSFTCEK, PLEELAYRRSLRVAL, IGWCVSLITDYRVRL, GFTVSLKSLKHFDCK, KGAESHILRAILKSRK,LEYYAADIS YPVRKS	91.56
PAPOLG	HLMPIITPA, CTIPTVVGR, KIFTFGSYR, RSDFFQSFF, YPNAAASTL, FRLTLRAVKLWAKRR, KYRHYIVLTASASTE, VESKIRVLVGNLERN, LRSLDIRCIRSLNGC, LGIIFRRVENAESVN	87.11
TPI1	HLMPIITPA, CTIPTVVGR, KIFTFGSYR, RSDFFQSFF, YPNAAASTL, GELIGTLNAAKVPAD, ESDELIGQKVAHALA, FHFAALYISGQWPRL,SKVVLAYEP VWAIGT, LRGWLKSNVDAVAQ	86.73
UBXD5	RTLEPIPLK, SAFEIFSTF, RLYPNGVPF, MTAEKFLNR, MAFMTRKLW, IPLKLYRNGIMMFDG, FPSELQRLYPNGVPF, KAALLLRARRAPKSS,EQAFLLMM QPDNTIG, DASAFEIFSTFPPTL	88.45

Conclusion

Therapeutic target were predicted for vaccine formation. These can be further tasted in vitro then could be used in personalised medicine.

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