Prediction of a Novel HLA-A2-restriced Cytotoxic T Lymphocytes Epitope of Tumor Antigen MAGE-12 by Bioinformatics Method*

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Abstract Objective To predict the HLA-A2-restricted CTL epitopes from melanoma antigen MAGE-12. **Methods** HLA-A2-restricted CTL epitopes of MAGE-12 were predicted by peptide supermotif prediction combined quantitative motif and extended motifs. **Results** Five HLA-A2-restricted CTL epitope candidates derived from the tumor antigen MAGE-12 were selected. **Conclusion** The combination of supermotif, quantitative motif and extended motifs can improve the prediction efficiency. The predicted epitopes provided useful hints to probe epitopes in MAGE-12 by experiments.

Key words: tumor-antigen; MAGE-12; cytotoxic T lymphocyte epitope; prediction

Recently, with the innovation of immunology methodology, molecular mechanisms of tumor antigen presentation and $CD8^+$ T cell activation have been gradually elucidated. Tumor antigens and their coding genes were identified successively. The breakthrough in theoretical tumor immunity accelerated the development of vaccine targeting on tumor antigen. The induction of antigen-specific CTL has been suggested to be highly efficacious in the prevention and treatment of various types of tumors. As it can induce specific CTL in vitro and in vivo to kill target cells, epitope peptide vaccine has been of particular interests. MAGE-12, a member of melanoma antigen (MAGE) gene family, which is expressed in most malignant tumors instead of normal tissues except for testis and placenta, has been used as an ideal target for tumor immunotherapy. In view of the fact that HLA-A2 is one of the most frequently expressed molecules in China [1].It is very valuable to identify the tumor antigen epitopes which are presented by HLA-A2 and able to induce epitope-specific CTL against tumor cells. In this study, we report a simple and efficient bioinformatics method to identify candidate HLA-A2 restricted CTL epitopes from the tumor antigen MAGE-12.

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MATERIALS AND METHODS

Materials

MAGE-12 (314aa) was selected as objective antigens in this study. The amino acid sequences of the tumor antigen were quoted from GENBANK database:
MPLEQRSQHC KPEEGLEAQG EALGLVGAQA PATEEQETAS SSSTLVEVTL REVPAAESPS PPHSPQGAST LPTTINYTLW SQSDEGSSNE EQEGPSTFPD LETSFQVALS RKMAELVHFL LLKYRAREPF TKAEMLGSVI RNFQDFFPVI FSKASEYLQL VFGIEVVEVV RIGHLYILVT CLGLSYAGLL GDNQIVPKTG LLIIVLAIIA KEGDCAPEEK IWEELSVLEA SDGREDSVFA HPRKLLTQDL VQENYLEYRQ VPGSDPACYE FLWGPRALVE TSYVKVLHHL LKISGGPHIP YPPLHEWAFR EGEE

Methods

SYFPEITHI prediction HLA-A2-restricted CTL epi-

Table1 Coefficients of the polynomial method(HLA-A2)

Res	1	2	3	4	5	6	7	8	9
A	-2.38	-3.22	-2.8	-2.66	-2.89	-2.7	-2.35	-3.07	-2.47
C	-2.94	-15.0	-2.58	-1.96	-3.29	-2.22	-2.97	-2.37	-15.0
D	-3.69	-15.0	-3.46	-2.71	-2.26	-2.63	-3.61	-3.03	-15.0
E	-3.64	-15.0	-3.51	-2.65	-3.39	-3.41	-3.21	-2.63	-15.0
F	-1.89	-15.0	-2.35	-2.5	-1.34	-2.43	-2.18	-1.71	-15.0
G	-2.32	-15.0	-3.04	-2.63	-2.56	-2.3	-3.13	-2.96	-15.0
H	-2.67	-15.0	-2.58	-2.58	-2.05	-3.32	-3.13	-2.16	-15.0
I	-1.65	-2.55	-2.8	-3.44	-2.74	-2.79	-2.2	-2.69	-2.1
K	-2.51	-15.0	-3.65	-2.93	-3.34	-3.77	-2.97	-3.27	-15.0
L	-2.32	-1.7	-2.09	-2.49	-2.71	-2.63	-2.62	-2.01	-2.74
M	-0.39	-1.39	-1.79	-3.01	-3.43	-1.38	-1.33	-0.97	-2.96
N	-3.12	-15.0	-3.31	-2.22	-2.36	-2.3	-3.14	-3.31	-15.0
P	-3.61	-15.0	-2.97	-2.64	-2.42	-2.31	-1.83	-2.42	-15.0
Q	-2.76	-15.0	-2.81	-2.63	-3.06	-2.84	-2.12	-3.05	-15.0
R	-1.92	-15.0	-3.41	-2.61	-3.05	-3.76	-2.43	-3.02	-15.0
S	-2.39	-15.0	-2.04	-2.12	-2.83	-3.04	-2.73	-2.02	-15.0
T	-2.89	-3.58	-2.6	-2.48	-2.17	-2.58	-2.67	-3.14	-3.7
V	-2.44	-2.64	-2.68	-3.29	-2.49	-2.25	-2.68	-2.8	-1.7
W	-0.14	-15.0	-1.01	-2.94	-1.77	-2.77	-2.85	-2.13	-15.0
Y	-1.46	-15.0	-1.67	-2.7	-1.92	-2.39	-1.35	-3.37	-15.0

topes of MAGE-12 were SYFPEITHI prediction according to previous study methods [2,3]. Database retrieval can be performed on any HTML-browser supporting JavaScript. The main page of the database (http://www.uni -tuebingen.de/uni/kxi/) offers three sections: Find Your Motif, Epitope prediction and Information. After a preselection of one or multiple MHC-types, the Epitope prediction section allows the user to predict candidate epitope from protein or its gene, the restriction element are available. The HLA-A*0201 type was chosen from the frame of Select MHC type. In this study, the nonamers (9aa) were chosen from the frame of Choose a nonamer due to the typical length of a class I ligand comprised 9 amino acids (nonamers). Following the amino acid sequences of the tumor antigen was inputted, the epitope prediction program was processed immediately. The values of all possible nonamers of a given sequence were added together and the fifteen high-scoring peptides of the antigen was selected as optimal T cell epitope for further study.

Polynomial method analysis The basic premise of this method is independent binding of individual side-chains (IBS) [4]. When residue R occurs at position i in the peptide, it is assumed to contribute a constant amount Ri to the free energy of binding of the peptide irrespective of the sequene of the rest of the peptide. Parameters Ri are estimated from a training set of 161 peptides by a method analogous to that used by epidemiologists to calculate risk factors for developing a disease [5]. All peptides in the training set contain the canonical motif for HLA-A2.1, so that they all contain the "correct" residue at the anchor positions (2 and 9). For i other than 2 and 9 (for the non-anchor positions), the average negative log10 of IC50 of all the peptides carrying R at position i is calculated and used as the estimate of Ri [6]. The values of the Ri terms of HLA-A2 specificity are shown in Table 1.

To calculate the polynomial method score of peptides in this study, the Ri values corresponding to the sequence of the given peptide were added together. If this sum exceeded a chosen threshold, the peptide was predicted to bind. The threshold was chosen as the number that gave a relatively clean separation between binders and non-binders in the training set. In the present study the threshold was -24.

Coefficients of the polynomial method. For each of

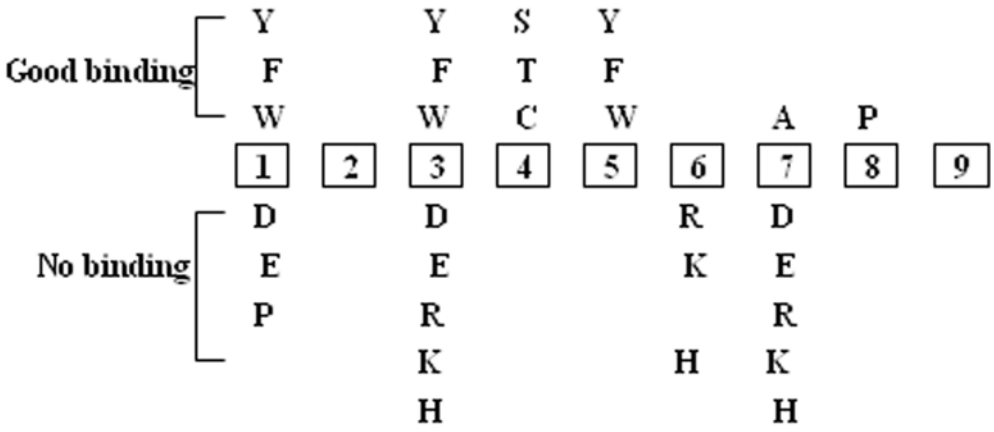

Fig 1 Residues associated with good binding or no binding to HLA-A2 molecules in 9-mer peptides

Table 2 The result of MAGE-12 epitope prediction by SYFPEITHI combined with polynomial methods

AApos	Sequence	SYFPEITHI Score	Coefficients score
112	KMAELVHFL	29	-21.89
201	**LLIIVLAII**	**28**	**-22.52**
108	ALSRKMAEL	27	-21.17
200	GLLIIVLAI	27	-22.33
271	**FLWGPRALV**	**27**	**-19.47**
174	HLYILVTCL	26	-22.22
220	KIWEELSVL	26	-23.01
159	**QLVFGIEVV**	**25**	**-22.70**
188	GLLGDNQIV	25	-20.04
176	YILVTCLGL	24	-22.10
181	CLGLSYAGL	23	-23.44
194	QIVPKTGLL	23	-24.43
153	KASEYLQLV	22	-20.80
285	KVLHHLLKI	22	-22.49
15	GLEAQGEAL	21	-24.57

A: the epitopes were reported; A: the prediction epitopes whose polynomial scores were less than -24.

the 20 amino acid residues, the amount they contribute to the polynomial score is shown (for all nine positions in which they may occur).

Extended motifs method analysis Potential binders for various MHC class I molecules are ranked according in the presence of primary and secondary anchor amino acids as well as favored and disfavored amino acids (Fig 1). Those with primary anchors and secondary anchors compose extended motifs [4]. Then perfect HLA-A2.1-binding epitopes were screened out from the primary predicted epitopes. The screening principle was that candidate epitopes didnt include disfavored amino acid residues or favored amino acid

residues were more than disfavored amino acid residues.

RESULTS

SYFPEITHI prediction

The SYFPEITHI prediction values of all possible nonamers of a given protein sequence were added together and the fifteen high–scoring peptides of each protein were selected for further analysis. Scores for all predicted epitopes specifically for HLA–A*0201 are given in Table 2. The SYFPEITHI predicted scores of these epitopes were usually higher than 20.

Polynomial method analysis

The primary predicted epitopes were compared with epitopes that were demonstrated in previous research. Three reported HLA –A2 restricted CTL epitopes MAGE–$3_{201-209}$ (LLIIVLAII), MAGE–$3_{271-279}$ (FLWGPRALV), MAGE–$n_{159-167}$ (QLVFGIEVV), were eliminated from the SYFPEITHI prediction results[7,8]. The epitopes which polynomial scores less than –24 were eliminated from the prediction results. Result, ten epitopes were selected for further extended motifs method analysis. The polynomial scores of predicted epitopes are shown in Table 2.

Extended motifs method analysis

Based on analysis for affect of other amino acid residues on affinity of peptide binding with HLA–A2 molecule, five 9–mer peptides (ALSRKMAEL$_{108-116}$, GLLIIVLAI$_{200-208}$, HLYILVTCL$_{174-182}$, GLLGDNQIV$_{188-196}$, CLGLSYAGL$_{181-189}$) were screened from above ten candidate epitope for further study.

DISCUSSION

Recent studies show that tumor antigens, especially TSA, which can induce tumor–specific CTL and damage tumor cells, are a major component of tumor vaccines. Induction of potent anti–tumor CTLs responses can result in regression and prevention of metastasis formation, as demonstrated in experimental model tumor systems [9,10]. Thus, efforts towards the development of cancer immunotherapy have recently focused on the generation of tumor–specific T cell responses.

The epitope prediction has been carried in tumor antigen specificity for CTL epitope identification in most studies recently. About 120 CTL epitopes presented by HLA–A, B, C molecules have been reported in recent study[11] and some epitopes have been used as peptide vaccine in animal and clinical experiments[12,13]. Schirle et al. reported that two new CTL epitopes of gastrointestinal tumor were identified by epitope prediction combined with acid eluted methods[14]. My colleague found that MAGE –n –derived peptide QLVFGIEVV was a new HLA–A2.1 –reatricted CTL epitope capable of inducing MAGE–n specific CTLs in vitro[15]. Identification of these CTL epitopes opens up the possibility of clinical applications of these peptides as cancer vaccines for patients with MAGE–n+/HLA–A2+ tumors.

The combination of epitope prediction, epitope reconstruction method and immunological methods can improve the efficiency and accuracy of CTL epitope studies [15]. The identification of human tumor–rejection antigens and the CTL epitopes of these antigens make it possible to develop new cancer vaccines because patients are likely to benefit from immunization with an identified CTL epitope. Among the identified tumor–rejection antigens, MAGE gene products are of particular interest owing to their wide expression in many tumors and their potential to induce tumor–specific CTL responses. Recent studies showed[16] that a vaccine with five common HLA–I restricted epitopes had function on 80~90% people and that with eight to nine common HLA–I restricted epitopes had function on almost all the people in the world. It is very valuable to identify the HLA–A2–restricted CTL epitopes from melanoma antigen MAGE–12.

The epitope prediction were constructed by immunologists in recent decades, In this study, the HLA–A2 restricted CTL epitope of MAGE–12 were predicted by the methods of the combination of supermotif, quantitative motif and extended motifs. Polynomial method is based on statistical parameter estimation assuming independent binding of the side –chain of residues [4]. Its sensitivity and positive predictive value

are better than the simple motif [5]. This and other [17,18] independent binding site methods that assign a score to a peptide have been called quantitative motifs [4]. Threshold is an integral part of the polynomial method. In the above analysis the threshold was set to -24. An extended motif taking into account secondary anchors increased the predictability of HLA-A2.1-binding epitopes to a level of 70%, underscoring the practical usefulness of extended motifs [4]. Then perfect HLA-A2.1-binding epitopes were screened by extended motifs out from the primary predicted epitopes. In this study, 5 candidate epitopes were selected for further immunology experiments by the peptide supermotif prediction combined, quantitative motif and extended motifs.

REFERENCES

1. Ming LH, Zhu YR, Harris CC, et al. Association of HLA-A02 genotype with hepatocellular carcinoma in a prevalent area of China. Chin J Immunol ,1999, 15: 305-310.
2. Rammensee HG, Bachmann J, Emmerich NPN, et al. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics, 1999, 50:213-219.
3. Dong Hailong, Sui Yanfang,YeJing, et al. Prediction, synthesis and identification of HLA-A2-restricted cytotoxic T lymphocyte epitopes of the tumor antigen MAGE-n. Zhonghua Yixue Zazhi (Natl Med J China), 2003, 83(12): 1080-1083.
4. Ruppert J, Sidney J, Celis E, et al. Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 Molecules. Cell, 1993, 74(5): 929-937.
5. Gulukota K, Sidney J, Settle A, et al.Two complementary methods for predicting peptides binding major histocompatibility complex molecules. J Mol Biol, 1997, 267:1258-1267.
6. Falk K, Rotzschke O, Steranovic S, et al. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature, 1991, 351:290-296.
7. Jia Zhengcai, Wu Yuzhang, Wan Ying, et al. Finding and identification of a novel HLA-A2-restricted CTL epitope derived from the tumor antigen MAGE-A3. Zhongguo Mianyixue Zazhi (Chin J Immunol), 2002, 18:26-29.
8. Vander Bruggen P, Bastin J, Boon T, et al. A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cell expressing MAGE-3. Eur J Immunol, 1994, 24: 3038-3043.
9. Marchand M, Van Baren N, Weynants P, et al. Tumor regression doserved in patients with metastic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. Int J Cancer, 1999, 80:219-230.
10. Horiguchi Y, Nukaya I, Okazawa K, et al. Screening of HLA-A24-restricted epitope peptides from prostate-specific membrane antigen that induce specific antitumor cytotoxic T lymphocytes. Clin Cancer Res , 2002, 8:3885-3892.
11. Feuerer M, Beckhove P, Bai L, et al. Therapy of human tumors in NOD/SCID mice with patient-derived reactivated memory T cells from bone marrow. Nat Med 2001, 7: 452-458.
12. Dagher R, Long LM, Read EJ, et al. Pilot trial of tumor-specific peptide vaccination and continuous infusion interleukin-2 in patients with recurrent Ewing sarcoma and alveolar rhabdomyosarcoma: an inter-institute NIH study. Med Pediatr Oncol, 2002, 38:158-164.
13. Schiavetti F, Thonnard J, Colau D, et al. A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. Cancer Res, 2002, 62: 5510-5516.
14. Schirle M, Keilholz W, Weber B, et al. Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. Eur J Immunol, 2000, 30: 2216-2225.
15. Dong Hailong, Li Zengshan, Ye Jing, et al. Identification of HLA-A2-Restricted CTL Epitope Encoded by the MAGE-n Gene of Human Hepatocellular Carcinoma. NA: Cancer Biol Ther, 2004, 3 :891-898.
16. Sidney J, Grey HM, Kubo RT, et al. Practical, bioche mical and evolutionary implications of the discovery of HLA class I supermotifs. Immunol Today,1996, 17:261-266.
17. Marshall KW, Wilson KJ, Liang J, et al. Prediction of peptide affinity to HLA DRBI*0401. J Immunol ,1995, 154: 5927-5933.
18. Parker KC, Bednarek MA, Coligan TE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol, 1994, 152: 163-175.
19. Hammer J. New methods to predict MHC-binding sequences within protein antigens. Curr Opin Immuno, 1995, 7263-269.