Cancer Immunity, Vol. 2, p. 14 (14 October 2002)

Can hTERT peptide$_{540-548}$-specific CD8 T cells recognize and kill tumor cells?

Daniel E. Speiser[1], Jean-Charles Cerottini[1,2], and Pedro Romero[1]

[1]Division of Clinical Onco-Immunology, Ludwig Institute for Cancer Research, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
[2]Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Epalinges, Switzerland

Keywords: human, cytotoxic T lymphocytes, HLA-A2, telomerase, peptide

Abstract

This commentary reviews the data on HLA-A2-restricted CD8 T cells specific for peptide$_{540-548}$ derived from hTERT (human telomerase reverse transcriptase). Several studies have reported the successful generation of such T cells (1, 2, 3). However, tumor recognition was observed in some, but not all, studies. More data are required to elucidate whether hTERT peptide$_{540-548}$-specific T cells can indeed recognize and destroy tumor cells. It would be highly useful if telomerase would emerge as a universal tumor antigen that can be targeted in the cancer immunotherapy of HLA-A2 positive patients.

Introduction

As potential targets of immunotherapy, tumor antigens need to confer efficient interactions between tumor cells and the corresponding antigen-specific T cells. Antigens that are expressed by many different tumor types but not in normal tissue are of great interest. Moreover, these antigens should be functionally involved in oncogenesis, resulting in the reduction of malignant potential if tumor cells escape T cell recognition by antigen downregulation or mutation.

Telomerase is a ribonucleoprotein complex responsible for the maintenance of the length of telomeres, which are shortened during cell division. Telomerase expression has been directly linked to tumor development, and inhibition of telomerase in human tumor cells may arrest growth (4, 5, 6). The human telomerase catalytic subunit hTERT is expressed in the vast majority of human cancer cells derived from multiple tissues, but infrequently in normal cells (7). Thus, hTERT is an attractive candidate target for tumor therapy.

To evaluate whether cytotoxic T cells can recognize and kill telomerase-expressing cells, one needs to identify peptide antigens that are presented by HLA class I antigens and recognized by CD8 T cells. A recent review has summarized the currently identified MHC class I binding peptide antigens from hTERT (8), and clinical trials are underway to test whether these antigens can elicit tumor-specific T cell responses (9).

Identification of the hTERT peptide$_{540-548}$

The hTERT peptide$_{540-548}$ was identified based on the computerized prediction of binding to HLA-A*0201 (1, 2). The peptide was synthesized and used to stimulate human peripheral blood mononuclear cells (PBMCs). Such lymphocyte cultures were tested in cytotoxicity assays. The studies demonstrated lysis of target cells loaded with specific peptide but not antigen negative targets, indicating the presence of peptide$_{540-548}$-specific cytotoxic T lymphocytes (CTLs). Subsequently, the same cultures were tested against various selected tumor cells. Positive correlations were found between tumor cell killing and the expression of telomerase / HLA-A*0201. The authors concluded that HLA-A*0201-restricted hTERT peptide$_{540-548}$-specific CD8 T cells could efficiently recognize and kill telomerase-expressing A*0201+ tumor cells (Table 1).

Table 1. Studies on the hTERT peptide$_{540-548}$[a].

T cells analyzed	hTERT transfection of target cells	HLA transfection of tumor cells	T cell recognition correlates with antigen / HLA expression	Reference
bulk	+	-	yes	1
bulk	-	-	yes	2
line and clones	+	-	no	3

[a]Abbreviations: "bulk", polyclonal lymphocyte cultures; "line", T cell line (culture with >99% CD8+ cells, >75% positive for the fluorescent tetramer HLA-A*0201/hTERT540-548); "clones", monoclonal CD8+ T cells; +, done; -, not done.

Inefficient antigen processing of the hTERT peptide$_{540-548}$

Recently, we have reassessed the usefulness of hTERT peptide$_{540-548}$ as a CTL-defined tumor antigen (3). In agreement with previous studies (1, 2), peptide$_{540-548}$-specific CTLs could be generated using PBMCs from HLA-A*0201 positive melanoma patients. CTL clones established from these cultures showed strong cytotoxicity

against HLA-A*0201 target cells in the presence of exogenously added peptide. However, no cytotoxicity was observed against melanoma and colon cancer cells, despite their expression of hTERT and HLA-A*0201. Transfection of melanoma cells with a plasmid containing an hTERT minigene resulted in recognition by the specific CTLs, but only for the minimal minigene (coding for hTERT amino acids 540-548). Longer minigenes with C- and/or N-terminal flanking regions did not confer recognition. Subsequently, a long hTERT precursor peptide (amino acids 534-554) was incubated with proteasomes and the product was analyzed by mass spectrometry and cytotoxic assays. The results suggested inefficient antigen processing of hTERT resulting in lack of peptide presentation on the cell surface, explaining why tumor cells were not recognized by the CTLs. This finding is in disagreement with previous data (1, 2).

T cell clones and transfected target cells as tools to validate tumor antigens

When stimulating PBMCs with MHC class I binding peptides, one can frequently observe an enrichment of antigen-specific CD8 T cells, while other CD8 T cells, CD4 T cells, B cells, and NK cells are diluted and progressively lost. However, even in cultures maintained for several weeks, significant percentages of cells other than specific CD8 T cells remain. These cells may account for "nonspecific" cytotoxic activity. In particular, NK cells can be cytotoxic against a variety of cells, whereby some but not all target cells are susceptible. After exclusion of NK cells, one still needs to consider the "nonspecific" cytotoxicity that can be exerted by T cells. This may be due to different receptors, such as the alphabetaTCR-mediated recognition of HLA-E positive peptides derived from viruses or allogeneic HLA class I signal sequences (10). Activatory and inhibitory NK receptors may also play a role (11, 12).

Therefore, for a direct demonstration of specific CTL-mediated cytotoxicity it is necessary to generate T cell clones (in which only one type of T cell receptor is present). Polyclonal T cell lines may also be used, provided that one can demonstrate that they are highly antigen-specific (e.g. using fluorescent HLA/peptide tetramers). Unfortunately, many studies on cancer epitopes have not been carried out with T cell lines and clones. Often, poorly defined bulk lymphocyte cultures are generated through peptide stimulation and directly tested for cytotoxicity against tumor cells.

It is possible that the bulk lymphocyte cultures used in the two studies (1, 2) may have contained (besides peptide$_{540\text{-}548}$-specific T cells) some cells with "nonspecific" cytotoxicity, killing some but not all tumor cells, which by coincidence correlated with antigen expression. Thus lysis may have been triggered by antigens and receptors other than telomerase and its cognate T cell receptors. Alternatively, antigen-specific CD8 T cells obtained in the two studies (1, 2) may have been of higher avidity than those used in our study (3). To assess T cell avidity, we have performed peptide titration experiments (3). It would be useful to do this also with the lymphocyte populations used in the first two studies (1, 2), to evaluate whether T cell avidity can possibly account for the discrepancy in tumor recognition.

An open question is whether dendritic cells (DCs) are required for the generation of efficient effector T cell populations. As compared to PBMCs, DCs are known to activate T cells more efficiently. However, T cells with low and high efficiency of antigen recognition ("avidity") can be obtained through both PBMC and DC stimulation, respectively.

Antigen positive and negative target cell pairs should be genetically identical, with the exception of the antigen or the HLA molecule in question, to ensure that all other parameters that could potentially influence the killing

(receptor density, adhesion and costimulatory molecules, HLA molecules and other ligands of natural killer receptors, etc.) are identical. This is not the case when differing tumor cells are compared as target cells. In the studies on peptides$_{540\text{-}548}$, tumor cells of different origins were typed for antigen and HLA expression, and a positive correlation was found with their susceptibility to lysis. Transfection of target cells was performed in two studies. The transfected target cells were tested with polyclonal lymphocytes (1) or with a T cell line and T cell clones (3). The latter study is the only one that combined the use of T cell clones and transfected target cells.

One strategy is to transfect cells with the gene coding for the antigen and to check for susceptibility to CTL-mediated lysis in comparison to cells transfected with irrelevant genes. Another approach is to transfect the restricting HLA molecule in cells endogenously expressing the antigen and to determine whether this results in susceptibility to lysis. The latter allows antigenicity to be assessed without manipulating the antigen providing the peptide epitope, while the former can test productive processing and cell surface presentation of the peptide antigen.

T cell activation in tumor lesions

High percentages of tumor antigen-specific T cells are frequently found in tumor infiltrating lymphocytes (TILs) and tumor-infiltrated lymph nodes (TILNs) (13). Indeed, the isolation of tumor reactive T cells from TIL(N)s was the basis for the identification of many tumor antigens (14, 15). Moreover, the fact that antigen-specific T cells are enriched (i.e. have proliferated) due to *in vivo* stimulation by endogenous tumor antigens is one of the best signs that the corresponding antigen is recognized *in vivo*. Enriched telomerase-specific CD8 T cells have so far not been observed *ex vivo*, even though telomerase is expressed by many tumors (16). Further studies should determine whether there are tumor lesions that may contain telomerase-specific T cells in detectable numbers, for instance using tetramer-based identification of antigen-specific T cells.

Conclusion

Although the computerized prediction of HLA class I binding and proteasome digestion is a powerful tool to identify candidate peptides (17, 18), the majority of these are not CTL-defined epitopes. At present it remains controversial whether hTERT peptide$_{540\text{-}548}$-specific T cells recognize tumor cells in humans. Lack of tumor cell recognition may be due to inefficient antigen processing and presentation, or because of low TCR avidity. *In vitro* specificity and efficacy of T cell recognition needs to be analyzed carefully to evaluate the potential for *in vivo* protective immunity and immunotherapy.

Abbreviations

hTERT, human telomerase reverse transcriptase

References

1. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM. The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. *Immunity* 1999; **10**: 673-9. (PMID: 10403642)

2. Minev B, Hipp J, Firat H, Schmidt JD, Langlade-Demoyen P, Zanetti M. Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. *Proc Natl Acad Sci U S A* 2000; **97**: 4796-801. (PMID: 10759561)

3. Ayyoub M, Migliaccio M, Guillaume P, Liénard D, Cerottini J-C, Romero P, Lévy F, Speiser DE, Valmori D. Lack of tumor recognition by hTert peptide 540-548 specific CD8+ T cells from melanoma patients reveals inefficient antigen processing. *Eur J Immunol* 2001; **31**: 2642-51. (PMID: 11536162)

4. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature* 1999; **400**: 464-8. (PMID: 10440377)

5. Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider CW, DePinho RA. Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse. *Cell* 1999; **97**: 515-25. (PMID: 10338215)

6. Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc Natl Acad Sci U S A* 1999; **96**: 14276-81. (PMID: 10588696)

7. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994; **266**: 2011-5. (PMID: 7605428)

8. Vonderheide RH. Telomerase as a universal tumor-associated antigen for cancer immunotherapy. *Oncogene* 2002; **21**: 674-9. (PMID: 11850795)

9. Marx J. Tackling cancer at the telomeres. *Science* 2002; **295**: 2350. (PMID: 11923505)

10. Romagnani C, Pietra G, Falco M, Millo E, Mazzarino P, Biassoni R, Moretta A, Moretta L, Mingari MC. Identification of HLA-E-specific alloreactive T lymphocytes: a cell subset that undergoes preferential expansion in mixed lymphocyte culture and displays a broad cytolytic activity against allogeneic cells. *Proc Natl Acad Sci U S A* 2002; **99**: 11328-33. (PMID: 12167676)

11. Ikeda H, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, Chambost H, Vitale M, Moretta A, Boon T, Coulie PG. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. *Immunity* 1997; **6**: 199-208. (PMID: 9047241)

12. Speiser DE, Pittet MJ, Valmori D, Dunbar R, Rimoldi D, Liénard D, MacDonald HR, Cerottini J-C, Cerundolo V, Romero P. In vivo expression of natural killer cell inhibitory receptors by human melanoma-specific cytolytic T lymphocytes. *J Exp Med* 1999; **190**: 775-82. (PMID: 10499916)

13. Romero P, Dunbar PR, Valmori D, Pittet M, Ogg GS, Rimoldi D, Chen JL, Lienard D, Cerottini JC, Cerundolo V. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytotoxic T lymphocytes. *J Exp Med* 1998; **188**: 1641-50. (PMID: 9802976)

14. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, Appella E, Rosenberg SA. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J Exp Med* 1994; **180**: 347-52. (PMID: 7516411)

15. Wolfel T, Van Pel A, Brichard V, Schneider J, Seliger B, Meyer zum Buschenfelde KH, Boon T. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. *Eur J Immunol* 1994; **24**: 759-64. (PMID: 8125142)

16. Schultze JL, Vonderheide RH. From cancer genomics to cancer immunotherapy: toward second-generation tumor antigens. *Trends Immunol* 2001; **22**: 516-23. (PMID: 11525943)

17. Kessler JH, Beekman NJ, Bres-Vloemans SA, Verdijk P, van Veelen PA, Kloosterman-Joosten AM, Vissers DC, ten Bosch GJ, Kester MG, Sijts A, Wouter Drijfhout J, Ossendorp F, Offringa R, Melief CJ. Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J Exp Med* 2001; **193**: 73-88. (PMID: 11136822)

18. Ayyoub M, Stevanovic S, Sahin U, Guillaume P, Servis C, Rimoldi D, Valmori D, Romero P, Cerottini JC, Rammensee HG, Pfreundschuh M, Speiser D, Levy F. Proteasome-assisted identification of a SSX-2-derived epitope recognized by tumor-reactive CTL infiltrating metastatic melanoma. *J Immunol* 2002; **168**: 1717-22. (PMID: 11823502)

Contact

Address correspondence to:

Dr. Daniel Speiser
Division of Clinical Onco-Immunology
Ludwig Institute for Cancer Research
Hôpital Orthopédique, Niveau 5, Aile Est
Av. Pierre-Decker 4
CH-1005 Lausanne
Switzerland
Tel.: + 41 21 314 01 82
Fax: + 41 21 314 74 77
E-mail: daniel.speiser@hospvd.ch