Review

Cancer Immunotherapy Targeting the Telomerase Reverse Transcriptase

Longfei Huo[1], Janice WS Tang[2], Junjian Huang[3], Peitang Huang[3], Cuifen Huang[3], Hsiang-fu Kung[2] and Marie C. Lin[1, 4]

The human telomerase reverse transcriptase (hTERT) is expressed in more than 85% of tumor cells but is usually not found in normal cells, which makes hTERT as an ideal tumor-associate antigen (TAA) to develop potential vaccine specifically destroying cancers without impairing normal tissues in human cancer immunotherapy. Here are reviewed the fundamental advances of studies on immunogenicity of hTERT or its peptides and the early clinical trials using the hTERT vaccine approach in the last decades. *Cellular & Molecular Immunology*. 2006;3(1): 1-9.

Key Words: cancer, telomerase reverse transcriptase, immunotherapy, lymphocyte

Introduction

Increased knowledge of immune system and tumor biology has allowed us to utilize the immune strategy in the treatment of human cancer, which is referred to cancer immunotherapy. In general, tumor immunotherapy is classified into passive and active approaches. Passive immunotherapy involves the supply of effectors such as cytokines, tumor-reactive antibodies and tumoricidal effector cells to the immune system of patients to directly attack the cancer cells. In contrast, active immunotherapy (tumor vaccines) is to elicit an endogenous antitumor immune responses through that immune cells are 'educated' to produce a long-lasting effect on generation of effective antitumor immunity (1).

IL-2, IL-12, IFN-α, IFN-γ and GM-CSF are among the various cytokines being currently used in cancer therapy (2-6). IL-2 and IFN-α have shown great promise in the treatment of renal cell carcinoma, melanoma and hairy cell leukemia (7-10). In fact it has been reported that the combination of IL-2 and INF-α is significantly more effective in treating metastatic renal cell carcinoma than when either cytokine is administered alone (11). IL-12 and GM-CSF are often included in cancer vaccines as adjuvant to augment the overall immunogenicity of the tumor antigen(s). For instance, IL-12 has been co-administered with MAGE-3 and Melan A-pulsed mononuclear cells to enhance specific $CD8^+$ T cells response in melanoma patients (12). It has also been shown that IL-12 can improve patients' response to gp100 and tyrosinase (13). In the case of GM-CSF, a phase I clinical trial has demonstrated its efficacy in inducing immune response against metastatic non-small-cell lung cancer (NSCLC) (14). Phase I clinical trial of human IFN-γ by intralesional injections of adeno-IFN-γ in patients with T- and B-cell lymphoma resulted in the regression of injected and noninjected lymphoma lesions (15). The major problems of cytokine therapy are the short half-life of cytokines delivered in body and significant side effects, e.g., dose-limiting toxicities (16).

While cytotoxicity and other side effects become the major drawbacks of using cytokines in cancer therapy, immunotherapeutic strategies that can specifically target tumor cells have been developed. For instance, antibodies (Abs) such as Rituximab, Alemtuzumab and Trastuzumab have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of B cell lymphoma and

[1]Open Laboratory of Chemical Biology of the Institute of Molecular Technology, Department of Chemistry, The University of Hong Kong, Pokfulam, Hong Kong, China;

[2]Li Ka Shing Institute of Health Sciences and Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Shatin, Hong Kong, China;

[3]Beijing Institute of Biotechnology, Beijing, China;

[4]Corresponding to: Dr. Marie Chia-mi Lin, Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery, Department of Chemistry, The University of Hong Kong, Hong Kong, China. E-mail: mcllin@hkusua.hku.hk.

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Abbreviations: hTERT, human telomerase reverse transcriptase; CTL, cytotoxic T lymphocyte; DC, dendritic cell; MHC, major histocompatibility complex; HLA, human leukocyte antigen; NK, natural killer; GM-CSF, granulocyte macrophage-colony stimulating factor.

breast cancer (17, 18). Adoptive transfer of tumor antigen-specific cytotoxic T lymphocytes (CTLs) is another promising option for cancer immunotherapy. Encouraging results obtained from animal studies have prompted scientists to look into the possibility of using this approach to treat cancer in human. Here, CTLs specific for tumor antigens are expanded *ex vivo* before they are infused into the recipient (19). In a phase I clinical trial, tumor elimination and regression was observed in 8 out of 10 melanoma patients who received MART/Melan A- and gp100-specific CTLs (20). However, autoimmune response to melanocyte could be observed with tumor regression in some patients (21, 22). Apart from CTLs, NK cells can also act as effective killer for tumor cells (23-26). For example, when IL-2 stimulated NK cells were infused into patients with renal cell carcinoma and melanoma, disease improvement was observed (27).

Cancer vaccine targeting tumor antigens has attracted much attention in recent years because of its more specificity and less toxicity than nonsurgical approaches, i.e., radio-therapy and chemotherapy (28, 29). Cancer vaccines can be categorized into peptide/protein vaccine, tumor cell vaccine, DNA vaccine, recombinant virus vaccine as well as dendritic cell (DC) vaccine (28). The rationale of using such vaccines in cancer treatment is that tumor antigen(s) or DNA encoding such antigen(s) will be taken up and processed by antigen-presenting cells (mainly DCs) when they are injected into the recipient. The antigen(s) is (are) then loaded onto major histocompatibility complex (MHC) molecules and is (are) recognized by T lymphocytes that possess cognate T-cell receptor (TCR) molecules for the MHC-peptide complex. The following will give a brief account about clinical trials of the various types of cancer vaccine.

Marchand et al. (30) vaccinated 39 patients with metastatic melanoma with MAGE-3 peptide in which tumor regression was observed in 7 patients. A new generation of peptide vaccine has been developed in which the immuno-genicity of the tumor antigen is enhanced by adjuvant such as incomplete Freund adjuvant (IFA), bacillus Calmette-Guerin (BCG) and GM-CSF (31-33). This is also true for tumor cell vaccine. In a Phase I clinical trial, Simons et al. (34) immunized 8 prostate cancer patients with irradiated autologous prostate tumor cells transduced with a retroviral vector expressing GM-CSF. The vaccine was shown to be potent in inducing specific B- and T-cell immune responses against prostate tumor antigens. In another study, CTL response was induced in 5 out of 9 patients who were vaccinated with melanoma cells engineered to produce GM-CSF (35); to evaluate the antitumor efficacy of a DNA vaccine, patients with B cell lymphoma were vaccinated with DNA encoding the lymphoma idiotype. Of the 12 patients who received this treatment, 7 developed specific B- and/or T-cell responses (36); recombinant virus vaccine consisted of vaccinia-CEA and avipox-CEA recombinant viruses have been demonstrated to stimulate specific CTL response in patients with advanced carcinoma (37, 38); lastly, DCs pulsed with MAGE-3A1 tumor peptide were inoculated into 11 patients who suffered from stage IV cutaneous malignant melanoma. At the end of the study, MAGE-3A1-specific CTL response was evident in 8 patients with tumor regressions in 6 patients (39). In a similar study, antigen-specific CTL response was induced in 13 out of 30 patients who were vaccinated with MAGE-3-loaded DCs (40).

Targeting human telomerase reverse transcriptase (hTERT) for cancer therapy

Tumor-associated antigens (TAA), which are recognized by the immune system and elicit antitumor immune response, are found on tumor cells and normal cells during fetal life and after birth in selected cells but at much lower concentration than on tumor cells. Immune responses to TAA may be suppressed because they may be considered as self-antigen by immune system. Examples of antigen that fall into this category include normal differentiation Ags (such as Melan-A/MART-1, gp100, etc.), tumor-specific Ags (such as MAGE-1, CKK-4, etc.) and universal Ags (such as hTERT, CyP1B1 etc) (41, 42). hTERT is an interesting example of universal TAA because it is the first tumor antigen identified to have a global expression in more than 85% of human cancer cells (43-46) and its continuing expression is necessary to the oncogenic process (47).

Telomeres are located at the distal ends of the chromosomes and the shortening of which with successive cycles of cell division leads to cell senescence and cell death (48, 49). Telomerase is a ribonucleoprotein that consists of two components, the telomerase reverse transcriptase (TERT) and an RNA template (47). Except germline cells, activated lymphocytes and some stem cell populations, most adult somatic cells do not express hTERT (50). In cells where telomerase is activated, hTERT synthesizes a TTAGGG sequence from the RNA template that is then added to the end of the shortening chromosome (51), thus saving the cells from death. The above mechanism is cleverly exploited by tumor cells to maintain their immortality (47, 50). Together with its universal expression, hTERT represents an ideal target for cancer therapy.

At present, cancer immunotherapy targeting hTERT is mainly focused on developing a strategy to elicit hTERT-specific T lymphocyte immune responses especially CTL responses that can specifically kill $hTERT^{+}$-tumor cells (Figure 1). An early step to trigger specific T cell response is to present antigens to the T cells in the context of MHC class I and II molecules. It is well known that T cells recognize antigens by binding their TCRs to short peptides with 8-10 amino acid residues (aa) (MHC I) or 12-20aa (MHC II) in length derived from specific intracellular processing of proteins (28, 33, 52). Recently, a number of such peptides have been identified in hTERT and their abilities to elicit T cell immune responses do present a promising approach for cancer immunotherapy (53-56). A list of hTERT peptides and the HLA molecules to which they are restricted to is shown in Table 1. Given that HLA-A2 is expressed by nearly 50% Caucasian, Asian and Hispanics and 33% African-Americans, eliciting T lymphocytes that can recognize hTERT-HLA-A2 complex would therefore be powerful weapons in attacking

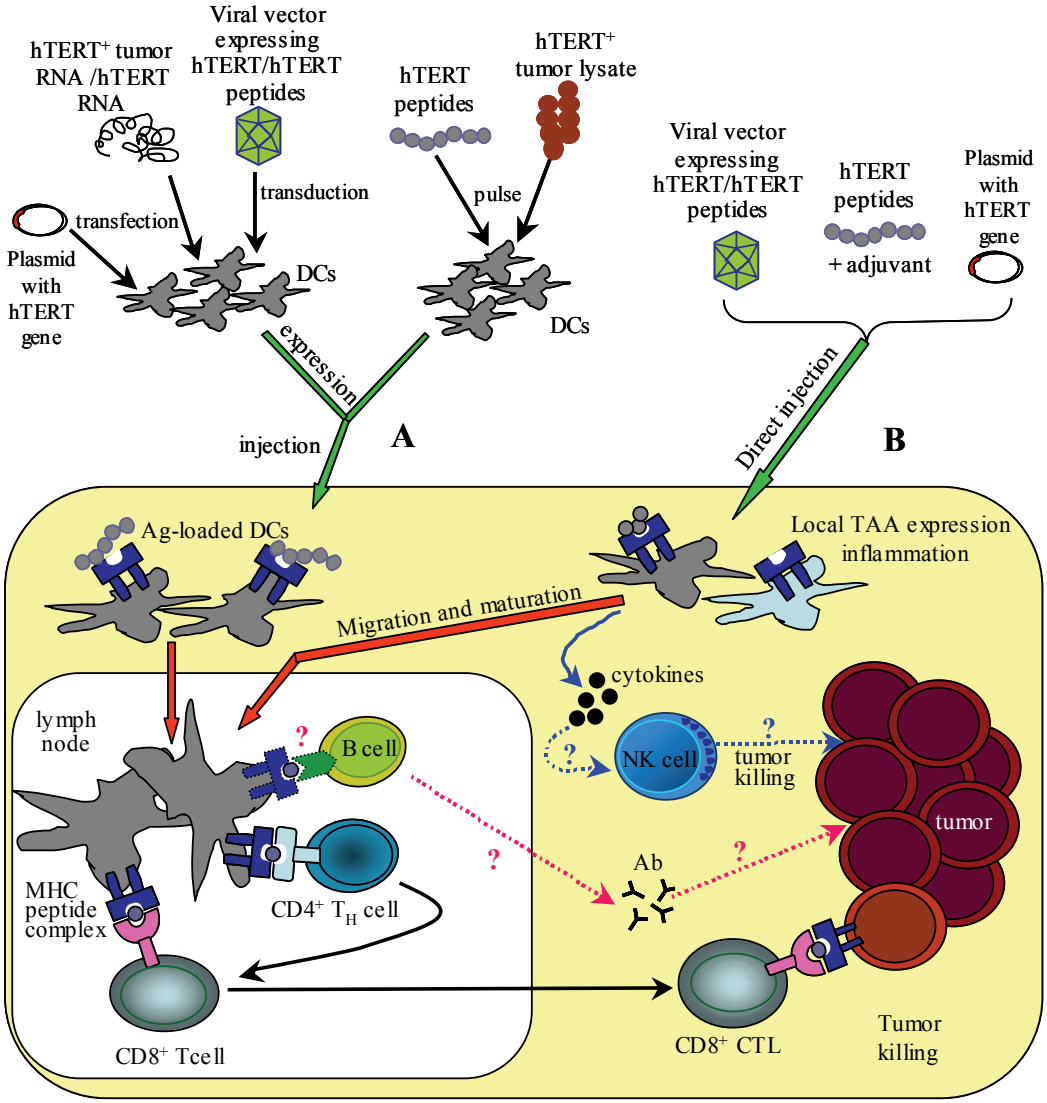

Figure 1. Schematic cancer immunotherapy targeting hTERT. Here DCs are exampled as antigen presentation cells (APCs). (A) DCs are prepared *ex vivo* and transfected with hTERT/tumor RNA or transduced with viral vector expressing hTERT peptides or directly pulsed with hTERT peptides/tumor lysate. After immunization, DCs migrate to draining lymph node where DCs induce hTERT-specific $CD4^+$ T_H cells and $CD8^+$ CTLs to trigger T-cell immunity against tumor cells. (B) Direct injection of hTERT peptides or viral vector expressing hTERT peptides induce inflammation and recruit immature DCs to capture expressed TAAs. Then the DCs become maturation and migrate to draining lymph node where to induce hTERT-specific $CD4^+$ and $CD8^+$ T cells to trigger T-cell immunity against $hTERT^+$ tumor cells. Dot lines suggest possible mechanisms that have not been demonstrated to date.

$hTERT^+$ tumor cells (57).

hTERT-peptide based immunotherapy

The first immunogenic TAA peptide targeting hTERT with intensive investigation is p540 (53). In this *in vitro* study, p540, demonstrated to be naturally processed by tumor cells and presented in an HLA-A2 restricted fashion, was able to elicit specific CTLs that could be generated from > 70% of individuals (53). These CTLs showed strong cytotoxicity against a broad panel of $hTERT^+$, $HLA\text{-}A2^+$ tumor cells including tumor cell lines, e.g., carcinoma, sarcoma, myeloma and melanoma, as well as freshly isolated lymphoma and leukemia cells (53). The endogenously process of p540 and its ability to elicit specific CTLs for killing $HLA\text{-}A2^+$ cancer cells were confirmed by another research group (55). Furthermore, similar CTLs were generated *in vivo* by immunization of transgenic HHD mice with the peptide (p540 + IFA) (55). Importantly, there is no hTERT-specific cytolysis of normal cells, e.g., $HLA\text{-}A2^+CD34^+$ blood cells observed in these p540 studies (53, 55), which imply the potential value of p540 used in anticancer immunotherapy. In

Table 1. hTERT epitopes recognized by $CD4^+$ and $CD8^+$ T lymphocytes

hTERT peptide	Sequence	Restriction element	Recognized by T cells
p540[53]	ILAKFLHWL	HLA-A2	$CD8^+$
p572[57, 60]	RLFFYRKSV	HLA-A2	$CD8^+$
p865[55]	RLVDDFLLV	HLA-A2	$CD8^+$
p988[57, 61]	DLQVNSLQTV	HLA-A2	$CD8^+$
p324[56]	VYAETKHFL	HLA-A24	$CD8^+$
p461[56]	VYHFVRACL	HLA-A24	$CD8^+$
p973[54]	KLFGVLRLK	HLA-A3	$CD8^+$
p672[58, 59]	RPGLLGASVLGLDDI	HLA-DR1, -7, -15	$CD4^+$
p766[59]	LTDLQPYMRQFVAHL	HLA-DR4, -11, -15	$CD4^+$

fact, the HLA-A2 restricted hTERT p540 peptide has been demonstrated to elicit specific CTLs in patients (29). In a phase I clinical trial, autologous DCs from PMBC of patients with advanced prostate and breast carcinoma were first pulsed with hTERT p540 peptide and then vaccinated with an adjuvant keyhole limpet hemocyanin (KLH) for several times by subcutaneous administration. p540-specific CTLs were induced in 4 out of 7 patients and the proportion of which in the PBMC population increased from 0.02-0.03% pre-vaccination to 0.9-6.3% post-vaccination. Most importantly, p540-specific CTLs primed in this way were able to kill tumor cells in an MHC-restricted fashion with maximum response being 25-fold higher than the baseline level (29).

Several other immunogenic hTERT peptides (Table 1) have also been described thereafter. Similar effects to p540 were reported for p572 (60), p865 (55, 62) and p973 (54). In the case of p973, the peptide strongly binds to HLA-A3 instead of HLA-A2 and triggers specific CTL lysis of $hTERT^+$ tumor cells (54), which suggests that there may be many different epitopes binding to different HLA alleles in hTERT. This idea is confirmed by identification of two HLA-A24-restricted peptides such as p324 and p461 (56) and another two HLA-DR-restricted peptides p672 and p766 (58, 59) in hTERT. Contrast to other HLA-restricted peptides to induce $CD8^+$ T cell response, the HLA-DR restricted peptides, i.e., p672 and p766 were demonstrated to induce specific $CD4^+$ T cell responses (58, 59). Considering the roles of $CD4^+$ T cells in adoptive immunity, the results from Schroers's group imply that class-II restricted peptides in hTERT may be applied in cancer immunotherapy combined with class-I restricted hTERT peptides to increase antitumor immune responses. The identified different HLA alleles in several studies suggest that different tumors prefer to process and load different hTERT epitopes onto their HLA molecules (62).

Given human tumors usually expressing multiple TAA epitopes recognized by T cells and the possibility of different time expression of these epitopes as well as the polymorphism of HLA in human populations, it is better to use polypeptide vaccine against multiple epitopes of tumor than single peptide vaccine in cancer immunotherapy (33). In fact, the immune effect of single peptide vaccine is sometimes uncertain. In the case of hTERT p540 peptide, for example, two groups found that p540-specific CTLs could not recognize tumor cell lines (melanoma, colon carcinoma, renal cell line, transformed B lymphocytes) that endogenously expressed hTERT (63, 64). They concluded that p540 may not be naturally processed by $hTERT^+$ tumor cells and therefore immunotherapeutic methods that target p540 will not be useful for cancer treatment. The reason for the discrepancy of p540-specific CTLs in targeting $hTERT^+$ tumor cells observed by several research groups is still unknown, although different immunization protocols between the research groups may be contributed to the discrepancy (29, 64).

Several other strategies can also be used to enhance or improve the immune response targeting hTERT peptide. For example, substitutions of the first amino acid residue of the peptides p572 and p988 with Tyr have been demonstrated to enhance the immunogenicity of the hTERT peptides, and the substitution does not reduce the specificity of hTERT-peptide elicited CTLs (60, 65). The activation of specific CTL response by peptide requires appropriate delivery of some inflammatory signals from monocytes or granulocytes recruited at the vaccination site, and the signal may not be provided by some adjuvants, e.g., IFA. Therefore, other adjuvants like synthetic oligodeoxynucleotides with unmethylated CpG dinucleotides (CpG-ODN) may be needed to stimulate the inflammatory signals (33).

DCs, the most efficient antigen- presenting cells (APCs), have been increasingly used in cancer immunotherapy to improve the immune responses (66-68). DCs are widely distributed throughout the body and abundantly found at the interfaces with the outer environments (e.g. mucosa and skin). DCs in peripheral tissues are immature and ready to capture antigens from exogenouse pathogens and endogenouse dead cells (67). These antigens are subsequently processed into small peptides while DCs become mature and move to the draining secondary lymphoid organs (68). In the secondary lymphoid organs, DCs directly present the processed peptides to naïve T cells to activate a series of cellular immune responses that involve both $CD4^+$ T help cells and cytolytic

$CD8^+$ T cells. DCs are also important to initiate humoral immune responses by activating naïve and memory B cells. In addition, DCs can activate both natural killer (NK) cells and natural killer T (NKT) cells (68), which have important antitumor effects (26, 69, 70).

hTERT-DCs based immunotherapy

DCs have been used in cancer immunotherapy targeting $hTERT^+$ tumors. In this case, DCs are simply pulsed with hTERT peptide (29, 53, 54) or genetic modified by transfection with plasmid containing hTERT cDNA (71), mRNA (72-74) or transduction with viral vector containing hTERT cDNA (71).

In an initial study, Nair et al. (72) transfected DCs derived from renal cell carcinoma patients with mRNA encoding the hTERT protein. The transfected DCs were then used to stimulate autologous CTLs *in vitro*. Their results showed that the transfected DCs were not only potent in inducing CTLs to kill renal tumor cells *in vitro* but also had an inhibitory effect on the metastasis of other tumors (lung, breast and bladder) when administered *in vivo* in humanized mice. Their results also indicated that immunization with DCs transfected with tumor mRNAs was generally more effective than immunization with those transfected only by TERT mRNA (72), suggesting the importance of including TERT and additional tumor antigens in DCs vaccine.

Thereafter, DCs transfected with plasmid containing hTERT gene or transduced with adenoviral vector encoding hTERT gene were also demonstrated to generate specific CTL responses targeting various $hTERT^+$ cancer cell lines *in vitro*. In this case, DCs transduced with adenoviral vector produced higher levels of hTERT expression than the DCs transfected with plasmid (71).

Except $CD8^+$ T cell response, antigen-specific $CD4^+$ T cell response can also be elicited by DCs trasnsfected by hTERT mRNA. Su et al. (73) reported that DCs transfected with mRNA encoding hTERT and a chimeric LAMP-hTERT could not only induce $CD8^+$ CTLs but also elicit hTERT-specific $CD4^+$ T cells *in vitro*. Furthermore, the LAMP-hTERT mRNA transfected DCs could elicit much higher $CD4^+$ T cells than those DCs transfected with hTERT mRNA (73). In follow clinic trial study, DCs transfected with hTERT mRNA or LAMP-hTERT mRNA, when immunized by intradermal injection, were also found to stimulate antigen-specific $CD4^+$ and $CD8^+$ T cell responses in patients with advanced prostate cancer (74). Here, patients with prostate cancer were immunized with autologous DCs transfected with mRNA encoding either hTERT protein or chimeric protein LAMP-hTERT. As compared to the group of patients who received DCs expressing hTERT only, patients who were vaccinated with DCs expressing the chimeric product had a significantly higher proportion of hTERT-specific $CD4^+$ T cells in their peripheral blood. That hTERT-specific CTLs derived from these patients had an enhanced capacity to lyse $hTERT^+$ target cells. More importantly, 9 of 10 patients received intradermal vaccination with the hTERT mRNA-transfected DCs showed transient clearance of circulating tumor cells (74), implying the promising efficacy of hTERT-DC vaccine.

Two interesting points are risen from Su's studies. First, the hTERT-specific T cell response elicited by DCs vaccine can be improved by modified hTERT template. In Su's studies, the attachment of LAMP-1 sequence to the hTERT template was used to direct hTERT to the lysosome so that hTERT would be easily digested and loaded onto MHC class II molecules and recognized by $CD4^+$ T cells. Second, stimulation of $CD4^+$ T cells can enhance the cytolytic activity of hTERT-specific CTLs. It is known that both antigen-specific CTLs and $CD4^+$ T helper cells are needed for eliciting potent antitumor immunity (75-77). $CD4^+$ T cells are important for the tumor specific cytotoxicity of CTLs by maintaining $CD8^+$ T-cell numbers, helping recruitment of CTLs to the tumor site (78, 79), and inducing a memory response (80, 81). Therefore, the hTERT-restricted antitumor vaccine should be designed to elicit strong CTL response as well as $CD4^+$ T cell response to get the maximal killing tumor effects.

Although vaccination of hTERT-DCs shows great promise, it is still in the early stage of development and there are still many questions and concerns about its widely use in clinic. The present patient autologous DCs preparation is not practical for clinic use as a universal vaccine in human population. Except that the DCs vaccine preparation is cost, time and labor intensive, parameters including source of DCs, maturation stage of DCs, frequency, dose, administration route, and adjuvant, etc., have also complicated the generation of a consensus regarding DC manufacturing (29). Among the parameters mentioned above, the maturation of DCs appears to be very crucial for DCs vaccine preparation, because 1) immature DCs may induce immune suppression (68, 82); 2) mature monocyte-derived DCs are likely to be more effective at eliciting immune responses than immature DCs (83, 84).

hTERT-recombinant virus based immunotherapy

There are few reports to date about the direct use of the recombinant virus that are engineered to express hTERT antigens as vaccine in animal and human studies. Recombinant adenovirus containing hTERT cDNA was constructed and then transfected into DCs that were used to elicit hTERT-specific CTLs from autologous T cells *in vitro*. In this study, hTERT-specific CTLs could be successfully elicited (71).

In another study, a 27 kD hTERT protein (hTERTC27) was constructed with the N-terminal and the central domain of hTERT deleted. Plasmid encoding this truncated product was packaged into a retroviral vector that was then used to transfect HeLa cells (85). Experiments demonstrated that ectopic expression of hTERTC27 in HeLa cells induced the cells to undergo a senescence-like growth arrest and apoptosis (85). hTERTC27 was also shown to be able to sensitize HeLa cells to H_2O_2-induced senescence (86). The observed effects were independent of the suppression of

telomerase activity and the shortening of telomere length. In a second round of experiment, HeLa cells transduced either with hTERTC27 or a control protein (EGFP) were administered to mice that were prone to develop solid tumor. Tumor was observed in all mice xenografted with HeLa cells expressing the control protein. In contrast, only 1 out of 4 mice xenografted with HeLa cells expressing the truncated product showed tumor development. The tumor so-formed was comparatively smaller in mass and with slower growth rate than those formed in the control group (85). Taken together, the results of these experiments suggest that hTERTC27 has antitumor properties, however, the mechanism of antitumor effects of hTERTC27 has not been reported. Considering the recombinant virus expressing hTERTC27 that contains p973 and p988, we suppose that antitumor immunity may be involved.

hTERT-targeted humoral immunity

Besides the cellular immune response elicited by hTERT molecule, humoral immune response may also be involved in tumor immunity. The presence of circulating anti-hTERT antibodies in the sera of cancer patients has been reported (87). More interestingly, the antibodies against hTERT were specific in cancer patients, and the Ab level was increasing with the development of cancer stage (87). Notwithstanding further studies are needed to forecast the importance of hTERT-specific Ab in cancer immunotherapy, this study, at least, implies that an antitumor vaccine against hTERT may be designed to improve both cellular and humoral immune responses in patients.

Safety concerns of the hTERT-targeted antitumor immunotherapy

Although hTERT is abundantly present on most human tumor cells, it is also expressed in some normal cells and tissues such as stem-cell precursors of the bone marrow, spermatogonia in the testis, activated lymphocytes and basal keratinocytes (42). Therefore, the major concerns about the anti-hTERT cancer immunotherapy are concentrated on the possible adverse effects on normal $hTERT^+$ somatic cells, e.g., germ cells, hematopoetic stem cells. Up to date, there is no obvious toxicity and autoimmune reaction observed in all the studies reported (29, 53-55, 58-61, 72, 74, 88).

hTERT-specific CTLs could effectively lyse target tumor cells but did not lyse $hTERT^+$ blood cells (53, 54) and activated B lymphocytes *in vitro* (59, 60). In animal model, studies suggested that immunization with hTERT vaccine did not show any evidence of autoimmunity and laboratory abnormalities, e.g., decreasing circulating B cells (55, 61) and other blood cells (61), and no evidence of inflammation or lymphocytic infiltrate within the liver, conlon or lymphatic tissues (61, 72). Encouragingly, similar results have been reported in several early clinical studies. Inoculation of cancer patients with 1.5×10^7 DCs pulsed with hTERT peptide did not induce serious adverse events (e.g. grade 3 or 4) or laboratory abnormalities including no histological changes in bone marrow and no reduce in Ig level & B cell number (29). The same research group performed further more detail studies recently and found no significant difference in colony-forming count (CFC) assay of bone marrow mononuclear cells even after long-term culture between pre- and post-vaccination of cancer patients. Moreover, bone marrow mononuclear cells from pre- and post-vaccinated cancer patients were then xenotransplanted into NOD/SCID mice by tail vein injection. Seven to eight weeks later, mouse bone marrow cells were prepared and the percentage of human bone marrow cells was analyzed by flow cytometry. Again, no significant difference of human bone marrow cells (leukocytes, B cells, myeloid cells and hematopoietic progenitors and stem cells) was observed in the level of human engraftment (% human $CD45^+$) between pre- and post vaccine samples (88). Similarly, no vaccine-related major toxicities or autoimmunity were observed in advanced prostate cancer patients vaccinated with hTERT-mRNA transfected DCs by intradermal injection (74). Results from other clinical phaseI/II trails also indicated that no adverse reaction could be found in vaccinated patients (89). All together, hTERT-derived vaccine for antitumor immunotherapy is safe, although there are further clinic trials needed.

Future prospects for hTERT-targeted immunotherapy

In view of the final aim of vaccine for human clinical use, the hTERT-targeted immunotherapy vaccine to be developed should be the one with 1) high specificity and efficacy; 2) the least risk of inducing adverse effects; 3) inexpensive and easy production; 4) feasible and convenient administration; 5) reliable and simple *ex vivo* assay to measure immune response and evaluate the vaccine effects. To reach the target, there are many hurdles to overcome. At present, increasing specificity and efficacy but reducing potential autoimmune toxicity of the hTERT-targeted immunotherapy is still a major focus. Introducing TAA-specific epitopes into vaccine can increase the specificity of hTERT-targeted immuno-therapy. In this case, identifying new highly hTERT-specific TAA peptide with high immunogenicity is very needed to improve the vaccine specificity for killing $hTERT^+$ tumor cells. The efficacy of hTERT-targeted vaccine can be improved by creating multi-epitopes vaccine with hetero-geneity but high immunogenicity in TAA expression. Adjuvants, e.g., cytokines (GM-CSF, IL-2 and IL-12) and chemokines (90, 91) can also be incorporated into the hTERT-targeted immunotherapy. Alternately, the amplitude of anti-hTERT responses can be synergistically enhanced by using more immunotherapeutic approaches (91), e.g., hTERT-DCs vaccine combined with gene-modified tumor cell vaccine. Respecting the important roles of $CD4^+$ T cells and NK cells in antitumor immunity, new generation of cancer vaccine for the hTERT-targeted immunotherapy should be generated to increase the immune response of multiple immune cells to get the maximal antitumor effects. In this

case, recombinant virus expressing hTERT antigens should be given much more attention in the future because recombinant viral vaccine is convenient to be engineered to coexpress hTERT antigens, other tumor antigens and immunostimulatory molecules as well as cytokines. In addition, it may be worth investigating the antitumor efficacy of the combination of the hTERT-targeted immunotherapy with surgical or non-surgical therapy approaches.

All in a word, Progress of cancer immunotherapy targeting hTERT can be expected to continuously expand fast in the future with the increasingly understanding of tumor-host interactions, which will bring on a new era of antitumor immunotherapy targeting $hTERT^+$ tumor cells.

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