Chapter 3
Antigen-Specific T Cell Responses in Hepatocellular Carcinoma

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3.1 Antigenicity and Immunogenicity of HCC

Hepatocellular carcinoma (HCC) arises as a result of (1) chronic hepatitis and liver cirrhosis due to infections with hepatitis B virus (HBV) or hepatitis C virus (HCV), (2) non-alcoholic steatohepatitis, or (3) alcohol-induced cytotoxicity in hepatocytes. Chronic liver inflammation and hepatocyte injury cause genetic and epigenetic changes that lead to formation of cancerous hepatocytes. The changes to the liver can also induce expression of several targets, such as oncofetal antigens and cancer/testis antigens, which are recognized by the host antitumor immune response, possibly leading to the formation of tumors with high antigenicity. In addition, a recent genome-wide association study demonstrated that nonsynonymous somatic mutations occur in solid tumors, including in HCC [1]. Therefore, there is evidence to suggest that HCCs have a relatively high antigenicity.

In addition, it has been reported that tumors in HCC patients contain a large number of lymphocytic infiltrates [2], and that the patients who have undergone surgical removal or liver transplantation show lower risk of recurrence [3]. These data suggest that HCC has high immunogenicity, leading to induction of the antitumor immune response that suppresses tumor progression in HCC patients.

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3.2 Identification of Tumor-Associated Antigens and Their T Cell Epitopes in HCC

In 1991, Boon and colleagues identified a gene that encodes a melanoma antigen named melanoma-associated antigen (MAGE) [4]. This pioneering study provided the first scientific evidence that the human immune system is capable of clearing tumors in the body by recognizing them as foreign entities. Since then, studies have uncovered the mechanisms of the antitumor immune response involving cytotoxic T lymphocytes (CTLs) that recognize peptides derived from tumor-associated proteins. CTLs recognize the peptides via an interaction between T cell receptors (TCRs) and major histocompatibility complex (MHC) class I molecule complexes that express the peptides on the cell surface.

In the past 10–15 years, several tumor-associated antigens (TAAs) for HCC have been identified, indicating the presence of T cell-mediated immune response in HCC patients (Table 3.1). The following section describes the known TAAs and their CTL epitopes for HCC.

3.2.1 α-fetoprotein (AFP)

Since the identification of CTL epitopes for AFP, the underlying immune recognition mechanisms have been studied. AFP is an oncofetal antigen synthesized during fetal development. Although its production is suppressed after birth, it is re-expressed in HCC. As AFP is a self-protein, it was unclear as to whether AFP-specific T cells can be produced and activated to induce an antitumor immune response. However, studies have identified AFP-derived HLA-A2-restricted CTL epitopes in humans, and have shown that CTLs specific to these epitopes can be induced in humans and in HLA-A2 transgenic mice [5]. Several immune-dominant AFP epitopes, including those that are HLA-A24-restricted, have also been identified [6, 7]. These studies demonstrate that the T cell repertoire includes self-antigen-specific CTLs that are not eliminated by central or peripheral tolerance mechanisms. Therefore, self-antigens are one of the attractive targets for immunotherapy against HCC.

3.2.2 Human Telomerase Reverse Transcriptase (hTERT)

Human telomerase reverse transcriptase (hTERT) is a catalytic enzyme required for telomere elongation. It is expressed in many cancers, including in HCC. hTERT expression is related to telomerase activity in several cell types including germ, stem and cancer cells, and is associated with the properties of these cells to maintain their cell division. hTERT is also expressed in cancer stem cells that are resistant to

Table 3.1 Tumor associated antigens related HCC and their cytotoxic T lymphocyte epitopes

Antigen	Frequency of expression	T cell epitope	HLA restriction	Year	Author, references
AFP	<80%	$AFP_{137–145}$, $AFP_{158–166}$, $AFP_{325–334}$, $AFP_{542–550}$	A2	2003	Butterfield et al. [5]
		$AFP_{357–415}$, $AFP_{403–411}$	A24	2006	Mizukoshi et al. [6]
NY-ESO-1	<50%	NY-ESO-$1_{157-165}$	A2	2004	Korangy et al. [32]
MAGE-A	<80%	MAGE-$1_{161-169}$	A1	2004	Zerbini et al. [11]
		MAGE-$3_{271-279}$	A2		
		MAGE-$10_{254-262}$	A2	2005	Bricard et al. [10]
SSX-2	<50%	SSX-2_{41-49}	A2	2005	Bricard et al. [10]
hTERT	<80%	$hTERT_{167–175}$, $hTERT_{324–332}$, $hTERT_{461–469}$, $hTERT_{637–645}$, $hTERT_{845–853}$	A24	2006	Mizukoshi et al. [8]
Glypican-3	<70%	$GPC3_{144–152}$	A2	2006	Komori et al. [9]
		$GPC3_{298–306}$	A24		
HCA661	unknown	$HCA661_{110–118}$, $HCA661_{246–254}$	A2	2007	Pang et al. [33]
MRP3	<55%	$MRP3_{503–511}$, $MRP3_{692–700}$, $MRP3_{765–773}$	A24	2008	Mizukoshi et al. [12]
HCA587	<70%	$HCA587_{140–149}$, $HCA587_{144–152}$, $HCA587_{248–256}$	A2	2008	Xing et al. [34]
SART2	100%	$SART2_{93–101}$, $SART2_{161–169}$, $SART2_{899–907}$	A24	2012	Mizukoshi et al. [14]
SART3	100%	$SART3_{109–118}$, $SART3_{315–323}$	A24	2017	Kaji et al. [35]

AFP alpha-fetoprotein, *MAGE* melanoma-associated antigen, *SSX-2* synovial sarcoma/X breakpoint-2, *hTERT* human telomerase reverse transcriptase, *HCA* hepatocellular carcinoma-associated antigen, *SART* squamous cell carcinoma antigen recognized by T cell

conventional chemotherapy. Therefore, it is an attractive target for cancer immunotherapy. hTERT-derived HLA-A2, A3 and A24-restricted CTL epitopes, and MHC class II-restricted helper T cell epitopes have been identified in many cancers, and measurable amounts of hTERT-specific CTLs have been isolated *ex vivo* from the peripheral blood of HCC patients [8].

3.2.3 Glypican-3 (GPC3)

Glupican-3 (GPC3) is a 65 kDa cell-surface protein consisting of 580 amino acids in the family of heparin sulphate proteoglycans. It was recently identified in a cDNA microarray as an oncofetal antigen expressed specifically in HCC. GPC-derived HLA-A2 and A24-restricted CTL epitopes have been identified, and CTLs that recognize these epitopes have been isolated from the peripheral blood of HCC patients [9].

3.2.4 Synovial Sarcoma X Breakpoint-2 (SSX-2)

Synovial sarcoma X breakpoint-2 (SSX-2) is a cancer/testis antigen overexpressed in HCC. Its CTL epitope was identified in melanoma, and CTLs that recognize the epitope have been isolated from the peripheral blood of HCC patients [10].

3.2.5 Melanoma-Associated Antigen A (MAGE-A)

Antigens in the MAGE-A family, first identified in melanoma, are expressed in many cancers. MAGE-A-derived CTL epitopes have been identified, and CTLs that recognize MAGE-A1 and A3-derived epitopes have been isolated from tumor-infiltrating lymphocytes (TILs) in HCC patients [11]. Studies have also identified CTLs that recognize MAGE-A10-derived epitopes [10].

3.2.6 Multidrug Resistance-Associated Protein 3 (MRP3)

Multidrug resistance-associated protein 3 (MRP3) is an ABC transporter that transports glucuronic acid conjugates as well as a variety of unconjugated organic anion compounds such as antibiotics and anti-inflammatory agents. It is expressed on the basolateral membrane of epithelial cells in the small intestine, where it is believed to be involved in absorption of drugs and bile acid. In liver, it is expressed on the basolateral membrane of hepatocytes along blood vessels, and plays an important role in efflux of unwanted substances from the liver. In addition to normal tissues, its expression has been found in many tumors. MRP3-derived HLA-A2 and A24-restricted CTL epitopes have been identified, and CTLs that recognize the epitopes have been isolated from many cancer patients, including from HCC [12].

In addition to those listed above, there are several TAAs that have been identified in HCC. They include NY-ESO-1, Cyclophyrin-B (Cyp-B), SART, p53, WT-1, β-Catenin and HSP70. CTL epitopes have been identified for some of them, for

which the evidence for specific CTL responses have been found in the peripheral blood of HCC patients. Peptides that express some of these CTL epitopes have been used as a vaccine for HCC patients in clinical trials, which will be discussed later.

3.3 Characteristics of Antigen-Specific T Cell Responses in HCC Patients

Identification of HCC-specific T cell epitopes can lead to the development of immunotherapy for HCC. HCC-specific T cell epitopes have also been studied to better understand the mechanisms of immune responses in HCC patients.

3.3.1 Antigen-Specific T Cell Responses in HCC Patients

In general, CTLs that recognize TAA-derived epitopes are obtained by stimulating peripheral blood mononuclear cells (PBMCs) or TILs of patients with candidate peptide epitopes, followed by 1–2 weeks of *in vitro* expansion. Many TAA-derived epitopes have been identified using this method. However, it does not fully recapitulate the T cell frequency and phenotype in patients as it requires long-term *in vitro* culture and stimulation with cytokines such as interleukin-2 (IL-2) or IL-12. This limitation can be overcome by using enzyme-linked immune spot (ELISPOT) or tetramer assays. However, the frequencies of TAA-specific T cells are low in the peripheral blood of cancer patients, including in HCC patients. Thus, there is a certain limit to fully characterize the behavior of TAA-specific T cells in HCC.

A study used the ELISPOT assay to study the frequency of TAA-specific T cells in HCC patients. By comparing a variety of TAA epitopes, the study demonstrated that the frequency of CTLs that are specific to TAA-derived epitopes is 10–60.5 cells/300,000 PMBCs in HCC patients, and that only 3–19% of the patients have CTLs specific to each epitope [13]. These values suggest a weaker host immune response against TAAs compared to that against virus-derived foreign antigens, indicating that there is an insufficient amount of TAA-specific T cells to eliminate tumors.

Studies comparing the immune response against TAA-derived CTL epitopes and the clinical background of HCC patients have generated new information about the host antitumor immune response. For example, it has been reported that different TAAs may elicit different host immune responses. While CTLs against AFP are found more frequently in advanced HCC, those against hTERT, SART and MRP3 can be found in peripheral blood at earlier stages [6, 8, 12, 14].

CTLs specific to some of the TAA epitopes have been obtained by tetramer assay in PMBCs of HCC patients to study their surface markers. For example, hTERT-specific CTLs were found in high frequencies in the peripheral blood of HCC

patients who had never been treated with antigen-specific immunotherapy such as peptide vaccines. The memory phenotype of hTERT-specific CTLs varied, with effector being the most frequent, followed by effector memory and central memory [15].

3.3.2 Insufficient Antigen-Specific T Cell Responses

Studies on TAA epitope-specific CTLs by ELISPOT and tetramer assays have identified so-called non-functional CTLs that bind to TAA epitopes but do not produce cytokines such as interferon-γ. Several mechanisms have been proposed to describe the role of non-functional CTLs and the insufficient host antitumor immune response in HCC. Recent studies have also identified a population of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and uncovered the underlying mechanisms of these cells that negatively regulate the antitumor response in HCC. Among different immunosuppressive cells, Tregs have been studied the most to understand the mechanisms of action in suppressing the antitumor immunity. In HCC patients, Tregs are found in PMBCs and TILs, and contribute to tumor progression [16, 17].

MDSC suppresses T cell function by upregulating arginase production that induces Foxp3 and IL-10 expression in $CD4^+$ T cells. Human MDSCs are heterogeneous, and can be divided into granulocytic $CD14^-$ and monocytic $CD14^+$ subtypes. Recent studies have demonstrated that there is an increased number of $CD14^+HLA\text{-}DR^{-/low}$ MDSCs in the peripheral blood of HCC patients [18, 19], and that the number of MDSCs in the peripheral blood after locoregional therapy is negatively correlated with the frequencies of TAA-specific T cells [20]. These studies indicate that the immunosuppressive cells lead to an insufficient TAA-specific immune response.

3.3.3 TAA-Specific T Cell Responses in HCC Treatments

Studies in several cancers have shown that tumors remaining from thermoablation regress spontaneously. This observation suggests that locoregional therapies may induce bystander effects resulting from the TAA-specific T cell response. Indeed, studies on HCCs demonstrated that radiofrequency ablation (RFA) and transarterial chemoembolization (TACE) induce tumor-specific immune responses [21, 22]. For example, an experimental study reported that RFA treatment of a tumor implanted on one side of a mouse led to growth delay in a contralateral tumor, suggesting that the bystander effect involves RFA-induced activation of immune cells such as $CD8^+$ T cells, $CD4^+$ T cells and macrophages [23].

Studies also demonstrated that, in HCC patients who were treated with RFA or TACE, the frequencies of TAA-specific CTLs increase in the peripheral blood after

treatment [20, 24]. In this study, however, the treatment-induced antitumor immune response was transient. This suggests that while treatments that induce cellular apoptosis or necrosis trigger antitumor immunity, the resulting antitumor response is insufficient to suppress tumor recurrence after treatment.

Recent studies have further demonstrated that the recurrence rate is significantly lower after RFA when the frequency of TAA-specific CTLs in the peripheral blood is higher after the treatment [20]. This evidence indicates that tumor recurrence may be suppressed when locoregional therapies are followed by immunotherapy in HCC.

3.4 Antitumor Immunity Induced by Antigen-Specific T Cell Therapies in HCC

The first step in establishing the treatment strategies exploiting the antigen-specific T cell immune response is to identify HCC-specific antigens and their T cell epitopes. As HCC is associated with liver dysfunction, immunotherapy should be targeted to tumor cells while sparing healthy hepatocytes for it to be safe and effective. As described earlier, many TAAs and their T cell epitopes for HCC have been identified over the past 10–15 years. Table 3.2 summarizes the clinical trials that made use of peptide vaccines including TAA-derived T cell epitopes.

3.4.1 Antigen-Specific T Cells Induced by Dendritic Cell Therapy

Dendritic cell therapy for HCC involves the use of dendritic cells that are (1) pulsed with TAA-derived peptides, or (2) isolated from patients' PBMCs and infused intratumorally. One such method used dendritic cells that were pulsed with HLA-A2-restricted AFP-derived peptides. While the treatment increased AFP-specific CTLs in 6 out of 10 HCC patients, there were no effects on reducing tumor size [25].

Dendritic cells are activated upon recognition of apoptotic cells. Upon activation, they differentiate into mature dendritic cells to enhance antitumor immunity. In HCC, dendritic cell therapy based on intratumoral infusion is being tested in patients who received treatment with TACE or RFA to investigate whether the therapy induces antigen-specific T cells. Although clinical evidence is limited, some studies demonstrated induction of CTLs that recognize antigen (e.g. AFP, hTERT)-derived epitopes by direct intratumoral infusion of dendritic cells, leading to a decreased recurrence rate after locoregional therapies [26].

Table 3.2 Clinical trials of peptide vaccines for HCC

Setting for peptides, HLA restriction	No. of patients	Responses	Year	Author, references
AFP-derived peptides + Montanide adjuvant, HLA-A2	6	No PR or CR	2003	Butterfield et al. [25]
hTERT-derived peptides + cyclophosphamide + GM-CSF multiple	40	No PR or CR	2010	Greten et al. [27]
GPC3-derived peptides + Montanide adjuvant, HLA-A24 and A2	33	1/33 PR and 19/33 SD	2012	Sawada et al. [28]
SART2-derived peptides + Montanide adjuvant, HLA-A24	12	Immune response	2012	Mizukoshi et al. [14]
hTERT-derived peptides + Montanide adjuvant, HLA-A24	14	Prolonged recurrence-free survival and immune response	2015	Mizukoshi et al. [15]
MRP3-derived peptides + Montanide adjuvant + HAIC, HLA-A24	12	1/12 PR and 9/12 SD	2015	Mizukoshi et al. [36]
SART3-derived peptides + Montanide adjuvant, HLA-A24	12	Immune response	2017	Kaji et al. [35]
AFP-derived peptides + Montanide adjuvant, HLA-A24	20	15 patients were assessed, 1/15 CR and 8/15 SD	2017	Mizukoshi et al. [29]

GM-CSF granulocyte-macrophage colony stimulating factor, *HCC* hepatocellular carcinoma, *AFP* alpha-fetoprotein, *hTERT* human telomerase reverse transcriptase, *GPC3* glypican-3, *SART* squamous cell carcinoma antigen recognized by T cells, *CR* complete response, *PR* partial response, *SD* stable disease

3.4.2 Antigen-Specific T Cells Induced by Peptide Vaccines

Since the discovery of the MAGE gene by Boon and colleagues, immunotherapy trials using tumor antigen-derived peptides have been conducted for many cancer types around the world. Although these clinical studies demonstrated antitumor effects, such as reduction of tumors and suppression of progressive cancer development, complete response (CR) or partial response (PR) has rarely been achieved. Clinical studies suggest that, while the efficacy against advanced cancers may be limited when used alone, peptide vaccines have a significant potential to prevent tumor recurrence, and to prolong survival and time-to-progression. As such, many clinical studies have been conducted to date to test the efficacy of peptide vaccines in several cancers.

Peptide vaccines have been evaluated in clinical trials for HCC. The combination of hTERT-derived peptide vaccines and cyclophosphamide was tested in 40 patients with advanced HCC. The study showed that the immune response was not triggered for the peptide, and that there was no case of CR or PR [27]. On the other hand, a study using HLA-A24-restricted hTERT-derived peptide demonstrated that

peptide-specific CTLs were induced in 71.4% of the patients. In this study, the effector memory phenotype was dominant in the induced CTLs [15].

Another study investigated the use of GPC3-derived peptide in 33 patients with advanced HCC. After the treatment, 1 patient achieved PR and 19 patients achieved stable disease (SD) for over 2 months [28]. Of the 19 patients with SD, 4 patients exhibited evidence for tumor necrosis and reduction of tumor size. The results of the study supported the notion of TAA-targeted immunotherapy for HCC.

A study using an HLA-A24-restricted AFP-derived peptide in advanced HCC patients demonstrated that CR and over 2 years of SD can be achieved [29]. This study was performed with patients who did not respond to conventional treatments, such as surgical resection, RFA, TACE, hepatic arterial infusion chemotherapy (HAIC) and sorafenib, demonstrating that the peptide vaccine approach is promising for HCC treatment. Furthermore, the frequencies of peptide-specific T cells increased in the peripheral blood after administration of the vaccine for those patients whose tumors responded to the AFP-derived peptide vaccine. Analysis of the peptide-specific TCRs revealed the presence of TCRs that have a strong binding affinity for AFP-derived CTL epitopes and are cytotoxic to target cells expressing the epitopes. Therefore, the efficacy of peptide vaccines likely depends on the number of TCRs induced by the vaccine as well as the number of TCRs with a high binding affinity to TAA-derived peptides.

3.4.3 Induction of Antigen-Specific TCRs

Adoptive T cell therapy using TILs has demonstrated clinical efficacy in some cancers including melanoma. However, the number of T cells that have tumor-specific TCRs and have antitumor effects is limited, making it challenging to isolate and expand the cell population. Thus, with some exceptions, the use of adoptive T cell therapy has been limited to the treatment of malignant melanoma.

To overcome this limitation, a technique was recently developed to enable manufacturing of a large number of tumor-specific T cells for adoptive T cell transfer. The technique involved transfer of TCR genes from tumor-specific T cells into lymphocytes collected from the peripheral blood of patients. Using this technique, a clinical trial demonstrated that adoptive transfer of MART-1 TCR transgenic T cells leads to reduction of tumor size [30].

Currently, TAA-specific TCRs have been obtained in many cancers. In HCC, TCR genes that recognize AFP, hTERT and GPC3-derived T cell epitopes have been cloned. Furthermore, previously uncharacterized TCRs can now be identified by a novel technique that enables the cloning of single cells. The technique involves a rapid cloning system which enables single-cell isolation of TAA-derived epitope-specific CTLs, cloning of the TCR gene and validation of the TCR specificity to the epitopes in less 10 days [31]. Using this method, AFP-specific TCRs were studied in healthy volunteers and in HCC patients receiving AFP-derived peptide vaccines. The study showed that although AFP-specific T cells existed in the peripheral blood

of healthy volunteers, there were only 1–2 TCR repertoires for different epitopes [29]. The number of TCR repertoires increased in patients whose tumors responded to the peptide vaccine. Lymphocytes from the peripheral blood of healthy volunteers were used to manufacture genetically modified T cells that express the AFP-specific TCR gene. The transgenic T cells were highly cytotoxic to target cells expressing the epitope, indicating that the adoptive therapy using AFP TCR transgenic T cells is promising.

In summary, immunotherapy for HCC is anticipated to have significant potential in HCC given that the HCC-specific TAAs have been identified and the antigen-specific T cell responses have been well characterized. Further development of immunotherapy in HCC will depend on the identification of highly immunogenic antigen-derived T cell epitopes, such as neoantigens, as well as a better understanding of the mechanisms underlying antitumor immunity. These studies should lead to the development of novel immunotherapies for HCC that presumably involve the combination of immunotherapeutic approaches engaging multiple mechanisms.

Conflict of Interest The authors disclose no conflicts of interest.

References

1. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339:1546–58.
2. Yoong KF, McNab G, Hubscher SG, Adams DH. Vascular adhesion protein-1 and ICAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma. J Immunol. 1998;160:3978–88.
3. Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology. 1998;27:407–14.
4. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254:1643–7.
5. Butterfield LH, Koh A, Meng W, Vollmer CM, Ribas A, Dissette V, et al. Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein. Cancer Res. 1999;59:3134–42.
6. Mizukoshi E, Nakamoto Y, Tsuji H, Yamashita T, Kaneko S. Identification of alpha-fetoprotein-derived peptides recognized by cytotoxic T lymphocytes in HLA-A24+ patients with hepatocellular carcinoma. Int J Cancer. 2006;118:1194–204.
7. Thimme R, Neagu M, Boettler T, Neumann-Haefelin C, Kersting N, Geissler M, et al. Comprehensive analysis of the alpha-fetoprotein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. Hepatology. 2008;48:1821–33.
8. Mizukoshi E, Nakamoto Y, Marukawa Y, Arai K, Yamashita T, Tsuji H, et al. Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma. Hepatology. 2006;43:1284–94.
9. Komori H, Nakatsura T, Senju S, Yoshitake Y, Motomura Y, Ikuta Y, et al. Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. Clin Cancer Res. 2006;12:2689–97.

10. Bricard G, Bouzourene H, Martinet O, Rimoldi D, Halkic N, Gillet M, et al. Naturally acquired MAGE-A10- and SSX-2-specific CD8+ T cell responses in patients with hepatocellular carcinoma. J Immunol. 2005;174:1709–16.
11. Zerbini A, Pilli M, Soliani P, Ziegler S, Pelosi G, Orlandini A, et al. Ex vivo characterization of tumor-derived melanoma antigen encoding gene-specific CD8+cells in patients with hepatocellular carcinoma. J Hepatol. 2004;40:102–9.
12. Mizukoshi E, Honda M, Arai K, Yamashita T, Nakamoto Y, Kaneko S. Expression of multidrug resistance-associated protein 3 and cytotoxic T cell responses in patients with hepatocellular carcinoma. J Hepatol. 2008;49:946–54.
13. Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, et al. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. Hepatology. 2011;53:1206–16.
14. Mizukoshi E, Fushimi K, Arai K, Yamashita T, Honda M, Kaneko S. Expression of chondroitin-glucuronate C5-epimerase and cellular immune responses in patients with hepatocellular carcinoma. Liver Int. 2012;32:1516–26.
15. Mizukoshi E, Nakagawa H, Kitahara M, Yamashita T, Arai K, Sunagozaka H, et al. Immunological features of T cells induced by human telomerase reverse transcriptase-derived peptides in patients with hepatocellular carcinoma. Cancer Lett. 2015;364:98–105.
16. Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology. 2007;132:2328–39.
17. Takata Y, Nakamoto Y, Nakada A, Terashima T, Arihara F, Kitahara M, et al. Frequency of CD45RO+ subset in CD4+CD25(high) regulatory T cells associated with progression of hepatocellular carcinoma. Cancer Lett. 2011;307:165–73.
18. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Kruger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+) CD25(+)Foxp3(+) T cells. Gastroenterology. 2008;135:234–43.
19. Arihara F, Mizukoshi E, Kitahara M, Takata Y, Arai K, Yamashita T, et al. Increase in CD14+HLA-DR −/low myeloid-derived suppressor cells in hepatocellular carcinoma patients and its impact on prognosis. Cancer Immunol Immunother. 2013;62:1421–30.
20. Mizukoshi E, Yamashita T, Arai K, Sunagozaka H, Ueda T, Arihara F, et al. Enhancement of tumor-associated antigen-specific T cell responses by radiofrequency ablation of hepatocellular carcinoma. Hepatology. 2013;57:1448–57.
21. Zerbini A, Pilli M, Penna A, Pelosi G, Schianchi C, Molinari A, et al. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. Cancer Res. 2006;66:1139–46.
22. Ayaru L, Pereira SP, Alisa A, Pathan AA, Williams R, Davidson B, et al. Unmasking of alpha-fetoprotein-specific CD4(+) T cell responses in hepatocellular carcinoma patients undergoing embolization. J Immunol. 2007;178:1914–22.
23. Iida N, Nakamoto Y, Baba T, Nakagawa H, Mizukoshi E, Naito M, et al. Antitumor effect after radiofrequency ablation of murine hepatoma is augmented by an active variant of CC Chemokine ligand 3/macrophage inflammatory protein-1alpha. Cancer Res. 2010;70:6556–65.
24. Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Mukaida N, Matsushima K, et al. Enhancement of tumor-specific T-cell responses by transcatheter arterial embolization with dendritic cell infusion for hepatocellular carcinoma. Int J Cancer. 2010;126:2164–74.
25. Butterfield LH, Ribas A, Dissette VB, Lee Y, Yang JQ, De la Rocha P, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. Clin Cancer Res. 2006;12:2817–25.
26. Nakamoto Y, Mizukoshi E, Kitahara M, Arihara F, Sakai Y, Kakinoki K, et al. Prolonged recurrence-free survival following OK432-stimulated dendritic cell transfer into hepatocellular carcinoma during transarterial embolization. Clin Exp Immunol. 2011;163:165–77.

27. Greten TF, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, et al. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. BMC Cancer. 2010;10:209.
28. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. Clin Cancer Res. 2012;18:3686–96.
29. Mizukoshi E. Association between high-avidity T-cell receptors, induced by alpha fetoprotein-derived peptides, and anti-tumor effects in patients with hepatocellular carcinoma. Gastroenterology. 2017;152:1395–406.
30. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314:126–9.
31. Kobayashi E, Mizukoshi E, Kishi H, Ozawa T, Hamana H, Nagai T, et al. A new cloning and expression system yields and validates TCRs from blood lymphocytes of patients with cancer within 10 days. Nat Med. 2013;19:1542–6.
32. Korangy F, Ormandy LA, Bleck JS, Klempnauer J, Wilkens L, Manns MP, et al. Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. Clin Cancer Res. 2004;10:4332–41.
33. Pang PH, Chan KT, Tse LY, Chan RC, Cheung YK, Sin FW, et al. Induction of cytotoxic T cell response against HCA661 positive cancer cells through activation with novel HLA-A *0201 restricted epitopes. Cancer Lett. 2007;256:178–85.
34. Xing Q, Pang XW, Peng JR, Yin YH, Li Y, Yu X, et al. Identification of new cytotoxic T-lymphocyte epitopes from cancer testis antigen HCA587. Biochem Biophys Res Commun. 2008;372:331–5.
35. Kaji K, Mizukoshi E, Yamashita T, Arai K, Sunagozaka H, Fushimi K, et al. Cellular Immune Responses for Squamous Cell Carcinoma Antigen Recognized by T Cells 3 in Patients with Hepatocellular Carcinoma. PLoS One. 2017;12:e0170291.
36. Mizukoshi E, Nakagawa H, Kitahara M, Yamashita T, Arai K, Sunagozaka H, et al. Phase I trial of multidrug resistance-associated protein 3-derived peptide in patients with hepatocellular carcinoma. Cancer Lett. 2015;369:242–9.