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Case Report

Rapid Dissemination followed by Spontaneous Regression of Metastatic Hepatocellular Carcinoma after Liver Radiofrequency Thermal Ablation: A Case Report with Correlative Immune Assay

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Keywords
Hepatocellular carcinoma · Spontaneous regression · Dissemination · Immune mechanism · Cytotoxic T cells · Case report

Abstract
Rapid intrahepatic and distant metastasis of hepatocellular carcinoma (HCC) after locoregional treatment for early stage tumor is very rare. Descriptions of spontaneous regression of HCC exist in case reports, but its true mechanism is unclear. Here, we describe a case of rapid dissemination with lung metastasis shortly after localized RFA treatment of HCC liver lesions, followed by spontaneous, sustained regression of those lung lesions. We also show the detection of cytotoxic T lymphocytes (CTLs) specific to hepatitis B antigens by immune assay in this patient. We propose immune-related destruction as the basis for spontaneous regression.

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Introduction

Hepatocellular carcinoma (HCC) is a cancer associated with viral hepatitis B or C and other non-viral chronic liver diseases [1]. Early stage HCC is treatable with a number of locoregional therapy options, such as surgery, trans-catheter arterial chemoembolization (TACE) [2], radiofrequency ablation (RFA) [3], stereotactic radiation [4], radioembolization using intra-arterial injection of yttrium-90 [5, 6], and liver transplant [7]. RFA is considered one of the main curative treatments for HCC of less than 5 cm in a cirrhotic liver [8]. By delivering a rapid electromagnetic pulse that causes thermal injury leading to coagulative necrosis of the liver tissue, it can achieve excellent control of liver lesions, particularly for lesions less than 5 cm [9]. RFA has been delivered to a vast number of patients, and multiple studies have shown that it is a well-tolerated procedure and is usually only associated with minor, self-limited side effects in about 5–10% of patients [10–13]. The reported common side effects are hematoma, liver abscess, pulmonary or gastrointestinal damage depending on the locations of the liver lesions.

Here we present an unusual case of rapid progression with lung metastasis shortly after localized RFA treatment of HCC, followed by spontaneous, sustained regression of lung metastatic lesions. We also present result from our correlative study, which showed the presence of CTLs specific to HBV antigens, suggesting an immune response as the underlying mechanism for the sustained tumor regression.

Case Presentation

The patient was 81 years old when she presented after a finding of a mass in the right hepatic lobe upon serial abdominal ultrasound evaluation due to the history of chronic active hepatitis B. She had been taking antiviral drug entecavir. MRI showed a 4.3 × 3.8-cm mass in segment 5 (close to the IVC) and a 2 × 1.4-cm mass in the periphery (segment 7). AFP was 50 μg/L, and HBV DNA was undetectable. Both of these masses exhibited image patterns consistent with HCC, as being heterogeneous, demonstrating arterial hyper-enhancement and washout on portal venous phase. A diagnosis of HCC was made based on clinical history and image characteristics after tumor board discussion. No tumor biopsy was taken. Past medical history included hypertension, diabetes, and type B aortic dissection which were all medically managed. Family history was significant for colon cancer in one of her sons. The patient declined surgery and was treated with TACE 1 month after diagnosis, with mild decrease in AFP and persistent mass. A second TACE was performed 3 months later, which did not result in AFP decline. Due to sclerosis of hepatic arteries, no further TACE was recommended. Her AFP continued to rise to 178, ten months after diagnosis, and MRI showed viable components in both lesions. Baseline labs at that time showed total WBC of 5.9 × 10^3/μL, absolute lymphocyte count of 3.8 × 10^3/μL, hemoglobin of 12 g/dL.

Eleven months after diagnosis, she underwent initial RFA for both lesions via laparotomy. She developed postoperative anemia with lowest hemoglobin of 8.5 g/dL, and CT scan 2 months after RFA showed a hematoma in one of the ablated cavities, which resolved on its own. It also showed development of numerous lung nodules consistent with metastases. Three months after RFA, her AFP rose to 3,308, with total WBC of 5.5 × 10^3/μL, absolute lymphocyte count of 3.2 × 10^3/μL, and a repeat CT reported multiple bilateral pulmonary nodules, which have markedly increased in size and number since 1 month ago (Fig. 1a). At that time, she had low energy, decreased appetite, and was mostly confined to the house, while her hemoglobin level already returned to her baseline (10.2 g/dL). Systemic treatment

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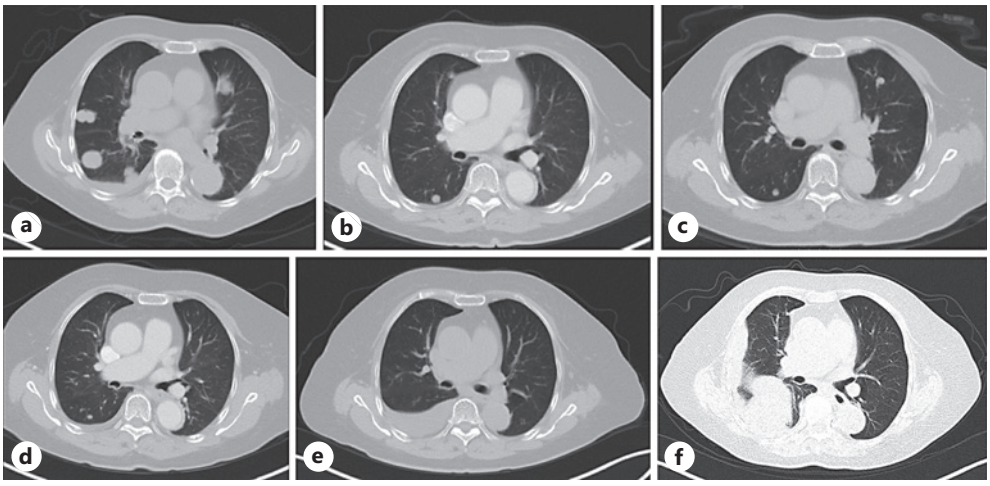

Fig. 1. Serial CT images showing the lung metastasis 3 months after RFA (**a**), 5 months after RFA (**b**), 8 months after RFA (**c**), 28 months after RFA (**d**), 39 months after RFA (**e**), and 41 months after RFA (**f**).

options including sorafenib or immunotherapy with nivolumab were offered and planned but she declined.

On a routine follow-up 5 months from RFA, she reported feeling well with greatly improved symptoms. AFP was 18, total WBC 5.9 × 10^3/μL, absolute lymphocyte count 3.4 × 10^3/μL, and hemoglobin 12 g/dL, and CT at that time showed significant decrease in size of the multiple lung nodules and the liver masses (Fig. 1b). Largest lung nodules measured 1.3 cm compared to 2.7 cm in diameter in the prior study (Fig. 1b). The patient denied taking any herbs or other alternative treatment. Monitoring without treatment continued. Repeat CT scan 8 months from RFA (Fig. 1c) and 14 months after RFA (data not shown) again showed further decrease in the size of the lung lesions.

Her AFP started to rise 18 months after RFA, and a CT scan showed increase in size of the hepatic dome lesion but stable appearance of the segment 7 lesion and the lung nodules and decrease in segment 5 lesion. Twenty-one months after RFA, an interventional radiology-guided RFA was performed to the hepatic dome lesion, which resulted in slow mild decrease in her AFP level (22 in 1/2018 before procedure and 36, then 25 after the procedure). Twenty-eight months after RFA, AFP increased to 74, and CT showed tumor recurrence in segment 7 liver lesion, while the bilateral lung nodules remained stable as subcentimeter lesions (Fig. 1d). The patient received another IR-guided RFA to the segment 7 lesion 31 months after initial RFA, and her AFP decreased to 18, and CT scan in March 2019 showed no more enhancing nodules in the segment 7 lesion.

Thirty-seven months after initial RFA, or 48 months from initial diagnosis, with rising AFP, MRI showed 2 new lesions in the left lobe of the liver, segment IVb and II. Systemic treatment was recommended which she declined. CT of the chest 39 months after initial RFA showed development of left pleural effusion (Fig. 1e). She presented to ER for shortness of breath 1 month later and was found to have large right-sided pleural effusion, which was drained. Cytology was negative for malignant cells. CT scan 41 months after initial RFA (Fig. 1f) showed tumor throughout the right pleural space, large right lower lobe necrotic tumor with active bleeding. The patient passed away shortly afterward.

We hypothesized that the spontaneous regression of the pulmonary metastatic lesions was a result of the self-anti-tumor immune response. A collaboration was established with Cellular Technology Limited (Shaker Heights, OH, USA), and cytotoxic T-cell (CTL) response

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was performed with the measurement by Enzyme-Linked Immune Absorbent Spot (ELISPOT) assay. IRB approval was obtained at both Cellular Technology, Ltd., and Maimonides Medical Center. The patient signed informed consent for the correlative studies. Ten milliliters of peripheral blood was collected from the patient, and peripheral blood mononuclear cell was isolated in CTL, cryopreserved, and stored in liquid nitrogen until testing. The ELISPOT assay was performed to detect IFN-γ-producing T cells from the patient's blood, after stimulation with HBV-associated whole protein antigens according to CTL standard operating procedure. Briefly, the cells were plated at 400,000 cells/well in triplicate wells; medium alone served as the negative control, and phytohemagglutinin (PHA) at 5 μg/mL served as the positive control. The whole protein antigens (hepatitis B core protein and hepatitis B surface antigen ayw) were titrated over the range of concentration from 40 μg/mL to 5 μg/mL in triplicate wells. The peptide pools (HBV capsid antigen and HBV large envelope protein) were titrated over the range of concentration from 2 μg/mL to 0.5 μg/mL in triplicate wells. The cells were incubated with the antigen for 24 h. Positive ELISPOTs were counted and reported as numbers above the background. The ELISPOT tests were performed at visit 1 (16 months after initial RFA) and visit 2 (20 months after initial RFA).

The patient's blood sample showed strong positive response to HBV capsid antigen, producing positive IFN-γ-producing spots (Table 1). At visit 1, the response appeared to be proportional to the dose of stimulating antigen concentration. At visit 2, the IFN-γ-producing spots were more and were similar at both 40 μg/mL and 10 μg/mL (Table 1). Upon stimulation of the sample with HBV surface antigen (ayw), there was no response at 40 μg/mL, showing inhibitory effect from the antigen at this concentration. However, there was a positive response at 20 μg/mL and 10 μg/mL levels consistently between the 2 experiments, confirming a weak but positive response (Table 2).

Discussion

This case is unique as it illustrated two rare phenomena: (1) an initial rapid dissemination of disease with metastasis to the lungs after RFA and (2) a secondary spontaneous regression of the pulmonary metastasis. The regression started at 5 months and lasted for 39 months. She eventually progressed both in the liver and in the lungs and passed away from the disease. We investigated whether immune response was the mechanism of regression and showed laboratory evidence that there was a specific CTL response to HBV core capsid antigen as well as surface antigen.

The phenomenon of disseminated intrahepatic or distant metastasis from a localized intrahepatic lesion occurs extremely infrequently but has been observed and reported [14]. Rapid intrahepatic dissemination was observed, and risk factors were high preoperative AFP values and location of the tumor near segmental portal branches. It is likely due to hematogenous spread, and there was no report on subsequent spontaneous regression.

On the other hand, regression of HCC in general has been widely reported and was a subject of multiple reviews [15–17]. Oquiñena et al. [16] reported 3 cases and performed a thorough literature review of 59 cases reported up to 2009. The authors proposed a number of possible mechanisms, such as tumor tissue ischemia due to previous hepatic artery thrombosis or rapid tumor growth; a distant bystander effect; cessation of alcohol consumption; or possible other medications, such as herbal remedies [18] or nonsteroidal anti-inflammatory drugs. No possible causes in 46% of the cases. The review by Sakamaki et al. [15] discussed multiple immune-related mechanisms. For example, marked inflammatory response, infiltration of CD8+ and CD4+ T cells, an increase in interleukin 18, tumor necrosis factor-alpha, and CD 163-positive macrophages were all reported [15]. Our study

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Table 1. ELISPOT numbers after in vitro stimulation with whole HBV capsid antigen

HBV capsid Ag	40 μg/mL Median±SD	10 μg/mL Median±SD	5 μg/mL Median±SD
Visit 1	132.5±41.7	92±21.6	11±4.2
Visit 2	324±NA	353±NA	N/A

demonstrated CD8+ CTLs against HBV antigen as a surrogate marker for immune response, which may be a first study of this result to be added to the literature.

Recent benchtop research and clinical observations on the treatment effect of immune checkpoint inhibitors in cancer treatment have broadened our understanding of the balance of the body's immune response and immune tolerance. Immune checkpoint inhibitors work well in tumors associated with viral infections such as HPV and EBVs [19]. Radiation followed by checkpoint inhibitor treatment showed augmented response [20], and the proposed hypotheses are (1) the release of neo-antigens and (2) radiation-induced cell death could be immunogenic and radiation can overcome T-cell exclusion [21]. A case of abscopal effect in a patient with melanoma who received radiation therapy and ipilimumab treatment was well studied and published [22]. In that study, antibody titers to a tumor antigen, NY-ESO-1, correlated with tumor regression. As immune checkpoint inhibitors act by releasing a brake on the suppression of the body's immune response, it is hypothesized that native immune responses to those viral antigens exist. The result of our study reinforces this hypothesis.

RFA generates region of necrosis and marked local inflammatory response with infiltration of a dense T-cell infiltrate [23]. Induction of antigen-specific T-cell response was characterized [24]. It was proposed that the release of a new antigen secondary to tumor destruction ensues after RFA [25]. In a prospective study in patients with HCC, activated, specific T-cell response was detected against tumor antigens derived from both the untreated HCC tissue as well as the necrotic tumor; in addition, T-cell responses to recall antigens were significantly augmented as well, indicating memory response [26]. In a recent review, the relationship of immune modulation effect on locoregional therapy and its potential synergy with immunotherapy has been further outlined. RFA can generate regions within the liver that lead to PD-L1 upregulation, increase in effector immune response cells, inhibition of immune-suppressive cells, and increased release of tumor antigens [27].

Immune response after HBV viral infection has been well studied [28]. CD4 T cells are robust producers of cytokines and are required for the efficient development of effector CD8 CTLs and B-cell antibody production. CD8 T cells clear HBV-infected hepatocytes through cytolytic and noncytolytic mechanisms, reducing the levels of circulating virus. This antiviral immune response is induced after acute HBV infection and leads to HBV control. In contrast, patients with chronic HBV infection fail to mount such an efficient antiviral response. After the infection is controlled, maturation of T-cell memory occurs and is associated with its functional exhaustion. In patients with chronic HBV infection, the HBV-specific T-cell response is extremely weak.

In this study, we tested immune response to HBV antigens and showed positive results. It is unclear whether this response can be considered as a surrogate result for antitumor response. Due to the lack of biopsy of tumor tissue, a direct measure of T-cell response to the tumor tissue itself could not be done. Our patient has been taking anti-HBV medication entecavir for a long time, and her HBV viral load was undetectable while she developed HCC. The positive response manifested in the study could be due to stimulation of the immune memory cells or be associated with a response the body mounted against the tumor cells which were infected by HBV and expressed HBV antigens, which led to regression of pulmonary metastases. This hypothesis will need further studies for confirmation.

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Table 2. ELISPOT numbers after in vitro stimulation with whole HBV surface antigen ayw

HBV sAg ayw	20 μg/mL Median±SD	10 μg/mL Median±SD	5 μg/mL Median±SD
Visit 1	13±2.7	11.7±5.5	9.3±7.5
Visit 2	17±9	22±10	N/A

The observations in this study have a number of limitations. We did not have samples to show her immune response at baseline; we also did not have samples from other patients who have chronic HBV infection to test if they harbor anti-HBV response. We did not have tumor tissue to design CTL assays for antitumor-associated antigen response. In addition, the initial diagnosis of lung metastasis was based on marked rise in AFP, not by biopsy, although the ultimate progression of pleural effusion and lung masses partially confirmed the original suspicion of malignant metastasis in the lungs. Throughout the disease course, the HCC diagnosis was clinical; we did not have pathology confirmation or pathology material for further molecular testing.

Conclusion

We have demonstrated the presence of positive anti-HBV T-cell response in a patient with HCC who showed rapid dissemination of metastatic disease followed by spontaneous regression, suggesting immune-related response as the potential underlying mechanism leading to the regression of the lung metastasis.

Acknowledgment

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Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed by the Maimonides Medical Center Institutional Review Board (IRB) and was deemed to be "Neither IRB approval nor a HIPAA Waiver/HIPAA Authorization is required for case report that involves no more than 3 patients and is part of a healthcare operations activity (e.g., resident training activity, performance improvement), provided the presentation or publication does not have identifiable information and the activity is not considered FDA regulated research." Approval No. 2016-11-17. Written informed consent was obtained from the patient's next of kin for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

Yiqing Xu received compensation for participating in community advisory boards with Abbvie, Inc, and AstraZneneca PLC. Ashrei Bayewitz and Magdalena Tary-Lehmann declared that they have no conflict of interest.

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None of the authors have a funding source to declare.

Author Contributions

Yiqing Xu and Ashrei Bayewitz wrote the manuscript; Magdalena Tary-Lehmann performed the laboratory test and explanations. Yiqing Xu, Ashrei Bayewitz, and Magdalena Tary-Lehmann approved the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Singal AG, El-Serag HB. Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. Clin Gastroenterol Hepatol. 2015 Nov;13(12):2140–51.
2 Maluccio MA, Covey AM, Porat LB, Schubert J, Brody LA, Sofocleous CT, et al. Transcatheter arterial embolization with only particles for the treatment of unresectable hepatocellular carcinoma. J Vasc Interv Radiol. 2008 Jun;19(6):862–9.
3 Huang J, Yan L, Cheng Z, Wu H, Du L, Wang J, et al. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. Ann Surg. 2010;252(6):903–12.
4 Sanuki N, Takeda A, Kunieda E. Role of stereotactic body radiation therapy for hepatocellular carcinoma. World J Gastroenterol. 2014 Mar;20(12):3100–11.
5 Kennedy A, Nag S, Salem R, Murthy R, McEwan AJ, Nutting C, et al. Recommendations for radioembolization of hepatic malignancies using yttrium-90 microsphere brachytherapy: a consensus panel report from the Radioembolization Brachytherapy Oncology Consortium. Int J Radiat Oncol Biol Phys. 2007 May;68(1):13–23.
6 Lobo L, Yakoub D, Picado O, Ripat C, Pendola F, Sharma R. Unresectable hepatocellular carcinoma: radioembolization versus chemoembolization: a systematic review and meta-analysis. Cardiovasc Intervent Radiol. 2016 Nov;39(11):1580–8.
7 Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996 Mar;334(11):693–700.
8 Nault JC, Sutter O, Nahon P, Ganne-Carrié N, Séror O. Percutaneous treatment of hepatocellular carcinoma: state of the art and innovations. J Hepatol. 2018 Apr;68(4):783–97.
9 European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012 Apr;56(4):908–43.
10 de Baère T, Risse O, Kuoch V, Dromain C, Sengel C, Smayra T, et al. Adverse events during radiofrequency treatment of 582 hepatic tumors. AJR Am J Roentgenol. 2003 Sep;181(3):695–700.
11 Livraghi T, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. Radiology. 2003 Feb;226(2):441–51.
12 Kasugai H, Osaki Y, Oka H, Kudo M, Seki T. Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3, 891 ablations in 2, 614 patients. Oncology. 2007;72(Suppl 1):72–5.
13 Takaki H, Yamakado K, Nakatsuka A, Yamada T, Shiraki K, Takei Y, et al. Frequency of and risk factors for complications after liver radiofrequency ablation under CT fluoroscopic guidance in 1500 sessions: single-center experience. AJR Am J Roentgenol. 2013 Mar;200(3):658–64.
14 Ruzzenente A, Manzoni GD, Molfetta M, Pachera S, Genco B, Donataccio M, et al. Rapid progression of hepatocellular carcinoma after radiofrequency ablation. World J Gastroenterol. 2004 Apr;10(8):1137–40.
15 Sakamaki A, Kamimura K, Abe S, Tsuchiya A, Takamura M, Kawai H, et al. Spontaneous regression of hepatocellular carcinoma: a mini-review. World J Gastroenterol. 2017 Jun;23(21):3797–804.
16 Oquiñena S, Inarrairaegui M, Vila JJ, Alegre F, Zozaya JM, Sangro B. Spontaneous regression of hepatocellular carcinoma: three case reports and a categorized review of the literature. Dig Dis Sci. 2009 May;54(5):1147–53.

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DOI: 10.1159/000527229

17 Parks AL, McWhirter RM, Evason K, Kelley RK. Cases of spontaneous tumor regression in hepatobiliary cancers: implications for immunotherapy? J Gastrointest Cancer. 2015 Jun;46(2):161–5.
18 Gordan JD, Chay WY, Kelley RK, Ko AH, Choo SP, Venook AP. And what other medications are you taking? J Clin Oncol. 2011 Apr;29(11):e288–91.
19 Gao P, Lazare C, Cao CH, Meng YF, Wu P, Zhi WH, et al. Immune checkpoint inhibitors in the treatment of virus-associated cancers. J Hematol Oncol. 2019 Jun;12(1):58.
20 Bhalla N, Brooker R, Brada M. Combining immunotherapy and radiotherapy in lung cancer. J Thorac Dis. 2018 May;10(S13):S1447–60.
21 Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. JAMA Oncol. 2015 Dec;1(9):1325–32.
22 Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012 Mar;366(10):925–31.
23 Hänsler J, Neureiter D, Strobel D, Muller W, Mutter D, Bernatik T, et al. Cellular and vascular reactions in the liver to radio-frequency thermo-ablation with wet needle applicators. Eur Surg Res. 2002 Sep–Oct;34(5):357–63.
24 Wissniowski TT, Hänsler J, Neureiter D, Frieser M, Schaber S, Esslinger B, et al. Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. Cancer Res. 2003 Oct;63(19):6496–500.
25 den Brok MHMGM, Sutmuller RPM, van der Voort R, Bennink EJ, Figdor CG, Ruers TJM, et al. In situ tumor ablation creates an antigen source for the generation of antitumor immunity. Cancer Res. 2004 Jun;64(11):4024–9.
26 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 Jan;66(2):1139–46.
27 Singh P, Toom S, Avula A, Kumar V, Rahma OE. The immune modulation effect of locoregional therapies and its potential synergy with immunotherapy in hepatocellular carcinoma. J Hepatocell Carcinoma. 2020 Feb;7:11–7.
28 Tan A, Koh S, Bertoletti A. Immune response in hepatitis B virus infection. Cold Spring Harb Perspect Med. 2015 Jul;5(8):a021428.