

Intratumoral Bicarbonate Functions as an Adjuvant to Potentiate PD-1 Blockade in Hepatocellular Carcinoma

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Abstract

AntiPD-1 immunotherapy improves survival in advanced hepatocellular carcinoma (HCC), but responses remain limited by the immunosuppressive, acidic tumor microenvironment (TME). We investigated whether intratumoral alkalization with sodium bicarbonate could enhance PD-1 blockade. Bicarbonate-induced intracellular alkalization disrupted mitochondrial membrane potential, triggered rupture, and activated the cGAS–STING pathway via cytosolic mitochondrial DNA release, while simultaneously inducing immunogenic cell death (ICD). In murine models, intratumoral bicarbonate recruited and activated dendritic cells and T cells, suppressing tumor growth and synergizing with antiPD-1 therapy. In a prospective clinical study, 28 patients with advanced-stage and 2 with intermediate-stage HCC received Tislelizumab plus intratumoral 5% sodium bicarbonate. The objective response rate was 93.3% (CR 53.3%, PR 40.0%); median progression-free survival was 31 months, and median overall survival was not reached. Treatment was well tolerated. Biopsies revealed significantly increased CD3⁺, CD4⁺, and CD8⁺ T-cell infiltration with combination therapy versus Tislelizumab alone. In line with our previous work, through this mitochondria-centered mechanism bicarbonate links metabolic reprogramming with innate and adaptive immune activation. Thus, intratumoral bicarbonate functions as a safe and accessible immunometabolic adjuvant that markedly enhances PD-1 blockade efficacy in HCC.

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, with limited options for advanced-stage disease (1, 2). Immune checkpoint inhibitors (ICIs), particularly antiPD-1/PD-L1 antibodies, have transformed treatment but benefit only a minority of patients, with landmark trials reporting objective response rates (ORRs) of 15–20% (3, 4).

Resistance to ICIs arises from an immunosuppressive tumor microenvironment (TME) marked by poor T-cell infiltration, upregulation of alternative checkpoints, and oncogenic pathway activation (5–8). Etiological heterogeneity also shapes immune responses; non-viral HCC often displays a “cold” TME enriched with regulatory T cells and myeloid-derived suppressor cells, contributing to primary resistance, while tumor immunoediting drives acquired resistance(9, 10).

Bicarbonate (HCO_3^-) is a fundamental physiological buffer that maintains systemic acid–base balance and contributes to extracellular and intracellular pH homeostasis. It also participates in ion exchange across membranes, influences membrane potential, and regulates cellular metabolism through its tight coupling with CO_2 hydration and transport. In normal tissues, bicarbonate helps preserve neutral pH in the extracellular milieu; however, solid tumors are typically immersed in an acidic interstitial environment, where glucose-driven lactic acidosis depletes available bicarbonate and suppresses effective immune surveillance. As a result, the equilibrium of acid–base balance is substantially altered, creating a hostile microenvironment that favors tumor progression and immune evasion.

Acidic intratumoral pH, largely due to lactic acidosis, promotes tumor progression and immune suppression. Bicarbonate therapy can alkalize tumor pH, suppress invasion and metastasis, and has shown safety in predictive models and preclinical studies (11, 12). We previously demonstrated that bicarbonate neutralizes lactic acidosis and markedly enhances the efficacy of transarterial chemoembolization in HCC (13–15). Specifically, we conducted both a retrospective controlled study and a randomized controlled trial (RCT) (13), with sample sizes determined by rigorous power analysis. In both studies, bicarbonate-integrated TACE (TILA-TACE) achieved an ORR of 100%, compared to 44.4% in the cTACE group of the retrospective cohort and 63.6% in the RCT. These findings were further validated in a large real-world study enrolling 413 patients, which showed an ORR of 99.01% for intrahepatic tumors, including 72.8% complete responses, with a median survival of 27 months in advanced-stage patients and a favorable safety profile(15). Collectively, these results establish neutralizing intratumoral lactic acidosis as a highly effective, safe, and translatable strategy for local control of HCC.

These findings prompted us to investigate whether bicarbonate could also enhance immunotherapy, acting as an immunometabolic adjuvant. Our previous work established that bicarbonate-induced alkalization of intracellular pH (pHi) actively disrupts cancer cell survival by collapsing mitochondrial oxidative phosphorylation and impairing autophagic flux(16). Building on this metabolic foundation, the current study demonstrates that bicarbonate-mediated pHi alkalization, by disrupting mitochondrial structure and function, activates cGAS–STING signaling and induces immunogenic cell death (ICD). Through this mitochondria-centered mechanism, bicarbonate links metabolic reprogramming with innate and adaptive immune activation, thereby reshaping the TME to favor immune infiltration and markedly enhancing antiPD-1 efficacy in murine HCC models. In a prospective clinical study, intratumoral bicarbonate combined with Tislelizumab achieved a 93.3% ORR, including 53.3% complete responses, supporting its potential as a clinically safe immunometabolic adjuvant in HCC (Fig. 1).

Materials and Methods

1. Cell lines and animals

Cell lines, including SK-HEP-1 (RRID:CVCL_0525), H22 (RRID:CVCL_H613), CT26 (RRID:CVCL_7254) and 4T1 (RRID:CVCL_0125), were purchased from the Cell Bank of the Shanghai Academy of Chinese Sciences. All the cell lines were maintained in RPMI-1640 medium (Gibco BRL, Rockville, MD) supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin and 10% fetal bovine serum (FBS; BioInd, Israel) and incubated at 37°C in a 5% humidified CO₂ atmosphere.

Six-week female BALB/c mice (RRID:MGI:2161072) we used were purchased from SLAC Laboratory Animal Co., Ltd (Shanghai, China), and housed in Zhejiang Chinese Medical University Laboratory Animal Research Center.

2. Cell-Counting Kit-8 (CCK-8) assay

To assess the cell viability under different concentrations of bicarbonate and IFN-I, cells were cultured in complete medium containing different concentrations of bicarbonate and IFN-I for the indicated time. Then, the cells were incubated with fresh medium containing 10% CCK-8 solution (Boster Bio, China) per well for 1 h at 37°C. The Spectramax i3 (RRID:SCR_026346, Molecular Devices, USA) was used to measure the optical density of the mixture at 450 nm.

3. Detection of IFN-I secretion with ELISA

The cells were placed in a 24-well plate (3×10^5 cells per well) for 24 h, Then, cells were incubated with 300 μ L of complete medium containing different concentrations of bicarbonate for the indicated time. Centrifuge the culture medium to collect the supernatant, and measure the IFN-I in the supernatant using IFN- β ELISA Kit (Proteintech, Cat# KE00195, China) and IFN- α 2a ELISA Kit (Proteintech, Cat# KE00207, China) according to manufacturer's instructions.

4. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells via TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Reverse transcription (RT) and quantitative PCR (qPCR) were performed via an Evo M-MLV RT Master Mix Kit (Accurate, China) and a Hieff qPCR SYBR Green Master Mix Kit (Yeasen, China) according to the manufacturers' instructions. Typically, amplification reactions were performed in a 10 μ L reaction system containing 2 $\times$ SYBR Green Premix, a 0.2 μ M primer mixture and moderate cDNA diluted with ddH₂O. The thermal cycling profiles for qRT-PCR included heating at 95°C for 30 s followed by 40 cycles at 95°C for 5 s and 60°C for 30 s, and the cDNA copy number was monitored via quantitative analysis of fluorescence emissions via a Roche LightCycler 480 II PCR instrument (RRID:SCR_018626, Basel, Switzerland). The threshold cycle (Ct) represents the refraction cycle number at which a positive amplification reaction was measured. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard control. Expression was quantified via the $2^{-\Delta\Delta Ct}$ method. The sequences of primers were shown in Table S1.

5. Western blotting (WB)

The cells were placed in a 6 cm dish (1×10^6 cells per well) for 24 h. After induction of bicarbonate or IFN-I for the indicated time and dose, the cells were harvested for WB analysis as follows. The treated cells were washed with PBS and lysed in M-PER lysis buffer (Thermo Fisher, USA) with phosphatase inhibitor cocktail and protease inhibitor cocktail (Sigma-Aldrich, Germany). The lysate samples were subsequently centrifuged at 12,000 $\times$ g for 15 min at 4°C. A BCA protein assay kit (Beyotime, China) was subsequently used to determine protein concentrations. Next, all the samples were electrophoresed through a 10% Bis-Tris polyacrylamide gel. After that, the protein in the gel was transferred to a 0.45 μ m polyvinylidene fluoride (PVDF) membrane (Millipore, USA). The PVDF membranes were blocked with TBST buffer containing 5% skim milk powder and incubated with the corresponding primary antibodies at 4°C overnight. The information of primary antibodies was shown in Table S2. The membrane was subsequently hybridized with an HRP-conjugated secondary antibody (Cat# D110058, RRID:AB_2940954

and Cat# D110087, RRID:AB_2940948; Sangon, China) for 60 min at room temperature with gentle rocking. An imaging system (Tanon 5200 Series Automatic Image Analysis System, RRID:SCR_027114, China) with a chemiluminescent ECL substrate (Fudebio, Cat# FD8030, China) was used to detect fluorescence signals on the PVDF membranes, and grayscale values were quantified via ImageJ (RRID:SCR_003070).

6. Immunofluorescence (IF)

After incubated with bicarbonate for the indicated time and dose, the cells were fixed with 4% polymethanol for 15 min, permeabilized with 0.2% triton X-100 for 15 min and blocked with 5% goat serum for 1 h. Then, the cells were incubated with primary antibodies at 4°C overnight. After that, the cells were incubated with coralite488 secondary antibodies (Cat# SA00013-2, RRID:AB_2797132 and Cat# SA00013-1, RRID:AB_2810983; Proteintech, China) at 37°C for 1 h. Nuclei were stained with DAPI (Beyotime, China). Images were acquired via confocal microscopy (RRID:SCR_018063, Zeiss, Germany).

7. mtDNA detection via IF and PCR

After incubated with bicarbonate for the indicated time and dose, cytosolic mtDNA was analyzed by IF and PCR. For IF, after incubated with Mito-Tracker Red CMXRos (Thermo Fisher, USA) for 20 min, the cells were fixed, permeabilized and blocked as described above. Then, the cells were incubated with anti-dsDNA primary antibody (Cat# ab27156, RRID:AB_470907, Abcam, USA) at 4°C overnight. After washed with PBS, the cells were incubated with coralite488-Goat Anti-Mouse secondary antibody (Cat# SA00013-1, RRID:AB_2810983, Proteintech, China) at 37°C for 1 h. Images were acquired via confocal microscopy (RRID:SCR_018063, Zeiss, Germany).

Cytosolic mtDNA was analyzed by general PCR as other studies described (17–20). Briefly, half of the cells were lysed by mild lysis buffer, and the other half was lysed by strong lysis buffer. Cells were cytoplasmically lysed with 0.1% NP-40 for 20 min on ice and centrifuged at $14,000 \times g$ for 20 min at 4°C. Cytosolic mtDNA from the supernatant cytosolic fraction and total mtDNA from the total lysis were purified by a QIAamp DNA micro Kit (QIAGEN, USA) according to manufacturer's instructions. The sequences of primers were shown in Table S1. General PCR followed by agar gelatin electrophoresis were used for DNA analysis normalized by the mtDNA in the total lysate.

8. Surface-exposed CRT detection

CRT on cell membrane was detected via flow cytometric (FC). Cells were harvested with trypsin. After washed with PBS, cells were fixed with 1% polymethanol for 10 min and washed twice. Then cells were blocked with 5% BSA for 30 min and incubated with anti-CRT antibody (Cat# 27298-1-AP, RRID: AB_2880835, Proteintech, China) at 37°C for 45 min. Next, cells were incubated with coralite488-Goat Anti-Rabbit secondary antibody (Cat# SA00013-2, RRID:AB_2797132, Proteintech, China) at 37°C for 45 min, and detected with a DxFLEX flow cytometer (RRID:SCR_027097, Beckman, USA).

9. Measurement of extracellular ATP

The cells were placed in 24-well plates (3×10^5 cells per well) with 1 mL of complete medium for 24 h. Then, cells were incubated with 300 μ L of complete medium containing different concentrations of bicarbonate for the indicated time. Centrifuge the culture medium to collect the supernatant, and measure the ATP in the supernatant using the luciferase-based Enliten ATP Assay (Promega, Cat# FF2000, USA) according to manufacturer's instructions.

10. Animal models

HCC and CRC xenograft models were developed via the subcutaneous injection of H22 cells and CT26 cells (5×10^5 cells per mouse) respectively into the left flank of mice. Breast tumor xenograft models were developed via injection of 4T1 cells (1×10^5 cells per mouse) into the mice mammary fat pad. When tumor size reached 4 to 6 mm in the largest diameter, mice were randomized to the experimental groups. Mice were given 200 mM bicarbonate in PBS intratumorally or/and 10mg/kg antiPD-1 monoclonal antibody (Cat# BE0146, RRID: AB_10949053, bio X cell, USA) in PBS i.p. every 2 days. The dosage of bicarbonate was calculated based on 50% of the tumor volume. The volume of the tumors was measured and calculated every 2 days according to $\pi/6 \times (\text{length} \times \text{width}^2)$. After treatment, the animals were sacrificed, and tumor tissues were collected for further analysis.

11. Histological analysis

The tumor tissues were fixed in 4% neutral buffered formalin for immunohistochemical (IHC) staining. To block endogenous peroxidase activity, the paraffin embedded tissue sections were incubated with 3% H_2O_2 for 20 min at room temperature. Then, 10% goat serum was used to block the sections for 30 min at room temperature. Next, the sections were incubated with different primary antibodies overnight at 4°C. The information of primary antibodies is shown in Table S2. Then, the sections were washed with PBS. After that, the sections were incubated with secondary antibody for 1 h at room temperature, and then incubated with DAB. After hematoxylin staining, the sections were observed via a microscope.

12. Prospective clinical study of combining hepatic arterial bicarbonate infusion with Tislelizumab in HCC patients

(1) Patients

Between November 2021 and May 2023, a total of 30 patients with HCC who were treated at the Second Affiliated Hospital, Zhejiang University School of Medicine were enrolled in this study. The study workflow is illustrated in Figure S1. The clinical characteristics of the patients are summarized in Table S3.

Patients were included based on the following specific criteria: (a) diagnosis of HCC confirmed by imaging or histopathology in accordance with the EASL clinical practice guidelines; (b) receipt of at least one cycle of hepatic arterial infusion of 5% sodium bicarbonate solution combined with anti-PD-1 immunotherapy; (c) no prior or concurrent cancer-related treatments before or during enrollment; (d)

Barcelona Clinic Liver Cancer (BCLC) stage B or C; (e) Child-Pugh class A liver function; (f) age > 18 years; (g) no other confirmed malignancies; and (h) availability of complete medical records and follow-up data. All laboratory serum data were collected within 7 days prior to the initial treatment. Radiological evaluations, including dynamic contrast-enhanced magnetic resonance imaging (MRI), were performed within one week before the initial treatment.

(2) Method of Hepatic Arterial Infusion of Sodium Bicarbonate Solution

The general principle of this procedure was to identify all tumor-feeding arteries and to fully perfuse the tumor with 5% sodium bicarbonate injection, thereby alkalinizing the local tumor microenvironment. The procedure was as follows: (a) Identify all tumor-feeding arteries (including extrahepatic arteries) using digital subtraction angiography (DSA) combined with C-arm CT; (b) embolize the extrahepatic tumor-feeding arteries with a mixture of iodized oil and PVA microspheres to ensure that tumor blood supply was completely redirected to the hepatic artery; (c) allocate the volume of 5% sodium bicarbonate injection to each tumor-feeding artery based on the supply area, with a total volume limited to within 300 mL per treatment session; (d) infuse the 5% sodium bicarbonate solution using a pulsatile technique (infusion rate: 1 to 1.5 mL/s), ensuring that the total infusion time for each artery exceeded 10 minutes; (e) if the tumor burden was high (diameter > 8 cm) or the tumor response after the previous bicarbonate infusion was unsatisfactory, a microcatheter was left in the hepatic artery on the affected side, allowing for an additional 200 mL of 5% sodium bicarbonate solution to be administered via micro-pump on the following day (infusion rate: 100 mL/h); (f) finally, arterial blood gas analysis was performed before and after treatment to ensure that metabolic alkalosis did not occur.

(3) Immunotherapy

Within 3 days prior to the hepatic arterial infusion of sodium bicarbonate solution, patients received anti-PD-1 antibody therapy. Anti-PD-1 antibody was administered intravenously at a standard dose (Tislelizumab injection, 200 mg, every three weeks).

(4) Discontinuation of Treatment and Other Therapies

Treatment discontinuation was determined based on disease progression, intolerable toxicity, patient withdrawal of consent, or changes in the treatment plan. For patients who showed suboptimal response after three cycles of the study protocol (hepatic arterial infusion of 5% sodium bicarbonate solution combined with anti-PD-1 antibody immunotherapy), additional local treatments such as TACE, HAIC, SBRT, or other systemic therapies could be administered to maximize patient benefit. However, for efficacy analysis, only outcomes after the first three cycles were included.

(5) Follow-up Schedule

After treatment initiation, contrast-enhanced MRI was performed every 4–6 weeks. Routine follow-up was conducted at intervals of 1–2 months. During this period, tumor viability was assessed using MRI or dynamic contrast-enhanced CT, alongside tumor-related biomarkers, liver and kidney function tests, and

routine blood tests. These assessments determined the need for further treatment. The final follow-up was completed on April 30, 2025.

(6) Efficacy Assessment

Tumor response was independently assessed by two radiology experts.

At day 30 after the first treatment and at subsequent follow-up visits, tumor response was evaluated using the modified Response Evaluation Criteria in Solid Tumors (mRECIST), with definitions as follows: complete response (CR) indicated complete tumor necrosis; partial response (PR) indicated a reduction in the viable portion of the tumor by $\geq 30\%$; stable disease (SD) referred to cases not meeting the criteria for PR or progressive disease (PD); and progressive disease (PD) was defined as an increase in viable tumor by $\geq 20\%$ or the appearance of new lesions.

(7) Overall Survival (OS)

OS was measured from the initiation of treatment until death from any cause or the last follow-up. The median follow-up duration was 24.5 months, ranging from 5 to 41 months (calculated from the first treatment to death from any cause or the last follow-up).

(8) Study Endpoints

The primary endpoint was the objective response rate (ORR), which was evaluated according to mRECIST. ORR was defined as the sum of complete response (CR) and partial response (PR). Disease control rate (DCR) was defined as ORR plus stable disease (SD). Secondary endpoints included progression-free survival (PFS) and OS.

(9) Treatment-Related Adverse Events

Monitored adverse events included fever, abdominal pain, nausea and vomiting, skin ulceration, liver enzyme abnormalities, bilirubin-related abnormalities, and immune-related adverse events such as immune-related pneumonitis and dermatitis. These adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, and all adverse events received timely symptomatic treatment.

13. Retrospective clinical study

We retrospectively collected clinical information, MR images and pathological sections of patients with liver cancer in the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, Zhejiang, China), during the year 2021–2024. The inclusion criteria for patients are as follows: patients with HCC diagnosed by pathological histology or who meet the clinical diagnostic criteria for HCC; at least one measurable intrahepatic lesion; adult patients aged 20 years or older. The exclusion criteria are: incomplete important clinical information; failure to follow up at the Second Affiliated Hospital, Zhejiang University School of Medicine; participation in other clinical trials; having autoimmune diseases

or immunodeficiency disorders; a history of organ transplantation or preparing for organ transplantation; currently using corticosteroids or other immunomodulatory drugs; other untreated cancer diseases; prior use of other treatments for liver cancer before enrollment in the study; receiving PD-1 monoclonal antibody or intra-arterial infusion of bicarbonate treatment simultaneously with other local or systemic therapies; other factors unsuitable for inclusion. The pathological slides were stained and provided by the Department of Pathology, the Second Affiliated Hospital, Zhejiang University School of Medicine. The assessment of treatment efficacy and the tumors response to the treatment was conducted by professional clinicians based on the mRECIST guideline.

14. Statistical analysis

The data were presented as the means $\pm$ standard deviations. Data in two groups were compared by two-tailed Student's t-tests. The repeated measures ANOVA (Two-way ANOVA) was used to analyze tumor volume changes *in vivo*. One-way ANOVA was used to compare data in multiple groups. GraphPad Prism 8 (RRID:SCR_002798) software was used for statistical analysis. The experimental group was the independent factor. Statistical significance was set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In prospective clinical study of combining hepatic arterial bicarbonate infusion with Tislelizumab in HCC patients, all statistical analyses were performed using R software (version 4.2.2, R Foundation for Statistical Computing, RRID:SCR_001905, Vienna, Austria), along with the use of MSTAT software (<https://www.mstata.com/>). The 95% CI for categorical variables was calculated using the Wilson method. Survival curves were estimated using the Kaplan-Meier method, and comparisons between survival curves were assessed using the log-rank test. A p-value of less than 0.05 was considered statistically significant.

Results

Bicarbonate upregulates IFN-I expression in SK-HEP-1 cells

We previously established that 20–200 mM bicarbonate increases pHi from 7.4 to 8.5 in SK-HEP-1 cells (16). To assess whether this alkalization activates cGAS/STING signaling, we measured type I interferon (IFN-I) expression, a downstream pathway readout.

SK-HEP-1 cells were treated with 20–200 mM bicarbonate, and culture supernatants were analyzed for IFN-I levels. Treatment with ≥ 80 mM bicarbonate significantly increased IFN- α and IFN- β secretion (Fig. 2A, B) and upregulated their transcripts (Fig. 2C, D).

These findings indicate that bicarbonate at 80–200 mM induces robust IFN-I production, suggesting cGAS/STING pathway activation.

Bicarbonate induces mtDNA leakage and activates the STING/TBK1/IRF3 pathway

We previously showed that pH_i alkalization triggers mitochondrial permeability transition pore (MPTP) opening, swelling, and rupture (16). Here, treatment of SK-HEP-1 cells with 120 mM bicarbonate caused marked mitochondrial swelling by electron microscopy (Fig. 2E), consistent with increased mitochondrial permeability.

Given that MPTP opening can cause cytosolic release of mitochondrial DNA (mtDNA) (16, 21), we hypothesized that bicarbonate-induced IFN-I production results from mtDNA leakage activating cGAS/STING signaling. Indeed, it was confirmed that 120 mM bicarbonate (4 h, cell viability $\leq$ 50%) increased cytosolic mtDNA levels via semi-quantitative PCR (Fig. 2F–H) and cytosolic DNA fluorescence staining (Fig. 2I).

We next examined pathway activation. Immunofluorescence revealed increased nuclear localization of IRF3 after 80 or 120 mM bicarbonate treatment (Fig. 2J, K). Western blotting showed elevated phosphorylation of STING, TBK1 and IRF3 in 120 mM–treated cells (Fig. 2L, M).

These findings indicated that bicarbonate-induced mitochondrial permeability promoted mtDNA leakage into the cytosol, thereby activating the cGAS/STING/TBK1/IRF3 pathway.

Bicarbonate induces IFN-I response via the mtDNA/cGAS/STING/TBK1/IRF3 pathway

To confirm that bicarbonate activates cGAS/STING signaling through mitochondrial swelling and mtDNA leakage, we impaired MPTP function by knocking down Cyclophilin D (CYPD) in SK-HEP-1 cells. Both CYPD wild-type (WT) and knockdown (KD) cells were treated with 120 mM bicarbonate.

Electron microscopy showed that bicarbonate-induced mitochondrial swelling was markedly reduced in CYPD KD cells (Fig. 3A). PCR and immunofluorescence analyses revealed significant increases in cytosolic mtDNA in CYPD WT but not KD cells after 120 mM bicarbonate treatment, compared to 20 mM (Fig. 3B–F).

Western blotting demonstrated strong phosphorylation of STING, TBK1, and IRF3 in CYPD WT cells after 120 mM bicarbonate treatment, with minimal changes in CYPD KD cells (Fig. 3G–I).

Immunofluorescence further showed enhanced nuclear translocation of IRF3 in WT but not KD cells (Fig. 3J, K).

Together with Fig. 2 findings, these results indicated that high-concentration bicarbonate induced mitochondrial swelling and mtDNA leakage in a CYPD-dependent manner, activating the cGAS/STING/TBK1/IRF3 pathway and driving IFN- α/β expression and secretion, thereby eliciting an IFN-I response in tumor cells (Fig. 3L).

Bicarbonate induces immunogenic cell death (ICD) in tumor cells

ICD is a form of regulated cell death that promotes antitumor immunity, characterized by surface exposure of calreticulin (CRT), extracellular release of ATP, secretion of IFN-I, and translocation of HMGB1 from the nucleus to the cytoplasm and extracellular space (22–27). Mitochondrial dysfunction is known to trigger ATP release, ER stress, and CRT exposure, all of which contribute to ICD (28).

Given that bicarbonate disrupts mitochondrial function and promotes IFN-I secretion in liver cancer cells, we tested whether it induces ICD across multiple tumor types. Breast cancer (4T1), colorectal cancer (CT26), and HCC (SK-HEP-1) cells were treated with bicarbonate.

Bicarbonate at 120–200 mM significantly increased IFN- β secretion in 4T1 and CT26 cells (Fig. 4A, B). It also elevated ATP release in SK-HEP-1, 4T1, and CT26 cells (Fig. 4C–E). Flow cytometry revealed increased CRT surface exposure at 80–120 mM bicarbonate in all three cell types (Fig. 4F–K). Additionally, 120 mM bicarbonate reduced nuclear HMGB1 and increased cytoplasmic HMGB1, indicating HMGB1 translocation (Fig. 4L–N).

These results demonstrate that bicarbonate induces hallmark ICD features in multiple tumor models, suggesting broad immunostimulatory potential.

Local injection of bicarbonate inhibits tumor growth and enhances anti-PD-1 immunotherapy

Given our *in vitro* findings that bicarbonate induces ICD and activates cGAS/STING signaling, we next examined its effects *in vivo*.

(1) Bicarbonate alone suppresses tumor growth and promotes immune cell infiltration

In an orthotopic 4T1 breast cancer model, mice bearing 4–6 mm tumors received intratumoral injections of either PBS or 200 mM bicarbonate every other day. Bicarbonate significantly slowed tumor growth and reduced tumor weight compared with PBS (Fig. S2A–C). Tumors from bicarbonate-treated mice exhibited elevated phosphorylation of STING, TBK1, and IRF3, along with increased IFN- α expression (Fig. S2D–F).

Mechanistically, IFN-I, ATP, HMGB1, and CRT induced by bicarbonate can collectively recruit and activate immune cells: IFN-I upregulates CXCL10/CCL5, ATP acts as a “find-me” signal for DCs, HMGB1 promotes DCs and T cell migration, and CRT exposure facilitates phagocytosis and antigen presentation(28–30). Consistent with this, IHC revealed significantly higher CD4⁺ and CD8⁺ T cell infiltration in bicarbonate-treated tumors (Fig. S2G–J).

These results indicate that intratumoral bicarbonate induces ICD, activates the cGAS/STING/TBK1/IRF3 pathway, and enhances immune cell infiltration into tumors.

(2) Bicarbonate synergizes with antiPD-1 therapy

While antiPD-1 antibodies restore T cell function by blocking PD-1/PD-L1 signaling, they do not directly increase immune cell infiltration—limiting efficacy in tumors with insufficient baseline immune infiltration. We hypothesized that bicarbonate could overcome this limitation and synergize with antiPD-1 therapy.

Murine models of breast (4T1), liver (H22), and colon (CT26) cancer were established, and mice were randomized to PBS, bicarbonate, antiPD-1, or combination treatment. In all models, bicarbonate or antiPD-1 monotherapy significantly slowed tumor growth versus PBS, but combination therapy achieved the greatest suppression (Fig. 5A–C). In the breast cancer model, combination therapy led to tumor shrinkage in six mice and complete regression in one, whereas antiPD-1 alone caused shrinkage in only one mouse (Fig. S3A). Tumor weight analysis showed the same pattern (Fig. 5D–I).

Western blotting confirmed increased phosphorylation of STING, TBK1, and IRF3 in the bicarbonate and combination groups, but not in the antiPD-1 group (Fig. 5J–L), supporting bicarbonate's role in pathway activation. Immune profiling revealed that combination therapy produced the highest infiltration of CD11c⁺ DCs (Fig. 5M–O; Fig. S3B; Fig. S4A; Fig. S5A) and CD8⁺/CD4⁺ T cells (Fig. 5P–U; Fig. S3C, D; Fig. S4B, C; Fig. S5B, C) across all tumor models. Bicarbonate alone increased infiltration compared with PBS, whereas antiPD-1 alone did not significantly increase DC infiltration (Fig. 5M - O; Fig. S3B; Fig. S4A; Fig. S5A).

Together, these findings indicated that bicarbonate enhanced immune cell recruitment into the tumor microenvironment, while antiPD-1 relieves T cell functional suppression. The complementary mechanisms produce superior antitumor effects when used in combination.

Translation to Clinical Application

Building on the preclinical findings, we investigated whether the immunomodulatory effects of bicarbonate—mitochondrial disruption, cGAS/STING/IFN-I activation, induction of ICD, and enhanced T-cell infiltration—could be reproduced in patients. The observed synergy between bicarbonate and antiPD-1 antibodies in animal models provided a strong rationale for clinical translation in HCC, where immune checkpoint blockade alone yields limited benefit. We therefore conducted a prospective study evaluating hepatic arterial bicarbonate infusion combined with antiPD-1 therapy (Tislelizumab) in intermediate to advanced HCC.

(1) Baseline Characteristics

From November 2021 to May 2023, 30 patients were enrolled (median age: 59 years; range 26–85; 76.7% male). HBV infection was the predominant etiology (90%), and 96.7% had Child–Pugh class A liver function. AFP exceeded 400 ng/mL in 66.7% of patients. Most tumors were large (≥ 5 cm in 100%; >10 cm in 63.3%) and advanced (BCLC stage C in 93.3%), with major vascular invasion in 93.3% and extrahepatic metastases in 13.3% (Table S3).

(2) Objective Response Rate

The combination therapy achieved an ORR of 93.3%, with CR in 53.3% and PR in 40.0% of patients (Table S4; Fig. 6A). Six patients (20%) underwent conversion surgery. MRI examples illustrate marked regression of both intrahepatic tumors and vascular tumor thrombi (Fig. 6B–F).

(3) Survival Outcomes

At data cutoff (April 2025), median OS was not reached; estimated 1-, 2-, and 3-year OS rates were 82%, 60%, and 53% (Table S5; Fig. 7A). Median PFS was 31 months, with 1- and 2-year PFS rates both at 55% (Table S6; Fig. 7B).

Subgroup analyses revealed better outcomes in patients with AFP ≤ 400 ng/mL (3-year OS 90% vs. 35%, $P = 0.012$; 2-year PFS 100% vs. 35%, $P = 0.0035$) and in those with single lesions (3-year OS 87% vs. 13%, $P = 0.00024$; 2-year PFS 83% vs. 28%, $P = 0.0024$) (Fig. 7C–F; Table S5 and S6). No significant differences were observed by age, tumor size, or tumor thrombus status (Table S5 and S6).

(4) Safety

All patients received ≥ 1 treatment cycle (median: 3; range: 1–7). Adverse events, mainly tumor necrosis–related (abdominal pain, fever, transient liver enzyme/bilirubin elevation), were manageable (Table S7). Child–Pugh classification remained stable in all patients.

Retrospective Analysis of Immunological Correlates

To explore immunological mechanisms underlying clinical response, we conducted a separate retrospective analysis of resected tumor specimens from three independent patient cohorts treated at our center during the same period:

- Combination group: 6 patients from the prospective trial who underwent conversion surgery after bicarbonate + antiPD-1 therapy.
- AntiPD-1 group: 8 patients who received Tislelizumab monotherapy before surgery among a total of 26 patients treated with Tislelizumab (2 CR, 4 PR, 2 SD).
- Surgery-only group: 11 patients undergoing resection without prior therapy.

MRI evaluation (mRECIST) showed higher CR rates in the combination group (66.7%) than in the antiPD-1 group (25%). The pathology showed that all combination therapy cases had tumor death, whereas 50% of the cases in the monotherapy group did not exhibit tumor death (Fig. 8A, B; Fig. S6 – S9).

IHC analysis of tumors containing viable cancer cells (antiPD-1 group, n = 7; combination group, n = 5; surgery group, n = 11) demonstrated significantly greater infiltration of CD3⁺, CD4⁺, and CD8⁺ T cells in the combination group compared to both monotherapy and surgery groups. No significant difference was seen between antiPD-1 monotherapy and surgery-only groups (Fig. 8C–F; Fig. S6 – S9).

Discussion

Our study indicates that bicarbonate fulfills the dual criteria of an immunometabolic adjuvant: (i) reprogramming tumor metabolism by disrupting mitochondrial and autophagic homeostasis(16), and (ii) simultaneously potentiating innate and adaptive immunity by coupling cGAS/STING activation with ICD. This dual metabolic–immunologic action distinguishes bicarbonate from conventional metabolic modulators or immune adjuvants and provides a compelling rationale for its development as a safe, accessible, and broadly applicable strategy to enhance immune checkpoint blockade, as further described below.

Our previous work provided the first mechanistic evidence that alkalization of intracellular pH (pHi) is not merely a buffering phenomenon but an active metabolic intervention that destabilizes cancer cell survival pathways(16). Specifically, bicarbonate-mediated alkalization reduced the mitochondrial pH gradient (ΔpH), membrane potential ($\Delta\Psi\text{m}$), and proton motive force (Δp), leading to collapse of oxidative phosphorylation (OXPHOS)(16). The resulting energy deficit elevated intracellular AMP and activated classical AMPK-mediated autophagy. However, this autophagic response was ultimately blocked by the alkalized lysosomal environment, impaired proton pump function, and reduced OXPHOS, resulting in defective autophagic flux(16). Simultaneously, bicarbonate induced persistent mitochondrial permeability transition (MPT) and mitochondrial damage, collectively driving tumor cell death(16). These findings established that bicarbonate-induced pHi modulation directly reprograms cellular metabolism and compromises mitochondrial integrity.

Building on this foundation, the current study demonstrates that the same mitochondrial disruption links metabolism to immunity. Bicarbonate-induced mitochondrial rupture leads to cytosolic leakage of mitochondrial DNA, which activates the cGAS/STING/TBK1/IRF3 axis and type I interferon production, a central driver of innate immunity. In parallel, mitochondrial dysfunction triggered by pHi alkalization promotes the hallmarks of ICD, including ATP and HMGB1 release and CRT exposure. Together, cGAS/STING activation and ICD generate complementary proinflammatory cues that recruit and activate DCs while simultaneously promoting T cell infiltration and priming. These processes reprogram the tumor microenvironment from immune-excluded to immune-infiltrated. In murine models, these effects translated into suppressed tumor growth and robust synergy with PD-1 blockade. In patients, intratumoral bicarbonate significantly increased T cell infiltration and markedly improved clinical responses to antiPD-1 therapy.

Clinically, the combination of hepatic arterial bicarbonate infusion and Tislelizumab achieved an ORR of 93.3% in 30 patients, with 53.3% CR—substantially higher than historical outcomes for antiPD-1

monotherapy (ORR 15–17%, CR ~ 1%) and some combination regimens (ORR ~ 20–46%)(31–34). The median PFS was 31 months, and the estimated 2-year and 3-year OS rates were 60% and 53%, respectively, comparing favorably with published benchmarks(35–39). Immunohistochemical analyses revealed significantly increased intratumoral CD3⁺, CD4⁺, and CD8⁺ T cells with combination therapy, whereas antiPD-1 alone did not significantly alter infiltration, underscoring bicarbonate's immunomodulatory role.

Importantly, the magnitude of clinical benefit observed is unlikely to be explained by chance. AntiPD-1 monotherapy achieves an ORR of ~ 20% in HCC, yet in our study 28 of 30 patients responded (93.3%). Under a binomial model, the probability of observing ≥ 28 responses if the true rate were 20% is 7.6×10^{-18} , essentially zero. The observed ORR corresponds to a 95% CI of ~ 78.7–98.2%, far above historical benchmarks, and implies nearly a fivefold higher response probability compared to antiPD-1 alone. This effect size strongly supports a genuine treatment benefit and provides a clear rationale for randomized validation.

Subgroup analyses suggested that patients with AFP ≤ 400 ng/mL or a single intrahepatic lesion derived the greatest benefit, consistent with prior prognostic associations. Notably, meaningful responses were also observed in patients with traditionally high-risk features, including large tumors (> 10 cm) and vascular invasion, suggesting that bicarbonate may mitigate resistance mechanisms in aggressive disease.

The combination therapy was well tolerated, with most adverse events being mild to moderate and attributable to tumor necrosis. No significant deterioration in liver function was observed, and the safety profile was consistent with that of PD-1 inhibitors and intra-arterial therapies.

Another basis for selectivity is the stark architectural difference between normal liver and tumor tissue. Normal hepatic parenchyma features an organized portal triad (portal vein, hepatic artery, and bile duct) with fenestrated sinusoids, intact lymphatics, and efficient perfusion–drainage balance. By contrast, HCC nodules lack a normal portal triad and exhibit chaotic, hyperpermeable microvessels, elevated interstitial fluid pressure, sparse or nonfunctional lymphatics, and dense, remodeled matrix—hallmarks of the “abnormal tumor vasculature” phenotype(40–46). These features impair convective clearance and favor retention of small buffers such as bicarbonate within the tumor interstitium, enabling more durable neutralization of lactic acidosis in tumors than in adjacent liver. The resultant localized alkalization intensifies mitochondrial and immunogenic stress in cancer cells while sparing normal parenchyma, providing a mechanistic basis for the observed therapeutic window(47, 48).

Our findings align with prior evidence that an acidic tumor microenvironment suppresses antitumor immunity and limits therapeutic efficacy. However, while most interventions aim to buffer extracellular acidity to support immune function, our approach targets intracellular alkalization within tumor cells, triggering mitochondrial and immunogenic stress. This mechanistic distinction may underlie the potency observed in our models and clinical cohort, and highlights bicarbonate as a unique immunometabolic adjuvant.

Limitations of this study include its single-arm, single-center design and modest sample size. While the magnitude and durability of responses are encouraging, randomized, multi-center trials are needed to confirm efficacy, define optimal dosing and delivery, and identify predictive biomarkers. Furthermore, whether this strategy is effective across other tumor types and in combination with additional immunotherapies remains to be explored.

Conclusion

Our integrated mechanistic, preclinical, and clinical findings establish bicarbonate as an immunometabolic adjuvant that reprograms tumor metabolism and immunity, markedly enhancing PD-1 blockade efficacy in HCC. By coupling mitochondrial disruption with innate and adaptive immune activation, bicarbonate offers a simple, cost-effective, and readily translatable strategy to boost cancer immunotherapy. These results support advancing bicarbonate-based immunometabolic adjuvants into randomized multicenter trials and exploring their potential across diverse cancer types.

Declarations

Ethics

The animal experiments in our study were reviewed and approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (Approval number: IACUC-20230918-11).

The clinical study of combining hepatic arterial bicarbonate infusion with Tislelizumab was conducted in accordance with the principles outlined in the Declaration of Helsinki, with written informed consent obtained from all patients, and approval from the hospital's Institutional Review Board of the Second Affiliated Hospital, Zhejiang University School of Medicine (Approval number 2021 – 1021)(ChiCTR registration number: ChiCTR2100053537).

The retrospective clinical study was approved and monitored by the hospital's Institutional Review Board of the Second Affiliated Hospital, Zhejiang University School of Medicine (Approval number: 2024 – 1593). For the use of MRI images and pathological slides, patients' consent was waived by the Institutional Review Board (The Second Affiliated Hospital, Zhejiang University School of Medicine).

Competing interests

All the authors declare that they have no conflicts of interest related to this work.

Author contribution

X.H., conception, study design, data analysis, paper writing; M.C., conception, study design, prospective clinical study leader; D. W., experimentation, data analysis, paper writing; C. Y., experimentation, data analysis; K. J., clinical study, data analysis. B. L., & G.Z., clinical study; L.C., immunohistological analysis of clinical samples; J.L., provided information of patients (group surgery, group anti-PD-1).

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

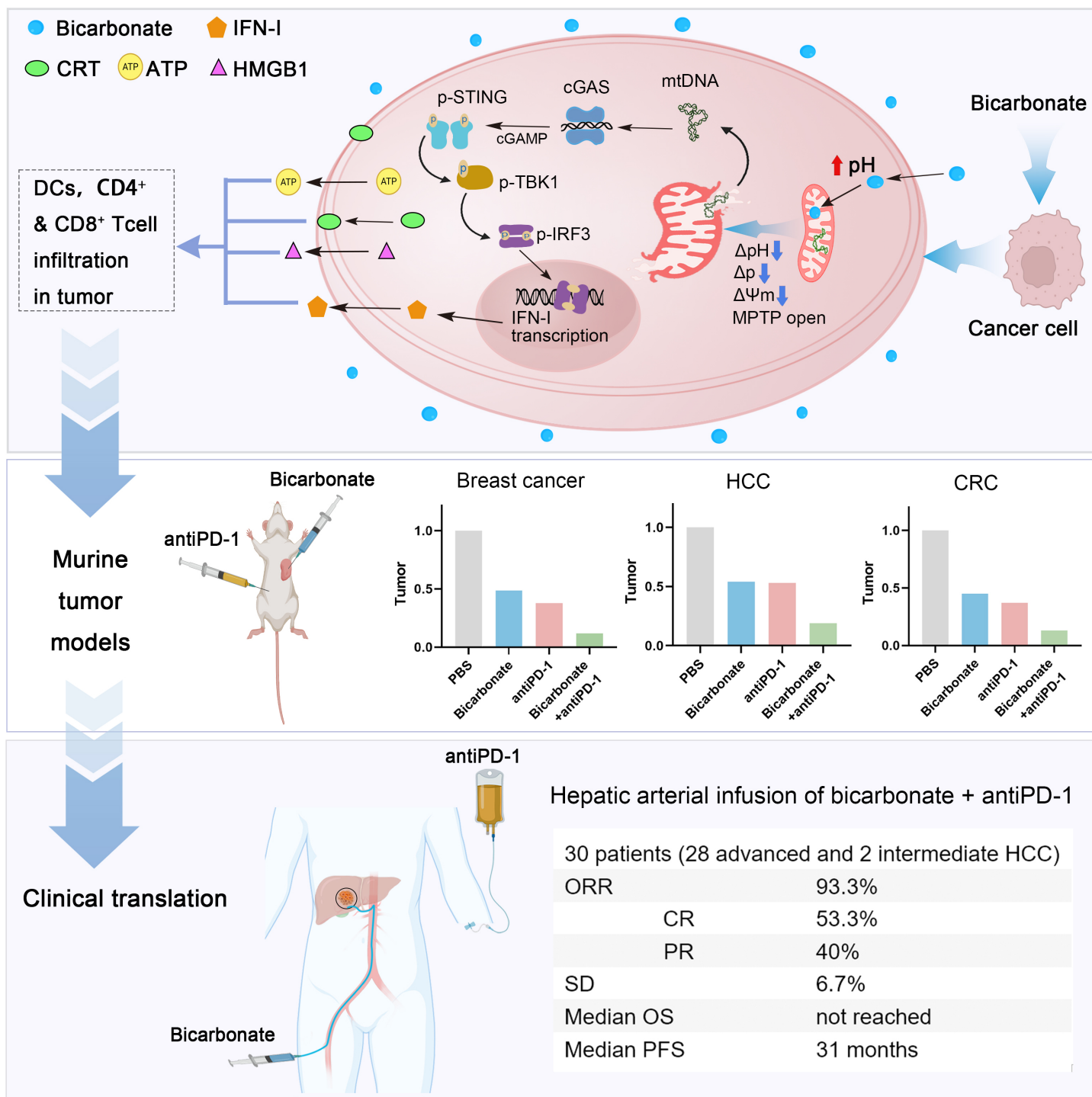


Figure 1

Bicarbonate as an immunometabolic adjuvant in HCC.

Bicarbonate-induced intracellular alkalization disrupts mitochondrial integrity, collapsing oxidative phosphorylation and inducing mitochondrial permeability transition. This metabolic stress triggers release of mitochondrial DNA and DAMPs, activating the cGAS/STING/type I interferon pathway and inducing immunogenic cell death (ICD). Together, these processes recruit and activate dendritic cells and T cells, converting immune-excluded tumors into immune-infiltrated ones. In preclinical models and

patients with HCC, intratumoral bicarbonate synergized with PD-1 blockade to achieve robust responses, highlighting its potential as a safe, accessible immunometabolic adjuvant.

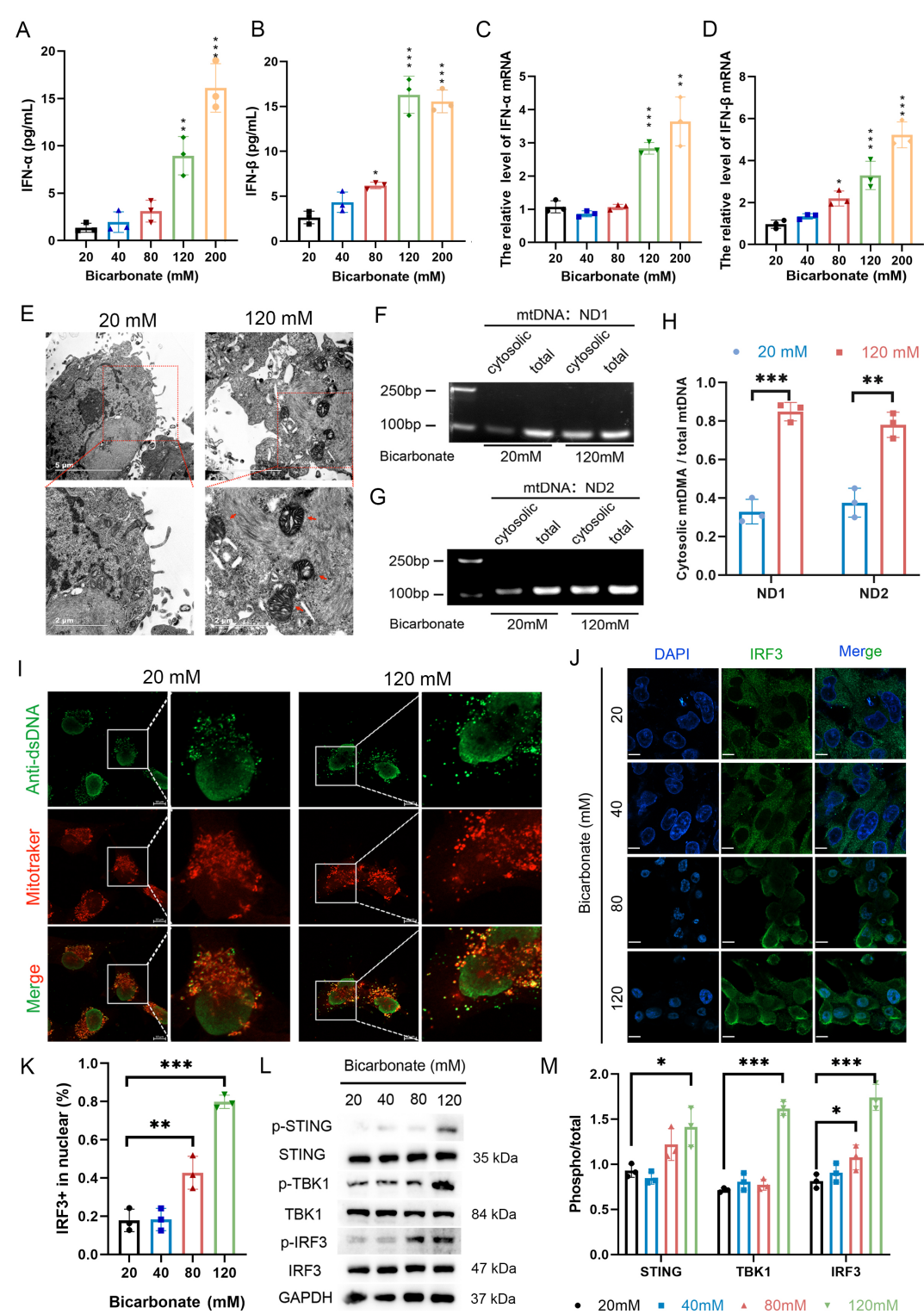


Figure 2

Bicarbonate induces SK-HEP-1 cell IFN-I response via mtDNA leakage and activating the cGAS/STING/TBK1/IRF3 pathway. SK-HEP-1 cells were cultured in RPMI-1640 medium supplemented

with various concentrations of bicarbonate, with 20 mM bicarbonate used as the control. **(A, B)** ELISA detection of IFN- α (A) and IFN- β (B) in culture supernatants after treatment with 200 mM bicarbonate for 2.5 h, 120 mM for 8 h, 80 mM for 16 h, and 40 mM or 20 mM for 72 h. **(C, D)** qRT-PCR analysis of IFN- α (C) and IFN- β (D) mRNA levels in cells treated with 200 mM bicarbonate for 1.5 h, 120 mM for 4 h, 80 mM for 12 h, and 40 mM or 20 mM for 48 h. **(E)** Representative electron microscopy images of SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. Mitochondrial swelling is evident in cells treated with 120 mM bicarbonate (indicated by red arrows). **(F, G)** Gel electrophoresis of cytosolic and total ND1 (F) and ND2 (G) mtDNA in SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. **(H)** quantitative analysis of cytosolic and total ND1 and ND2 mtDNA. **(I)** Representative immunofluorescence images of anti-dsDNA and mitochondrial co-staining in SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. Scale bar = 10 μ m. **(J, K)** Representative immunofluorescence images of IRF3 (J) and quantification of nuclear IRF3-positive cells (K) after treatment with different bicarbonate concentrations. Scale bar = 10 μ m. **(L, M)** Phosphorylation levels of STING/TBK1/IRF3 in SK-HEP-1 cells treated with different bicarbonate concentrations; (L) shows representative WB images, and (M) shows quantitative analysis. Data are presented as mean $\pm$ SD ($n = 3$) of 3 independent experiments. Two-tailed Student's t-test was used for (H), and one-way ANOVA for others, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

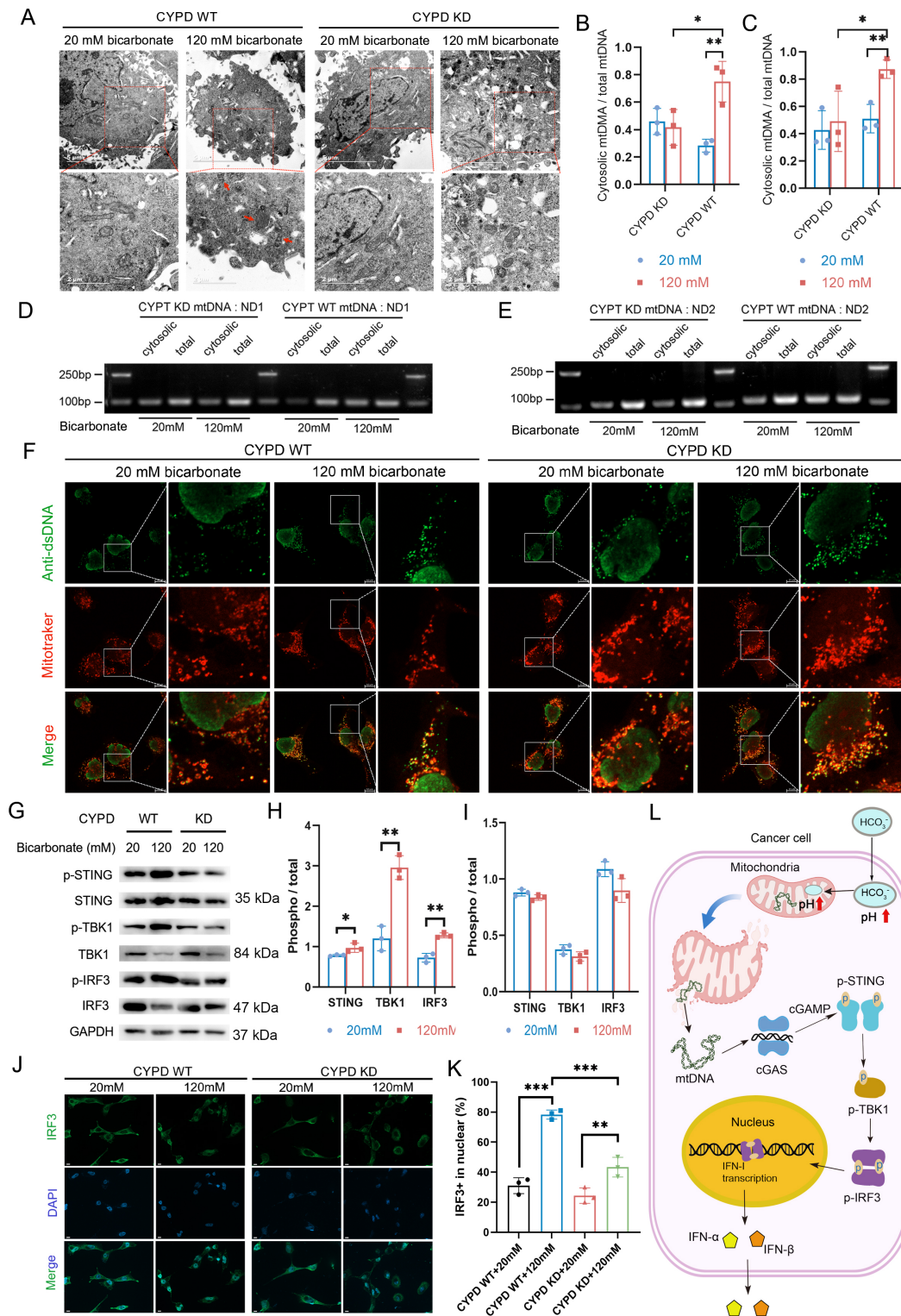


Figure 3

120 mM bicarbonate induces IFN-I response in tumor cells via the mtDNA/cGAS/STING/TBK1/IRF3 pathway. (A) Representative electron micrographs of SK-HEP-1 cells (CYPD WT and KD) treated with 20 mM and 120 mM bicarbonate. Mitochondrial swelling is evident in CYPD WT cells treated with 120 mM bicarbonate (indicated by red arrows), while CYPD KD cells show no obvious swelling under the same conditions. (B, C) Quantitative PCR analysis of cytosolic and total ND1 (B) and ND2 (C) in CYPD WT and

KD SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. **(D, E)** PCR electrophoresis images of cytosolic and total ND1 (D) and ND2 (E) in CYPD WT and KD SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. **(F)** Representative immunofluorescence images of anti-dsDNA and mitochondrial colocalization in CYPD WT and KD SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. Scale bar = 10 μ m. **(G)** WB analysis of phosphorylation levels of STING, TBK1, and IRF3 in CYPD WT and KD SK-HEP-1 cells treated with different concentrations of bicarbonate. **(H, I)** Semi-quantitative analysis of WB results in CYPD WT (H) and KD (I) cells from panel (G). **(J)** Representative immunofluorescence images of IRF3 in CYPD WT and KD SK-HEP-1 cells treated with 20 mM and 120 mM bicarbonate. Scale bar = 10 μ m. **(K)** Quantification of nuclear IRF3-positive cells in CYPD WT and KD SK-HEP-1 cells following treatment with 20 mM and 120 mM bicarbonate. **(L)** Schematic illustration of the mechanism by which high-concentration bicarbonate induces IFN-I response in tumor cells. Data are reported as mean $\pm$ SD (n = 3). Two-tailed Student's t-test for (H) and (I), and one-way ANOVA for other comparisons. *p < 0.05, **p < 0.01, ***p < 0.001.

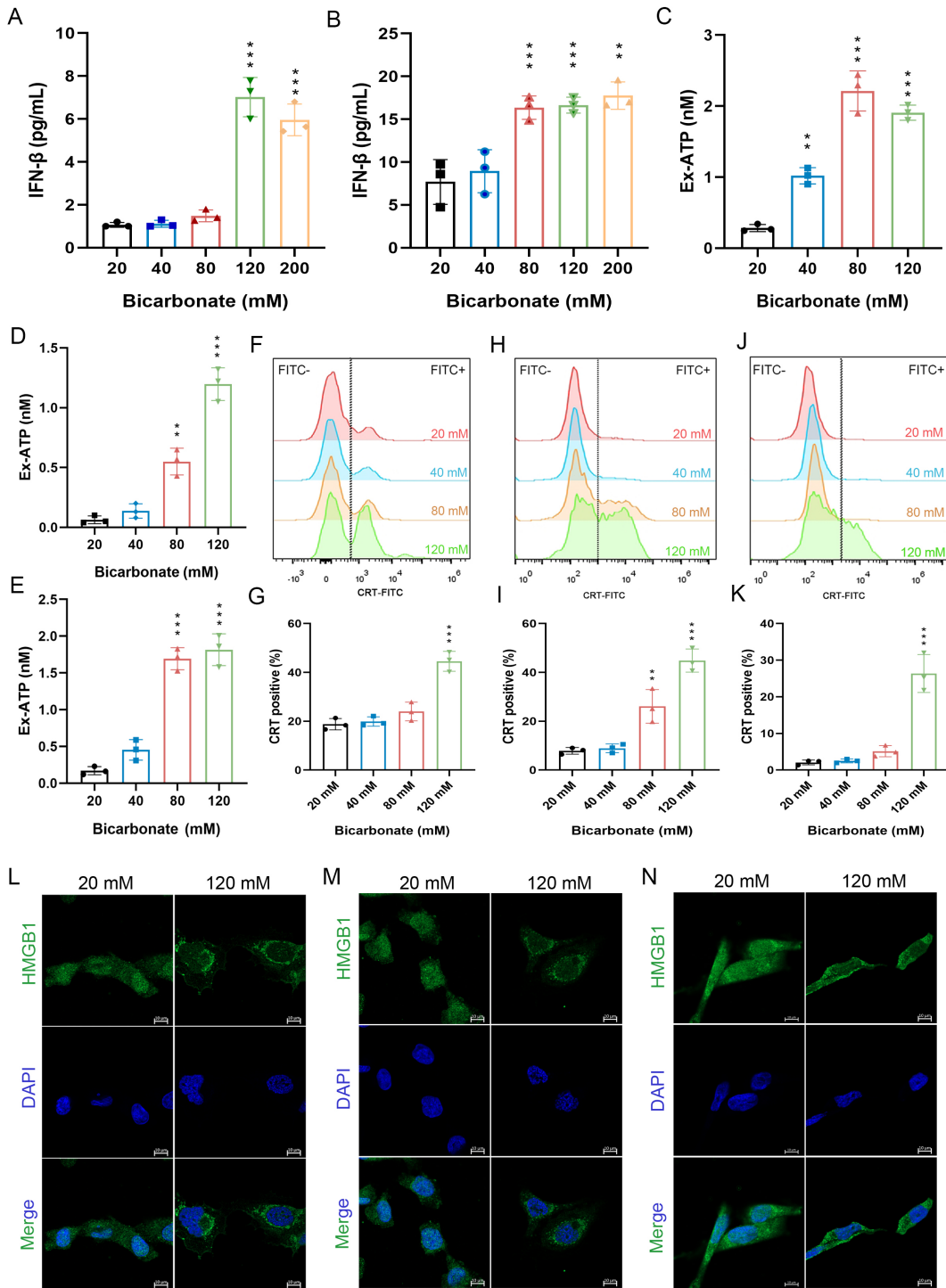


Figure 4

Bicarbonate induces ICD in tumor cells. (A, B) IFN- β levels in the supernatants of 4T1 (A) and CT26 (B) cells treated with different concentrations of bicarbonate. (C - E) ATP levels in the supernatants of SK-HEP-1 (C), 4T1 (D) and CT26 (E) cells treated with different concentrations of bicarbonate. (F, G) FC analysis of CRT levels on the cell surface of SK-HEP-1 cells treated with different concentrations of bicarbonate. (F) is representative image and (G) is quantitative analysis. (H, I) FC analysis of CRT levels

on the cell surface of 4T1 cells treated with different concentrations of bicarbonate. (H) is representative image and (I) is quantitative analysis. (J, K) FC analysis of CRT levels on the cell surface of CT26 cells treated with different concentrations of bicarbonate. (J) is representative image and (K) is quantitative analysis. (L - N) Representative immunofluorescence images of intracellular HMGB1 in SK-HEP-1 (L), 4T1 (M) and CT26 (N) cells treated with 20 mM and 120 mM bicarbonate. HMGB1 is shown in green, nuclei in blue. Scale bar = 10 μ m. Data are reported as mean $\pm$ SD (n = 3). One-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001.

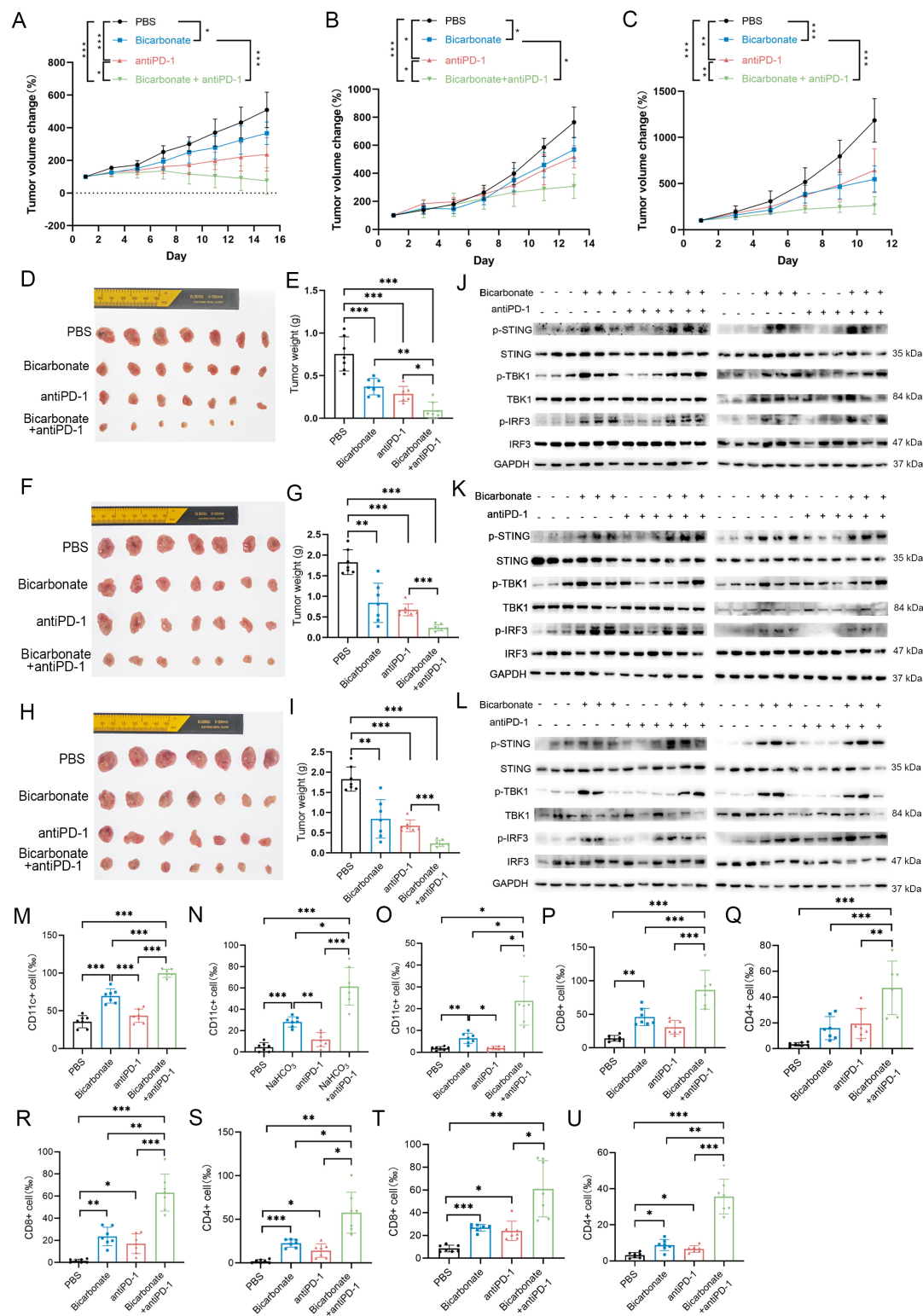


Figure 5

Intratumoral Injection of bicarbonate Combined with AntiPD-1 Therapy for Cancer. Mouse orthotopic breast cancer models were established using 4T1 cells. Mice xenograft models of HCC and CRC was established using H22 and CT26 cells, respectively. When tumor size reached 4 to 6 mm in the largest diameter, mice were divided into four groups: PBS, intratumoral injection of 200 mM bicarbonate, intraperitoneal injection of antiPD-1 antibody, and combination treatment with 200 mM bicarbonate + antiPD-1. **(A - C)** Tumor growth curves of the breast cancer (A), HCC (B) and CRC (C). **(D, E)** Images and weight of excised breast tumor tissues. **(F, G)** Images and weight of excised HCC tissues. **(H, I)** Images and weight of excised CRC tissues. **(J - L)** WB analysis of STING/TBK1/IRF3 phosphorylation in breast tumors tissues (J), HCC tissues (K) and CRC tissues (L). **(M - O)** IHC analysis of CD11c⁺ cells in breast tumors tissues (M), HCC tissues (N) and CRC tissues (O). **(P, Q)** IHC analysis of CD8⁺ cells (P) and CD4⁺ (Q) cells in breast tumors tissues. **(R, S)** IHC analysis of CD8⁺ cells (R) and CD4⁺ (S) cells in HCC tissues. **(T, U)** IHC analysis of CD8⁺ cells (T) and CD4⁺ (U) cells in CRC tissues. Data are reported as means $\pm$ SD. Two-way ANOVA was used for (A - C), and one-way ANOVA for the others. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

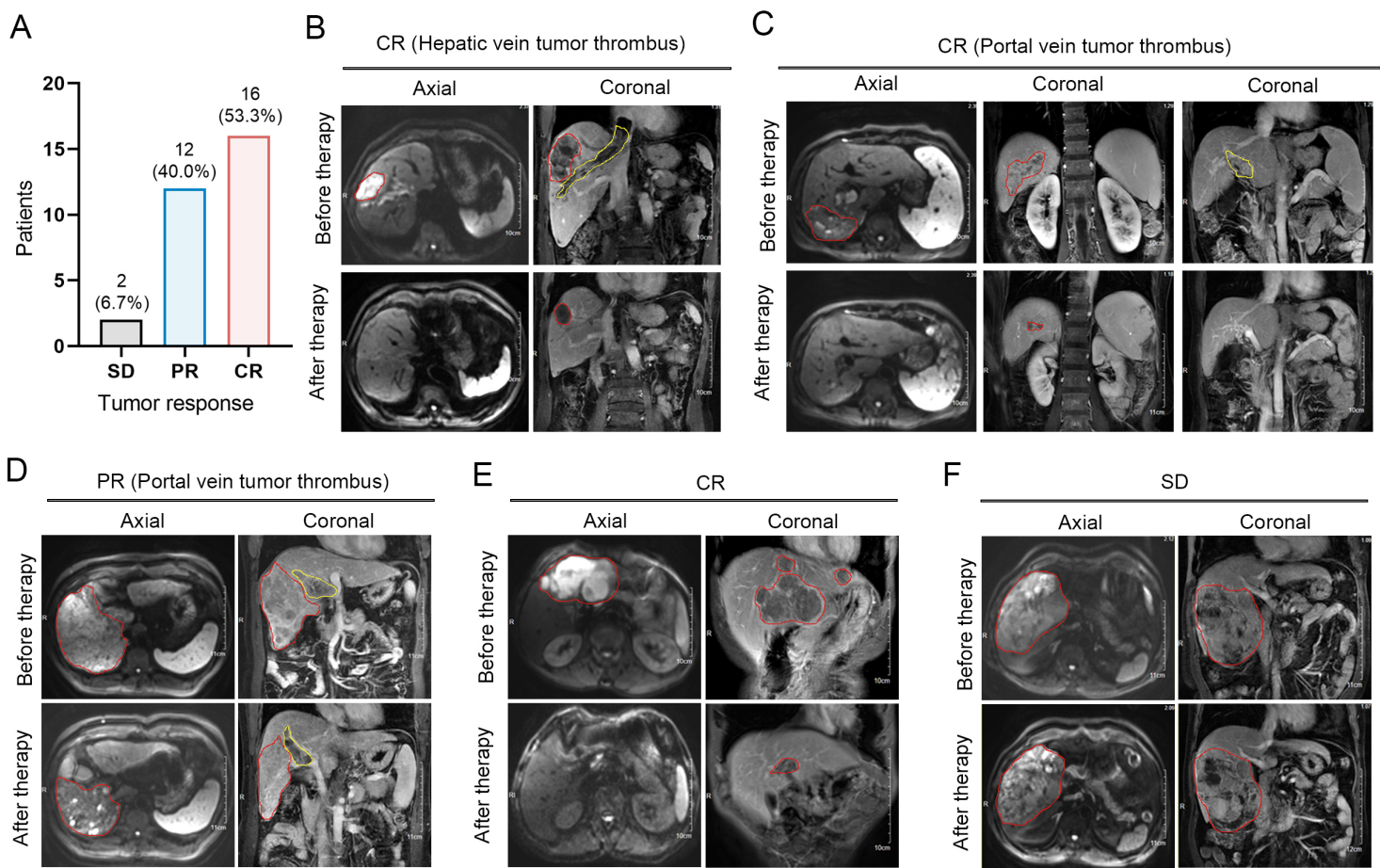


Figure 6

Tumor response rate to arterial infusion of bicarbonate combined with anti-PD-1 therapy. (A) Tumor response rate following treatment (n = 30). **(B–F)** Representative MRI images of HCC from five patients before and after treatment. The tumor is outlined with red, and the tumor thrombus in the vein is outlined with yellow. **(B)** A hyperintense mass on T2-weighted and diffusion-weighted imaging (T2W/DWI) in segments V and VIII of the right liver, with marked enhancement and tumor thrombus in the right hepatic vein. At 24-month post-treatment, both the primary tumor and hepatic vein thrombus showed normalized T2W/DWI signals and no enhancement, indicating CR. **(C)** Diffuse patchy hyperintense lesions with marked enhancement on T2W/DWI in segments VI and VII of the right liver, with a tumor thrombus in the main trunk of the right portal vein. After four treatment cycles (5 months), the primary lesion and the thrombus had nearly completely resolved, indicating CR. **(D)** A large hyperintense mass with marked enhancement on T2W/DWI occupying most of the right liver, with a tumor thrombus extending into the main trunk of the right portal vein. After two treatment cycles (2 months), the T2W/DWI signal was markedly reduced, with scattered punctate hyperintensities forming a “mosaic” pattern. The tumor and thrombus were significantly reduced in size and showed decreased enhancement, indicating PR. **(E)** A large hyperintense mass on T2W/DWI in the left liver with marked enhancement, along with multiple hypervascular nodules in the left hepatic parenchyma. After four treatment cycles, the primary tumor markedly shrank with no enhancement, and the disseminated lesions disappeared, indicating CR. **(F)** A large hyperintense mass with marked enhancement on T2W/DWI in segments IV and V of the right liver, with involvement and disappearance of the anterior branch of the right portal vein. After three treatment cycles, the lesion exhibited a “mosaic” pattern of scattered punctate hyperintensities, but no significant change in tumor size or enhancement was observed, indicating SD.

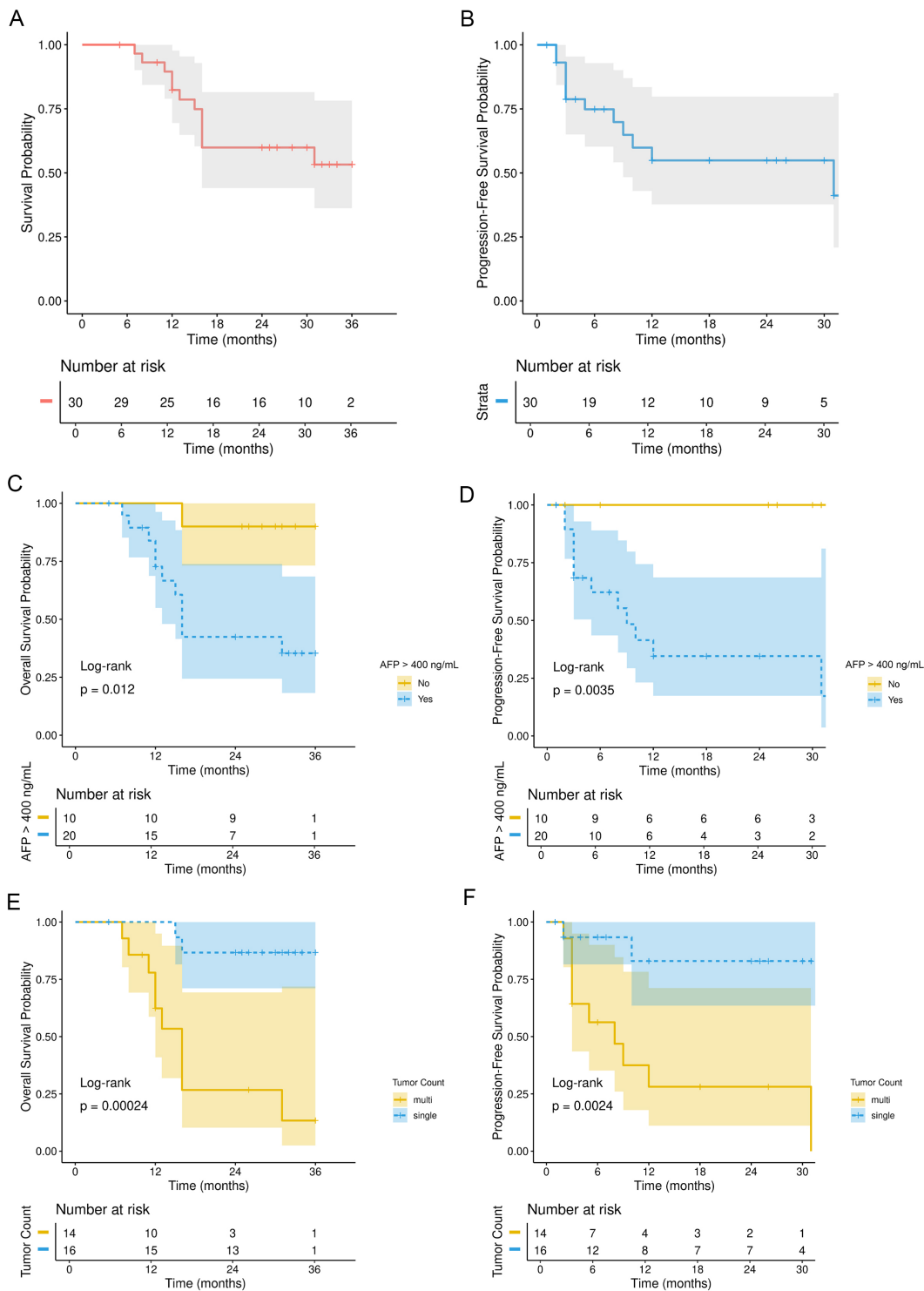


Figure 7

Kaplan-Meier analysis of survival. (A) Overall survival curve of 30 patients. (B) PFS curve of 30 patients. (C, D) Survival curves (C) and PFS curves (D) of patients with different AFP levels. AFP ≤ 400 ng/mL, n = 10; AFP > 400 ng/mL, n = 20. (E, F) Survival curves (E) and PFS curves (F) of patients with different number of intrahepatic lesions. Single: number of intrahepatic lesions = 1, n = 16; multi: number of intrahepatic lesions ≥ 2, n = 14. Log-rank test.

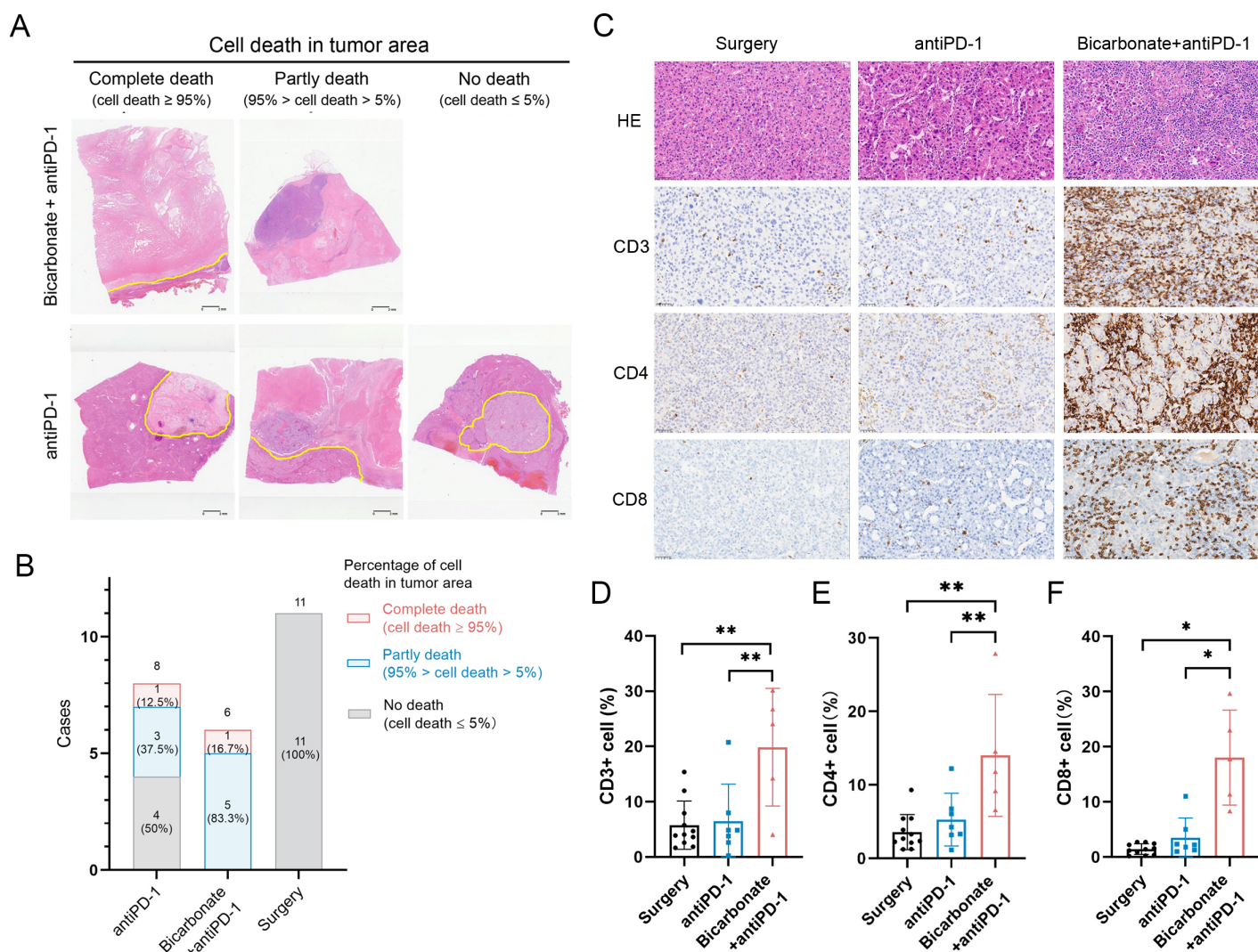


Figure 8

Immunological Correlation to Clinical Response. (A) The representative images of the different degrees of cell death in the tumor regions on pathological slices of patients in the bicarbonate + antiPD-1 group and the antiPD-1 group. (B) The proportion of patients with different degrees of cell death in the tumor regions based on pathological slices. (C) The representative IHC images of CD3, CD4, and CD8 in the pathological slices of patients with residual viable tumor cells. (D - F) The percentage of CD3⁺ cells (D), CD4⁺ cells (E), and CD8⁺ cells (F) in the tumor region of pathological slices. The data are reported as the means $\pm$ SDs. One-way ANOVA, * $p < 0.05$, ** $p < 0.01$.

Supplementary Files

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