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Modulatory Effect of Gingerols on Type I Interferon (IFN-I) and PI3K/AKT Signaling Pathways in Hepatocellular Carcinoma (HCC)

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Abstract

Hepatocellular Carcinoma (HCC) is a leading cause of cancer mortality globally, with poor survival rates and limited therapeutic options. Racial disparities in HCC outcomes, particularly among African American (AA)/Black patients, highlight the need for alternative treatments targeting molecular pathways unique to these populations. Current therapies often fail to address these disparities. Our previous study identified statistically significant activation of the type I interferon (IFN-I) signaling pathway in AA/Black patient samples and HCC cell lines derived from Caucasian white (HepG2), (AA)/Black (Hep3B and O/20) and Asian (HuH-7) patients. Nonetheless, treatment with ginger extract has been shown to inhibit cell proliferation and reduce the phosphorylation of IFN-I signaling pathway in HCC cell lines. This study further investigated the effects of pure ginger compounds, including 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and zingerone, on HCC cell lines from diverse racial backgrounds including Caucasian white (HepG2), (AA)/black (Hep3B and O/20) and Asian (HuH-7) patients.

The cytotoxic effects of the 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and zingerone, were evaluated using the WST-1 assay on Hep3B, O/20, HuH-7 and HepG2 HCC cell lines.

The molecular signaling pathways including JAK/STAT and AKT/PI3K were evaluated using western blot analysis.

8-Gingerol, 10-gingerol, and 6-shogaol had significant anti-proliferative effects, reducing cell viability in a dose-dependent manner, whereas zingerone and 6-gingerol showed minimal impact. The western blot analysis revealed that 6-shogaol and gingerols effectively inhibited key signaling pathways, including IFN-I and PI3K/AKT pathways, by reducing phosphorylation of critical mediators such as STAT1, STAT2, and PI3K.

Our findings demonstrate the potential of ginger compounds as adjunct therapies for HCC, particularly in mitigating disparities in AA/Black patients. This study underscores the importance of integrating natural bioactive compounds into HCC treatment strategies to improve patient outcomes.

Keywords: Hepatocellular carcinoma (HCC), Type I interferon signaling pathway (IFN), PI3K/AKT pathway, Gingerols, Anti-proliferative effect

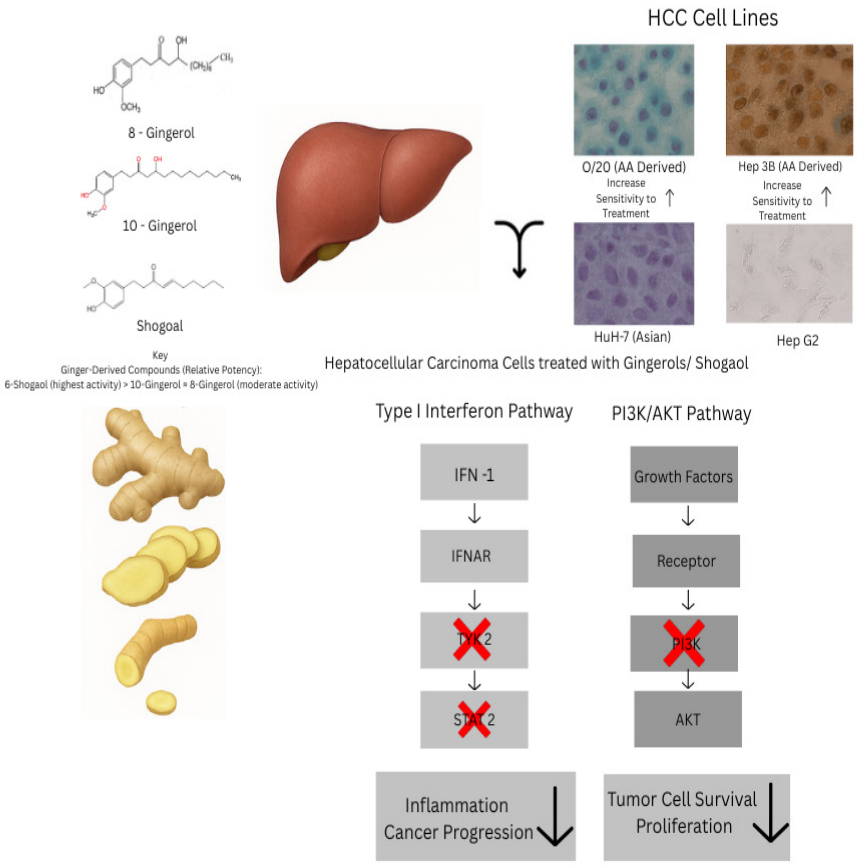
Introduction

Liver cancer remains a pressing concern worldwide because of its increasing incidence and persistently high mortality rate [1,2] Projections suggest that by 2025, the number of new cases could surpass one million annually [3]. Although diagnostic and treatment modalities have improved, survival rates remain unacceptably low. Globally, liver cancer ranks among the leading causes of cancer-related deaths, second only to pancreatic cancer, with over 830,000 deaths reported each year [4]. The outlook for patients is generally poor, as indicated by a five-year survival rate that lingers around 18% [5]. Hepatocellular Carcinoma (HCC), a primary liver malignancy, constitutes the majority of these cases, representing roughly three-quarters to nearly 90% of all liver cancer diagnoses.

The development of HCC is associated with a range of risk factors, including chronic infections caused by Hepatitis B (HBV) and Hepatitis C (HCV), cirrhosis, exposure to aflatoxin B1, iron overload, and metabolic conditions such as alcoholic liver disease, diabetes, and Nonalcoholic Steatohepatitis (NASH) [6,7]. While surgical resection and liver transplantation remain the most effective curative options, the majority of HCC cases are detected at advanced stages. Advanced HCC is challenging to treat due to the limited availability of curative interventions and the intrinsic resistance of tumor cells to conventional therapies, such as chemotherapy and radiotherapy. Moreover, coexisting liver conditions, such as cirrhosis, often complicate treatment approaches, further narrowing therapeutic possibilities.

Current treatments for Hepatocellular Carcinoma (HCC) show varying toxicity profiles across stages [8]. Approved treatment options for advanced HCC include systemic therapies such as tyrosine kinase inhibitors (sorafenib, lenvatinib, regorafenib, and cabozantinib), anti-angiogenic antibodies (ramucirumab), and immune checkpoint inhibitors [9,10]. Surgical resection and

Graphical Abstract



liver transplantation for early-stage HCC carry risks like liver failure, bile leakage, and infections. Locoregional therapies, such as thermal ablation and TACE, often cause pain, fever, and liver dysfunction. The combination of atezolizumab and bevacizumab has emerged as one of the most effective therapies for advanced, nonresectable HCC, showing an overall response rate of 27% [11]. However, despite these therapeutic advances, there remains a pressing need to investigate the molecular drivers of HCC and develop innovative, targeted treatment strategies to improve outcomes.

Racial and ethnic disparities play a critical role in HCC incidence, survival, and treatment responses [12]. African American/ Black populations have been reported to experience higher rates of HCC and worse survival outcomes than non-Hispanic White populations [13]. Compared to HCC in Asian or Western populations, African HCC tends to present at more advanced stages with poorly differentiated tumors and limited access to early diagnostic and therapeutic interventions, resulting in poorer treatment outcomes. These disparities may stem from both genetic variations and differences in molecular pathways, which can influence treatment efficacy [14]. This underscores the importance of identifying novel therapeutic approaches that address these molecular differences and improve outcomes for African American/Black patients. Chemotherapy, radiation, and targeted therapies add to the burden of side effects such as nausea, vomiting, fatigue, and alopecia. Immune checkpoint inhibitors can trigger rash, liver enzyme elevation, diarrhea, and endocrine disorders. Careful monitoring and management are critical to balance treatment efficacy with patient quality of life.

The significant adverse effects of existing HCC therapies have prompted researchers to explore alternative treatment options with fewer side effects. Natural products, including medicinal herbs and phytochemicals, have gained attention as promising candidates for managing chronic liver diseases because of their therapeutic potential and minimal toxicity. The evidence suggests that these natural agents, either alone or in combination with conventional therapies, can effectively inhibit tumor growth, reduce oxidative damage, and suppress tumorigenesis [15-17].

The rhizome of Zingiber officinale commonly known as ginger, has been integral to both traditional medicine and culinary practices around the world for centuries. Used extensively in herbal medicine systems, ginger is considered safe by several regulatory bodies and is frequently included in natural remedies for ailments such as colds, fevers, nausea, and gastrointestinal discomfort [18]. It is also traditionally employed to stimulate appetite, alleviate headaches, and inflammation through its diverse bioactive profile [18-21].

Ginger contains numerous bioactive compounds that contribute to its well-known biological activities [22]. These compounds include phenolic compounds, terpenes, lipids, and carbohydrates [23]. Among them, phenolic compounds including gingerols, shogaols and paradols have been identified as the principal contributors to the biological efficacy of ginger [24-25]. In particular, gingerols, a group of volatile phenols, are largely responsible for the well-documented antioxidant, anti-inflammatory, anticancer, and hepatoprotective activities of ginger [25-28]. The characteristic sharpness of fresh ginger is primarily due to the presence of gingerols, with 6-gingerol being the most abundant and potent contributor to its pungent flavor [25]. Other gingerols, including 4-, 8-, 10-, and 12-gingerol, occur in lower concentrations. However, these compounds are thermally unstable and readily convert into shogaols, especially 6-shogaol, during drying or heat processing [25]. Notably, among shogaols, 6-shogaol has been shown to possess greater biological activity than its precursor, which can be credited to the presence of an -unsaturated ketone moiety which is known to enhance electrophilic reactivity and facilitate interactions with key cellular targets [29]. Gingerols and shogaols, especially shogaol-6, have demonstrated potent anticancer properties across multiple malignancies, including hepatic, pulmonary, cervical, breast, colon and colorectal cancers, through modulation of key pathways involved in tumor growth, apoptosis, angiogenesis, and metastasis, including p-PI3K, p-AKT, and p-mTOR [30-34].

In our previous work, ginger extract was found to modulate the IFN-I signaling pathway, particularly in African-derived HCC cell lines [11]. Continuing this line of investigation, we extended our research to explore the effects of purified compounds from ginger on HCC cell lines. Specifically, we examined how individual ginger components influence cell proliferation and molecular pathways including IFN-I and PI3K/AKT, signaling pathways in HCC cell lines derived from Caucasian White (HepG2), American/African Black (Hep3B and O/20), and Asian (HuH-7) patients. These results hold promise for integrating ginger bioactive components

Methods

Cell Lines and Chemicals

HepG2 and Hep3B cells were acquired from the American Type Culture Collection, whereas HuH-7 cells were received from the Japanese Collection of Research Bioresources Cell Bank. O/20 cells were grown as previously described [35]. Ethanol (HPLC grade), 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, zingerone, and Roche Cell Proliferation Reagent WST-1 were procured from Sigma-Aldrich (St. Louis, MO, USA). The culture medium, Eagle's Minimum Essential Medium (EMEM), supplemented with 1% antibiotic/antimycotic, 10% Fetal Bovine Serum (FBS), 0.25% trypsin, and 1 mM EDTA, was acquired from Invitrogen. Mini-PROTEAN TGX gels and Precision Plus Protein Dual Color Standards (1610374) were obtained from Bio-Rad (Hercules, CA, USA).

Measurement of Cell Proliferation by WST-1 Assay

O/20, Hep3B, HuH-7, and HepG2 cells were grown in Eagle's Minimum Essential Medium (EMEM) containing 1% antibiotic/ antimycotic solution and 10% Fetal Bovine Serum (FBS). Cells were incubated in an incubator at 37°C until reached confluency. Cell viability assay was assessed with WST-1 following the manufacturer's protocol (Roche Applied Science, Germany).

Briefly, cells (1 × 104) were seeded in 96-well plates and incubated at 37°C for 24 hours. Cells were then treated with various concentrations of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and zingerone. Controls received vehicle (EtOH) at a concentration equal to that used in compound-treated cells. After adding 10 µl of WST-1, plates were incubated at 37°C for 3 h. Finally, the absorbance was measured at 450 nm using a microplate reader.

Western Blot Analysis

Western blotting was carried out as described in our previous study [11]. Briefly, cells (2 × 105) were cultured in a six-well plate and then treated with 8-gingerol, 10-gingerol, or 6-shogaol for 24 h. To perform the cell lysis step, RIPA buffer containing protease/phosphatase was added to the cells, and the cell lysates were collected after centrifugation at 13,000 × g. The Bradford method (Pierce, Rockford, IL, USA) was used to quantify the protein concentration. After adding 2x Laemmli sample buffer (Bio-Rad), the samples were heated at 100°C for 10 min. Equal amounts of protein were loaded and separated on SDS polyacrylamide gels (4–15%), after which the protein was transferred to a PVDF (Millipore, Bedford, MA, USA) membrane. To carry out the blocking step, 5% skim milk was used, after which the membranes were incubated with the indicated primary and secondary antibodies (anti-rabbit IgG-HRP conjugates). The STAT1 (D1K9Y) rabbit mAb, phospho-STAT1 (Tyr1034/1035) (D9J7L) rabbit mAb, STAT2 (D9J7L) rabbit mAb, phospho-STAT2 (Tyr690) (D3P2P) rabbit mAb, JAK1 (6G4) rabbit mAb, phospho-JAK1 (Tyr1034/1035) (D7N4Z) rabbit mAb, TYK2 (D4I5T) rabbit mAb, phospho-TYK2 (Tyr1054/1055) (D7T8A) rabbit mAb, (13E5) rabbit mAb, AKT (pan) (C67E7) rabbit mAb, phospho-AKT (Ser473) (193H12) rabbit mAb, PI3 kinase p85, phospho-PI3 kinase p85 (Tyr458)/p55 (Tyr199) (E3U1H) rabbit mAb and anti-rabbit HRP-linked antibodies were purchased from Cell Signaling. Proteins were visualized via an enhanced chemiluminescence (ECL) system using Super Signal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, 34095) detection reagent and then imaged and quantified via Analytik Jena UVP BioImaging Systems.

Statistical Analysis

All values are presented as mean ± standard deviation. Sigma Plot version 15.1.1.26. was used for statistical analysis. One-way Analysis of Variance (ANOVA) followed by Holm-Sidak method tests was employed to determine statistical significance. Differences between groups were considered statistically significant at *p*-values less than 0.05.

Results

Gingerols, 6-Shogoal and Zingerone Inhibited the Proliferation of HCC Cell Lines

We evaluated the anti-proliferative effects of five compounds namely, 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and zingerone on four HCC cell lines derived from African American (O/20 and Hep3B), White (HepG2), and Asian (HuH-7) patients. The data presented here shows the percent cell viability compared to that of the control group (EtOH) [Figure 1]. The concentrations of the treatments varied from 1 to 500 µM. As depicted in figure 1, treatment of the HCC cell lines with these compounds for 24 hours reduced/inhibited cell viability in a dose-dependent manner. Among the compounds tested, gingerols and 6-shogaol demonstrated significant anti-proliferative activity in a dose-dependent manner, while zingerone showed no anti-proliferative activity, even at relatively high concentrations [Figure S1]. Of the gingerols, 6-gingerol was the least effective, with high IC50 values of 277 µM (O/20), 310 µM (Hep3B), 318 µM (HuH-7), and 260 µM (HepG2) [Figure S1]. 8-Gingerol and 10-gingerol exhibited comparable anti-proliferative effects across all cell lines, with IC50 values of 40 µM for O/20, 163 µM and 70 µM for Hep3B in 8-gingerol and 10-gingerol treated groups, and 80 µM for both compounds in HuH-7 and HepG2 cell lines. 6-Shogaol exhibited more potent inhibitory effects on the viability of all four HCC cell lines. 6-Shogaol-treated HepG2, O/20, and Hep3B cells presented IC50 values of 19 µM, 30 µM, and 35 µM, respectively, while HuH-7 was effective.

The morphology of the HCC cells was altered following treatment with gingerols, particularly 6-shogaol. At higher concentrations of these compounds, most treated cells exhibited cell shrinkage and detachment from the culture surface. In contrast, control cells maintained their normal morphology and remained firmly attached to the culture plates. However, the groups treated with zingerone or 6-gingerol presented minimal morphological changes at the tested doses. On the basis of these findings, we selected 8-gingerol, 10-gingerol, and 6-shogaol for further biological assays, with selection criteria based on the IC50 values determined from the cell viability and literature search [36-38].

Effects of 8-gingerol, 10-gingerol and 6-shogaol treatment on JAK/STAT signaling pathway

Our previous study confirmed the activation of canonical IFN-I signaling pathway in HCC cell lines. Nonetheless, ginger extract treatment had a modulatory effect on the IFN-signaling pathway by reducing the phosphorylation of IFN signaling genes. Henceforth, we investigated the effects of gingerols on these downstream effector molecules to determine whether they could inhibit the activation of canonical IFN-I signaling pathway in HCC cell lines. The unphosphorylated TYK2, JAK1, STAT1 and STAT2 were expressed in all four HCC cell lines [Figure 2 & Figure S2]. The control (untreated) group has shown detectable expression of p-TYK2, p-JAK1, p-STAT1 and p-STAT2 in all cell lines, which was variably reduced upon gingerols treatment in most of the

Figure 1

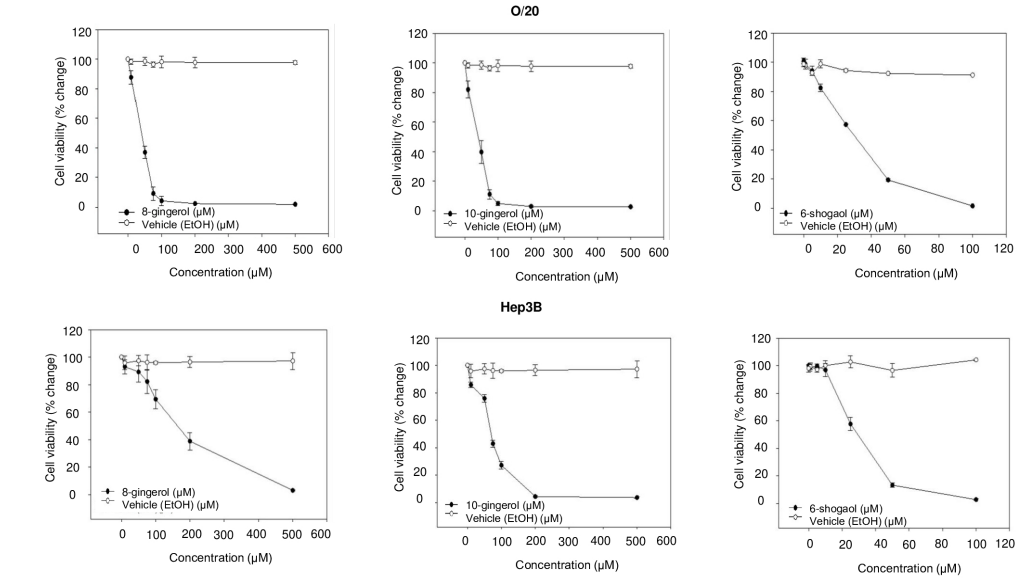


Figure 1

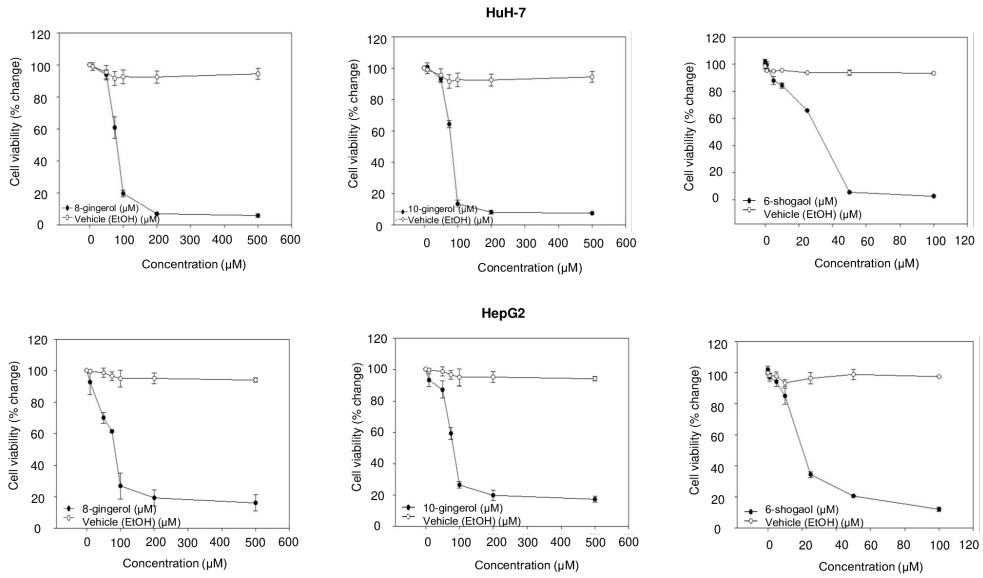
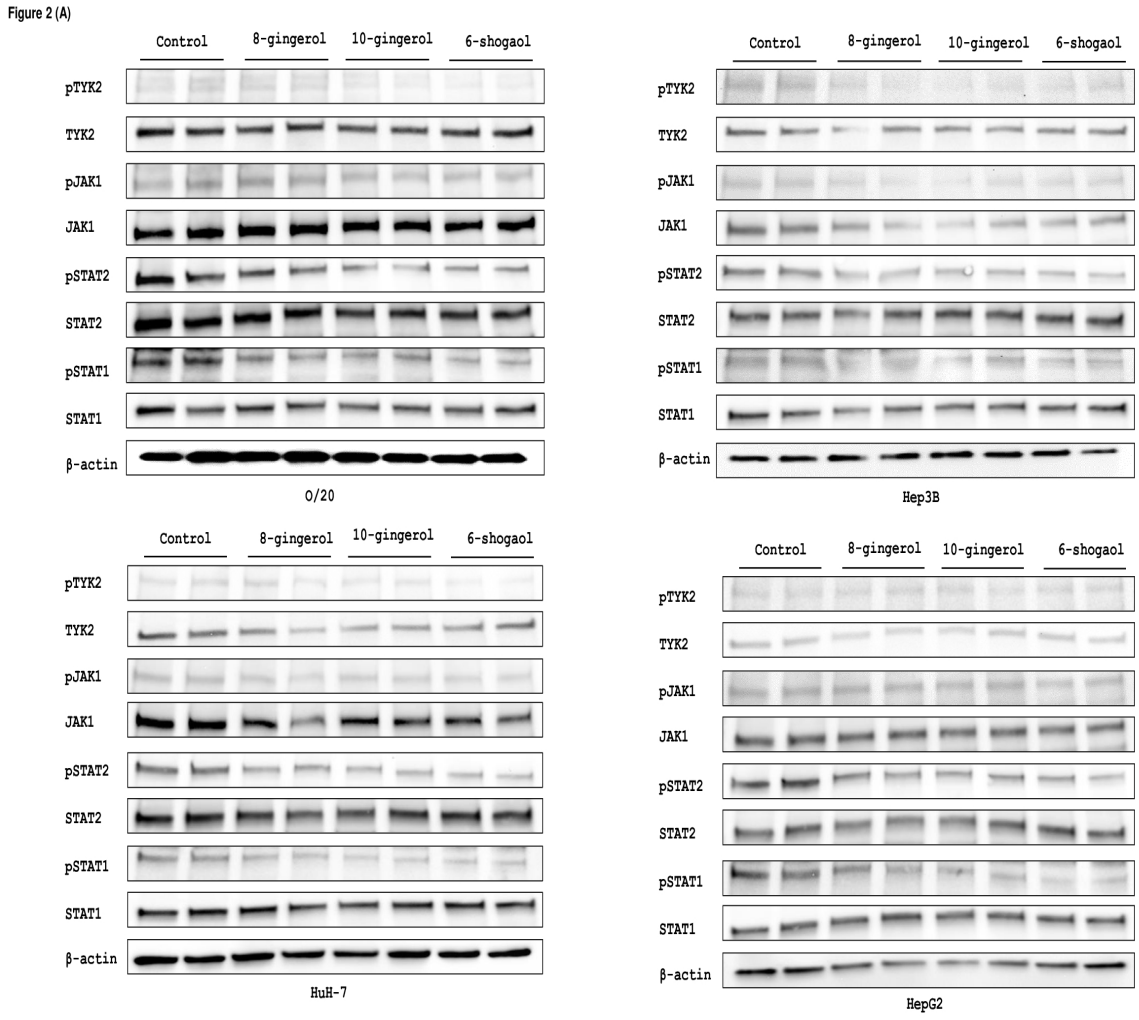


Figure 1: Effects of 8-gingerol, 10-gingerol and 6-shogaol on cell viability of 0/20, Hep3B, HuH-7 and HepG2 cells. The percentage of viable cells was calculated as a ratio of 450 nm of treated cells versus control cells (treated with EtOH vehicle). Data were acquired from three independent experiments. Data are shown as mean ± SD from three independent experiments each performed in triplicate.

treated groups [Figure 2]. Among gingerols, 10-gingerol and 6-shogaol have shown the highest modulatory activities and significantly inhibited the phosphorylation of STAT-1 and STAT-2 in all four cell lines. p-JAK-1 and p-TYK2 expression was variable in the 8-gingerol, 10-gingerol and 6-shogaol treated groups across all four cell lines. 8-Gingerol treatment resulted in a significant reduction of p-STAT1 and p-STAT2 in most cases. There was a significant reduction of p-STAT1 in 0/20 and HepG2 cell lines in 8-gingerol treated group, but not in Hep3B and Huh-7 groups.

Effects of 8-Gingerol, 10-Gingerol and 6-Shogoal Treatment on PI3K/AKT Signaling Pathway

One of the key tumorigenesis regulatory pathways includes PI3K and AKT signaling pathways which influence the activation state of numerous downstream effector molecules [39]. We investigated the effects of 8-gingerol, 10-gingerol and 6-shogaol on the activation of PI3K and AKT in HCC cell lines. Our findings revealed higher expression levels of p-PI3K and p-AKT in the untreated cells compared to the gingerols and 6-shogaol treated HCC cells [Figure 3 & Figure S3]. 8-gingerol, 10-gingerol and 6-shogaol-treated cells have markedly inhibited the phosphorylation of PI3K and AKT at different intensities. 0/20 and HuH-7 cells presented a significant reduction of p-PI3K in 8-gingerol, 10-gingerol and 6-shogaol-treated cells, whereas no significant change in p-AKT levels was detected compared to the untreated group across all four HCC cell lines. The total protein expression of PI3K and AKT, were expressed in all four HCC cell lines and no significant Change was observed in the HCC cells [Figure 3]. Overall, our findings suggested the activation of the PI3K/AKT pathway in HCC cells, which was variably inhibited after gingerols and shogoal treatment.



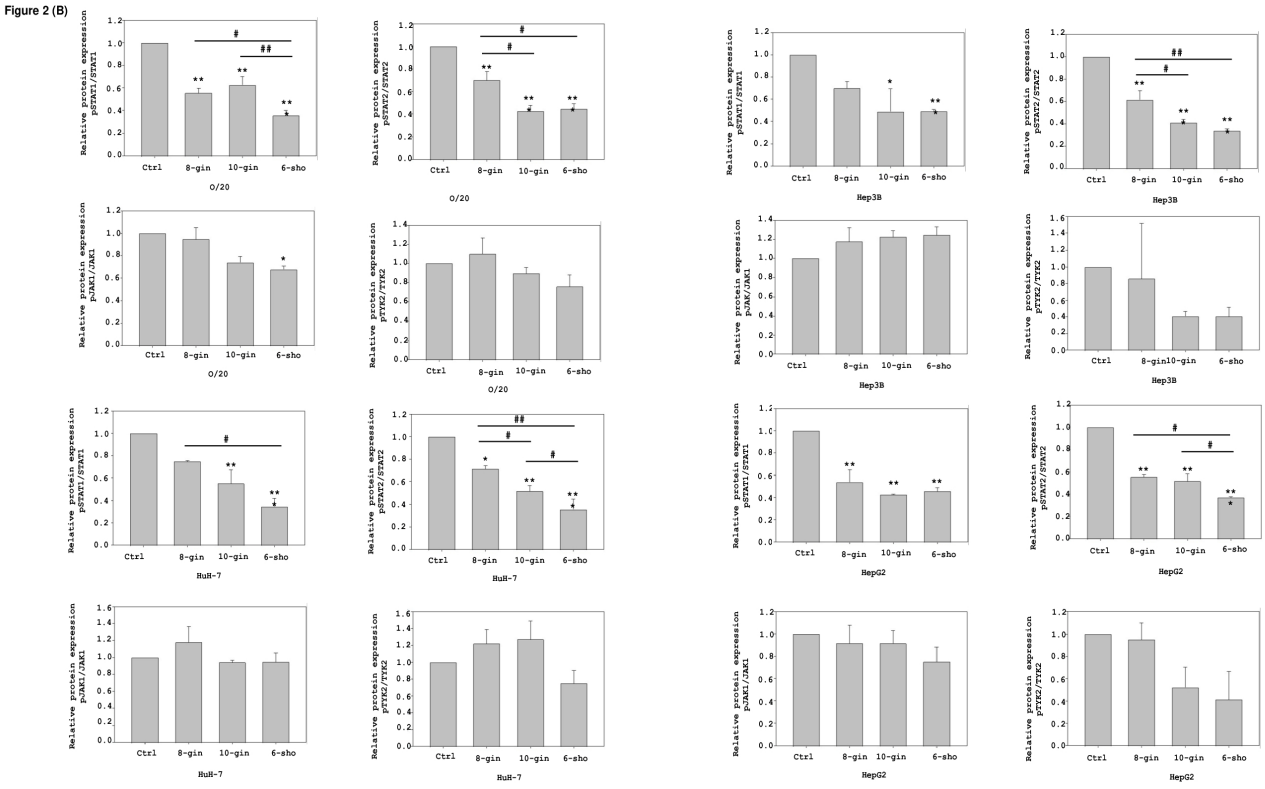
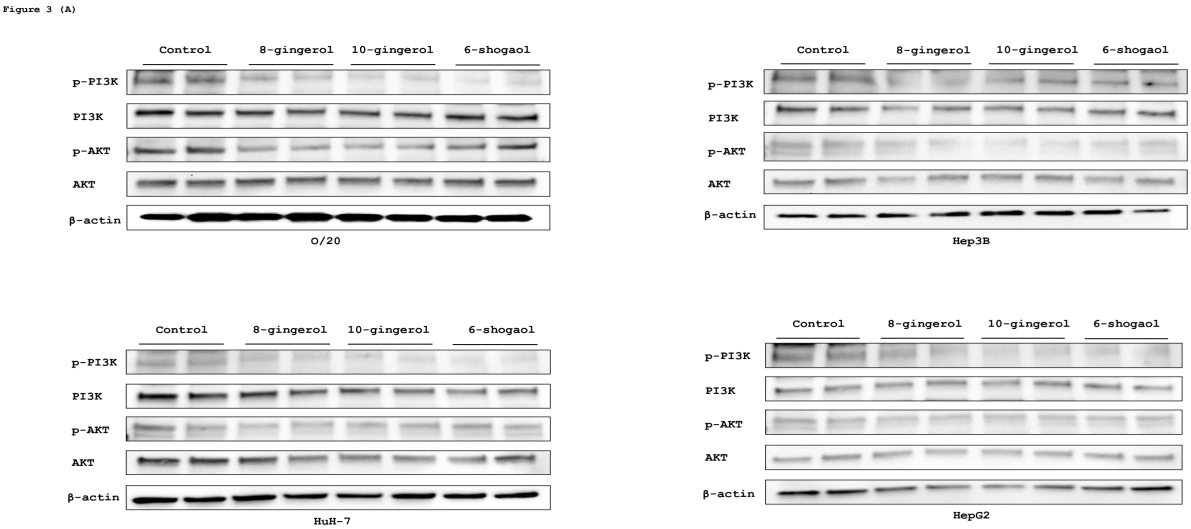


Figure 2: Gingerols and 6-shogaol suppress the JAK/STAT signaling pathway in HCC cells. (A) Representative blots of phospho- and unphospho-TYK2, JAK1, STAT2, STAT1 expression in HCC cells after treatment with 8-gingerol, 10-gingerol and 6-shogaol. β-Actin was used as a loading control. The full western blots images are presented in Figure S2.

(B) Quantitative analysis of protein expression. Data are expressed as mean ± SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control, # $p < 0.05$, ## $p < 0.01$, $p < 0.001$ versus 8-gingerol vs 10-gingerol vs 6-shogaol for each gene in each cell line. The data were acquired from three independent experiments, each performed in duplicate and is expressed as mean ± SEM.



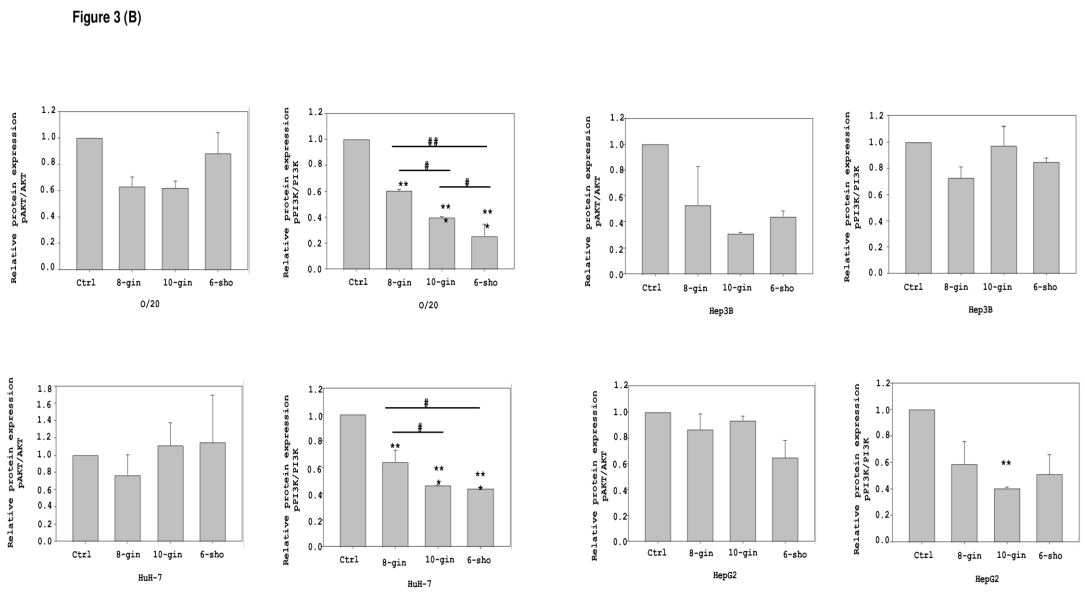


Figure 3: Effects of gingerols and 6-shogaol on PI3K/AKT expression in HCC cell lines. (A) Representative blots of phospho- and nonphospho- PI3K/AKT signaling pathway expression after treatment with 8-gingerol, 10-gingerol and 6-shogaol in HCC cell lines. β-Actin was used as a loading control. Western blot full images are presented in Figure S3. (B) Quantitative analysis of protein expression. The data was acquired from three independent experiments, each performed in duplicate and is expressed as mean ± SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control, # $p < 0.05$, ## $p < 0.01$, $p < 0.001$ *vs* 8-gingerol *vs* 10-gingerol *vs* 6-shogoal for each gene in each cell line.

Discussion

Hepatocellular Carcinoma (HCC) continues to pose a significant global health challenge, with disproportionately higher incidence and mortality rates observed among African American (AA)/Black populations compared to non-Hispanic Whites and Asians. Although socioeconomic factors, healthcare access, and genetic predispositions contribute to these disparities, emerging evidence highlights the role of molecular differences, particularly in immune-related pathways, as critical factors [11].

In the present study, we explored the effects of bioactive constituents of ginger, specifically 6-shogaol, 8-gingerol, and 10-gingerol on HCC cell lines derived from AA/Black (Hep3B, O/20), White (HepG2), and Asian (HuH-7) patients. Our findings highlight the potent anti-proliferative effects of these compounds, particularly 6-shogaol, and their ability to attenuate key oncogenic signaling pathways, including the Type I interferon (IFN-I) and PI3K/AKT pathways. These findings support the potential use of ginger-derived compounds as complementary therapeutic agents for HCC, particularly in AA/Black populations, which may exhibit heightened sensitivity to these treatments [11].

Consistent with previous research [19][34][40], our results revealed that 6-shogaol had the greatest potency in reducing HCC cell viability, with its effects observed in a clear dose-dependent manner across all four cell lines. The superior efficacy of 6-shogaol relative to gingerols is well-documented and attributed to its structural features that enhance bioactivity. Given the chemo-resistant nature of HCC, the potent effects of 6-shogaol make it a promising candidate for novel therapeutic strategies.

Notably, the O/20 cell line, derived from an AA/Black patient and characterized by a more aggressive proliferative phenotype, exhibited greater sensitivity to all three ginger compounds compared to Hep3B, HepG2, and HuH-7 cells. This differential sensitivity suggests that ginger-based therapeutics may hold particular promise in addressing racial disparities in HCC outcomes [11].

The type I interferon (IFN-I) signaling cascade is critical for orchestrating immune responses; however, its persistent activation in malignancies such as Hepatocellular Carcinoma (HCC) has been implicated in promoting tumor immune evasion [11].In the present study, we observed that ginger-derived compounds, particularly 6-shogaol and gingerols, suppressed the phosphorylation of key IFN-I pathway mediators, including STAT1 and STAT2. Among the compounds tested, 10-gingerol and 6-shogaol exhibited the greatest inhibitory effects, with the most pronounced reductions in p-STAT1 and p-STAT2 observed in the O/20 cell line, followed sequentially

Figure S1

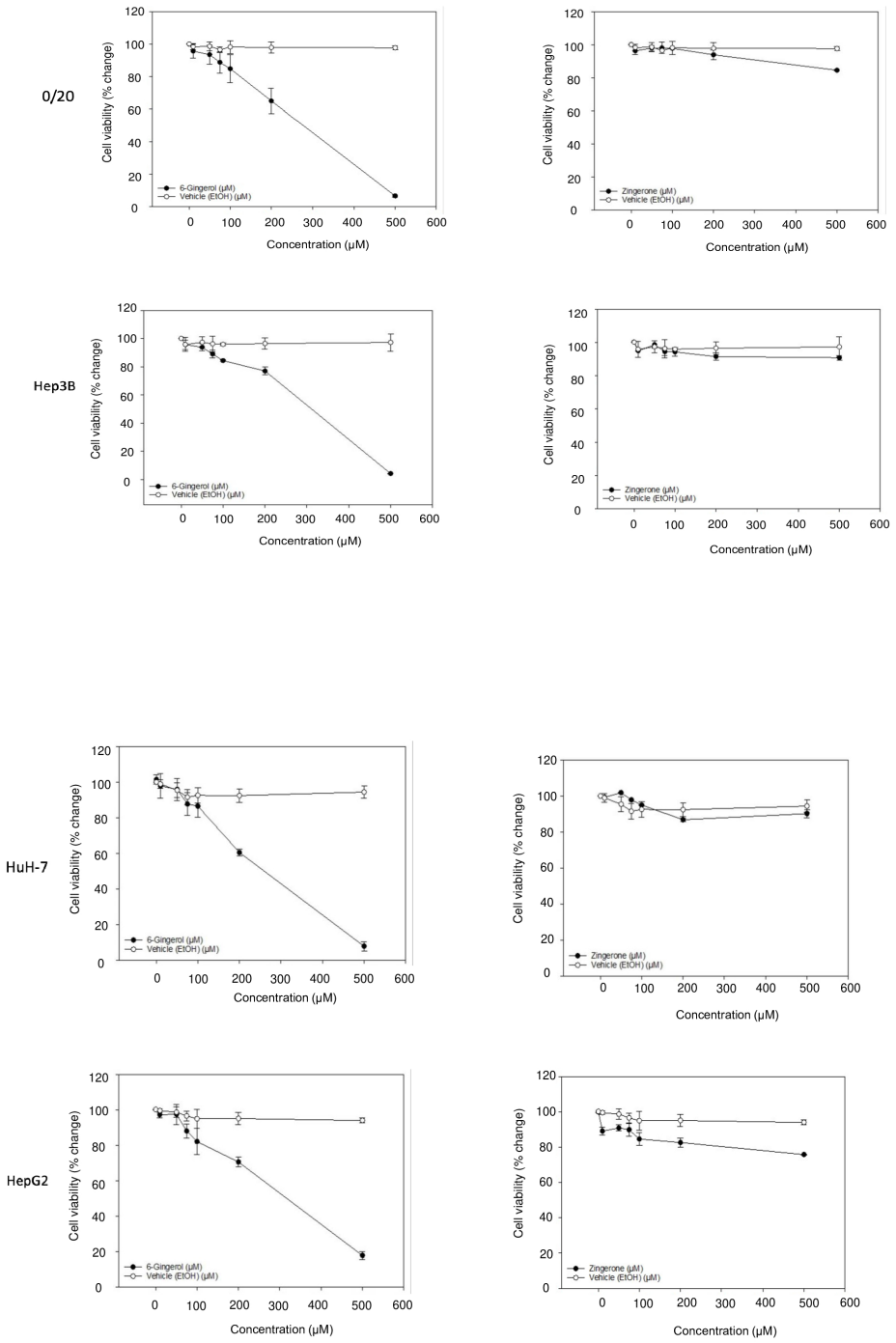
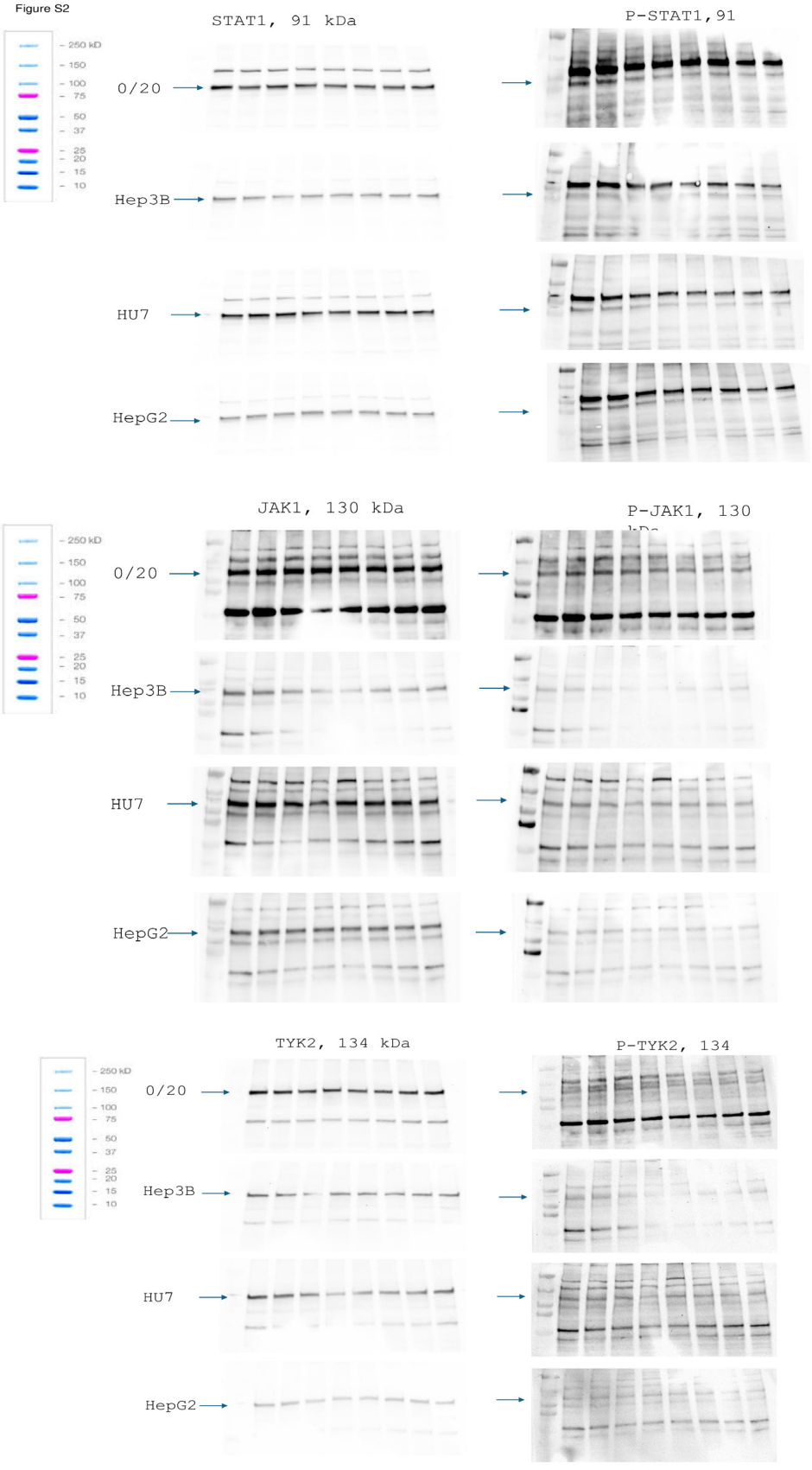


Figure S1: Effects of 6-gingerol, and zingerone on the cell viability of 0/20, Hep3B, HuH-7 and HepG2 cells

Figure S2



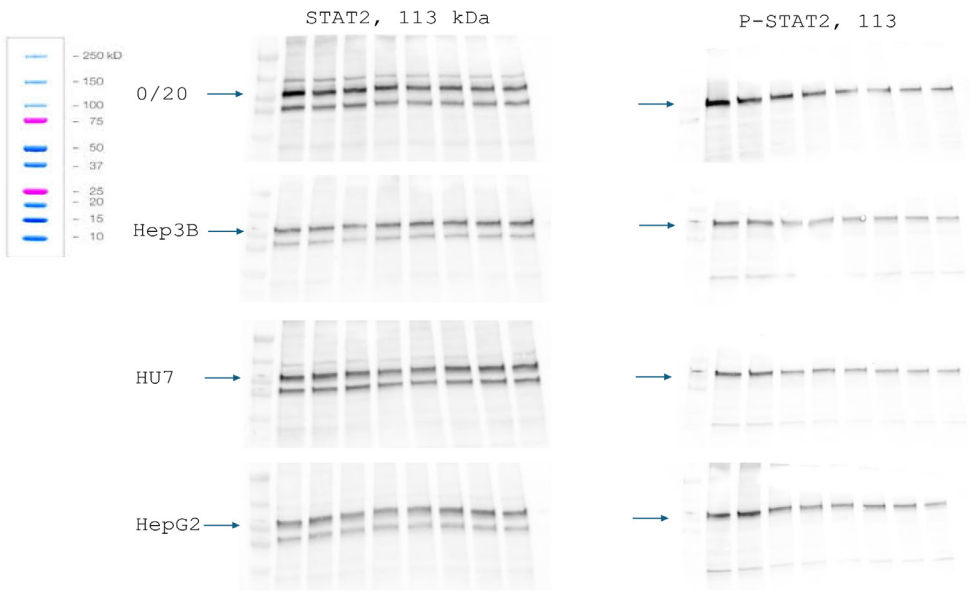


Figure S2: Full pictures of the Western blots

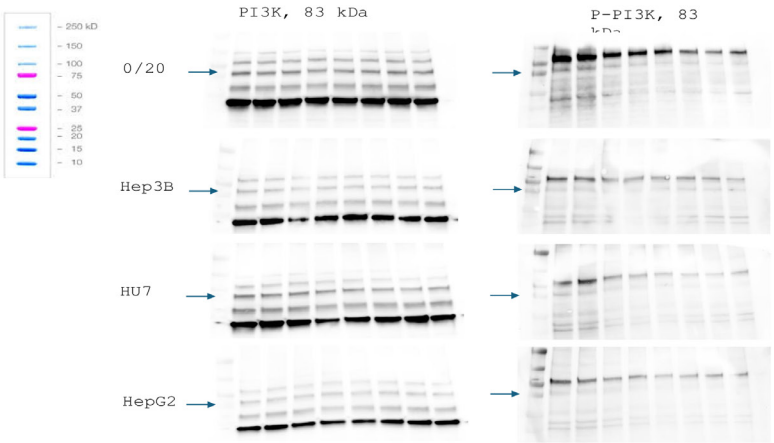


Figure S3: Full pictures of the Western blots

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It is worth highlighting that the reduction in STAT phosphorylation observed following treatment may reflect an overall suppression of IFN-I pathway activity. Since persistent activation of IFN-I signaling contributes to the creationof a pro-inflammatory tumor microenvironment that supports immune evasion, its inhibition could be beneficial in curbing hepatocellular carcinoma progression. These findings align closely with those previous studies, which demonstrated that ginger-derived bioactive compounds can disrupt inflammation-driven oncogenic pathways, ultimately fostering a tumor microenvironment that is more conducive to immune-mediated clearance [41, 42].

The PI3K/AKT pathway is crucial for regulating cell survival, and growth in numerous cancers, including hepatocellular carcinoma

Conclusions

In conclusion, Hepatocellular Carcinoma (HCC) remains a major global health issue, with racial disparities contributing to worse outcomes, especially among African American/Black populations. Our study investigated the effects of ginger-derived compounds, 6-shogaol, 8-gingerol, and 10-gingerol, on HCC cell lines from African American, White, and Asian patients. These compounds, particularly 6-shogaol, effectively reduce cell proliferation and modulate key pathways such as the IFN-I and PI3K/AKT pathwas, with African American-derived cell lines (O/20, Hep3B) being more sensitive to treatment. These findings suggests that ginger compounds could provide targeted therapeutic benefits, particularly for African American HCC patients, potentially overcoming resistance to conventional therapies. While the study demonstrated promising results, further research, including genetic validation, is needed to confirm the exact mechanisms through which these compounds exert their effects. This work lays the foundation for developing more personalized and effective treatment strategies for HCC.

Declarations

Availability of Data and Materials

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' Contributions

Conceptualization, S.K., and R.A.S.; methodology, S.K., S.A. and J.Z.; software, S.K.; resources, R.A.S.; writing-original draft preparation, S.K.; writing-review and editing, J.Z., and S.A.; supervision, R.A.S.; project administration, R.A.S., S.K.; funding acquisition, R.A.S. All authors have read and agreed to the published version of the manuscript.

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