Network Pharmacology and *In Vitro* Validation of Brazilin as a Potential Apoptosis-Inducing Agent in HBV-Related Hepatocellular Carcinoma

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Abstract

Hepatitis B virus (HBV) reactivation-related hepatocellular carcinoma (HCC) presents a significant threat due to its potential to cause liver failure and mortality. Consequently, the discovery of novel treatments that offer anticancer efficacy and liver protection is urgently needed. Brazilin, a natural compound, has previously been reported to possess cytotoxic and liver-protective properties. This research aimed to investigate the potency of brazilin in suppressing the growth of HCC cells through *in silico* and *in vitro* approaches. Hep3B cells, which harbor integrated HBV DNA, were selected as the HCC model, with PGV-1 utilized as a positive control. The *in silico* study used network pharmacology to predict brazilin's potential gene targets. Cytotoxicity was evaluated using the CCK-8 assay, and apoptosis detection was carried out using Annexin V/PI staining followed by flow cytometry. The analysis predicted that brazilin targets key genes such as *SRC, EGFR, AKT1, GRB2, IGF1, ESR1, STAT1, MMP9, JAK2*, and *PPARG* involved in cancer proliferation and metastasis. Proteins such as *SRC, GRB2*, and *MMP9* are overexpressed in *TP53*-mutated HCC and linked to low survival. Brazilin showed moderate cytotoxicity with an IC_{50} value of 17 μM at 72 h and significantly induced apoptosis in Hep3B cells. These findings suggest that brazilin is a promising apoptosis-inducing agent for HBV-related HCC.

Keywords: *brazilin, Hep3B cell lines, network pharmacology, cytotoxic, apoptosis.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and deadly types of liver cancer worldwide, with a rising incidence in many countries (Kim, 2024). In Indonesia, the burden of HCC continues to grow, with 2,159 reported deaths in 2022 (Kemkes, 2024). The majority of HCC

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cases, both globally and in Indonesia, are caused by chronic infections with the hepatitis B virus (HBV) infection at 62.6%, followed by the hepatitis C virus (HCV) infection at 18.8% (Hasan, *et al*., 2023). Chronic HBV infection often progresses silently to cirrhosis and eventually HCC, with many patients being diagnosed at an advanced stage when only palliative treatment options remain (Bozza, *et al*., 2016). The high burden of HCC in Indonesia, particularly those associated with chronic HBV infection, presents major challenges for cancer therapy development due to late-stage diagnosis, limited treatment responsiveness, and the risk of HBV reactivation during systemic chemotherapy.

Therapies with radiation or surgery are only recommended for HCC patients in the early stages, with liver function assessed as being in good condition (Child-Pugh A category) (Kemkes, 2022). Systemic HCC treatment is currently limited to sorafenib, which has been reported to cause resistance and recurrence after three months of chemotherapy treatment and can also result in HBV reactivation, which increases the risk of liver failure, treatment resistance, and death (Jiang, *et al*., 2023). Thus, more effective and safe HCC therapy is needed. One of the herbal plants known to have properties in treating cancer is *Caesalpinia sappan* (Wijayakusuma, 2004). Infused water of *Caesalpinia sappan* wood is commonly consumed as a typical Indonesian herbal drink, "wedang secang," to increase immune function (Nirmal, *et al*., 2015). According to Hung, *et al*., (2014), different types of Vietnamase *Caesalpinia sappan* extracts demonstrated varying degrees of cytotoxicity against HepG2 hepatoblastoma cells, with the ethanolic extract showing the strongest activity (IC_{50}>30 µg/mL), followed by the methanolic extract (IC_{50}=65 µg/mL), and the aqueous extract (IC_{50}=78 µg/mL). The superior cytotoxicity of the ethanol-based extract might be linked to its brazilin content, as ethanol is widely used to extract and isolate this compound (Hangoluan, 2011). Brazilin, the active compound in *Caesalpinia sappan*, has been widely studied for its immune-modulating and anticancer properties (Nirmal, *et al*., 2015). While *Caesalpinia sappan* is a well-known source of brazilin, *Caesalpinia brazilwood* has been reported to contain even higher levels of this bioactive compound (Dapson and and Bain, 2015). Brazilin has demonstrated hepatoprotective effects *in vivo* by reducing $BrCCl_3$-induced liver injury in rat hepatocytes (Moon, *et al*., 1992). *In vitro* studies of brazilin on MDAMB-231, T47D, and MCF7/HER2 cancer cells showed moderate cytotoxic effects and apoptosis modulation (Handayani, *et al*., 2020; Jenie, *et al*., 2020; Jenie, *et al*., 2018). Based on these reports, brazilin has the potential to treat HCC.

Although brazilin has shown moderate anticancer activity against breast cancer cells, consuming it directly or through *Caesalpinia sappan* extracts requires large quantities due to various other constituents in *Caesalpinia sappan*, such as flavonoids and alkaloids (Wijayakusuma, 2004). This research aims to specifically investigate the impact of purified brazilin on an HCC cell line containing an integrated HBV genome (Hep3B cells) using *in silico* predictions and *in vitro* experiments. As a comparison, the curcumin-derived compound pentagamavunon-1 (PGV-1) was employed as a positive control, given its previously reported strong anticancer effects on multiple cancer cell types, including HUH-7 cells harboring the HCV genome (Lestari, *et al*., 2019; Moordiani, *et al*., 2023; Rifai, *et al*., 2024; Wulandari, *et al*., 2021). Computational methods, including network pharmacology and bioinformatics, were utilized to identify genes potentially targeted by brazilin in Hep3B cells. To assess cytotoxicity, the CCK-8 assay was used to determine cell viability and calculate the IC_{50} value. Meanwhile, apoptosis induction was analyzed via Annexin V and PI staining, followed by flow cytometric analysis.

MATERIALS AND METHODS

Compounds

Brazilin (SML-2132-25MG) was acquired from Sigma, while PGV-1 (positive control) was synthesized by the Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Bioinformatic and Network Pharmacology Study

The target genes of brazilin and PGV-1 were identified by PharmMapper (https://www.lilab-ecust.cn/pharmmapper/), while Gene Expression Omnibus was used to determine the target genes of Hep3B cells (GEO accession: GSE49994). Furthermore, Interactivenn (https://www.interactivenn.net/) was utilized to analyze and identify potential target genes of brazilin or PGV-1 in Hep3B cells. The obtained target genes were then analyzed for their effects on biological processes, cellular components, molecular functions, and KEGG through ShinyGO 0.82 with an FDR cut-off setting of 0.05. Each protein's expression and survival patterns in hepatocellular carcinoma with *TP53* mutation characteristics through UALCAN.

Cell Culture

Hep3B cells were provided by the Chiba Cancer Center Research Institute (CCCRI), Japan. The cells were maintained in Dulbecco's Modified Eagle Medium (Gibco, Invitrogen, USA), enriched with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin 10,000 U/mL (Gibco), under standard culture conditions.

Cytotoxic Assay

Cells (5×10^3 per well) were plated into 96-well plates and allowed to attach overnight. They were treated with varying concentrations of brazilin (1–100 µM) and PGV-1 (0.01–10 µM) for 24, 48, and 72 h. Post-treatment, the medium was replaced with 10 µL of CCK-8 reagent (Dojindo) and 90 µL of 1× PBS. The cells were incubated for another 3 to 4 h, and absorbance was measured at 450 nm using a Thermo Scientific™ microplate reader. Cell viability data were used to compute IC_{50} values.

Apoptosis Detection via Annexin V/PI Staining

To assess apoptosis, 3×10^5 cells per well were seeded into 6-well plates and incubated overnight. The following day, cells were treated with brazilin or PGV-1 for 24 h. Post-treatment, cells were harvested using TrypLE™ (Gibco), washed twice in 1 mL PBS, and centrifuged at 2,500 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in PBS, centrifuged again, and then stained with Annexin V and Propidium Iodide (PI; BD Pharmingen). Samples were incubated for 30 minutes at 37 °C in the dark. Apoptotic cells were quantified by flow cytometry using a FACSCalibur instrument.

Statistical Analysis

All quantitative results were analyzed using GraphPad Prism version 9.0. Statistical differences among treatment groups were evaluated via one-way ANOVA followed by Tukey's post hoc test, with significance set at $p<0.05$.

RESULTS

Potential Target Genes

Evaluating brazilin's potential in HCC cells began with identifying its target genes in Hep3B cells. Hep3B is an HCC cell line characterized by high proliferative capacity due to *TP53* mutation and the integration of the hepatitis B virus (HBV) genome. To further investigate brazilin's anticancer potential, we compared its target genes with those of PGV-1 in Hep3B cells. Brazilin was predicted to interact with 627 potential target genes, while PGV-1 was associated with 289 and 214 targets from two different datasets. According to mRNA expression data from the GEO database, Hep3B cells expressed

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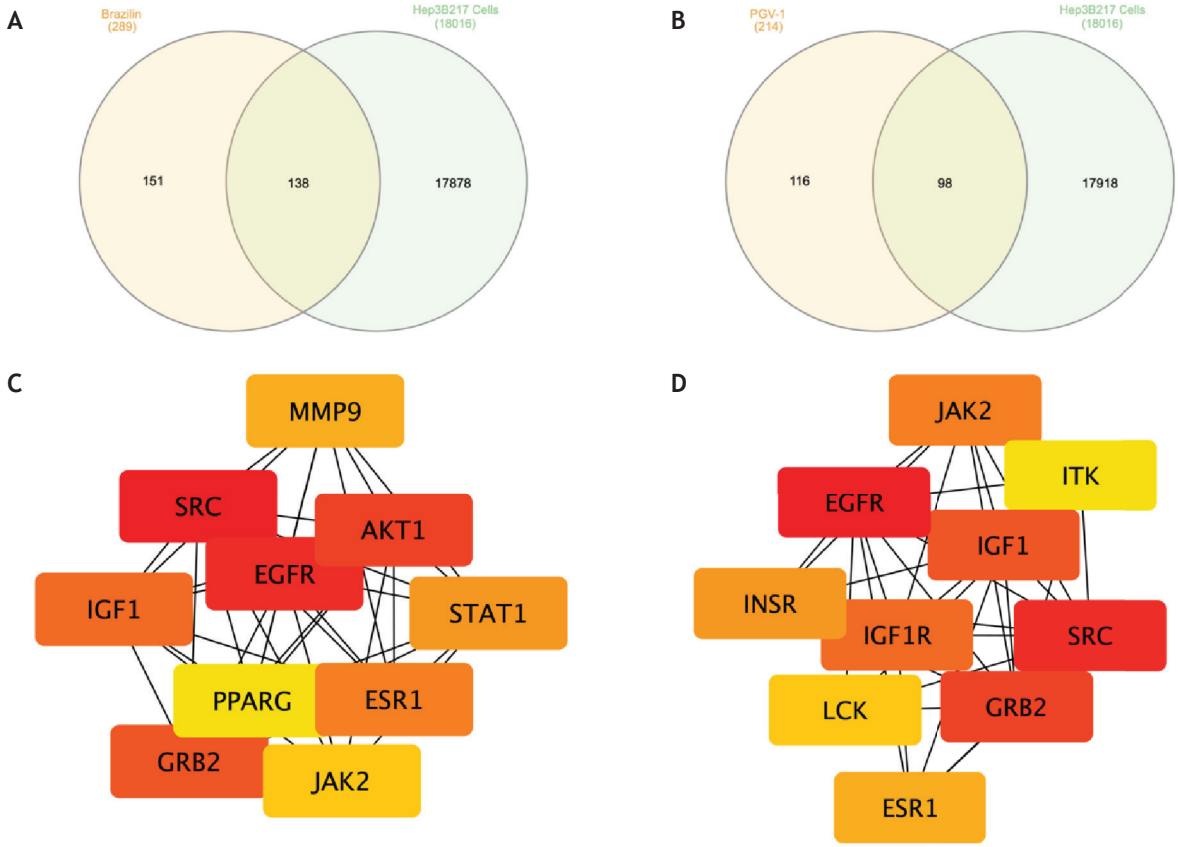

Figure 1. Distinct target genes of brazilin and PGV-1 in Hep3B cells. A Venn diagram (A) illustrates the genes predicted to mediate the inhibitory actions of brazilin (A) and PGV-1 (B) on Hep3B cells. All predicted targets underwent protein-protein interaction (PPI) analysis via STRING version 12.0 with a confidence threshold set at 0.700. These genes were then analyzed using Cytoscape with the Cytohubba plugin to determine the top 10 hub genes based on maximal clique centrality (MCC) scores, shown separately for brazilin (C) and PGV-1 (D). Genes with higher MCC values are marked in red, whereas those with lower scores are colored yellow.

Table 1. Topology analysis based on MCC scores identified the top ten potential gene targets of brazilin in Hep3B cells.

Ranking	Gene name	Score
1	*SRC*	4,226
2	*EGFR*	3,850
3	*AKT1*	2,783
4	*GRB2*	2,474
5	*IGF1*	2,432
6	*ESR1*	2,087
7	*STAT1*	1,681
8	*MMP9*	1,646
9	*JAK2*	1,608
10	*PPARG*	1,460

Table 2. Top ten potential gene targets of PGV-1 in Hep3B cells based on topology analysis via Cytoscape-CytoHubba with MCC score.

Ranking	Gene name	Score
1	*EGFR*	1,502
2	*SRC*	1,485
3	*GRB2*	1,331
4	*IGF1*	1,262
5	*IGF1R*	984
6	*JAK2*	894
7	*INSR*	720
8	*ESR1*	443
9	*LCK*	186
10	*ITK*	144

18,016 genes. Of these, 138 genes overlapped with brazilin's predicted targets, whereas PGV-1 shared 98 target genes with the Hep3B expression profile (Figure 1A-B).

Moreover, to identify crucial genes involved in suppressing HCC progression, hub gene analysis was performed on the overlapping gene sets using Maximal Clique Centrality (MCC) scores. The top ten hub genes targeted by brazilin were S*RC, EGFR, AKT1, GRB2, IGF1, ESR1, STAT1, MMP9, JAK2*, and *PPARG* (Figure 1C, Table 1). In comparison, PGV-1's top ten hub genes were *EGFR, SRC, GRB2, IGF1, IGF1R, JAK2, INSR, ESR1, LCK*, and *ITK,* ranked in descending order of MCC score (Figure 1D, Table 2). Brazilin's main target was identified as *SRC*, while PGV-1 primarily targeted EGFR. Despite these differences, both compounds shared six common targets (*SRC, EGFR, GRB2, IGF1, JAK2,* and *ESR1*) according to network pharmacology results. All candidate genes from brazilin and/or PGV-1 were extensively examined to explore their roles in biological processes, cellular locations, molecular functions, and their participation in multiple pathways listed in the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Functional Enrichment Analysis of Predicted Target Genes

The progression of hepatocellular carcinoma involves complex molecular networks that influence various biological functions and signaling cascades. The hub genes targeted by brazilin and PGV-1 demonstrate different molecular functions. Brazilin's key genes are mainly linked to biological activities such as promoting angiogenesis by controlling vascular smooth muscle cell growth, regulating programmed cell death via apoptosis, and modulating intracellular signaling pathways essential for tissue growth and communication. These functional roles correspond to gene targets in specific cellular structures, including euchromatin regions, the plasma membrane's external components, and specialized lipid raft domains.

Moreover, brazilin shows strong enrichment in molecular functions such as growth factor receptor binding, transcription coactivator binding, and kinase activity, which may modulate key signaling pathways and molecular functions. KEGG pathway analysis further indicates that brazilin is enriched in prolactin signaling and EGFR tyrosine kinase pathways, both of which play significant roles in cancers such as breast, endometrial, and colorectal

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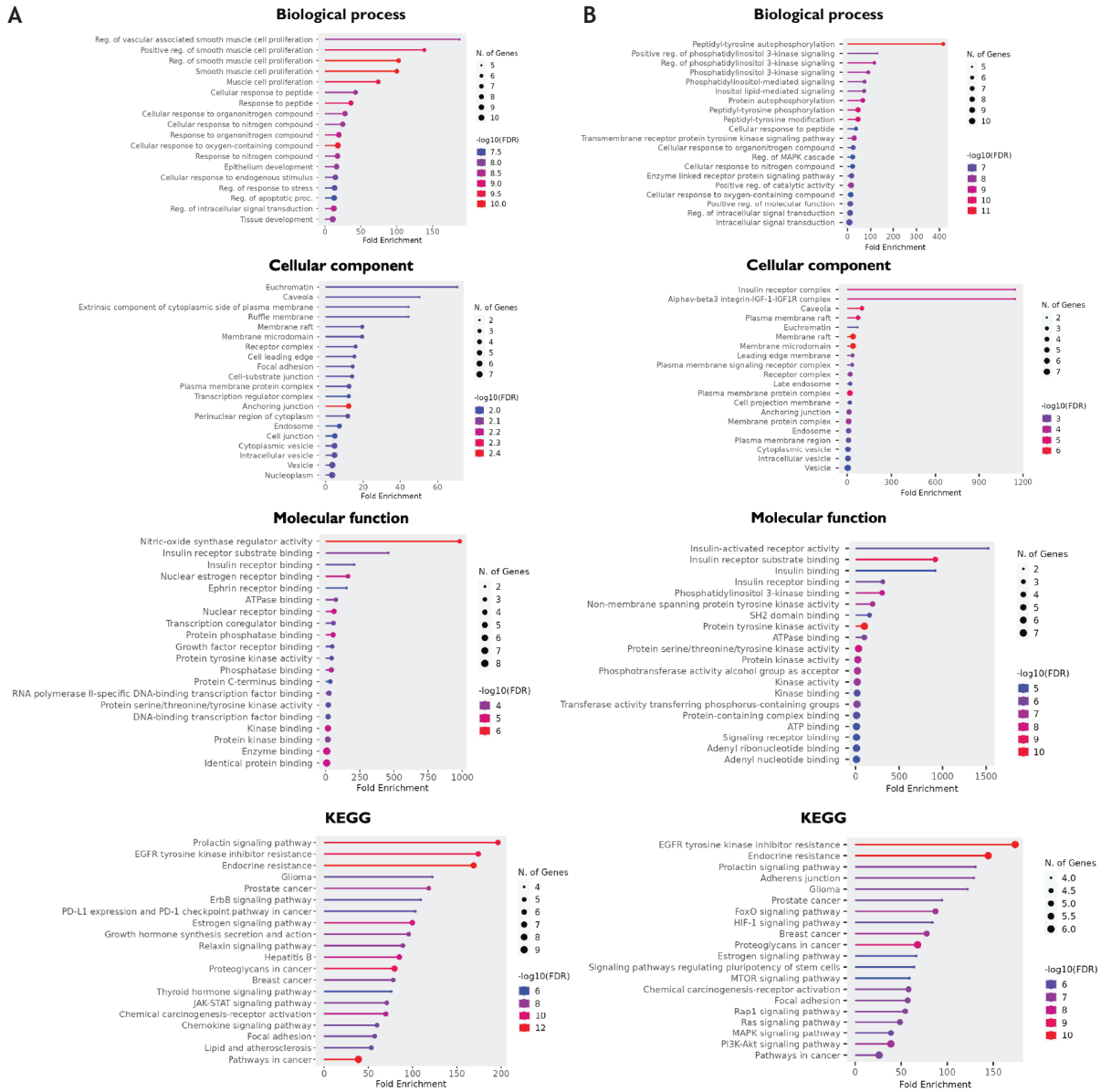

Figure 2. Functional enrichment analysis of Brazilin and PGV-1 gene targets in Hep3B cells. Predicted hub genes of brazilin (A) and PGV-1 (B) in Hep3B cells were analyzed using ShinyGO version 0.80.

cancer (Figure 2A). These findings suggest that brazilin targets explicitly multiple signaling pathways, including those related to cell growth, hypoxia response, and steroid hormones.

Conversely, PGV-1 mainly influences biological activity by blocking tyrosine kinase signaling pathways, as shown by significant enrichment in processes like regulation of peptidyl-tyrosine autophosphorylation and transmembrane receptor protein tyrosine kinase signaling. The effects of PGV-1 also extend to cellular components, molecular functions, and KEGG pathways through kinase binding, phosphotransferase activity, and interaction with insulin receptor substrates. These functions are linked to critical pathways including EGFR tyrosine kinase, insulin signaling, PI3K/Akt,

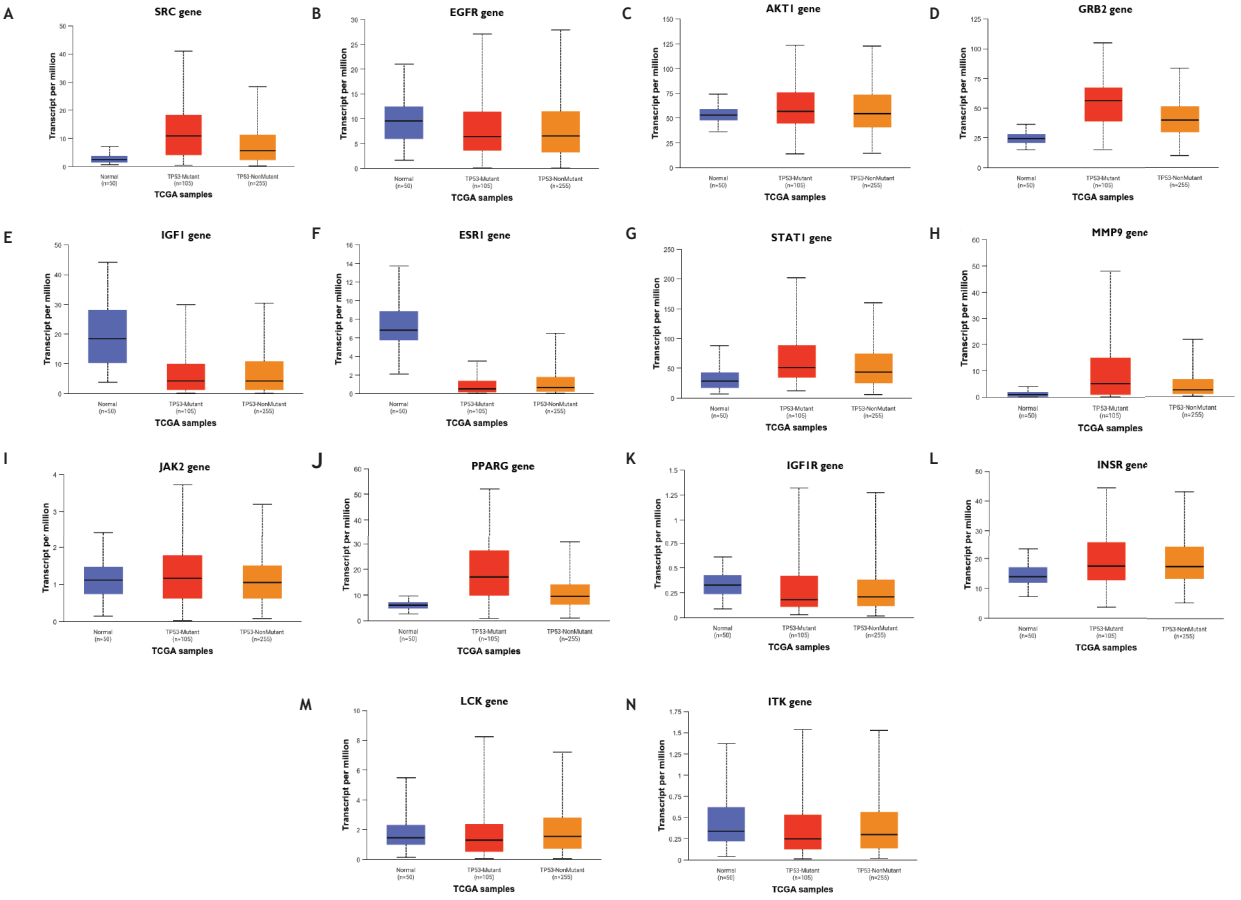

Figure 3. Expression level of predicted target genes. The predicted target genes of brazilin and PGV-1, each encoding proteins potentially involved in their biological activity, were assessed for expression patterns. Gene expression levels were analyzed using the UALCAN portal in hepatocellular carcinoma (HCC) patients harboring *TP53* mutations. The genes evaluated include: (A) *SRC*, (B) *EGFR*, (C) *AKT1*, (D) *GRB2*, (E) *IGF1*, (F) *ESR1*, (G) *STAT1*, (H) *MMP9*, (I) *JAK2*, (J) *PPARG*, (K) *IGF1R*, (L) *INSR*, (M) *LCK*, and (N) *ITK*.

and MAPK (Figure 2B). Although their mechanisms differ, both brazilin and PGV-1 interfere with signaling pathways that govern cancer cell growth, survival, and resistance to treatment, underscoring their promise as anti-cancer agents.

Expression and Survival Probability

When mutated, genes regulating the expression of specific proteins can lead to altered cell growth characteristics, ultimately affecting cancer patient survival rates (Mercadante and Kasi, 2025). One key factor in Hep3B cells exhibiting a highly proliferative phenotype is the *TP53* mutation (Pomo, *et al.*, 2016). Mutations in *TP53* disrupt this function, leading to abnormal cell growth and dysregulation of proliferation-related genes (Pomo, *et al.*, 2016). Mutant *TP53* proteins often accumulate in the nucleus and exert a dominant-negative effect, further impairing cellular regulation (Wang, *et al.*, 2023).

A total of 14 previously predicted genes showed differential expression in HCC patients with or without *TP53* mutations (Figure 3). Proteins, including *SRC, GRB2, MMP9, JAK2, PPARG,*

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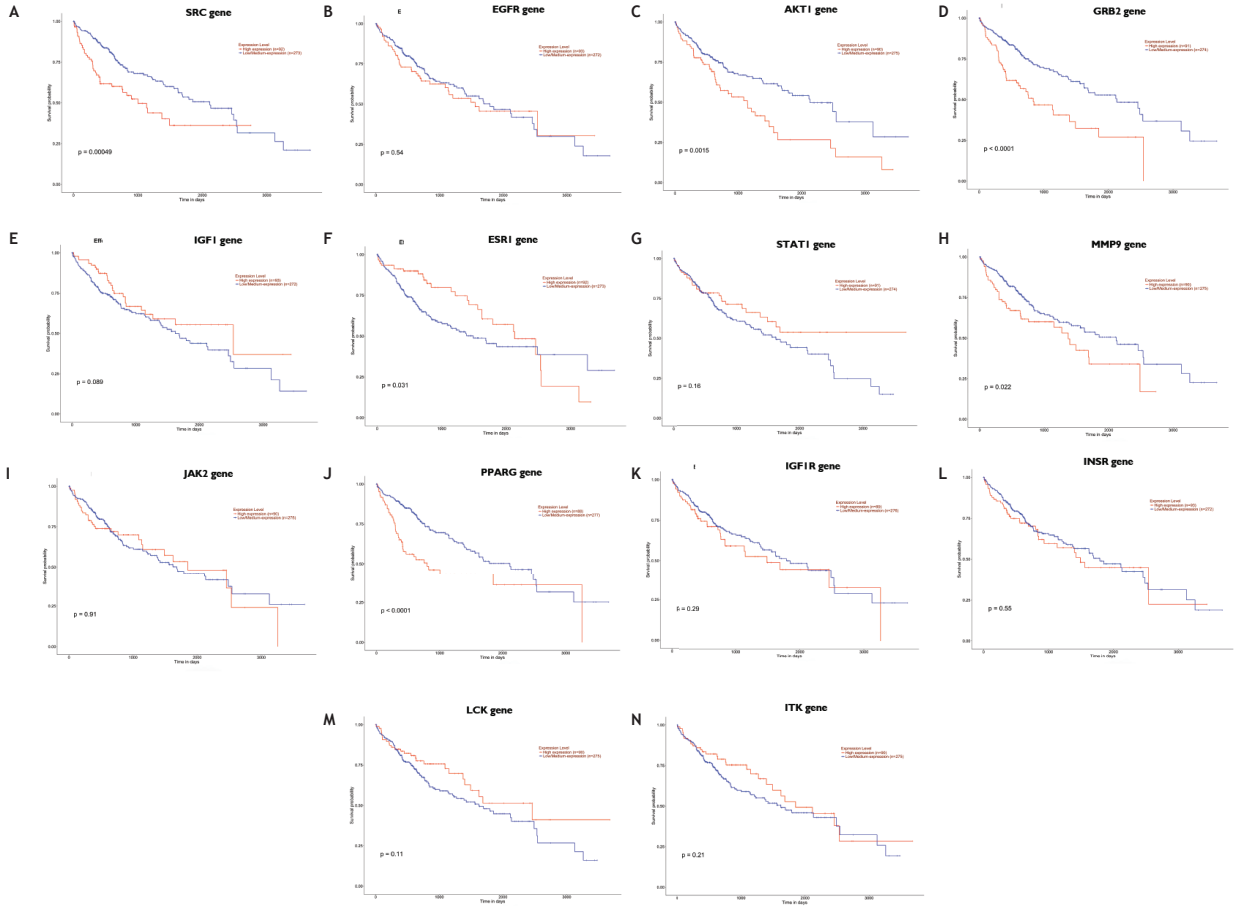
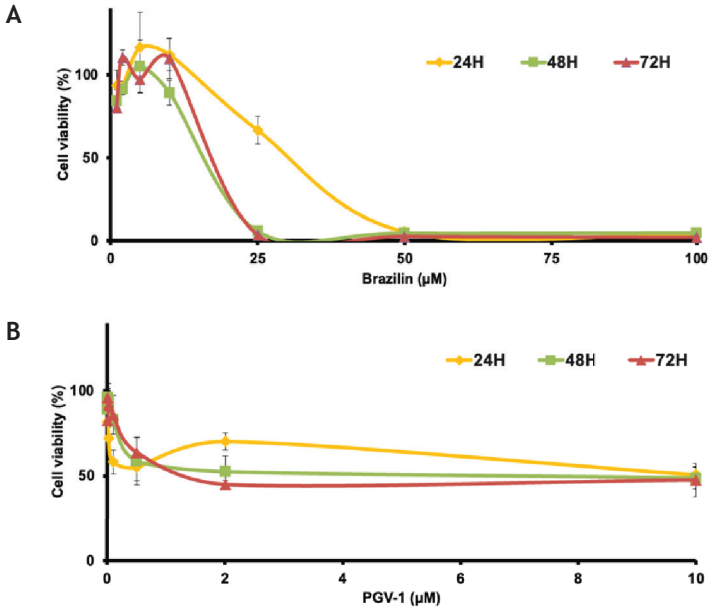


Figure 4. Survival analysis of predicted target genes. Survival analysis of the predicted targets of brazilin and PGV-1 was conducted on genes encoding proteins potentially involved in their biological activity. Using the UALCAN portal, overall survival over a 3,000-day period was analyzed in HCC patients with *TP53* mutations. The genes evaluated include: (A) *SRC*, (B) *EGFR*, (C) *AKT1*, (D) *GRB2*, (E) *IGF1*, (F) *ESR1*, (G) *STAT1*, (H) *MMP9*, (I) *JAK2*, (J) *PPARG*, (K) *IGF1R*, (L) *INSR*, (M) *LCK*, and (N) *ITK*.

IGFR1, and *INSR* were significantly overexpressed in HCC patients with TP53 mutations ($p<0.05$). *EGFR, AKT1, STAT1, LCK,* and *ITK* were highly expressed in *TP53*-mutant and *TP53*-non-mutant patients. In contrast, *IGF1* and *ESR1* were consistently downregulated in both patient groups. Survival analysis indicated that elevated *SRC, GRB2,* and *MMP9* expression was significantly associated with reduced survival times, specifically less than 3,000 days (equivalent to approximately 8 years and 2 months) (Figure 4). Shorter survival is often linked to uncontrolled, abnormal cell proliferation. These results highlight the importance of *SRC, GRB2,* and *MMP9* in HCC with *TP53* mutation. Their high expression in *TP53*-mutant HCC cells suggests they could serve as critical markers or therapeutic targets in the progression of HCC.

Cytotoxic Profile

To validate the results of the previous bioinformatics investigation, we evaluated the cytotoxic effects of Brazilin and PGV-1 on Hep3B cells using the CCK-8 assay over 24, 48, and 72 h. Brazilin exhibited IC_{50} values of 30, 15, and 17 µM at 24, 48, and 72 h, respectively (Figure 5A).



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Compound	IC_{50} (μM)		
	24H	48H	72H
Brazilin	30.0 ± 2.1	15.4 ± 1.35	16.7 ± 2.26
PGV-1	> 10	5.8 ± 0.54	4.1 ± 0.20

Figure 5. Cytotoxicity of brazilin and PGV-1 in Hep3B cells. The cytotoxic effects of brazilin (A) and PGV-1 (B) on Hep3B cells were assessed after 24, 48, and 72 h of treatment, followed by incubation with the CCK-8 reagent for 3 to 4 h. Absorbance readings were taken at 450 nm using a microplate reader, and the reduction in cell viability was used to calculate the IC_{50} values ± standard deviation (C).

In contrast, PGV-1 displayed IC_{50} values of >10, 6, and 4 μM over the same time points (Figure 5B). Both compounds demonstrated lower IC_{50} values at 72 h compared to 24 and 48 h, indicating increased cytotoxicity over time (Figure 5C). However, PGV-1 showed significantly greater cytotoxic effect than brazilin in Hep3B cells. Additionally, the cytotoxicity profile revealed that brazilin was moderately cytotoxic, whereas PGV-1 exhibited potent cytotoxicity (Ren, *et al*., 2018).

Cell Death Modulation via Apoptosis

Based on previous bioinformatics and network pharmacology studies, potential gene targets of brazilin were known to affect the apoptosis level of Hep3B cells. The death response through apoptosis was then evaluated using flow cytometry using ½ IC_{50} of brazilin and PGV-1 in 24 h, which were 9 μM and 2 μM, respectively (Figure 6A). Brazilin and PGV-1 showed the ability to induce apoptosis. The induction of apoptosis by brazilin was higher than that of PGV-1 in Hep3B cells (Figure 6B). These findings confirmed the effect of brazilin in inducing apoptosis in Hep3B cells.

DISCUSSION

This study aimed to investigate the ability of brazilin to suppress the growth of rapidly dividing HCC cells harboring integrated hepatitis B virus (HBV) by *in silico* and *in vitro* approaches using Hep3B cell lines. HCC with chronic hepatitis B infection remains a significant concern due to its potential to cause liver failure and its high mortality rate. Therefore, current explorations in anti-HCC drug development often utilize compounds with immunomodulatory and hepatoprotective properties to halt HCC progression while maintaining liver function (PPHI, 2017). Brazilin is among the compounds known to provide such benefits.

From our *in silico* study, we found that the low survival rate in HCC patients with *TP53*

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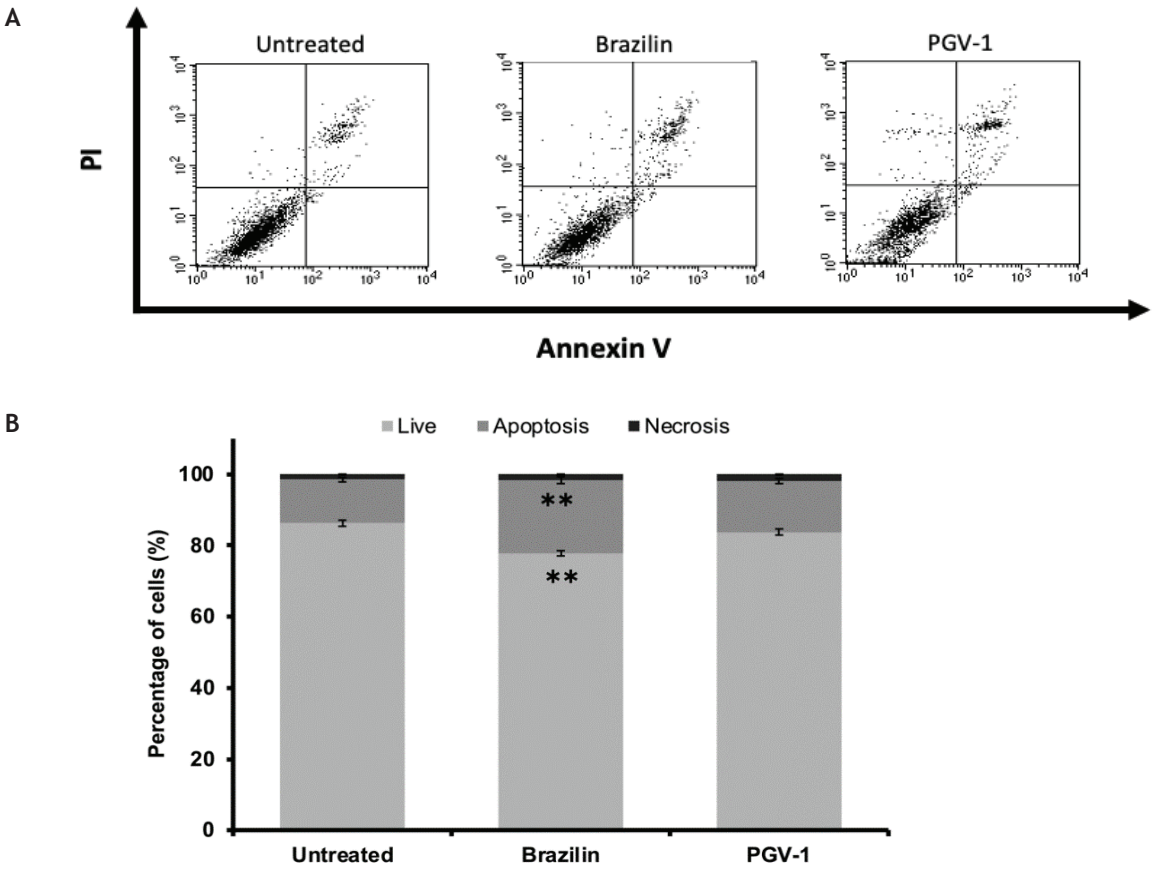

Figure 6. Apoptosis effect of Brazilin and PGV-1 on Hep3B cells. Hep3B cells were exposed to brazilin and PGV-1 for 24 h, stained with Annexin V/PI and analyzed by flow cytometry to assess apoptosis. The flow cytometry results showing apoptotic cell counts are presented in panel (A), while panel (B) displays the proportions of viable, apoptotic, and necrotic cells. Data represent the mean ±SD. Statistical significance was evaluated using one-way ANOVA with Tukey's post hoc test at a 95% confidence interval. ** indicates $p<0.01$.

mutations, as modeled by Hep3B cells, was influenced by overexpression of *SRC, GRB2*, and *MMP9* (Figure 3). These three genes are indeed known to be highly expressed in HCC cells with *TP53* mutations (Hidalgo, *et al.*, 2024; Lu, *et al.*, 2013; Tang, *et al*., 2023). The SRC proto-oncogene regulates proliferation, differentiation, cell motility, and angiogenesis and is frequently activated in cancer cells, contributing to tumor aggressiveness (Yang, *et al*., 2021). This finding is consistent with our previous data showing that brazilin also targets SRC in HCC JHH-4 cells integrated with hepatitis C virus (HCV) (data on publication process). When SRC is activated, it will phosphorylate GRB2 at the Src homologous (SH2) domain (Wang, *et al*., 2024). Furthermore, activated GRB2 triggers the Ras/MAPK pathway, critical in cell proliferation and differentiation in the EGFR tyrosine kinase signaling pathway (Xiao, *et al*., 2021). Interestingly, brazilin also targets EGFR. On the other hand, MMP9 plays a critical role in mediating metastasis (Li, *et al*., 2017). These findings suggest that brazilin modulates multiple signaling pathways observed in HCC by integrating the HBV and/or HCV genomes.

Targeting these pathways results in proliferation inhibition. However, with extended incubation up to 72 h, the cytotoxic effect of brazilin in Hep3B cells remained moderate, with an IC_{50} value approximately four times higher than PGV-1. This data indicates that Hep3B cells were more sensitive to PGV-1. Our current cytotoxicity effect of PGV-1 is consistent with previous results in HUH-7 HCC cells, where the incubation period was prolonged to 96 h to observe its effects (Moordiani, *et al*., 2023). The decision for extended incubation is supported by the high proliferation rate of HCC cells with HBV integration, such as in Hep3B cells (Llovet, *et al*., 2021). This fact is supported by the visible cytotoxic effect of brazilin and its stable cytotoxicity after 48 and 72 h of observation. Meanwhile, on JHH-4 cells, which are HCC cells with HCV integration, the same effect of brazilin was seen after 24 h. The data showed that the proliferation rate characteristic of the two HCC cells affects the cytotoxic potential of brazilin.

Based on our *in silico* findings, brazilin targets different genes than PGV-1 in targeting key pathways in Hep3B cells. Specifically, brazilin targets the genes *AKT1, STAT1, MMP9*, and *PPARG* in Hep3B cells. This reduction in cell viability may be linked to the induction of apoptosis due to the interaction of brazilin and AKT1. Moreover, the apoptotic mechanism was also one of the biological processes influenced by brazilin *in silico* studies (Figure 2). AKT1 plays a crucial role in promoting cell survival by inhibiting apoptosis (Sun, *et al*., 2020). AKT1 signaling is activated through the PI3K/AKT pathway, where phosphoinositide 3-kinase (PI3K) phosphorylates PIP2 to PIP3, which in turn recruits AKT1 to the plasma membrane for activation via phosphorylation (He, *et al*., 2021). Once activated, AKT1 phosphorylates and inhibits several pro-apoptotic proteins, such as BAD and caspase-9, and also upregulates anti-apoptotic proteins like Bcl-2, suppressing the apoptotic response and promoting cell proliferation and survival (He, *et al*., 2021). Our study revealed that brazilin induced a higher apoptosis than PGV-1 after 24 h incubation (Figure 5). This effect may involve the modulation of AKT1 signaling. However, we still need to confirm this hypothesis by observing the expression of its downstream effector, such as phosphorylated BAD, Bcl-2, or caspase-9. Apoptosis in Hep3B cells occurs through a p53-independent pathway due to TP53 mutations (Mitry, *et al*., 1997). This mode of cell death is considered more favorable, as it minimizes inflammation and tissue disruption (Fink and Cookson, 2005). Limiting inflammation is crucial for maintaining homeostatic conditions, which in turn helps preserve liver morphology and function (Robinson, *et al*., 2016). Brazilin has been reported to protect rat hepatocytes from damage induced by the hepatotoxic agent $BrCCl_3$ (Moon, *et al*., 1992). These findings suggest that brazilin could be developed as a potential therapeutic agent against HCC by inducing apoptosis without triggering liver damage.

Meanwhile, *MMP9, STAT1*, and *PPARG* are involved in cell migration (Marchi, *et al*., 2017). In HCC cells with HCV integration (JHH-4 cells), brazilin reduced both inactive and active forms of MMP9 (data on publication process). In particular, HCC cells with HBV integration have been shown to express higher levels of MMP9 than those without HBV (Kim and Kim, 2004). Marchi, *et al*. (2017) reported that *STAT1* and *PPARG* are among the prognostic biomarkers of penile carcinoma, along with eight other genes (*AR, BIRC5, DNMT3B, ERBB4, FGFR1, PML, RB1*, and *TNFSF10*). Thus, PPARG plays an important role in inhibiting inflammation and angiogenesis by inhibiting the activity of various transcription factors, one of which is STAT (Lv and Yao, 2022). These findings warrant further investigation of the anti-migratory effects of brazilin in HCC, particularly in Hep3B cells, to better understand its therapeutic potential against metastatic HCC. Although in this study we did not perform migration assay, there are several studies reporting the ability of brazilin to

inhibit migration in breast cancer cells specifically through FAK inhibition in MDA-MB-231 cells and suppression of MMP2 and MMP9 in MCF7/HER2 cells (Hernández-Moreno, *et al*., 2025; Jenie, *et al*., 2018). Overall, our findings support the potential of brazilin as a multitarget agent that not only inhibits proliferation via *SRC, GRB2*, and *EGFR* but also induces apoptosis through modulation of AKT1 signaling and suppresses cancer cell migration by targeting *MMP9, STAT1*, and *PPARG*. These dual effects highlight the promise of brazilin as a therapeutic candidate for HCC, particularly in p53-independent cancer models. However, further studies are needed to validate its molecular targets *in vivo*. A deeper understanding of brazilin's pharmacokinetics, bioavailability, and safety profile is also essential to advance toward clinical application.

CONCLUSION

Brazilin and PGV-1 are predicted to share two top target genes, *SRC* and *EGFR*, in Hep3B cells. In addition, brazilin is specifically predicted to target genes involved in apoptosis regulation, including *AKT1*, *STAT1*, *MMP9*, and *PPARG*. While brazilin exhibits only moderate cytotoxic activity against Hep3B cells, it induces a higher proportion of apoptotic cells compared to PGV-1.

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