Turk J Pharm Sci 2025;22(3):170-177
DOI: 10.4274/tjps.galenos.2025.46837

ORIGINAL ARTICLE

Effect of PGV-1 on Apoptosis *via* Mitotic Arrest and Senescence in Polyploid Giant Cancer Cells of Hepatocellular Carcinoma JHH4

Nadzifa NUGRAHENI[1], Beni LESTARI[2], Edy MEIYANTO[3], Rohmad Yudi UTOMO[4]*

[1]Universitas Gadjah Mada, Graduate School of Biotechnology, Yogyakarta, Indonesia
[2]Universitas Gadjah Mada, Faculty of Medicine, Department of Pharmacology and Therapy, Division of Public Health and Nursing, Yogyakarta, Indonesia
[3]Universitas Gadjah Mada, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Laboratory of Macromolecular Engineering, Yogyakarta, Indonesia
[4]Universitas Gadjah Mada, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Laboratory of Medicinal Chemistry, Yogyakarta, Indonesia

ABSTRACT

Objectives: Senescent cells release a senescence-associated secretory phenotype, promoting polyploid giant cancer cells (PGCCs) to emerge, fostering tumor heterogeneity and resistance. Pentagamavunone-1 (PGV-1) emerges as a promising agent inducing senescence and prometaphase arrest, resulting in permanent cytotoxicity. This study was aimed to investigate the effect of PGV-1 in dysregulating mitosis through the modulation of PGCCs and senescence in low MYCN-expressing hepatocellular carcinoma (HCC) cells JHH4.

Materials and Methods: To assess the physiological effects of PGV-1, several *in vitro* tests were done including the MTT assay, cell cycle assay, May-Grünwald-Giemsa staining, senescence associated-β-galactosidase (SA-β-Gal) assay, and apoptosis assay. The protein levels of the apoptosis regulatory protein were evaluated using western blot analysis. The interaction of PGV-1 toward the protein that plays a role in PGCCs formation was simulated by molecular docking and molecular dynamics (MD).

Results: The cytotoxic assay revealed that PGV-1 inhibited the proliferation of JHH4 liver cancer cells permanently. Inhibition of cell proliferation by PGV-1 was associated with the modulation of G2/M phase, particularly mitotic arrest and formation of PGCCs. The SA-β-Gal verified that PGV-1 induced senescence in cells (p<0.01), inducing PGCCs formation. Apoptotic mechanisms were validated *via* Annexin V staining, which showed the level of cleaved poly (ADP-ribose) polymerase (p<0.001). Molecular docking and MD simulations suggested that PGV-1 could interfere with the conformation of the chromosomal passenger complex (CPC) protein, particularly disrupting essential interactions within the inner centromere protein, Survivin, and Borealin.

Conclusion: PGV-1 induced strong cytotoxicity in HCC cells by disrupting mitosis leading to PGCC formation, senescence, and subsequent apoptotic cell death.

Keywords: PGV-1, polypoid giant cancer cells, senescence, apoptosis, hepatocellular carcinoma

INTRODUCTION

Polyploid giant cancer cells (PGCCs) and cellular senescence play multifaceted roles in the cell cycle machinery of hepatocellular carcinoma (HCC). PGCCs are characterized by abnormal polyploidization, leading to errors in mitotic spindle assembly and chromosomal segregation during mitosis.[1] Aberrant centrosome function in PGCCs contributes to the enlarged cell size and genomic instability, defining the characteristic morphology of PGCCs.[2,3] PGCCs exhibit a senescence-like phenotype, contributing to chemoresistance by evading the cytotoxic effects of chemotherapy drugs.[4] Simultaneously, the irreversible growth arrest characteristic of cellular senescence can create a microenvironment rich in senescence-associated secretory phenotype (SASP) factors,

***Correspondence:** rohmad.yudi.utomo@ugm.ac.id, **ORCID-ID:** orcid.org/0000-0003-4803-9417
Received: 20.05.2024 , **Accepted:** 02.06.2025 **Publication Date:** 01.08.2025

Cite this article as: NUGRAHENI N, LESTARI B, MEIYANTO E, UTOMO R. GV-1 promotes apoptosis *via* mitotic arrest and senescence in polyploid giant cancer cells of hepatocellular carcinoma JHH4 Turk J Pharm Sci. 2025;22(3):170-177

influencing the behavior of neighboring cancer cells, including the emergence of PGCCs.[5,6] The dynamic interaction between PGCCs and cellular senescence promotes heterogeneity within the tumor, fostering adaptability and resistance mechanisms.[7] Understanding the crosstalk between PGCCs and senescence is crucial for developing chemotherapeutic agents targeting cell cycle machinery in HCC.

The induction of senescence and dysregulation of mitosis represent two primary physiological effects of pentagamavunone-1 (PGV-1) (Figure 1a) in various cancer cells. PGV-1 triggers senescence through transient elevation in reactive oxygen species (ROS) by interacting with several ROS-metabolizing enzymes.[8] PGV-1 promotes prometaphase arrest, leading to permanent cytotoxic effects on leukemia.[8] In both triple-negative breast cancer (TNBC) and non-TNBC cells, PGV-1 increased polyploid cells as an indication of mitotic catastrophe.[9-11] Functional network analysis with molecular docking studies revealed that PGV-1 could target several mitotic checkpoints such as aurora kinase A (AURKA), aurora kinase B (AURKB), polo-like kinase 1 (PLK1), and cyclin-dependent kinase 1 (CDK1).[12] AURKB is a member of the chromosomal passenger complex (CPC) localized at the centromere from prophase to the metaphase-anaphase transition. The CPC consists of four proteins: the AURKB, the scaffold protein inner centromere protein (INCENP) and two targeting proteins, Survivin and Borealin. Reducing or disrupting the activity of any component within CPC results in cytokinesis defects, leading to subsequent multinucleation, resembling the phenotype of PGCCs.[13-15] The effect of PGV-1 on the CPC complex remains unexplored.

Dysregulation of mitosis and induction of senescence by PGV-1 also persist in MYCN-expressing HCC, including HepG2 and JHH7 cells.[15,16] During mitosis, aberrant MYCN expression contributes to centrosome duplication, spindle formation, and chromosome segregation in HCC.[17] MYCN also activates CDK1 to promote mitosis through Aurora A-PLK1 cascades.[18] However, PGV-1 does not affect the mRNA level of MYCN, suggesting that PGV-1 targets different proteins in the dysregulation of centrosomes during mitosis in HepG2 and JHH7.[16] These distinctive phenotypic changes induced by PGV-1, especially in the cell cycle machinery of HCC, provide attractive attributes for targeting PGCCs.

In this study, we aimed to investigate the effect of PGV-1 in dysregulating mitosis through the modulation of PGCCs and senescence in low MYCN-expressing HCC, JHH4 cells. Similar to other cancer types, PGV-1 is expected to exhibit potent cytotoxic effect in JHH4 cells. We identified changes in the phenotype of JHH4 cells based on the cell cycle profile, mitotic phase profile, the presence of senescent cells, and predicted CPC-related targets through computational analysis.

MATERIALS AND METHODS

Compound

PGV-1 was obtained from our compound collection and synthesized as previously reported.[19] For each biological assay, PGV-1 is dissolved in dimethyl sulfoxide to prepare a stock solution. These stock solutions are then diluted for various concentrations for specific experiments.

Cell culture and reagents

JHH4 cells were kindly provided by Professor Yoshitaka Hippo and Dr. Yusuke Suenaga (JRBC® CRL-2359) from Chiba Cancer Center Research Institute, Japan. The cells were maintained under standard conditions (37 °C, 5% CO^2) in high glucose Dulbecco's Modified Eagle Medium (Gibco USA) enriched with HEPES (Sigma), 10% fetal bovine serum (Gibco USA), and 1% penicillin-streptomycin (Gibco USA).

MTT assay

A 96-well plate was seeded with 1×10^4 cells per well, and the cells were cultured until reaching 80% confluency. PGV-1 (0.1-10 μM) was then administered to the cells in repeated doses, followed by incubation for 24 and 48 hours. After the incubation period, MTT (Sigma) reagent was added, and absorbance was measured at 595 nm using a multi-plate reader (BioRad). The absorbance values were converted into percentages of cell viability using Hill's equation to determine the half-maximal inhibitory concentration (IC_{50}) value.[20]

Cell cycle analysis

In brief, JHH4 cells were cultured and treated with PGV-1 for 24 hours. The cells were trypsinized and washed with cold 1X phosphate-buffered saline (PBS). Fixation was performed by adding 70% ethanol for 30 minutes, followed by replacement with 1X PBS. The cells were incubated with a staining solution containing 200 μL 1X propidium Iodide and RNase, prepared according to the kit protocols (Abcam, ab139418), for 20-30 min. The distribution of the cell cycle BD fluorescence-activated cell sorting Calibur flow cytometer.

May-Grünwald Giemsa staining

JHH4 cells were seeded in a 6-well plate at a density of 2.5×10^4 cells per well and incubated with PGV-1 for 24 hours. After removing the medium and washing with 1X PBS, the cells were stained with May-Grünwald stain solution for 5 min, followed by two washes with 1X PBS. Additional staining was performed using 1:20 *v/v* Giemsa solution, followed by air drying. The mitotic phase was evaluated based on the chromosomal formation observed under a phase-contrast microscope.[10]

Senescence associated-β-galactosidase (SA-β-gal) assay

PGV-1 was applied to JHH4 cells that had been seeded at a density of 3×10^6 cells per well on a 6-well plate and incubated for 24 hours. After two washes with 1X PBS, the cells were fixed for 10 to 20 minutes and then washed again with 1X PBS. Cells were stained using an X-gal solution for 72 hours at 37 °C. The presence of senescence was indicated by the green color under the microscope.[21]

Apoptosis assay

Apoptosis assays were performed according to the manufacturer's instructions (Apoptosis Detection Kit I, Cat. 556547). JHH4 cells were treated with PGV-1 for 24 hours. After treatment, cells were harvested using trypsin and washed

with cold 1x PBS. The cells were then resuspended in 1x binding buffer, and equal amounts of fluorescein isothiocyanate Annexin V and propidium iodide were added. The cells were gently vortexed and incubated at 25 ˚C in the dark for 15 minutes. Apoptotic cells were detected using the FACSCalibur flow cytometer.

Western blotting

Briefly, JHH4 cells were seeded at a density of 1 × 10^6 cells per well in a 6-well plate and treated with or without PGV-1 for 24 hours. After treatment, cell lysates were collected using a radioimmunoprecipitation assay buffer. The samples were separated by Sodium dodecyl sulfate – polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. After blocking with 5% bovine serum albumin for 2 hours, the membranes were incubated with primary antibodies: anti-poly(ADP-ribose) polymerase (PARP) antibody (Cell Signaling Technology, PARP (46D11) Antibody #9532) at a 1:1000 dilution, and anti-β-actin antibody (Cell Signaling Technology, #3700) at a 1:5000 dilution, which served as the housekeeping protein. The membranes were probed overnight with primary antibodies at 4 ˚C with gentle agitation. After washing with PBST, the membranes were incubated with an anti-rabbit immunoglobulin G (IgG) secondary antibody (anti-rabbit IgG, horseradish peroxidase-linked Antibody #7074) for 1 hour at room temperature. Chemiluminescent signals were detected using the ImageQuantTM TL with enhanced chemiluminescence Western Blotting Substrate (Bio-Rad). ImageJ software was then used to semi-quantify and normalize the intensity of each band relative to the housekeeping protein (anti-β-actin antibody).[16]

Molecular docking

Molecular docking was performed to predict the binding interaction between PGV-1 and CPC protein, which is among the proteins involved in PGCC generation. The crystal structure of CPC was obtained from the PDB (ID: 2QFA). The binding site for the molecular docking simulation was identified based on the core of CPC, which consists of the interacting amino acids among INCENP, Borealin, and Survivin.[22] The molecular docking simulation was carried out using the default protocol in molecular operating environment, with slight modifications.[23] The conformation of PGV-1 with the lowest docking score was selected for binding interaction analysis.

Molecular dynamics

The stability and conformational changes of PGV-1 in complex with CPC were evaluated by molecular dynamics (MD) simulation. Nanoscale molecular dynamics 2.14 was used for the MD simulation, and the resultant trajectories were visualized with visual molecular dynamics 1.9.4.[24,25] The ligands and protein were parameterized using CGenFF and CHARMM36, accessed through the CHARMM-GUI web server.[26] The preparation included adding water molecules in a cubic box with 20-Å padding for solvation and neutralization, followed by the introduction of K^+ and Cl^- ions. The complex then underwent 70 ps of minimization and a 1000 ps equilibration simulation. To further refine the MD simulation, a 1000 ps production run was performed under the NPT ensemble, maintaining a temperature of 303 K and pressure of 1 atm. The MD results were analyzed based on conformational changes and root mean square deviation (RMSD).

Statistical analysis

The data are expressed as the mean ± standard deviation or standard error (SE) from three independent experiments. Statistical analysis was performed using analysis of variance, followed by the Student's t-test with a 95% and 99% confidence level ($p<0.01$; $p<0.001$). All analyses were conducted using GraphPad Prism software, version 9.0 (GraphPad, San Diego, CA, USA).

RESULTS

PGV-1 permanently inhibits proliferation by inducing G2/M arrest

The establishment of a permanent cytotoxic effect has proven to be a crucial factor in the advancement of chemotherapeutic agents, addressing the challenge of relapse incidence upon the discontinuation of treatment.[27] Based on wash-out experiments, PGV-1 (Figure 1a) exerted strong cytotoxic effects, particularly after withdrawal, in chronic myeloid leukemia, breast cancer, colon cancer, and hepatocellular carcinoma (HCC), with an IC_{50} range of 1-7 μM for 72 h treatment.[8,15,28] However, in high MYCN HCC JHH7, PGV-1 exhibited strong cytotoxicity only after 96 h without affecting MYCN mRNA levels.[16] We are then interested in evaluating the cytotoxicity of PGV-1 toward HCC with low expression of MYCN, the JHH4 cell line. The MTT assay revealed the dose-dependent cytotoxic effect of PGV-1, with IC_{50} values of 10 μM and 1.5 μM for 24 and 48 hours, respectively (Figure 1b). Upon the withdrawal of PGV-1 after three days, the potent cytotoxic effect of PGV-1 remained until six days of observation, indicating the permanent inhibition of proliferation (Figure 1c). The potent cytotoxicity of PGV-1 in JHH4, as reported in other types of cancer cells, confirmed its potential novel phenotypic cytotoxic effect. The induction of a persistent cytotoxic effect has the potential to disrupt the cell cycle. Cell cycle modulation of PGV-1 is associated with G2/M arrest in various cancer cells.[8,15,28,29] We found that PGV-1 consistently induced G2/M arrest in JHH4 and increased the sub-G1 population (Figure 1d). In addition, PGV-1 increased the number of hyperdiploid cells in a dose-dependent manner (Figure 1e). The presence of hyperdiploid cells could indicate an abnormality of genetic material, which could lead to cancer survival or death.[2]

PGV-1 triggers multiple arrests in mitosis and increases senescence cells

The precise regulation of mitosis plays a pivotal role in the action of chemotherapeutic drugs, as aberrations in cell division are a hallmark of cancer.[30] In MYCN-expressing HCC, PGV-1 specifically demonstrated prometaphase and metaphase arrest.[15,16] The May-Grünwald Giemsa staining was used to analyze the mitosis phase affected by PGV-1. At low concentrations, PGV-1 induced chromatin condensation resulting

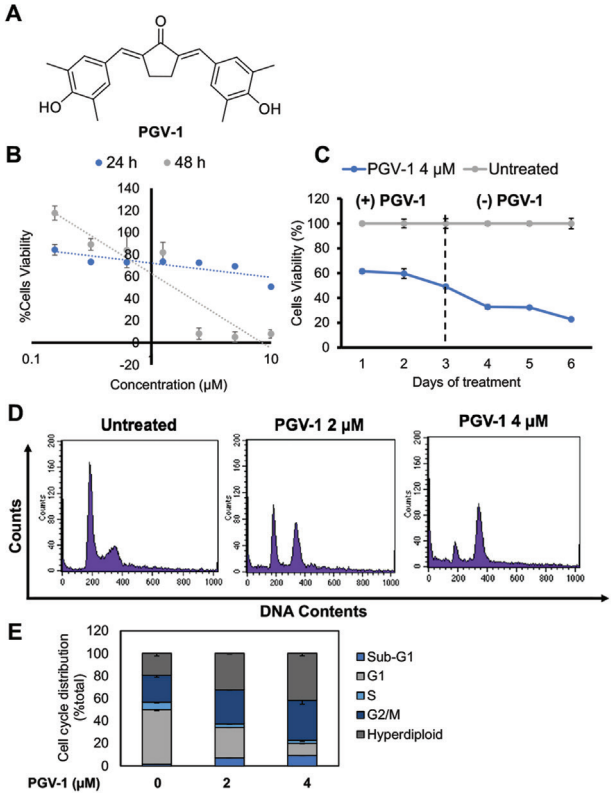

Figure 1. Cytotoxicity of PGV-1 toward JHH4 cells. (A) Chemical structure of PGV-1. (B) Dose-dependent cytotoxic effect in JHH4 cells after PGV-1 is incubated for 24 h and 48 h. (C) JHH4 cells were treated with PGV-1 (4 μM) for 3 days; then the compounds were removed from the medium. Cells viabilitiy was determined at indicated days. (D) JHH4 cells ($2.5x10^5$ cells/mL) were treated with PGV-1 for 24 h, then subjected to propidium iodide-staining flow cytometry for cell cycle analysis. (E) The percentage of cells in each phase as in panel A. Data were expressed on average with SEM (n=3)

SEM: Scanning electron microscope, PGV-1: Pentagamavunone-1

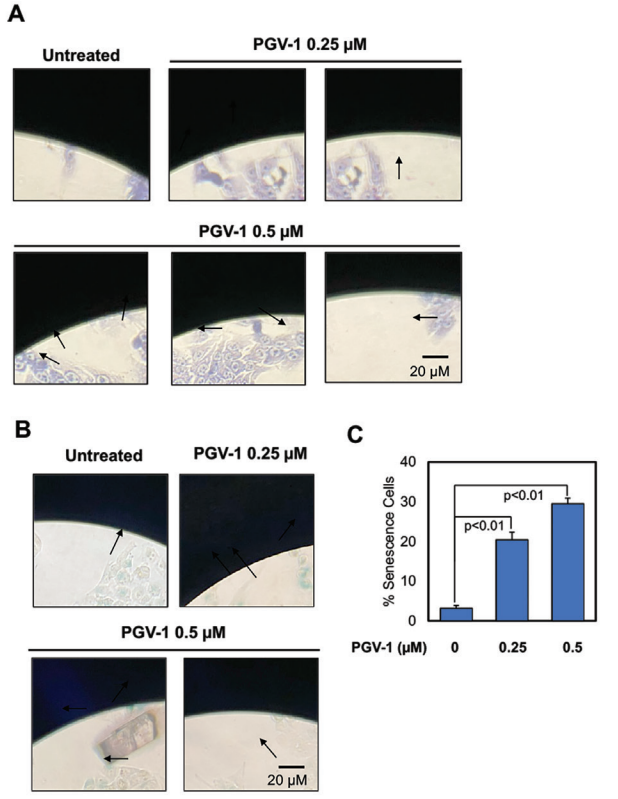

Figure 2. PGV-1 induced metaphase arrest and senescence. (A) JHH4 (3×10^5 cells/well) cells were treated with PGV-1 for 24 h, then stained with a May-Grünwald Giemsa solution. PGV-1 at a concentration of 0.25 μM induced chromatin condensation (left) and mitotic catastrophe (right). At a concentration of 0.5 μM, PGV-1 induced metaphase arrest (left), abnormal anaphase (middle), and PGCCs (right). (B) The percentage of metaphase cells. (C) JHH4 cells (3×10^5 cells/well) were treated with PGV-1 for 24 hours, then stained with an X-gal solution. Senescent cells were marked with arrows. (D) The percentage of senescence cells. Data was expressed on average with SEM (n=3)

SEM: Scanning electron microscope, PGV-1: Pentagamavunone-1

in mitotic catastrophe (Figure 2a). Higher concentrations of PGV-1 triggered metaphase arrest, anaphase abnormality, and PGCCs (Figure 2a). The emergence of PGCCs, characterized by an abnormal number of chromosomes, suggested a unique phenotypical change in PGV-1 in JHH4 cells.

Uncontrolled mitotic activity, which triggers genomic instability, could lead to senescence as a protective mechanism to curb the unbridled proliferation of cancer cells.[31] A reduced prevalence of senescent cells has been implicated in the progression of hepatocarcinogenesis within HCC. Conversely, an elevated abundance of senescent cells is associated with the activation of anti-tumoral mechanisms.[6] The involvement of PGV-1 in eliciting metaphase arrest associated with the senescence arrest in HCC is reported.[16] We found, by SA-β-gal assay, that a low level of cellular senescence was observed in untreated cells and significantly increased in PGV-1-treated cells in a dose-dependent manner (Figure 2b, c). Notably, senescent cells were also identified within the PGCCs, suggesting a connection between genomic rearrangements and the effects of PGV-1 treatment.

The apoptosis mechanism as the final step of the permanent cytotoxic effect by PGV-1

Evidence of senescence in PGCCs facilitated genetic rearrangement in cancer, which led to cancer chemoresistance.[3] The failure of PGCCs regulation could lead to abnormality in cell division, resulting in cell death.[32] We conducted an annexin-V staining to confirm the apoptosis induction of PGV-1, which triggers the PGCCs and senescence in JHH4. PGV-1 increases the incidence of early and late apoptosis (Figure 3a, b). Apoptosis induction by PGV-1 was also identified by the increased protein level of cleaved PARP (Figure 3c, d). Our results confirmed that the induction of senescence and apoptosis by PGV-1 acted as mechanisms to restrict the proliferation of PGCCs, preventing further genomic instability and tumor progression.

Binding interaction of PGV-1 in the chromosomal passenger complex

AURK-PLK1 cascades involving CEP192 and TPX2 tightly regulate the transition of prometaphase to metaphase in mitosis. During preparation to enter anaphase, PLK1 and AURKB

form a complex with CPC, consisting of INCENP, Survivin, and Borealin, to regulate chromosome attachment to the mitotic spindle and ensure proper chromosome segregation (Figure 4a).[18] We hypothesize that the persistent metaphase arrest caused by PGV-1, leading to mitotic catastrophe and PGCCs, was associated with the disruption of the CPC complex. Our molecular docking simulation, focusing on the core of CPC complex, showed PGV-1 interacting with INCENP by forming hydrogen bonding with Gln38 and hydrophobic bonding with Asp27. In addition, PGV-1 formed hydrophobic bonding with Thr127 on survivin (Figure 4b). The multiple interactions of PGV-1 on the core structure might affect the conformational stability of the CPC.

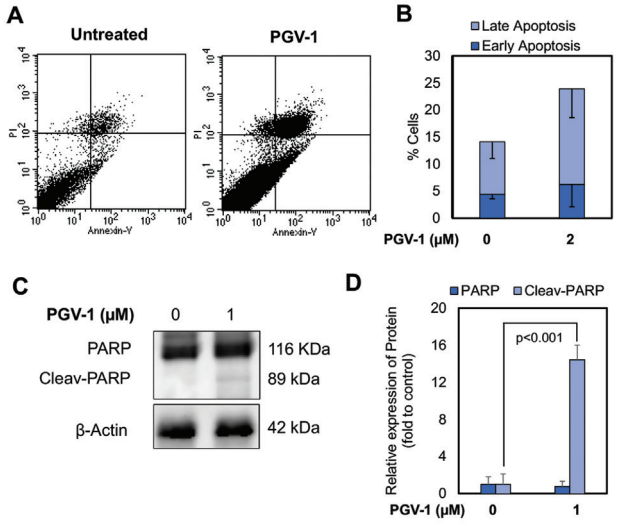

Figure 3. (A) PGV-1 induced apoptosis. JHH4 cells (2.5 x 10^5 cells/mL) were treated with PGV-1 (4 μM) for 24 h, then subjected to Annexin V-staining for apoptosis assay. (B) The percentage of cells undergoing apoptosis as in panel A. Data was expressed on average with SEM (n=3). (C) Protein expression level of PARP in JHH4 after treatment with PGV-1 determined by western blotting. (D) Quantitative analysis of C, PARP, and Cleav-PARP normalized against β-actin. Data was expressed on average with SEM (n=3)

SEM: Scanning electron microscope, PGV-1: Pentagamavunone-1, PARP: Poly (ADP-ribose) polymerase

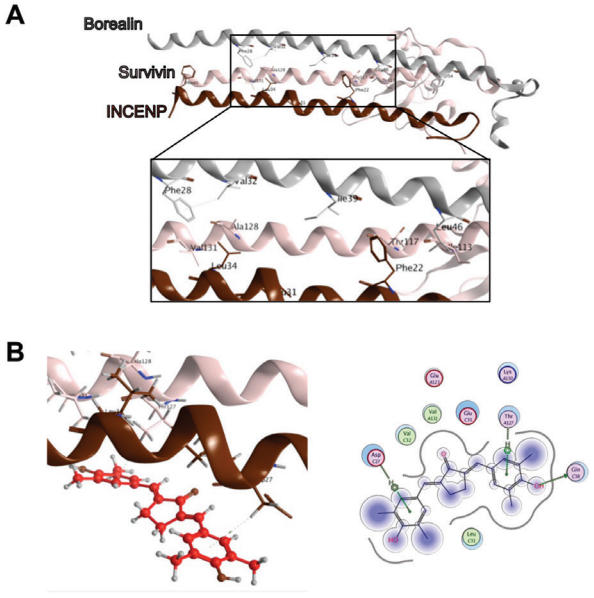

Figure 4. Visualization of binding interaction of PGV-1 in the core of CPC. (A) The core of CPC consisted of the essential interaction between the complex of INCENP, Survivin, and Borealin. (B) PGV-1 interacted with the complex between Survivin and INCENP through hydrogen bonding with Gln38 and hydrophobic bonding with Asp27 and Thr127

CPC: Chromosomal passenger complex, PGV-1: Pentagamavunone-1

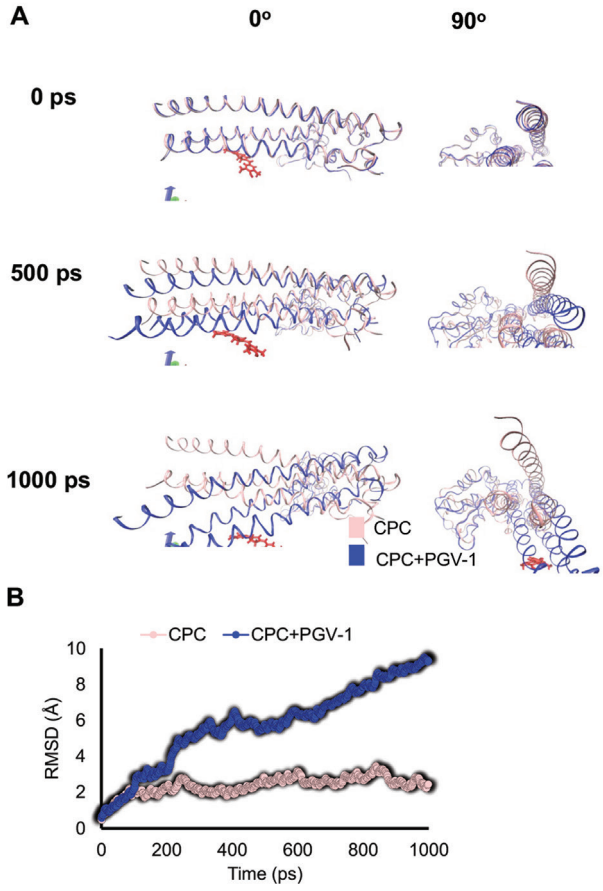

Figure 5. PGV-1 induced conformational change identified by molecular dynamic simulation. (A) Superimposed visualization of the CPC with or without PGV-1 after 1000 ps simulation (B) Comparison of RMSD profile of CPC with or without PGV-1 shown in 1000 frames

CPC: Chromosomal passenger complex, PGV-1: Pentagamavunone-1, RMSD: Root mean square deviation

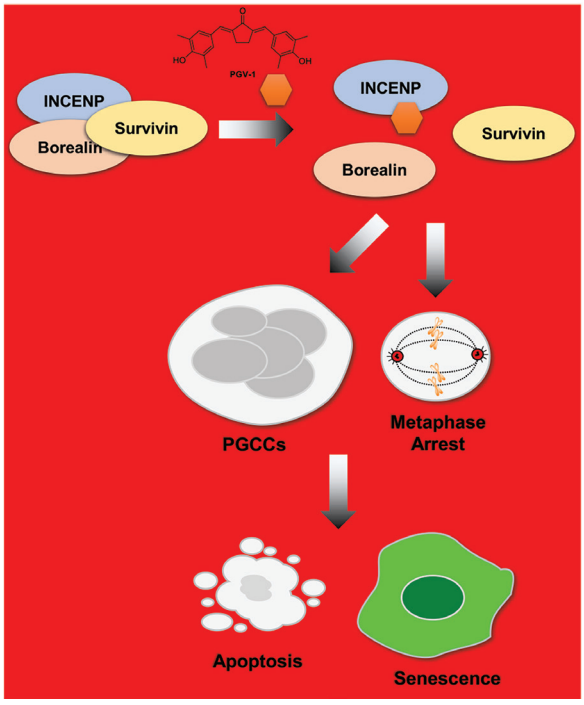

Figure 6. Plausible anticancer mechanism of PGV-1 in JHH4 cells
PGV-1: Pentagamavunone-1

MD simulations were conducted to investigate the stability and potential conformational changes of the CPC complex further. At 500 ps, PGV-1 slightly modified the conformation of the CPC complex (Figure 5a). CPC showed a significant conformational change in the presence of PGV-1 after 1000 ps (Figure 5a). The conformational change was located mainly in the helical structure of INCENP, Survivin, and Borealin, suggesting the binding interaction promoted destabilization of the CPC complex (Figure 5a). The destabilization of CPC in the presence of PGV-1 was also indicated by an increase in RMSD value up to 9.2 Å (Figure 5b). Our MD simulations suggest that PGV-1 promotes a conformational change in the CPC complex, potentially contributing to the dysregulation of chromosome localization during the transition from metaphase to anaphase.

DISCUSSION

PGV-1 has previously exhibited potent cytotoxicity in HepG2 and JHH7 cells without affecting the mRNA level of MYCN.[15,16] This study explored the distinctive phenotypic changes induced by PGV-1 in JHH4 cells with low MYCN expression, contributing valuable foundational knowledge for the development of chemotherapeutic agents for HCC. Examining these phenotypic changes is crucial for elucidating potential signaling pathways activated by PGV-1 in HCC. The utilization of cells with low MYCN expression enables us to distinguish the correlation between MYCN/NCYM signaling regulation and the mechanism of action of PGV-1. While the specific target of PGV-1 remains elusive, our study highlights a cluster of essential targets regulated by PGV-1 in HCC.

HCC is classified as a malignant cancer with only a 10% survival rate for patients within five years of diagnosis.[33] The high rate of recurrence is a major factor contributing to the poor prognosis of HCC compared to other liver cancers.[34] Given the limited treatment options for HCC, there is a critical need for potent and effective cytotoxic chemotherapeutic agents. PGV-1 exhibited potent cytotoxicity in JHH4, greater than in HepG2 and JHH7 after a 48-hour treatment, suggesting that MYCN signaling may not be the primary target of PGV-1. Our results also confirm the persistent cytotoxicity of PGV-1 even after removal from the culture medium, aligning with previous reports.[8,15,16] This observation could indicate the ability of PGV-1 to overcome the recurrence phenomenon in HCC. Revealing the molecular mechanism of the permanent cytotoxic effect of PGV-1 would be essential for further development of chemotherapeutic agents for HCC. The typical cytotoxic effect of PGV-1 in HCC and other cancer types is associated with G2/M arrest, especially in prometaphase or metaphase.[15,16,28] We consistently found that metaphase arrest in JHH4-treated PGV-1 led to mitotic catastrophe. Results from various cancer cells highlight the shared protein targets of PGV-1 in promoting metaphase arrest. Previous bioinformatic and molecular docking studies propose AURKA, AURKB, PLK1, CDK1, KIF11, and WEE1 as possible protein targets of PGV-1.[9,10,12] Elucidating the specific role of metaphase arrest is a crucial requirement for uncovering the precise cytotoxicity of PGV-1.

Metaphase arrest in cancer could either lead to cell death mechanisms or facilitate the promotion of PGCCs for cancer survival. Constitutive endoreplication and endomitosis at metaphase arrest resulted in centrosomal dysregulation and genomic rearrangement, generating PGCCs.[1,35] We found that PGV-1 promotes PGCCs alongside metaphase arrest cells, indicating a connection between both processes. Upon committing to mitosis, it was crucial that subsequent events, such as chromosome segregation and cytokinesis, proceeded without errors. We consider CPC as another target of PGV-1. CPC emerged as a pivotal coordinator overseeing chromosome condensation, orchestrating spindle assembly, rectifying aberrant microtubule-kinetochore interactions, impacting chromosome alignment, signaling to the spindle checkpoint, and aiding in the completion of cytokinesis. Furthermore, disrupting the interaction within the CPC emerged as a promising strategy to impede HCC growth. A prior study demonstrated that the absence of either Survivin or Borealin resulted in HCC regression after hepatocyte mitotic arrest, hypertrophy, and genome polyploidy.[36,37] mirroring the effect of PGV-1 on JHH4.

By performing molecular docking and molecular dynamic simulation of PGV-1 on the complex of CPC, we found that PGV-1 interacted with the essential amino acid sequence that contributes to the interaction of INCENP, Borealin, and Survivin protein-protein interaction, resulting in destabilization of the complex formation. New approaches in anticancer strategies, aiming to restrict CPC function by degrading CPC members or destabilizing the CPC complex, show promise.[38] Interestingly, a previous investigation emphasized the pivotal role of targeting INCENP or interrupting the interaction between INCENP and Aurora B kinase

in efficiently restraining the growth of neuroblastoma cell lines in both MYCN-WT and MYCN-amp cells.[39] Our findings align with this report, showcasing a MYCN-independent impact.

PGV-1 also induces senescence, particularly in PGCCs of JHH4. To control abnormal cell division, PGCCs enter senescence to reorganize cell division for the release of daughter cells that are more resistant to chemotherapeutic agents.[6,35] In contrast, PGV-1 triggers apoptosis in JHH4, as evidenced by the increase in cleaved PARP. These findings reveal the mechanism behind the lasting cytotoxic impact of PGV-1 in JHH4. This involves inducing mitotic arrest, forming PGCCs, increasing cellular senescence, and facilitating ultimately hindering the progression of HCC (Figure 6). Notably, no reported studies have demonstrated the promotion of PGCCs associated with irreversible cytotoxic effects.

Our findings highlight PGV-1 as a promising chemotherapeutic agent with multifaceted mechanisms that address key challenges in cancer treatment, including drug resistance, tumor recurrence, and metastasis. Unlike conventional therapies, PGV-1 demonstrates permanent cytotoxicity, even after drug withdrawal. This durable cytotoxic effect is important to improve survival rates among cancer patients, particularly in highly recurrent malignancies like HCC. As research advances toward clinical trials, PGV-1 holds promise not only for extending survival but also for transforming the therapeutic landscape of aggressive and recurrent cancers.

CONCLUSION

In conclusion, the low MYCN level in JHH4 cells established a crucial model for investigating the distinctive cytotoxic effects of PGV-1 in HCC, focusing on the observation of phenotypic changes. Our consistent findings highlighted the robust cytotoxic effect of PGV-1 in HCC cells, manifested by the induction of arrests across various mitotic phases. This suggested a potential interference with the CPC, resulting in multiple defects during mitotic arrest and the formation of PGCCs. The revelation that PGV-1 initiated senescence in PGCCs, ultimately leading to apoptotic cell death, is noteworthy. These novel insighted into the unique attributes of PGV-1 in JHH4 cells contribute to the understanding of potential therapeutic strategies, particularly in the pursuit of inducing permanent PGCCs death in HCC.

Ethics

Ethics Committee Approval: The ethical committee approval was obtained from the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada - Dr Sardjito General Hospital (approval number: KE/FK/1045/EC/2022, dated: 10.08.2022).

Informed Consent: Not required.

Acknowledgments

This research was funded by the Ministry of Education, Culture, Research and Technology through the "Penelitian Pascasarjana Magister" 2023 scheme under number 3253/UN1/DITLIT/Dit-Lit/PT.01.05/2023 to RYU. NN also would like to thank the Indonesia Endowment Fund for Education (LPDP), Ministry of Finance of the Republic of Indonesia, through the Indonesian Education Scholarship (BPI) program [202209091880]. The authors would like to thank the scientific publication and journal department, Universitas Gadjah Mada, for their assistance in English language editing through Enago (https://www.enago.com).

Footnotes

Authorship Contributions

Concept: E.M., R.Y.U., Design: E.M., R.Y.U., Data Collection or Processing: N.N., E.M., R.Y.U., Analysis or Interpretation: B.L., E.M., R.Y.U., Literature Search: B.L., E.M., R.Y.U.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Zhou X, Zhou M, Zheng M, Tian S, Yang X, Ning Y, Li Y, Zhang S, 2022. Polyploid giant cancer cells and cancer progression. Front Cell Dev Biol. 2022;10:1017588.
2. Zhang S, Mercado-Uribe I, Xing Z, Sun B, Kuang J, Liu J, 2014. Generation of cancer stem-like cells through the formation of polyploid giant cancer cells. Oncogene. 2014;33:116-128.
3. Niu N, Zhang J, Zhang N, Mercado-Uribe I, Tao F, Han Z, Pathak S, Multani AS, Kuang J, Yao J, Bast RC, Sood AK, Hung MC, Liu J, 2016. Linking genomic reorganization to tumor initiation *via* the giant cell cycle. Oncogenesis. 2016;5:e281.
4. Bharadwaj D, Mandal M, 2020. Senescence in polyploid giant cancer cells: a road that leads to chemoresistance. Cytokine Growth Factor Rev. 2020;52:68-75.
5. Mosieniak G, Sliwinska MA, Alster O, Strzeszewska A, Sunderland P, Piechota M, Was H, Sikora E, 2015. Polyploidy formation in doxorubicin-treated cancer cells can favor escape from senescence. Neoplasia. 2015;17:882-893.
6. Cai X, Guillot A, Liu H, 2022. Cellular senescence in hepatocellular carcinoma: the passenger or the driver? Cells, 2022;12:132.
7. Song Y, Zhao Y, Deng Z, Li Y, Hu C, Zhang Q, Liu Z, Tan H, 2021. Stress-induced polyploid giant cancer cells: unique way of formation and non-negligible characteristics. Front Oncol. 2021;11:724781.
8. Lestari B, Nakamae I, Yoneda-Kato N, Kato JY, Meiyanto E, 2019. Pentagamavunon-1 (PGV-1) inhibits ROS metabolic enzymes and suppresses tumor cell growth by inducing M phase (prometaphase) arrest and cell senescence. Sci Rep. 2019;9:14867.
9. Hasbiyani NAF, Wulandari F, Nugroho EP, Hermawan A, Meiyanto E, 2021. Bioinformatics analysis confirms the target protein underlying mitotic catastrophe of 4T1 cells under combinatorial treatment of PGV-1 and galangin. Scientia Pharmaceutica. 2021;89:38.
10. Musyayyadah H, Wulandari F, Nangimi AF, Anggraeni AD, Ikawati M, Meiyanto E, 2021. The growth suppression activity of diosmin and PGV-1 co-treatment on 4T1 breast cancer targets mitotic regulatory proteins. Asian Pac J Cancer Prev. 2021;22:2929-2938.
11. Rahmawati DR, Nurrochmad A, Jenie RI, Putri H, Sari DP, Meiyanto E, 2023. The synergistic cytotoxic effect of pentagamavunon-1 (PGV-1) and curcumin correlates with cell cycle arrest to induce mitotic catastrophe in 4T1 and T47D breast cancer cells. Indones Biomed J. 2023;15:318-327.

12. Meiyanto E, Novitasari D, Utomo RY, Fatmawati S, Putri H, Murwanti R, Astari D, Sari N, 2020. Bioinformatic and molecular interaction studies uncover that CCA-1.1 and PGV-1 differentially target mitotic regulatory protein and have a synergistic effect against leukemia cells. Indones J Pharm. 2020;11:145-158.
13. Schumacher JM, Golden A, Donovan PJ, 1998. AIR-2: An Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in Caenorhabditis elegans embryos. J Cell Biol. 1998;143:1635-1646.
14. Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, Nigg EA, Gerloff DL, Earnshaw WC, 2004. Borealin. J Cell Biol. 2004;166:179-191.
15. Novitasari D, Kato J, Ikawati M, Meiyanto E, 2023. PGV-1 permanently arrests HepG2 cells in M phase and inhibits liver carcinogenesis in DMH-induced rats. J Appl Pharm Sci. 2023;8:204-211.
16. Moordiani M, Novitasari D, Susidarti RA, Putri H, Meiyanto E, 2023. Curcumin analogs PGV-1 and CCA-1.1 induce cell cycle arrest in human hepatocellular carcinoma cells with overexpressed MYCN. Indones Biomed J. 2023;15:141-149.
17. Qin XY, Gailhouste L, 2021. Non-genomic control of dynamic MYCN gene expression in liver cancer. Front Oncol. 2021;10:618515.
18. Joukov V, De Nicolo A, 2018. Aurora-PLK1 cascades as key signaling modules in the regulation of mitosis. Sci Signal. 2018;11:eaar4195.
19. Sardjiman SS, Reksohadiprodjo MS, Hakim L, Noor Z, Tanjung M, Yuwono M, 1997. 1,5-Diphenyl-1,4-pentadiene-3-ones and cyclic analogues as antioxidative agents: synthesis and structure-activity relationship. Eur J Med Chem. 1997;32:625-630.
20. Mosmann T, 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55-63.
21. Debacq-Chainiaux F, Borlon C, Pascal T, Royer V, Eliaers F, Ninane N, Carrard G, Friguet B, de Longueville F, Boffe S, Remacle J, Toussaint O, 2005. Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. J Cell Sci. 2005;118:743-758.
22. Jeyaprakash AA, Klein UR, Lindner D, Ebert J, Nigg EA, Conti E, 2007. Structure of a Survivin-Borealin-INCENP core complex reveals how chromosomal passengers travel together. Cell. 2007;131:271-285.
23. Lestari B, Utomo RY, 2022. CEP55 inhibitor: extensive computational approach defining a new target of cell cycle machinery agent. Adv Pharm Bull. 2022;12:191-199.
24. Humphrey W, Dalke A, Schulten K, 1996. VMD: visual molecular dynamics. J Mol Graph. 1996;14:33-38.
25. Phillips JC, Hardy DJ, Maia JDC, Stone JE, Ribeiro JV, Bernardi RC, Buch R, Fiorin G, Hénin J, Jiang W, McGreevy R, Melo MCR, Radak BK, Skeel RD, Singharoy A, Wang Y, Roux B, Aksimentiev A, Luthey-Schulten Z, Kalé LV, Schulten K, Chipot C, Tajkhorshid E, 2020. Scalable molecular dynamics on CPU and GPU architectures with NAMD. J Chem Phys. 2020;153:044130.
26. Jo S, Kim T, Iyer VG, Im W, 2008. CHARMM-GUI: A web-based graphical user interface for CHARMM. J Comput Chem. 2008;29:1859-1865.
27. Lee KH, 2010. Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach. J Nat Prod. 2010;73:500-516.
28. Wulandari F, Ikawati M, Widyarini S, Meiyanto E, 2023. Tumor-suppressive effects of curcumin analogs CCA-1.1 and pentagamavunone-1 in colon cancer: *In vivo* and *in vitro* studies. J Adv Pharm Technol Res. 2023;14:317.
29. Meiyanto E, Putri H, Larasati YA, Murwanti R, Sardjiman S, Fitriasari A, Kusmardi W, Utomo RY, 2019. Anti-proliferative and anti-metastatic potential of curcumin analogue, pentagamavunon-1 (PGV-1), toward highly metastatic breast cancer cells in correlation with ROS generation. Adv Pharm Bull. 2019;9:445-452.
30. Hanahan D, 2022. Hallmarks of cancer: new dimensions. Cancer Discov. 2022;12:31-46.
31. Sherr CJ, DePinho RA, 2000. Cellular senescence. Cell. 2000;102:407-410.
32. Amend SR, Torga G, Lin K, Kostecka LG, Wajapeyee N, Van Houten B, Pienta KJ, 2019. Polyploid giant cancer cells: unrecognized actuators of tumorigenesis, metastasis, and resistance. Prostate. 2019;79:1489-1497.
33. Venook AP, Papandreou C, Furuse J, de Guevara LL, 2010. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. Oncologist. 2010;15(Suppl 4):5-13.
34. Zeng H, Zheng R, Guo Y, Zhang S, Zou X, Wang N, Zhang L, Tang J, Chen J, Wei K, Huang S, Sun K, He J, Gu X, Jia L, Yang X, Li H, Chen W, 2015. Cancer survival in China, 2003-2005: a population-based study. Int J Cancer. 2015;136:1921-1930.
35. Zhang X, Yao J, Li X, Zhang D, Gao Y, Ding Y, Han C, 2023. Targeting polyploid giant cancer cells potentiates a therapeutic response and overcomes resistance to PARP inhibitors in ovarian cancer. Sci Adv. 2023;9:eadf7195.
36. Li L, Li D, Tian F, Zhang X, Ji S, Deng H, Zhang Y, 2016. Hepatic loss of Borealin impairs postnatal liver development, regeneration, and hepatocarcinogenesis. J Biol Chem. 2016;291:21137-21147.
37. Yue L, Li L, Li D, Ji S, Zhang Y, Deng H, 2017. High-throughput screening for Survivin and Borealin interaction inhibitors in hepatocellular carcinoma. Biochem Biophys Res Commun. 2017;484:642-647.
38. Gohard FH, St-Cyr DJ, Tyers M, Heck MM, Earnshaw WC, 2014. Targeting the INCENP IN-box-Aurora B interaction to inhibit CPC activity *in vivo*. Open Biol. 2014;4:140163.
39. Sun M, Veschi V, Bagchi S, Scher D, Annunziata M, Zhou W, Lee DW, Bollard CM, Wayne AS, Cripe TP, Houghton PJ, 2019. Targeting the chromosomal passenger complex subunit INCENP induces polyploidization, apoptosis, and senescence in neuroblastoma. Cancer Res. 2019;79:4937-4950.